1994 ANNUAL PROGRESS REPORT

concerning

COLONIAL BENTGRASS (AGROSTIS TENUIS) SIBTH.

BREEDING AND CULTIVAR DEVELOPMENT

Submitted by:

Dr. Bridget Ruemmele
Assistant Professor
Turfgrass Improvement

Dr. Joel Chandlee
Assistant Professor
Molecular Biology

University of Rhode Island

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EXECUTIVE SUMMARY

1994 Annual Colonial Bentgrass Progress Report

Principle Investigators: Dr. Bridget Ruemmele
Dr. Joel Chandlee

Technical Support: Ms. Pei-Yu Zeng
Graduate Research Assistant

Research Period: 1 November 1993 to 31 October 1994

Accessions continue to be added to the current germplasm collection. Four private companies have been actively involved with cooperative acquisition and assessment of materials introduced from this program.

More than 600 plants were set in the field in fall 1993, including up to five clones of each accession. A cold, snowy winter and cold spring stressed young plants set in the field in late fall. Most survived the stress, despite their late establishment. These plants were evaluated for growth characteristics. Seed collection was not possible with most accessions in spring 1994. Additional material was propagated for greenhouse assessment and planting in the adjacent field plots in 1994.

Ms. Pei-Yu Zeng, an M.S. degree student, conducted preliminary and advanced greenhouse screening trials for Rhizoctonia sp. (brown patch) resistance in bentgrasses. From a start of eight strains of this fungus, the three most virulent strains were determined for use in the larger screening of the entire germplasm collection later in the year. Differences among the fungal strains were confirmed by Polymerase Chain Reaction (PCR) technology.

Cooperative collection efforts with private industry are forging associations which will enhance cultivar distribution of improved materials. Progeny from 69 initial collections planted for turf trial evaluation last fall in Rhode Island showed varied degrees of establishment and survival this spring, as well as possible resistance to weed invasion. The highest rated material has been planted in polycross this fall for seed production next spring in Oregon. This material was also included in the brown patch screening in Rhode Island.

Molecular efforts have included successful preparation of both creeping and Colonial bentgrass in tissue culture suitable for gene transfer. We are currently trying to determine which genes are available and useful for this process.

Additional support was provided by private turf industries, plant royalties, the State of Rhode Island, and Federal Hatch funding.

I. INTRODUCTION

The Colonial bentgrass breeding and cultivar development program was initiated by Emeritus Professor C. R. Skogley. Dr. Skogley received partial support for his breeding efforts from the USGA prior to the arrival of Dr. Bridget Ruemmele. After Dr. Ruemmele's arrival, the USGA increased its financial support beginning 1 February 1993 to encourage greater efforts in developing new Colonial bentgrass cultivars. The USGA was instrumental in providing access to germplasm from Dr. William Rumball's program in New Zealand from seed sent directly from New Zealand and plantings maintained at Rutgers University by Dr. Reed Funk. Primary emphasis of the project is improving Rhizoctonia spp. (brown patch) resistance, with additional goals to develop cultivars with improved cold hardiness, darker green leaf color, low maintenance requirements (reduced cultural inputs including fertilizers, pesticides, water), close mowing tolerance, recuperative ability and wear tolerance, retention of desired turf-type characters (fine texture, density, uniformity, evenness of growth), and hybridization with related species to improve traits noted above.

II. TECHNICAL SUPPORT PERSONNEL

Dr. Bridget Ruemmele, an assistant professor, was hired April 1991 to continue the program initiated by Dr. C. R. Skogley. She has a three-way appointment in research, extension and teaching with primary emphasis on plant improvement. Her approximate effort on this project is 20%.

Dr. Joel Chandlee, an assistant professor with research and teaching efforts in molecular biology, devotes approximately 5% effort on this project.

Ms. Pei-Yu Zeng began as a technical assistant in early 1993. She officially became a Master's student of this project in Fall 1993 when her support from USGA funding also began, continuing for one year. Due to regulations regarding College tuition funding, Ms. Zeng's assistantship technically came from USDA funds. Three other full-time turf employees who would normally be paid from the USDA funds received partial payment from the USGA grant in an amount equivalent to Ms. Zeng's stipend. The College of Resource Development provided her tuition. Her research concentrates on brown patch screening in relation to cultivar improvement of Colonial bentgrasses. Ms. Zeng is supported for the 1994-95 school year by a Graduate Research Assistantship from the University of Rhode Island College of Resource Development. At the present time, her efforts on the project are approximately 75 percent.

Ms. Stephanie Legare was hired fall 1994 to replace Ms. Grace Wojcik and Ms. Kirsten Thornton. Ms. Legare is employed for 40 hours per week on USGA funding. Her responsibilities include bentgrass germplasm and plot establishment and maintenance, record- keeping, data collection and processing. Her effort on this project is 100 percent.

Ms. Grace Wojcik provided technical assistance to the breeding program for several summers preceding the employment of Dr. Ruemmele. Ms. Wojcik continued working with Dr. Ruemmele for the past 3 1/2 years, including transplanting and maintaining germplasm field space plantings and greenhouse collections, seed harvesting, and data collection. She has received support from private product testing and was on USGA funding for 28 hours per week for approximately 1 year from fall 1993 through summer 1994. Her effort on this project ranged from approximately 25 to 75 percent, depending on other personnel employed at any given time to assist with the project.

Ms. Kirsten Thornton was an undergraduate plant science student on USGA funding for the spring semester in 1994. Her primary assistance was with germplasm maintenance and propagation. She also assisted with data collection. Her effort on bentgrass research was 100 percent for a maximum of 8 hours per week.

Mr. Greg Fales, Mr. Donald Timpson, and Mr. Barry Prefontaine provide technical support in the field. Mr. Fales oversees all operations, including additional student labor to maintain turf plots and prepare additional space for field plantings. Mr. Timpson assists Mr. Fales. Mr. Prefontaine is a full-time mechanic who has been instrumental in constructing a wear machine for use in turf trials. He also provided expertise in getting the polyhouse functional for increased greenhouse work as well as maintaining all turf equipment and the field irrigation system. Mr. Timpson spent numerous hours this past winter in repair of the polyhouse which suffered from neglect prior to my arrival in Rhode Island. These employees are supported by the State of Rhode Island. The combined effort of field support personnel on bentgrass research was 20%.

Ms. Lisa Rowley is employed by Dr. Noel Jackson, U.R.I. turf pathologist, who has graciously permitted Ms. Rowley to assist in preparing materials for the brown patch screening efforts and providing assistance as needed to Ms. Zeng in conducting her brown patch screening. Ms. Rowley's M.S. from the University of Massachusetts dealt with this disease. Her efforts on the bentgrass project are intermittent, making assigning a percentage difficult. She is supported by private contributions.

Ms. Jane Knapp is employed by Dr. Chandlee as a research assistant. She has successfully prepared tissue cultures of Colonial and creeping bentgrass for gene introduction through molecular techniques. Her efforts on the bentgrass project are also intermittent, making assigning a percentage difficult. Ms. Knapp is supported by the University of Rhode Island.

III. ADDITIONAL FUNDING

This project has received support from several programs and members of the turf industry. In addition to the USGA grant, a $5,000 Faculty Development Grant from the University of Rhode Island was used to support Ms. Sardha Suryapperuma's efforts in the summer of 1992 and for germplasm collection trips in New England and New York.

USDA, private turf companies, and College of Resource Development funding have provided summer employment for three high school students in 1992, two students in 1993, and three students in 1994. Part of their efforts included planting, plot maintenance and seed harvesting bentgrasses.

As noted under the technical support section, the State of Rhode Island supports several field personnel as well as the salaries of Dr. Ruemmele and Dr. Chandlee. State funding also provides facilities and money for facility upkeep,

Federal Hatch funds are used primarily for equipment acquisition and maintenance and supplies.

The New England Golf Course Superintendents' Association has enthusiastically supported construction of a USGA specification green. Although initially planted to creeping bentgrasses, we hope to test Colonial bentgrasses in the future as they near release.

Numerous private turf-related industries support the University turfgrass research program through product testing or direct grants. This support enables us to employ needed personnel as well as to acquire equipment and supplies.

Royalties from previous cultivar releases provide similar benefits afforded by industry support. The University of Rhode Island

expects the hiring of Dr. Ruemmele will increase funding from this source.

In addition to monetary support, donations supplement turfgrass research. These include plot maintenance equipment, growth media for greenhouse testing, fertilizers and seed for greenhouse and field plantings, and pesticides.

The National Plant Germplasm System of the United States, which supports plant explorations, will be approached for funding Colonial bentgrass collection in Eastern Europe. As additional grant opportunities become available, appropriate funding requests will be prepared.

IV. PROGRESS AND RESULTS

A. GERMPLASM ACQUISITION

Primary emphases in the initiation of the program included assessment of existing germplasm and acquisition of additional plants to increase the germplasm base. Existing material came primarily from Dr. Skogley's collection efforts in New England. The collection included 175 bentgrasses classified as 'Colonial'. Additional plants were obtained from the New Zealand collections of Dr. William Rumball and collection trips from Maine to Pennsylvania and all states in between except Vermont. Much area remains to be covered, including collections further south along the East Coast of the United States where higher humidity and heat conditions may be more favorable for obtaining brown patch resistant Colonial bentgrasses. The sites of origin in Europe could also provide beneficial breeding material.

Since arriving at the University of Rhode Island, Dr. Ruemmele has conducted several collection trips to golf courses, cemeteries, parks, old home lawns, and roadsides. At most sites, there was no

known overseeding done within the past thirty years, or in many cases, ever. The variability in material was encouraging. Some collections may actually be hybrids between bentgrass species.

One goal for the first year of this research was to acquire germplasm from native or naturalized sites as well as from the New Zealand collection located at Rutgers University. Material at Rutgers was evaluated three times in 1993. Selections were plugged from the site twice for evaluation in Rhode Island, with many currently planted in field space plantings as well as maintained in a greenhouse collection.

Including all species collected (bentgrasses and fine fescues), from 1 to 143 collections were obtained per site. The following list indicates the number of sites sampled within each state:

Connecticut	25
Maine	12
Massachusetts	23
New Hampshire	2
New York	13
Pennsylvania	2
Rhode Island	38

Greenhouse facilities were greatly expanded with the addition of approximately 1000 square foot polyhouse almost exclusively used for the turfgrass improvement program. This has facilitated the ability to increase plantings from seed or vegetative propagules for field and greenhouse evaluations as well as providing a location for maintaining the germplasm collection. Most recently acquired germplasm has been propagated into deepots for preservation of purity. This material was increased in 1993 and 1994 for field planting and greenhouse disease screening, both of which began in late fall 1993 and continue to the present time.

B. FIELD EVALUATION

Goals include continued field screening of current space plant or close-seeded plot germplasm not assessed for at least two years for desirable turf traits and establishing space plant progeny rows and close-seeded plots of seed harvested from advanced selections in 1991 or earlier. Close-seeded plots are maintained under conditions simulating ultimate management practices and evaluated for quality as well as disease resistance.

Initial germplasm provided by Dr. Skogley has been evaluated for seed production, leaf color and texture, and growth habit. Natural infestations of brown patch have been lacking in the field the past two years, making greenhouse screening efforts and field inoculations more critical. The entire space planting was relocated in the summer of 1992 to make room for a new USGA specification green. Seed harvested from the original site was accumulated from two or more years' harvests to provide enough seed for turf plot evaluations. Three selections planted in turf trials in 1992 continue to be evaluated. One is more promising than the other two. Additional seed was harvested in 1993 and 1994 from selected original space planting material for use in progeny space plantings and close-seeded plots.

Cooperative collection efforts with private industry are forging associations which will enhance cultivar distribution of improved materials. One association has already produced progeny from 69 initial collections (Appendix 1). Seed planted in the fall of 1993 at the University of Rhode Island for turf trial evaluation in close-seeded plots survived a cold and snowy winter with varied degrees of success. Differential infestation of crabgrass is currently under assessment for potential allelopathy. The highest quality plants were planted in polycross nurseries in Oregon in 1994. Vegetative material from each plot has also been screened for brown patch resistance in greenhouse screening procedures.

The goal to complete single and polycrosses and self pollination of selected superior genotypes in the first year was not entirely successful. Single and self pollinations were unable to be completed due to limited labor at time of pollination. Some flowering occurred during the winter in the greenhouse. Selected materials were bagged together for cross-pollination (Appendix 2). This seed will be planted in late 1994. Controlled pollinations will continue in 1995.

One of the most severe droughts in the region occurred during the summer of 1993. The non-irrigated space plantings from the original bentgrass collection were examined for tolerance to this drought. Selections were grouped into one of three categories: low, medium, or high drought resistance. One group of each classification, containing three to five parents each, was established in a polycross nursery for seed production 1994. Progeny will be evaluated for brown patch resistance and potential association to drought resistance.

Germplasm collected since 1991 was increased vegetatively for field space planting. Some plugs were planted, but the bulk of the material awaited preparation of adequate land (1-2 acres) to accommodate them. The site was finally ready for planting in fall 1993. More than 600 plants were set in the field, including up to five clones of each accession. These plants will be evaluated for growth characteristics and seed collection. Additional material was propagated for greenhouse assessment and planting in the adjacent field plots in 1994. Another severe summer of high heat and low moisture in 1994 further stressed plants from the original bentgrass collection by Dr. Skogley, as well as stressing the new plantings from the fall of 1993. Differential responses to these stresses were noted among both groups of bentgrasses.

Re-treatment of the original plants was a part of the second experiment (Appendix 5). Despite good mycelial growth in the second experiment, heat may have been a greater factor than the fungus in killing plants in this experiment. Three isolates produced more fungal growth than the others.

Four isolates produced the most plant damage in experiment three, with one superior to the other three (Appendix 6). Nine bentgrass clones were used in this experiment.

The fourth preliminary experiment (Appendix 7) included 27 bentgrass clones. Isolates 4, 5, and 8 produced the most severe damage to grasses.

Based on the four preliminary experiments, isolates 4, 5, and 8 were selected for advanced brown patch screening. The first three advanced experiments used either 33 or 36 bentgrass clones. Each plant was inoculated with 10 seeds of infected perennial ryegrass and covered by plastic covers for the next 96 hours. Quality notes were recorded to determine the degree of plant damage caused by the disease. Plants with the most resistance were retained for planting in field trials. Approximately 130 additional Colonial bentgrasses are currently undergoing or about to undergo the brown patch screening process.

D. VERIFICATION OF FUNGAL ISOLATES AND THEIR UNIQUENESS

The fungus was re-isolated from inoculated plants to confirm infection by the original strains. Treatments 1, 2, 6, 7, and 8 were collected from infected blades of three bentgrass clones used in experiment three. Grass blades were cut into small pieces and sterilized with 10% bleach before culturing on potato dextrose agar medium. After retransfers on 13 and 14 April, pure re-isolates were obtained, confirming disease transmission onto grasses.

C. GREENHOUSE EVALUATION

During the first year, plans included conducting greenhouse screening of current germplasm for brown patch resistance. Ms. Pei-Yu Zeng, a Master's Degree student, officially began working with this project in fall 1993. Her thesis concentrates on Rhizoctonia sp. (brown patch) resistance screening in bentgrasses.

Plants were increased between 1992 - 1994 for screening as well as field planting (Photographs 1 through 5). Four replications were used in brown patch screening for most accessions.

The greenhouse expansion has facilitated the ability to increase plantings from seed or vegetative material for field and greenhouse evaluations as well as providing the requisite area for doing the actual greenhouse screening. Seed collected in 1993 was germinated in 1994 from 57 bentgrass selections for progeny evaluations.

Eight isolates of Rhizoctonia solani (Appendix 3) were incubated for inoculating greenhouse materials beginning in late 1993 through early 1994 using infected grains as the inoculum source. A meeting among Dr. Ruemmele, Ms. Zeng, and Ms. Rowley in November established the inoculation procedure and timing for screening bentgrasses.

Ms. Zeng conducted four preliminary experiments using 8 to 13 genotypes inoculated with each of the eight brown patch isolates plus a control that was not inoculated (Appendices 4 through 7). Each treatment was replicated four times in each experiment.

The first experiment determined that more inoculum than originally planned may be necessary to induce enough disease for plant resistance assessment (Appendix 4). One isolate was most severe, with three others in the second most severe group.

Re-treatment of the original plants was a part of the second experiment (Appendix 5). Despite good mycelial growth in the second experiment, heat may have been a greater factor than the fungus in killing plants in this experiment. Three isolates produced more fungal growth than the others.

Four isolates produced the most plant damage in experiment three, with one superior to the other three (Appendix 6). Nine bentgrass clones were used in this experiment.

The fourth preliminary experiment (Appendix 7) included 27 bentgrass clones. Isolates 4, 5, and 8 produced the most severe damage to grasses.

Based on the four preliminary experiments, isolates 4, 5, and 8 were selected for advanced brown patch screening. The first three advanced experiments used either 33 or 36 bentgrass clones. Each plant was inoculated with 10 seeds of infected perennial ryegrass and covered by plastic covers for the next 96 hours. Quality notes were recorded to determine the degree of plant damage caused by the disease. Plants with the most resistance were retained for planting in field trials. Approximately 130 additional Colonial bentgrasses are currently undergoing or about to undergo the brown patch screening process.

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Polymerase chain reaction (PCR) was conducted to confirm the identity of the inoculum and re-isolations as the same, as well as to determine that the eight strains were different from each other. Three protocol were used, with success occurring only by the third procedure described below. Since the sequence of the fungal genome was unknown, several primers were used to obtain the most complementary types, including: UBS: 302 (80% GC), 317 (70%), 347 (60%), 338 (70%), 336 (70%), 359 (60%), 326 (50%), 384 (70%), OPA17, and OPA18.

Results with UBS 331 and 338 showed that the re-isolates and isolates of the three worst strains of brown patch fungi were identical to each other respectively. All other primers did not produce distinct bands. Some DNA samples produced clear samples, but this did not occur consistently with all samples. Attempting to improve the PCR method, DNA samples were cleaned using the spermidine ppt method. A set of increasing DNA concentrations were tested, including: 20ng, 30ng, 45ng, 75ng, and 90ng per 4.5ug DNA samples. The primer used was POA17. The clearest bands were obtained using the 90ng concentration.

Additional isolates may be sought from Drs. Phil Colbaugh and Henry Wilkinson, turf pathologists with collections of particularly virulent Rhizoctonia spp.

E. MOLECULAR EVALUATION

Random Amplified Polymorphic DNA (RAPD) technology was used to analyze numerous genomic DNA preparations. The objective was to identify genetic markers which could be used to 'fingerprint' plants and assist breeding efforts by identifying genes associated with morphological or physiological traits. Initial molecular identification screening was difficult due to contamination in field plots. Fine fescues provided clearer results and have become the basis for the Ph.D. of Ms. Sardha Suryapperuma, who conducted this research. Another research assistant in Dr. Chandlee's lab,

Ms. Jane Knapp, has successfully prepared both creeping and Colonial bentgrass tissue cultures suitable for gene transfer via bombardment. We are currently trying to determine available and suitable genes for this process.

F. USGA SPECIFICATION GREEN

The New England Golf Course Superintendents' Association has enthusiastically supported construction of a USGA specification green. Although initially planted to creeping bentgrasses, we hope to test Colonial bentgrasses in the future as they near release.

V. FUTURE WORK PLANNED

1. Continue acquisition of germplasm from native or naturalized sites, particularly along the Southeast U.S. coastline. A cooperative trip in New England with a breeder from a private company is scheduled for the first week of November 1994. Work cooperatively with Dr. Bill Rumball, who has contacted this program regarding this matter. Plan germplasm collection trip to Eastern Europe -- a private company has expressed interest in a European collection trip. Germplasm acquisition is critical to obtain the most diverse representation of available material. Expanding the germplasm base increases the opportunity to improve Colonial bentgrasses, particularly with respect to brown patch resistance.

2. Continue field screening of current and additional space plant or close-seeded plot germplasm not assessed for at least two years for desirable turf traits. The best plots will be selected for expanded trials and/or going back to original parental material to use for further species improvement in single or multiple crosses.

3. Establish space plant progeny rows and close-seeded plots of seed harvested from pollination and advanced selections chosen in the previous year. Conduct greenhouse screening of germplasm not

previously screened for brown patch resistance. Additional beneficial greenhouse and laboratory screening for desirable genotype improvement will be incorporated as they become available. Progeny selected from space plantings, close-seeded plots, or screening procedures will be placed in polycross nurseries or isolated for self or single outcrosses for seed production. Greenhouse-screened material will also be field planted if grown from seed which was never grown under field conditions.

4. Complete single and polycrosses and self pollination of additional selected superior genotypes. Continue screening for brown patch resistance and plant in field trials for turf characteristic evaluation.

5. If enough seed is available, establish regional turf trials of advanced superior genotypes.

By the completion of the third year, additional brown patch resistant material present in the URI collection will be determined in addition to previously screened material. Advanced material currently in field production will be thoroughly evaluated and documented for turf potential. Additional seed harvests from current plantings will be used to initiate new cycles of plot evaluations. Selections deemed advanced enough to warrant seed production trials will be sent to Oregon. New plant acquisitions will be subjected to the rigors of evaluation to continue the process of cultivar enhancement.

VI. EXPENDITURES

As of my last report from the URI accounting office regarding officially recorded expenditures, the following are indicated. Additional money has been encumbered, but not recorded as spent for the labor of Ms. Legare. This lack of recording is due to the

slow process of completing paperwork at this University.

Ms. Zeng's Graduate Research Assistant Stipend (exchanged with another employee on USDA funding as indicated in section II; 93-94 academic year)	$9,550	
Ms. Grace Wojcik, hourly salary	7,200	
Ms. Kirsten Thornton, hourly salary	2,800	
Employee benefits	3,914	
Salaries and benefits		$23,464
Office expenses	9	
Greenhouse building supplies	500	
Greenhouse and field supplies	195	
Operating expenses		704
Germplasm collection trips (encumbered, actual not submitted to date)		312
University overhead		5,425
Total committed for first two years		29,905
Total remaining for second year		11,095

Due to payroll procedures, I cannot employ Ms. Legare for longer than 4 months at a time. Her current 4-month encumbrance was not recorded as of the printing date of the current expenses. For the period between 09-26-94 and 02-01-95 she will receive approximately $5,100 in salaries and benefits. I expect to expend similar amounts to continue her employment or find a suitable replacement at the end of the current period.

Appendix 1. 9 October 1993 Colonial Bentgrass Seeding. Two foot X two foot plots.

Plant	No. plots	Seed wt./plot (g)	July 94 * = best
304	2	3.55	4
2827	2	4.34	4
2828	2	3.88	5
2829	2	4.40	6
2830	2	3.72	5
2831	2	3.71	5
2832	1	4.56	5
2833	1	6.88	6
2834	1	6.66	6
2835	1	4.24	4
2836	1	5.21	4
2837	1	3.62	8
2838	1	6.06	4
2839	1	3.84	6
2840	1	3.38	4
2841	2	3.62	6
2842	1	3.68	5
2843	1	6.89	5
2844	1	4.08	5
2845	1	4.48	5
2846	3	3.98	5
2847	2	4.87	5
2848	1	6.67	4
2849	3	4.48	6
2850	2	4.48	4
2852	1	3.01	4
2853	2	4.49	5
2854	2	5.14	5
2855	1	2.82	5
2856	1	5.04	5
2857	2	5.13	5
2858	1	6.96	5
2859	1	2.66	5
2860	1	2.02	5
2861	1	6.49	5
2862	1	4.76	4
2863	1	0.90	4
2864	1	3.39	4
2865	2	3.98	6
2866	1	3.47	7
2867	2	4.20	5
2868	1	5.22	5
2869	1	6.17	5
2902	1	3.82	5
2903	1	4.32	5

2904	1	6.80	5
2905	2	3.64	6
2922	2	5.16	6
2923	3	3.76	5
2924	1	4.39	4
2925	1	3.50	4
2926	1	4.65	4
2927	1	3.64	4
2928	1	5.35	5
2929	1	4.54	5
2930	1	3.94	5
2931	1	5.08	5
2932	2	3.45/4.07	5
2933	1	1.80	5
2934	1	6.73	7
2935	1	2.99	4
2936	1	5.87	6
2937	2	3.88	5
2938	1	2.11	4
2939	1	2.48	4
2940	1	0.54	3
2941	1	2.16	4
2942	1	4.64	4
2943	1	5.54	4

Appendix 2. Cross-pollinations attempted in greenhouse during spring 1994. Diallel groupings, '*' = pollination attempted.

	NORWALK VILLAGE GREEN 11	BP POLO 1ST 5	BERGEN PT L1 2	4 BR 1564 1	URI 92-32 4
NORWALK VILLAGE GREEN 11	*	*	*	*	
BP POLO 1ST 5	*	*	*	*	
BERGEN PT L1 2	*	*	*	*	*
4 BR 1564 1	*	*	*	*	*
URI 92-32 4			*	*	*

	BRANFORD CT CEM 3	URI 92-17 2	URI 92-17 3	BRANFORD CT CEM 13	BP POLO 1ST 9
BRANFORD CT CEM 3	*	*	*	*	*
URI 92-17 2	*	*	*	*	*
URI 92-17 3	*	*	*	*	
BRANFORD CT CEM 13		*	*	*	
BP POLO 1ST 9		*	*		

	BP POLO 1ST 6	BERGEN PT L1 5	NORWALK VILLAGE GRN 10	86 BR 1627 1	EAST GREENW. CC POND	BERGEN PT L1 4
BP POLO 1ST 6	*	*	*	*	*	*
BERGEN PT L1 5	*	*	*			
NORWALK VILLAGE GRN 10	*	*	*		*	*
86 BR 1627 1	*			*	*	*
EAST GREENW. CC POND	*		*	*	*	*
BERGEN PT L1 4			*	*		*

	URI 92-14 1	BERGEN PT L1 3	SPRING-GROVE CEM 1	CEM 4 WOODS-HOLE	URI 92-14 3	4 BR 1564 2
URI 92-14 1	*	*		*	*	
BERGEN PT L1 3	*	*	*			
SPRING-GROVE CEM 1		*	*			
CEM 4 WOODS-HOLE	*			*		
URI 92-14 3					*	
4 BR 1564 2		*				*

	JAMESTOWN CEM 138 1	URI 92-18 1	JAMESTOWN CEM 138 2	URI 92-14 4	BERGEN PT L1 1
JAMESTWN CEM 138 1	*			*	
URI 92-18 1		*	*		*
JAMESTWN CEM 138 2	*	*	*		
URI 92-14 4		*	*	*	
BERGEN PT L1 1		*			*

	URI 92-22 4	URI 92-15 2	URI 92-15 1	URI 92-32 4	URI 92-32 5
URI 92-22 4	*	*		*	
URI 92-15 2	*		*		*
URI 92-15 1		*			
URI 92-32 4	*				
URI 92-32 5		*			

	NEWPORT CC 27	BRANFORD CT CEM 11	BRANFORD CT CEM 4
NEWPORT CC 27	*	*	*
BRANFORD CT CEM 11	*	*	*
BRANFORD CT CEM 11	*	*	*

	URI 92-15 3	URI 92-32 2
URI 92-15 3	*	*
URI 92-32 2	*	*

	BRANFRD RD CT CEM 14	BP POLO 1ST 10	URI 92-17 4	NEWPORT CC 27	BERGEN PT L1 6	SPRING-GROVE CEM 5
BRANFRD RD CT CEM 14	*		*	*		
BP POLO 1ST 10			*			
URI 92-17 4	*	*				
NEWPORT CC 27	*			*	*	*
BERGEN PT L1 6	*			*	*	
SPRING-GROVE CEM 5				*		

URI 92-17 * URI 92-14 2

BRANFORD CT CEM 2 * CEM WOODSHOLE 1

SPRINGGROVE CEM N. CT 5 * CEM WOODSHOLE 5

Appendix 3. Cultures of Rhizoctonia solani used in brown patch screening in greenhouse.

Eight isolates of Rhizoctonia solani obtained January 19, 1994 from the collection of Dr. Noel Jackson:

Isolate	Group	Grass source	Collection site
1	AG1 (?)	creeping bentgrass	Penncross sod
2	AG1 (?)	creeping bentgrass	Wallace's sod
3	AG2-2	velvet bentgrass	Turf farm
4	AG1 (?)	Kentucky bluegrass	New England Turf
5	AG1 (?)	perennial ryegrass	Segregansett G.C.
6	AG2-2	creeping bentgrass	Turf farm
7	AG1 (?)	unknown	Turf farm
8	AG1 (?)	tall fescue, cv. ISI-ATK	URI NTEP plots

Cultures of each isolate were grown in 90mm petri dishes containing 10 ml sterilized potato dextrose broth. After retransfers on 19, 22, 28 January and 4 February, pure cultures with no contamination were obtained.

Appendix 4. Summary for first preliminary experiment of brown patch screening.

1. Thirty-six plants each of eight bentgrasses were propagated 27 December 1993. They include:
   a) URI 92-2
   b) BAR FRIDAY 5
   c) NEWPORT CC 20
   d) 25
   e) URI 92-35 17
   f) 14-1
   g) URI 92-14
   h) 1137 BR 1445

2. Eight isolates of Rhizoctonia solani were inoculated in 250 ml erlenmeyer flasks containing sterilized perennial ryegrass seeds and distilled water (1:1 ratio) at room temperature 19 February.

3. Grasses were cut to 1 cm 19 February and arranged in a randomized complete block design with four plants per treatment. Treatments included the control or one of the eight isolates noted in Appendix 1. After inoculation with six perennial ryegrass seeds per plant, plants were placed inside plastic bags which was then sprayed 10 times with a hand mist sprayer. Bags were sealed to maintain high humidity and placed under the greenhouse bench during the day to avoid overheating. Bags were placed atop benches during the night.

4. By 1 March, mycelia grew well on the grass blades, yet the plants were still green. Twenty-two hours after bagging, plants were removed from the bags. Some leaves turned brown, but crown were not killed. Two of the four replications were re-bagged to assess for additional damage.

5. Two weeks after treatment, the following results were noted:
   a) treatment 7 caused the most damage, with treatments 4, 5, and 8 the next worst, in order from most to least.
   b) grass leaves were damaged; crowns were not damaged.
   c) all grasses began to recover one week after inoculation.
   d) plants of replications 1 and 2 were more damaged than in replications 3 and 4, indicating the longer bagging induced more damage.

6. Conclusions: More inoculum per plant and/or longer coverage after inoculation may be advantageous.

Appendix 5. Summary for second preliminary experiment of brown patch screening.

1. The following 13 bentgrasses were cloned 36 times 21 February:
   a) URI 92-20
   b) CAPE COD SALT POND 3
   c) BGB 4
   d) NEWPORT CC 5
   e) HIGH FAIRWAY 16
   f) 1 BR 1566 2
   g) MT GROVE CEM STRATFORD 5
   h) NEWPORT CC 36
   i) VB 92-1 20
   j) MEADOWEDGE 5
   k) OAK HILL 90/22/92
   l) 5849 BR 159 2
   m) 17

2. The eight plants treated in the first preliminary experiment recovered and were retreated as a part of this second preliminary experiment.

3. Eight isolates of Rhizoctonia solani were inoculated in 250 ml erlenmeyer flasks containing sterilized perennial ryegrass seeds and distilled water (1:1 ratio) at room temperature 17 March.

4. Grasses were cut to 1 cm 1 April and arranged in a randomized complete block design with four plants per treatment. Treatments included the control or one of the eight isolates noted in Appendix 1. After inoculation with eight perennial ryegrass seeds per plant, plants were placed under plastic covers made to fit the plant trays. From 1-4 April, plants were placed under the greenhouse benches.

5. Mycelia grew well on the plants, with some lesions forming on leaves. Despite the damage, plants retained green color.

6. On 5 April, plants were returned to benches. Although the soil was still wet, plants wilted. The concern was that the plastic covers allowed temperatures to become excessive. Covers were removed to allow grass recovery.

7. Summary: Treatments 2, 4, and 5 caused more damage than other treatments or the control, although the most damage apparently resulted from excessively high temperatures.

Appendix 6. Summary for third preliminary experiment of brown patch screening.

1. Nine bentgrasses were cloned 36 times 28 February.

2. Eight isolates of Rhizoctonia solani were inoculated in 250 ml erlenmeyer flasks containing sterilized perennial ryegrass seeds and distilled water (1:1 ratio) at room temperature 17 March.

3. Grasses were cut to 1 cm 8 April and arranged in a randomized complete block design with four plants per treatment. Treatments included the control or one of the eight isolates noted in Appendix 1. After inoculation with eight perennial ryegrass seeds per plant, plants were placed under plastic covers made to fit the plant trays. During the day, plants were placed under the greenhouse benches to avoid overheating.

4. Covers were removed 11 April, 84 hours after treatments were initiated. Control plants were in good condition, indicating damage resulted from the inoculum rather than heat stress. Treatment 5 inoculum was contaminated by other fungi and had to be disregarded.

5. Conclusions: The amount of inoculum and treatment duration are acceptable. Treatment 8 caused the most damage, followed by treatments 2, 6, and 7, in order.

Appendix 7. Summary for fourth preliminary experiment of brown patch screening.

1. The following 27 bentgrasses were cloned 36 times 5 March:
   a) URI 92-20
   b) 105 BR 1296 10
   c) NEWPORT CC 114
   d) 105 BR 1296 5
   e) NEWPORT CC 26
   f) URI 92-21 4
   g) URI 92-23 4
   h) URI 92-34 1
   i) 104 BR 1296 7
   j) URI 92-20 1
   k) URI 92-19 3
   l) URI 92-24 6
   m) LIDO CC L1 3
   n) 105 BR 1296 8
   o) NEWPORT CC 29
   p) URI 92-20 4
   q) NEWPORT CC 113
   r) NEWPORT CC 30
   s) BERGEN PT LI 2
   t) 105 BR 1296 6
   u) BRANFORD CT CEM 6
   v) URI 92-22 2
   w) URI 92-19 1
   x) 105 BR 1296 4
   y) URI 92-22 4
   z) 104 BR 1296 3
   aa) NEWPORT CC 112

3. Eight isolates of Rhizoctonia solani were inoculated in 250 ml erlenmeyer flasks containing sterilized perennial ryegrass seeds and distilled water (1:1 ratio) at room temperature 11 April.

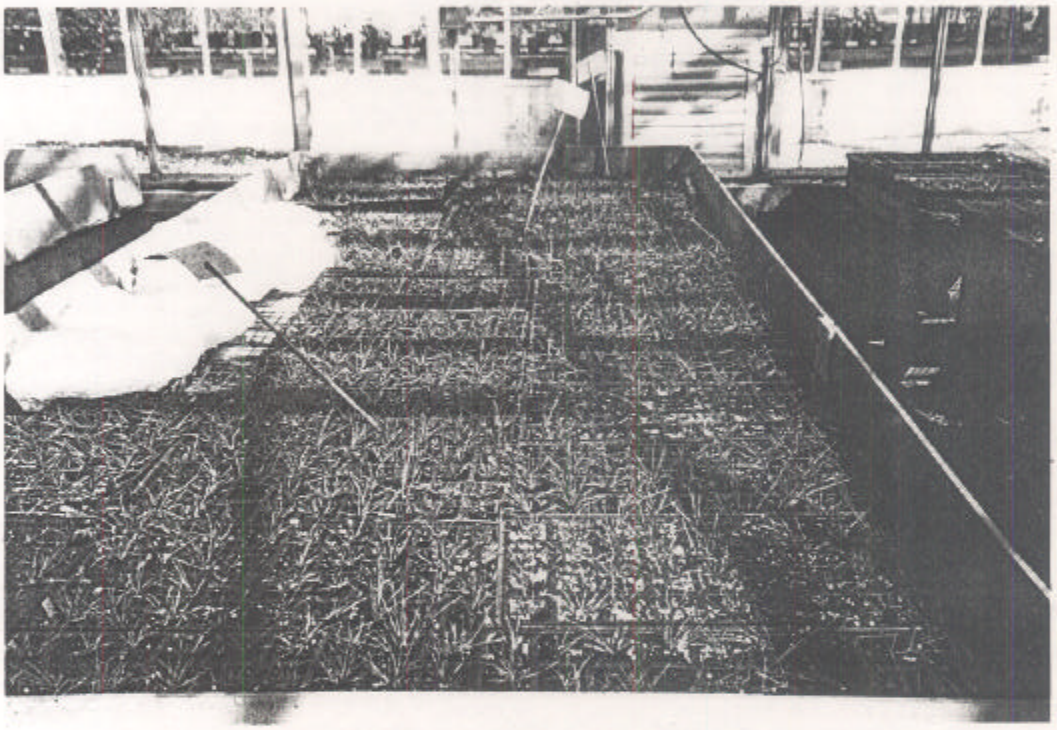
3. Grasses were cut to 1 cm 29 April and arranged in a randomized complete block design with four plants per treatment. Treatments included the control or one of the eight isolates noted in Appendix 1. After inoculation with eight perennial ryegrass seeds per plant, plants were placed under plastic covers made to fit the plant trays at 0700.

4. Covers were removed 3 May, 84 hours after initiation of treatments.

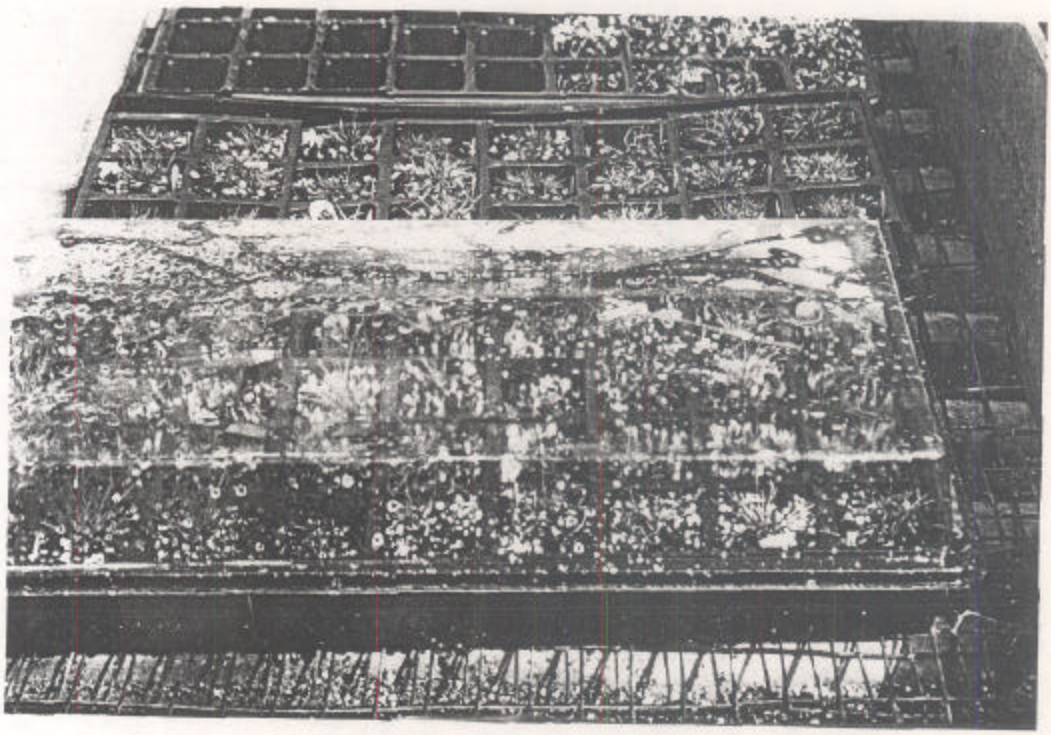
5. Plants were scored for quality every 1 or 2 days.

Conclusions of preliminary testing for three most damaging brown patch isolates:

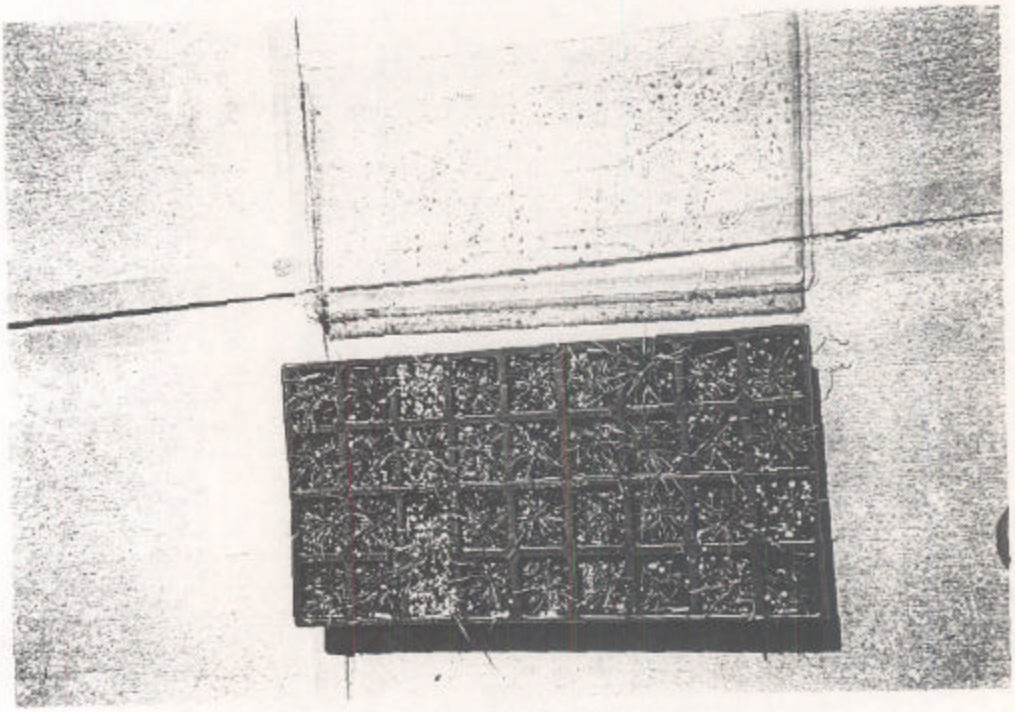
1. **#4 = Kentucky bluegrass from New England Turf.**
2. **#5 = perennial ryegrass from Segregansett G.C.**
3. **#8 = tall fescue from URI NTEP plots**



Photograph 1. Bentgrass clones during establishment phase prior to inoculattion.

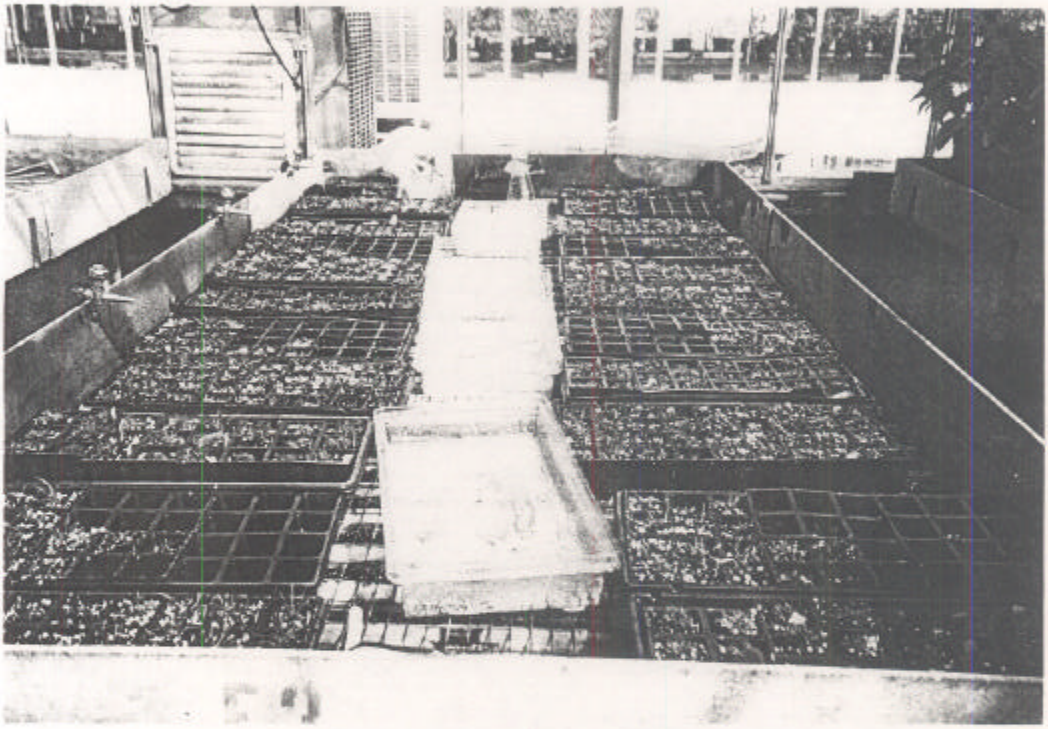


Photograph 2. Treated bentgrass plants sealed by plastic covers during period of disease induction.



Photograph 3. Control plants after disease induction phase. Plants were undamaged, including any damage possible due to high heat.

Photograph 4. Bentgrass plants damaged by brown patch fungus, prior to recovery phase.



Photograph 5. Bentgrass plants in recovery phase after receiving brown patch treaments.