Biological Control of Turf Pests: Isolation and Evaluation of Nematode and Bacterial Pathogens

This research has centered on the isolation of new strains and species of entomopathogenic nematodes and bacteria, evaluation of these new isolates against Japanese beetle and masked chafer larvae, and characterization of those with the greatest activity. Since the general public, golf course superintendents, and governmental agencies all favor increased utilization of reliable biological agents, the thrust of this project has been well received. This research is a cooperative effort between the U. S. Golf Association, Green Section Research and a team of USDA, Agricultural Research Service and University entomologists/nematologists in Ohio, New Jersey, and California.

The emphasis during the first years of this project has been on the isolation of new organisms. We successfully recovered new strains of the two major genera of entomopathogenic nematodes from all three states. Results from our laboratory examinations and field plots have shown that several of these new strains are more effective in controlling Japanese beetle and masked chafer larvae than were nematodes which were commercially available. As a result, these nematodes have been licensed to industry for future commercial development. Rutgers University and the USGA will share royalties from this work. Other nematodes isolated by this team are being shared with industry under biological transfer agreements, and continue to be utilized in molecular biology studies on the taxonomy of entomopathogenic nematodes in the U.S., Argentina, and Ireland.

We have also been successful in isolating bacteria from Japanese beetle and masked chafer larvae with signs and symptoms of amber disease. Bacteria causing this disease in the New Zealand grass grub, have been commercially developed in that country. Over 75 bacterial isolates have been obtained from the digestive tracts of scarab larvae in Ohio, West Virginia, Indiana, and California utilizing a selective media. More than 35 isolates have been identified as being in the same genus as the casual agent of amber disease. About half of these isolates appear to be closely related to the NZ pathogen. A complete characterization of all the organisms isolated in this project is continuing. Preliminary information indicate that larvae feeding in inoculated soil died more quickly than those in untreated soil. In addition, signs of amber disease were present in test larvae, and bacteria were reisolated from cadavers.

During the remainder of this project we will establish the identity and pathogenicity of nematode and bacterial isolates already obtained. We will also continue alliances with commercial partners to make promising pathogens available to all.

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Annual Report for USGA Turfgrass Research Project Biological Control of Turf Pests: Isolation and Evaluation of Nematode and Bacterial Pathogens

M. G. Klein, R. Gaugler, & H. K. Kaya

Progress

During the past year, Specific Cooperative Agreements between the USDA-ARS and Rutgers University, and The University of California, Davis have been renewed and the funds for research at those two institutions have been transferred. All but $1000 of the money for both Rutgers and UC Davis has been made available to those institutions. Since those Specific Cooperative Agreements have been funded to their maximum level, the additional money will be made available for supplies through Wooster. Because of the long delays in getting those Cooperative Agreements implemented, the Agreements will not terminate until August of 1994. As noted before, the USDA - USGA CRADA supporting this project is set to expire January 31, 1994. Because no funds were available for the Wooster location in the first year of the project, the money was needed to cover Specific Cooperative Agreements, it would be desirable to extend the CRADA, with no additional funds, to the end of the fiscal year in October, 1994, or at least to the termination of the SCAs in August of that year.

Nematodes - The results from the survey and field trials in New Jersey have been published in the Journal of Economic Entomology and in Biological Control. Copies of those publications have previously been shared with USGA. Laboratory bioassays and small scale field tests have shown that a New Jersey isolate of Steinernema glaseri is superior to the strains previously available. Agreements were signed between Rutgers University and biosys to allow experimental production of selected strains. Those production runs were very successful, and provided large quantities of nematodes for use by members of this team, as well as other interested scientists. Additional negotiations between Rutgers and biosys have resulted in a license for the future commercialization of those nematodes. Arrangements were worked out to provide the USGA with part of the royalties from this license. Heterorhabditid nematodes isolated in New Jersey will be shared with Ecogen under a Biological Transfer Agreement.

Nematodes which were isolated in California have been made available to biosys for experimental production and storage in liquid nitrogen. California considers the nematodes to be in the public domain, and has no plans for licensing them at this time.

Nematodes from Ohio are still in storage at Wooster and may be transferred to biosys for storage while tests on their pathogenicity continue. During the past year additional isolates

of both heterorhabditid and steinernematid nematodes have been obtained from scarab larvae Ohio. The Ohio heterorhabditids are providing key information in basic studies conducted in Ireland on the taxonomy of nematodes utilizing molecular biology techniques. Results from this work were presented by our Irish collaborator at a recent Meeting of the Society for Invertebrate Pathology. A manuscript on this taxonomic work is also being prepared and will be shared with the USGA in the near future.

Numerous field trials were conducted this past year with the S. glaseri from biosys. Results from both large and small plot test in New Jersey, California, and the Azores (treated by Drs. Klein and Kaya) are not yet available, but will be finalized in the next few weeks. Three tests have been completed in Ohio this fall. The S. glaseri nematodes gave excellent control (70-80%) of Japanese beetle and northern masked chafer larvae in replicated $9m^2$ plots at two locations. These results were equivalent to those obtained with the standard insecticide (Turcam). In addition, we had 35% control of Japanese beetle larvae in a large ($840m^2$) unreplicated field plot.

Bacteria - Since the start of this project we have isolated 47 strains of bacteria from Japanese beetle larvae with signs and symptoms of amber disease. In addition, 5 isolates have been obtained from the northern masked chafer, Cyclocephala borealis

in Ohio and Indiana, and 23 from masked chafers, C. hirta, in California. We have attempted to identify these bacteria in a number of different ways. They were isolated on a Serratia selective media developed in New Zealand. However, it appears that only about half of them are in the genus Serratia, which contains the bacteria that cause amber disease in the NZ grass grub. We have employed the use of the Enterotube II system of identification for our cultures and found that 44 isolates came out as Serratia species. The remaining bacteria were all Enterobacteriacaea in the genera Acinetobacter, Enterobacter, Escherichia, Klebsiella, Providencia, Salmonella, Shigella, and Yersinia. This system is designed for identification of human pathogens and does not include the species causing amber disease in New Zealand. We have also recorded the reactions of our bacteria on DNAse Agar, Adonitol Agar and Itaconate Agar to further characterize the isolates and compare them with the New Zealand Serratia. Results of all these tests, as well as hosts, isolation dates and location, are summarized in a table which accompanies this report.

We have recently obtained and set up another diagnostic tool to help us characterize the bacteria obtained in this project. Biolog(R) utilizes a series of 95 (compared to 15 for Enterotube) biochemical reactions in a standardized cell well. Results are entered into an accompanying computer program, and bacteria are identified. So far, there has been fair agreement between the

Enterotube and Microlog identifications. Although S. entomophila, the causative agent of amber disease, is included in this data bank, we have not yet been able to identify any of our isolates as S. entomophila. It is likely that any amber disease causing bacteria isolated here may be new and different species than those from New Zealand.

We have tested several of the isolates characterized as being Serratia for pathogenicity against late 2nd and early 3rd instar Japanese beetle larvae in the laboratory. Cultures were prepared by adding bacteria to nutrient broth, incubating for two days, and then mixing the broth and bacteria in sterile soil. Larvae placed in the soil were examined for mortality and symptoms of amber disease. In the first bioassay, larvae feeding in six of nine Serratia inoculated soils died more quickly than those fed in soil with nutrient broth only, or in untreated soil. We also noted signs and symptoms of amber disease in test larvae, and Serratia were reisolated from cadavers. In the second bioassay, only one of ten Serratia cultures resulted in increased mortality. However, Serratia were reisolated from cadavers showing amber disease signs. Confirmation of these results and feeding tests against masked chafers will be conducted when larvae become available. We will also be looking for a more sensitive test to indicate when larvae cease feeding.

In addition to the isolates from the United States, we were able to obtain several bacteria from soil in China, and a white grub (Anomala sp.) in Japan. These have been identified as Serratia spp., Klebseilla sp. and Enterobacter. All of these isolates will be further characterized with the Biolog system and those confirmed as Serratia will be tested for pathogenicity.

The preliminary results of the bacterial isolation, identification, and pathogenicity were presented by Dr. Klein at the SIP Meeting in Ashville during August and the abstract of that talk (Klein and Kaya, 1993) was published in the Meeting Program. The support of the USGA was acknowledged during that presentation.

Proposed Research

Nematodes - Only limited activity is planned with nematodes between now and the end of this project. Information on the effects of fall applications of S. glaseri will be obtained from field plots recently established in California, New Jersey, and the Azores. We will also obtain results from other researchers who are conducting field tests with the S. glaseri which has been licenced to biosys. This information will be critical in obtaining commercial production of this nematode. In addition,

laboratory evaluations will continue on newly isolated nematodes from scarab larvae in the field. Efforts will also continue to interest industry partners in licensing and producing the most promising nematode isolates.

Bacteria - The major effort during the remainder of this project will focus on obtaining a more complete identification of those bacteria now identified as Setrratia. A newly obtained bacterial identification system - Biolog, is now up and running and will be utilized to further characterize these bacteria. As in this past year, emphasis will be placed on establishing the pathogenicity of isolates identified as being close to Serratia entomophila or S. proteamaculans. Those isolates obtained from masked chafers will be tested against both the northern masked chafer in Ohio, and C. hirta in California. Larvae will be reared from eggs in the laboratory to provide susceptible 2nd instar larvae. We will also be evaluating a number of bioassay methods to try and develope a more reliable method for testing bacteria against Japanese beetle and masked chafer larvae.

1 November 1992 - 31 October 93 Expenditures

During the past year, Dr. M. G. Klein, Research Entomologist, USDA-ARS, Wooster, Ohio has spent approximately 10% of his time on this project in preparing yearly CRIS and USGA Reports, overseeing the research conducted in Ohio, and facilitating transfer of funds to Rutgers and UC Davis.

Specific co-op agreement with Rutgers, University	8/31/93	$4850
Specific co-op agreement with Univ. California, Davis	7/31/93	4650
Biological safety cabinet	8/31/93	1310
Broadform co-op agreement with OSU	10/25/93	17490
Total costs for the year		$28300

BACTERIAL ISOLATES FROM SCARAB LARVAE 1990 - CURRENT

ISOLATE	HOST	LOCATION	ISO DATE	ENTEROTUBE II RESULTS	DNA	AD	ITAC	COLOR
*BHK-1	C. hirta	Calif	10/17/90	S. marcescens	+	+y	-	cream
BMK-1	JB	storage	12/18/90	A cinetobacter				cream
BMK-2A	JB	storage	12/18/90	Yersinia ; Shigella				yellow
BMK-2B	JB	storage	12/18/90	Shigella ; Enterobacter				cream
BMK-3	JB	storage	12/18/91	(isolate lost)				
*BMK-4	C. borealis	storage	12/18/91	S. liquefaciens ; Salmonella ; Shigella ; Yersinia	-	+b/g	-	cream
GBMK-1A	JB	storage	05/29/91	Klebsiella	+	+y	+	cream
*GBMK-1B	JB	storage	05/29/91	S. liquefaciens	+	+y	+	cream
*GBMK-2	JB	storage	05/29/91	S. liquefaciens ; Salmonella	+	+b/g	+	cream
*GBMK-3A	JB	storage	05/29/91	S. liquefaciens ; Escherichia ; Salmonella	+	+b/g	-	cream
GBMK-3B	JB	storage	05/29/91	Klebsiella	+	+y	+	cream
*GBMK-4	JB	Ohio	05/29/91	S. liquefaciens	+	+y	+	cream
GBMK-5	JB	Ohio	05/29/91	(isolate lost)				
GBMK-6A	C. borealis	Ohio	10/02/91	(isolate lost)				
GBMK-6B	C. borealis	Ohio	10/02/91	(isolate lost)				
*GBMK-30	JB	W.Va	??	S. liquefaciens ; Salmonella ; Shigella ; Yersinia	+	+b/g	+	cream
GBMK-34	JB	W.Va	??	Klebsiella	+	+y	+	cream
GBHK-1	C. hirta	Calif	10/02/91	Shigella ; Enterobacter				yellow
GBHK-2A	C. hirta	Calif	10/02/91	Salmonella	+	+b/g	+	cream
GBHK-2B	C. hirta	Calif	10/02/91	(isolate lost)				
*GBHK-3	C. hirta	Calif	10/02/91	S. liquefaciens ; Salmonella	+	+b/g	-	cream
GBJB-A	JB	W.Va	10/02/91	Shigella ; Enterobacter				cream
*GBJB-B	JB	W.Va	10/02/91	S. liquefaciens ; Enterobacter	+	+b/g	+	cream
*GBJB-C	JB	W.Va	10/02/91	S. liquefaciens	+	+y	+	cream
*GBG-1	JB	W.Va	??	S. liquefaciens ; Escherichia	+	+y	+	cream
CH-2	C. hirta	Calif	??	Enterobacter				cream
CH-3	C. hirta	Calif	??	Enterobacter				cream
WCCH-A	C. hirta	Calif	10/02/91	A cinetobacter				yellow
*WCCH-B	C. hirta	Calif	10/02/91	S. marcescens	+	+y	+/-	cream
WCCH-C	C. hirta	Calif	10/02/91	Salmonella				cream
*WCCH-D	C. hirta	Calif	10/02/91	S. marcescens	+	+y	+	cream
*WCCH-E	C. hirta	Calif	10/02/91	S. liquefaciens ; Salmonella	+	+b/g	+	cream

ISOLATE	HOST	LOCATION	ISO DATE	ENTEROTUBE II RESULTS	DNA	AD	ITAC	COLOR
CJB-A1	JB	Ohio	10/02/91	Enterobacter				yellow
CJB-A2	JB	Ohio	10/02/91	Shigella ; Salmonella ; Klebsiella				cream
CJB-B	JB	Ohio	10/02/91	Yersinia ; Shigella	weak +	+b/g	+	yellow
*CJB-C	JB	Ohio	10/02/91	S. liquefaciens ; Salmonella	-	+b/g	-	cream
*C1	soil	China	07/11/92	S. marcescens	+	+y;+b/g	+	cream
C2	soil	China	07/12/92	Klebsiella	-			cream
C4	soil	China	07/17/92	Enterobacter ; Providencia				cream
*J1	white grub	Japan	08/04/92	S. liquefaciens	+	+y	-	cream
OA	C. hirta	Calif	09/08/92	S. marcescens ; S. liquefaciens	+	+y	-	cream
*OB-A	C. hirta	Calif	09/08/92	S. liquefaciens ; Salmonella	+	+y	-	cream
OB-B	C. hirta	Calif	09/08/92	S. marcescens ; S. liquefaciens	+	+y	-	cream
CMV-1A	C. hirta	Calif	10/27/92	(isolate lost)				
CMV-1B	C. hirta	Calif	10/27/92	S. liquefaciens ; Enterobacter	+	+y	-	cream
CMV-2	C. hirta	Calif	10/27/92	S. liquefaciens	-	+y	+	
CD-1A	C. hirta	Calif	10/27/92	S. liquefaciens ; Klebsiella	v.weak +	+b/g	+	cream
CD-1B	C. hirta	Calif	10/27/92	S. rubidea ; Klebsiella	weak +	+b/g	-	cream
CHR	C. hirta	Calif	09/16/92	Enterobacter				cream
CS	C. hirta	Calif	10/27/92	S. liquefaciens ; Salmonella	+	+y	-	cream
CM	C. hirta	Calif	09/16/92	Klebsiella				cream
MCFW-1	Cyclocephala	Indiana	09/09/92	Enterobacter				cream
MCFW-2	Cyclocephala	Indiana	09/09/92	Enterobacter				cream
*JBD	JB	diet exp	12/23/92	S. liquefaciens ; S. marcescens	+	-	+	red
*JBFW-1	JB	Indiana	09/09/92	S. liquefaciens	+	+y	+	cream
*JBFW-2	JB	Indiana	09/09/92	S. liquefaciens ; Salmonella	+	+y	+	cream
*JBFW-3	JB	Indiana	09/09/92	S. liquefaciens	+	+b/g	+	cream
*JBFW-4A	JB	Indiana	09/09/92	S. liquefaciens ; Salmonella	+	+b/g	+	cream
*JBFW-4B	JB	Indiana	09/09/92	S. liquefaciens ; Klebsiella	+	+b/g	+	red
*JBFW-5A	JB	Indiana	09/09/92	S. liquefaciens ; Salmonella	+	+b/g	+	cream
*JBFW-5B	JB	Indiana	09/09/92	S. liquefaciens ; Klebsiella	+	+b/g	+	red

ISOLATE	HOST	LOCATION	ISO DATE	ENTEROTUBE II RESULTS	DNA	AD	ITAC	COLOR
*JBT-1	JB	Ohio	03/08/93	S. liquefaciens	+	+b/g	-	cream
*JBT-2	JB	Ohio	03/08/93	S. marcescens	+	+y	-	cream
JBT-3	JB	Ohio	03/08/93	Enterobacter				cream
JBT-4	JB	Ohio	03/08/93	Enterobacter				cream
JBT-5	JB	Ohio	03/08/93	2K-1				cream
*JBT-6	JB	Ohio	03/22/93	S. liquefaciens ; Enterobacter	weak +	+y	+	cream
JBT-7	JB	Ohio	03/22/93	Enterobacter ; Providencia				cream
*JBT-8	JB	Ohio	03/22/93	S. liquefaciens	v.v. weak +	+y	+	cream
*JBT-9	JB	Ohio	03/22/93	S. liquefaciens ; S. marcescens	+	+y	-	cream
*JBT-10	JB	Ohio	03/22/93	S. liquefaciens	v.v.weak +	+y	-	cream
*JBS-1	JB	Ohio	04/26/93	S. liquefaciens	+	+y	+	cream
*JBS-2	JB	Ohio	04/26/93	S. liquefaciens	+	+y	+	cream
*JBS-3	JB	Ohio	04/26/93	S. marcescens	weak +	+y	+	cream
JB-A	JB	Ohio	06/04/93	Yersinia ; Enterobacter	+	+y;+b/g	+	cream
*JB-B	JB	Ohio	06/04/93	S. liquefaciens	+	+y;+b/g	-	cream
JB-C	JB	Ohio	06/04/93	S. liquefaciens	v.weak +	+y	_	cream
*MHJB	JB	Ohio	08/24/93	S. liquefaciens; S. marcescens	+	+y	+	rd/cr
*JBD-1	JB	lab reared	07/27/93	S. marcescens; Hafnia	+	+y	+	red

* = isolate has been used in feeding experiment

VKR 10/13/93

BIOASSAY #1 - Evaluation of feeding mortality on 2nd instar Japanese beetle larvae contained in Serratia spp. infested soil.

ISOLATE NAME	WEEK OF LAST DEATH OR PUPATION	TOTAL NO. OF LARVAL DEATHS	TOTAL NO. OF PUPATIONS	ENTEROTUBE® II IDENTIFICATION	MICROLOG® IDENTIFICATION
GBJB-B	16	10	0	S. liquefaciens	
GBMK-3A	18	10	0	S. liquefaciens	S. liquefaciens
GBG-1	22	8	1	S. liquefaciens	S. marcescens
GBMK-1B	22	10	0	S. liquefaciens	
GBMK-30	22	7	2	S. liquefaciens	S. liquefaciens/ grimesii
GBJB-C	22	10	0	S. liquefaciens	S. marcescens
NB control	23	9	1	S. liquefaciens	-----
H_2O control	24	10	0	S. liquefaciens	-----
GBMK-4	26	8	2	S. liquefaciens	S. marcescens
CJB-C	26	8	1	S. liquefaciens	
GBMK-2	27	8*	2	S. liquefaciens	S. liquefaciens/ grimesii

* 1 larva died from milky spore disease

First pupation - Week 17
Last pupation - Week 28

Larvae were field collected 2nd instars

6 out of 9 treatments died more quickly than nutrient broth, or untreated controls.

Serratia spp. was reisolated from cadavers exhibiting amber-disease symptoms.

Set up: 9/92
Terminated: 4/93
Total duration: 27 weeks

VKR 10/93

BIOASSAY #2 - Evaluation of feeding mortality on 2nd instar Japanese beetle larvae contained in Serratia spp. infested soil.

ISOLATE NAME	WEEK OF LAST DEATH OR PUPATION	TOTAL NO. OF LARVAL DEATHS	TOTAL NO. OF PUPATIONS	ENTEROTUBE® II IDENTIFICATION	MICROLOG® IDENTIFICATION
JBFW-1	10	3	7	S. liquefaciens	S. marcescens
H_2O control	12	10*	0		
JBT-2	12	9	0	S. marcescens	
JBT-1	14	1	8	S. liquefaciens	S. liquefaciens
C1-B	15	6**	3	S. marcescens	S. marcescens
NB control	17	6	4		
JBT-9	18	5	5	S. marcescens; S. liquefaciens	
JBD	22	8	1	S. marcescens; S. liquefaciens	S. marcescens
J1-B	24	5	5	S. liquefaciens	Klebsiella
JBFW-4A	25	5	5	S. liquefaciens; Salmonella	S. liquefaciens S. grimesii
JBFW-5A	in progress			S. liquefaciens; Salmonella	S. liquefaciens; S. grimesii
J1-A	in progress			S. liquefaciens	Klebsiella

* 2 larvae died from fungus
** 1 larva died from fungus

First pupation - Week 7
Last pupation - in progress

Larvae were lab reared 2nd instars

1 treatment died more quickly than the H_2O control
4 treatments + H_2O control died before nutrient broth control

Serratia spp. was reisolated from cadavers exhibiting amber-disease symptoms.

Set up: 4/93
Terminated: in progress
Total duration: in progress

VKR 10/93