ANNUAL PROGRESS REPORT TO USGA TURFGRASS RESEARCH COMMITTEE

1993 PROJECT YEAR

Project Title: Investigation of Turf Disease Decline for Potential Development of Biological Control Methods

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EXECUTIVE SUMMARY

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Public concerns regarding the environmental and human health impact of chemical pesticide usage has led to increasing restrictions on the use of these materials. This situation requires a shift in emphasis from chemical control toward alternative disease control methods. This project is aimed at developing biocontrol of turfgrass patch diseases using microbial antagonists of the pathogens. The project is based on the hypothesis that sites where disease has occurred but has subsequently declined or disappeared (particularly under conditions otherwise favorable to the pathogen) may indicate the activity of natural antagonists of the pathogen, and therefore be a source of potential biocontrol agents from among the natural microbial antagonist population.

We previously reported on inhibition of growth of *R. solani* and *S. rolfsii* by bacteria and fungi isolated from disease "decline" sites. This past year these microorganisms were tested for the ability to inhibit the growth of *L. korrae* in culture: 12 bacteria inhibited growth of *L. korrae* more than 40%. Inhibition of growth could indicate sensitivity of *L. korrae* to antibiotics produced by the test microorganism or more successful competition for nutrients by the test microorganism. Although 32 test fungi inhibited radial growth of L. korrae, their relatively rapid growth interfered with a clear demonstration of antibiosis in these tests. The role of competition was addressed by inclusion of several growth media. Although not a sure indication of biocontrol ability, inhibition of growth in culture tests serve to prioritize microorganisms for further testing of their biocontrol potential.

Our priority for this past year has been to test the biocontrol agents for control of spring dead spot (*L. korrae*) of bermudagrass and brown patch (*R. solani*) and southern blight (*S. rolfsii*) of perennial rye in the greenhouse. The results of these experiments are inconclusive due to lack of uniform disease throughout the experiments. In last year's annual report, there was an indication that one of the bacterial biocontrol agents, JA78, appeared to enhance growth of perennial rye. Additional experiments monitoring dry weight of grass clippings at weekly intervals have shown no growth enhancement by this or any other biocontrol agent tested.

One field test to control spring dead spot has been performed. The site contained bermudagrass that was naturally infected with *L. korrae* (spring dead spot). Treatments were a bacterial biocontrol agent (JT80), a fungal biocontrol agent (KA159), a combination of JT80 and KA159, and three chemical fungicides (Rubigan, Lynx, and Bayleton). The number of infected areas, total area infected and health (0 - 5 rating) showed no significant differences at α=0.05. Lynx at both rates did show significantly better recovery (0 - 5 rating) than the control or the biocontrol agents at α=0.05.

Background. Public concerns regarding the environmental and human health impact of chemical pesticide usage has led to increasing restrictions on the use of these materials. This situation requires a shift in emphasis from chemical control toward alternative disease control methods. This project is aimed at developing biocontrol of turfgrass patch diseases using microbial antagonists of the pathogens. Microbial antagonists include organisms that limit the growth, reduce the population, or interfere with the disease-causing ability of a pathogen. The project is based on the hypothesis that sites where disease has occurred but has subsequently declined or disappeared (particularly under conditions otherwise favorable to the pathogen) may indicate the activity of natural antagonists of the pathogen, and therefore be a source of potential biocontrol agents from among the natural microbial antagonist population.

Laboratory experiments with potential biocontrol agents. Last year (1992 Annual Report) we reported on inhibition of growth of *R. solani* and *S. rolfsii* by bacteria and fungi isolated from disease "decline" sites. This past year these microorganisms were tested for the ability to inhibit the growth of *L. korrae* in culture. In these experiments, 12 bacteria and 32 fungi inhibited growth of *L. korrae* more than 40% (Tables 1a and 1b). Inhibition of growth could indicate sensitivity of *L. korrae* to antibiotics produced by the test microorganism, more successful competition for nutrients by the test microorganism, or predation/parasitism in the case of test fungi (for rapidly growing test fungi, a clear demonstration of antibiosis is difficult in these tests). The role of competition was addressed by inclusion of several growth media: PDA, a relatively rich medium; CMA, a relatively poor medium; PAF, for production of bacterial siderophores (iron scavenging compounds); PAF + $FeCl_3$ to eliminate growth inhibition due to unavailability of iron due to bacterial siderophores. Differences in radial growth of *L. korrae*, in the presence of test microorganisms, between PDA compared to CMA, or PAF compared to PAF + $FeCl_3$, would suggest a possible involvement of competition for nutrients; however, antibiotic production or sensitivity might be influenced by the growth medium.

Microorganisms that exhibit antagonism of a pathogen in culture will not necessarily be effective biological control agents. On the other hand, potentially effective biological control agents may not demonstrate the antagonism of a pathogen under the particular cultural conditions used for the laboratory experiments. With this in mind, however, inhibition of growth in culture tests serve to prioritize microorganisms for further testing of their biocontrol potential.

Greenhouse experiments. Our priority for this past year has been to test the biocontrol agents for control of spring dead spot (*L. korrae*) of bermudagrass and brown patch (*R. solani*) and southern blight (*S. rolfsii*) of perennial rye in the greenhouse. The results of these experiments are inconclusive due to our inability to induce uniform disease throughout our experiment. Considerable effort has gone into solving the problems and we now believe we are in a position to obtain conclusive results.

In last year's annual report, there was an indication that one of the bacterial

biocontrol agents, JA78, appeared to enhance growth of perennial rye. Additional experiments monitoring dry weight of grass clippings at weekly intervals have shown no growth enhancement by this or any other biocontrol agent tested.

Field experiment. One field test to control spring dead spot has been performed. The site contained bermudagrass that was naturally infected with *L. korrae* (spring dead spot). Treatments were a bacterial biocontrol agent (JT80), a fungal biocontrol agent (KA159), a combination of JT80 and KA159, and three chemical fungicides (Rubigan, Lynx, and Bayleton). In this initial field trial, biocontrol agents were limited to one bacterium, one fungus and the combination of the two to work out procedures and solve unforseen problems prior to setting up a more complex field experiment. No. infected areas, total area infected and health (0=poor to 5=healthy) showed no significant differences at α=0.05 (Table 2). Lynx at both rates did show significantly better recovery (0=none to 5=highly recovered) than the control or the biocontrol agents at α=0.05 (actually, the difference between Lynx and JA80 treatments was just less than significant, LSD=1.836). The biocontrol agents in this trial were applied in a manner similar to fungicide application. In an effort to enhance their effectiveness, future experiments with biocontrol agents will include multiple applications and nutrients to increase biocontrol activity.

Table 1a. Results of laboratory tests for inhibition of growth of *Leptosphaeria korrae* in culture by test bacteria isolated from spring dead spot "decline" site. Media used were potato dextrose agar (PDA), corn meal agar (CMA), Pseudomonas agar F (PAF) and PAF + 500 μM $FeCl_3$. Test microorganisms were placed at four spots, 4 cm from a central plug of *L. korrae* mycelium. Diameter of initial plug of *L. korrae* was subtracted from colony diameters measured 10 days after beginning of experiment to determine radial growth relative to control colony diameter; a colony with the same colony diameter as the control is indicated below as 1.00.

Test bacterium	Colony diameter (relative to control)			
	PDA	CMA	PAF	PAF+Fe
JT1	1.00	0.96	0.74	0.53
JT2	0.88	0.96	0.68	0.12
JT3	0.83	0.84	0.47	0.35
JT4	0.96	0.92	0.53	0.59
JT5	1.00	0.76	0.63	0.62
JT6	0.46	0.44	0.79	0.65
JT8	0.67	0.84	0.68	0.76
JT9	0.79	0.84	0.84	1.00
JT10	0.79	0.80	0.79	1.06
JT11	1.00	0.96	0.74	0.53
JT13	0.88	0.96	0.89	0.94
JT14	0.71	0.96	0.74	0.74
JT15	0.83	0.96	0.84	1.00
JT16	1.00	0.96	0.95	1.00
JT17	0.83	0.96	0.95	0.76
JT19	0.79	0.94	0.58	0.06
JT21	0.88	0.96	0.84	0.82
JT22	0.88	0.88	0.68	0.82

JT23	0.79	0.96	0.63	0.82
JT25	0.46	0.20	0.21	0.12
JT26	0.83	0.82	0.71	0.65
JT28	1.00	0.96	0.28	0.82
JT34	0.79	0.96	0.84	0.65
JT35	0.38	0.32	0.37	0.24
JT37	0.67	1.00	0.58	0.38
JT39	1.00	0.96	0.79	0.82
JT41	0.88	0.96	1.00	1.06
JT43	0.79	0.96	0.84	0.53
JT44	1.00	0.96	0.95	0.94
JT46	1.00	1.00	0.95	0.94
JT47	0.67	0.56	0.58	1.06
JT50	0.88	0.84	0.74	0.94
JT51	0.88	0.84	0.58	0.88
JT52	0	0.12	0.16	0.12
JT53	0.79	0.96	1.11	0.76
JT54	0	0.64	0.37	0.39
JT55	0.87	0.96	0.74	0.86
JT57	0.71	0.88	0.74	0.71
JT58	0.83	1.00	0.68	1.07
JT59	0.89	0.96	0.74	0.86
JT60	1.00	0.96	0.74	1.00
JT63	0.67	0.84	0.55	0.25
JT67	0.79	0.96	0.47	0.79
JT68	0.08	0.12	0.05	0.43

JT71	0	0.08	0.10	0.21
JT72	0.83	0.96	0.63	0.86
JT73	0.67	0.96	0.78	1.00
JT74	0.75	0.96	0.74	1.00
JT77	1.00	0.96	0.79	1.07
JT78	0.12	0.56	0.29	0.11
JT79	0.67	1.00	0.58	0.14
JT80	0	0.16	0.16	0
JT81	0.83	1.00	0.53	0.21
JT82	0.75	0.96	0.74	0.71
JT83	1.00	0.96	0.84	1.29
JT84	0.75	0.96	0.74	0.93
JT85	0	0.16	0.21	0.07
JT86	0.25	0.32	0.32	0.18
JT87	1.00	1.00	0.58	0.35
JT88	0.88	0.96	0.58	0.65
JT89	0.67	0.96	0.58	0.76
JT90	0.63	0.96	0.63	0.59
JT91	0.79	0.96	0.68	1.06
JT93	0.67	0.80	0.89	0.88
JT95	0.69	1.00	0.97	0.79
JT96	0.60	1.00	1.11	0.65

Table 1b. Same as Table 1a, but for test fungi isolated from spring dead spot "decline" site.

Test fungus	Colony diameter (relative to control)	
	PDA	CMA
KT3	0.82	0.82
KT4	0.55	0.72
KT5	0.77	0.83
KT6	0.54	0.32
KT7	0.79	1.03
KT8	0.71	0.85
KT9	0.35	0.54
KT10	0.59	0.33
KT11	0.68	0.74
KT12	0.65	0.82
KT13	0.73	0.85
KT14	0.67	0.74
KT15	0.15	0.82
KT16	0.59	0.79
KT17	0.74	0.82
KT18	0.85	0.90
KT19	0	0
KT20	0.15	0.13
KT21	0.09	0.13
KT22	0.35	0.51
KT23	0.71	0.82
KT24	0	0

KT25	0.33	0.11
KT26	0.58	0.38
KT27	0.74	0.67
KT28	0.50	0.67
KT29	0.06	0.05
KT30	0.83	0.94
KT31	0.15	0.36
KT32	0.12	0.33
KT33	0.09	0.08
KT34	0.09	0.13
KT35	0.06	0.05
KT36	0.12	0.18
KT37	0.25	0.26
KT38	0.85	0.87
KT39	0.27	0.28
KT40	0.21	0.25
KT41	0.25	0.21
KT42	0.21	0.85
KT43	0.21	0.30
KT44	0.67	1.02
KT45	0.35	1.02
KT46	0.60	0.55
KT47	0.83	0.31
KT48	0.58	1.00
KT49	0.82	0.90
KT50	0.83	1.02

KT51	0.35	0.34
KT52	0.87	1.02

Table 2. Results of spring dead spot trial, Miller residence, Fresno, CA, 1992-93. Treatments were arranged in a randomized complete block design, 4 replicate 10 x 10 ft plots per treatment. Rubigan (11.6% AS) was applied at 4 oz per 1000 sq ft; Lynx (25% DF) at 2 or 3 oz per 1000 sq ft; Bayleton (25% DF) at 4 oz per 1000 sq ft. Biocontrol agents, *Pseudomonas* JT80 and *Trichoderma* KA159, were applied as an aqueous suspension, 2 gallons per plot, at the rate of 10^{12} cfu of JT80 or $3x10^{10}$ conidia of KA159 per plot.

	Means			
Treatment	No. infected areas	Total area infected (%)	Health	Recovery
Control (non-treated)	5.000	11.250	3.000	1.000
Rubigan	7.188	32.500	2.000	1.750
Lynx (2 oz/1000 sq ft)	4.000	21.250	3.000	2.250
Lynx (3 oz/1000 sq ft)	4.938	23.750	2.750	3.250
Bayleton	4.250	23.750	3.125	3.250
JT80	5.500	16.250	2.625	1.500
KA159	4.000	25.000	2.625	1.000
JT80 + KA159	4.025	32.500	1.750	1.000
LSD (α=0.05) for column	3.985	26.372	1.547	1.836