USDA, Rutgers University, University of California

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

INVESTIGATORS:

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CLIMATIC REGION: Cool Humid
USGA REGION: Northeastern/Mid-Atlantic/Western

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

The objectives of this project are to obtain new strains and species of entomopathogenic nematodes and bacteria, and to characterize those with the greatest activity against scarab larvae. The current need for better biological control agents for use against grubs such as the Japanese beetle and masked chafers creates an opportunity to licence promising new pathogens to industries for development and commercialization. This cooperative effort between the U. S. Golf Association and a team of U. S. Department of Agriculture and University scientists in Ohio, New Jersey, and California has generated interest from the media and resulted in increased visibility for the USGA's Environmental Research Program.

During the first two years, we have recovered more than 125 strains and potential new species of entomopathogenic nematodes. Four described species, and several possible new species have been isolated by both Ohio and New Jersey Scientists from golf course turf and scarab larvae. Additional strains and possible new species from the two major genera of entomopathogenic nematodes have been identified from California. Results from field plots in New Jersey and California indicated that recently isolated strains were more effective in controlling Japanese beetle and masked chafer larvae than were commercially available nematodes. The greater pathogenicity of the recent isolates may be due to an increase in the presence of the symbiotic, pathogenic bacteria in those strains. In addition, the new nematode isolates have proven useful in molecular biology studies on the taxonomy of entomopathogenic nematodes in the U.S. and Ireland.

Isolation of bacteria similar to those causing amber disease in New Zealand grass grub larvae has resulted in the discovery of over 35 strains of bacteria from Ohio, New Jersey, West Virginia, California, Japan and China. Fourteen isolates have been characterized in the same genus as the New Zealand bacteria. Feeding tests with those strains have been initiated against Japanese beetle larvae in the laboratory. Additional tests to identify recently isolated bacteria are underway.

Major emphasis during the next year will be to establish the identity and pathogenicity of nematode and bacterial isolates already obtained as a result of this project. In addition, efforts to obtain new isolates of both nematodes and bacteria from infected white grubs in golf course turf in Ohio, California, and New Jersey will continue.

Dr. Michael G. Klein
Dr. Randy Gaugler
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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, new Specific Cooperative Agreements between the USDA and Rutgers University, and The University of California, Davis have been established and the funds intended for those two institutions have been transferred. This project continues to draw interest from the news media. A recent press release in the USDA - ARS Quarterly Report has been picked up by several sources. Most recently it was reprinted in the Newsletter of the International Organization for Biological Control (IOBC). The efforts of the USGA to help control turf pests, now and in the future, is being recognized.

Nematodes - The results of the extensive New Jersey nematode survey conducted during 1991 have been summarized in a manuscript entitled "Large-scale inoculative releases of the entomopathogenic nematode Steinernema glaseri: assessment 50 years later" now accepted for publication in the journal Biological Control (abstact attached). Twenty four isolates of steinernematids and 42 of heterorhabditids were recovered. Heterorhabditis bacteriophora was the most common species found. In addition, 13 isolates of Steinernema glaseri, and four

each of S. carpocapsae and S. feltiae were recovered. Four isolates of Heterorhabditis spp., and two of Steinernema spp. are possibly new species since they did not match any known species description. There was no evidence that the extensive colonization efforts with this nematode during 1939-42 were successful.

Results of last years New Jersey field trials are reported in a second manuscript "Comparative evaluation of entomopathogenic nematode strains against Japanese beetle, Popillia japonica (Coleoptera: Scarabaeidae)" accepted for publication in the Journal of Economic Entomology (abstract attached). New isolates performed as well as, or better than, the chemical standard and commercially available nematodes. These results may be explained by the fact the New Jersey strain of S. glaseri used in the tests retained about four times more bacteria per infective juvenile, and that more nematodes retained bacteria when compared to the commercial strain of S. glaseri. No similar increase in bacterial association was noted with strains of H. bacteriophora.

Field tests conducted in 1992 produced similar results (Figure 1). The New Jersey strain, and a selected strain of S. glaseri gave control that was equal to the chemical standard, and better than commercially available S. glaseri. Additional tests

revealed that the presence or absence of irrigation was more critical than the time of day at which application occurred. If nematodes are to be utilized by golf course managers, it is important that application can occur over a broad time spectrum.

During the past year additional isolates of heterorhabditids have been obtained from Japanese beetle larvae in Ohio and Indiana, and steinernematids have been recovered from field infected larvae in Ohio, Indiana, and North Carolina. In addition, two new isolates of Heterorhabditis species have been recovered from Cyclocephala hirta and one from C. pasadenae in California. Field tests in California during the fall of 1992 with various nematodes have again demonstrated increased activity of recent isolates over those now in commercial production.

Nematodes discovered during this project have also proved useful in more basic studies on the taxonomy of nematodes utilizing molecular biology techniques. The Heterorhabditis isolate from Davis has been partially characterized using randomly amplified ploymorphic DNA (RAPD) analysis and found to be closely related to a Heterorhabditis species identified from Argentina. It is not identical and may represent a new species. Heterorhabditids from Ohio are being utilized in molecular biology studies in Ireland in an attempt to more accurately characterize H. megidis as a species. H. megidis was originally described from Japanese

beetle larvae in Ohio, and similar nematodes have subsequently been found in Europe. Our nematodes are proving pivotal in more clearly defining this species.

Bacteria - During the first year we isolated almost 20 strains of bacteria from Japanese beetle larvae with symptoms of amber disease. In addition, two isolates have been made from the northern masked chafer, C. borealis in Ohio, and 12 from masked chafers, C. hirta, in California. Although these bacteria were isolated on a media developed in New Zealand which is selective for species of Serratia, the cause of amber disease in the grass grub, only about half of the isolates appear to be in that genus. Utilization of the Enterotube II system of identification for Enterobacteriacaea found 14 isolates were Serratia species. This rapid identification system relies on a series of 15 biochemical reactions in a standardized, self contained, tube (see Appendix 1). Positive reactions to the series of media are recorded on a form as numerical values. Those values correspond to specific bacteria in an accompanying Computer Coding System. Since the species causing amber disease in New Zealand are not included in the coding system, additional diagnostic tests are needed. We have 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 those comparisons are not yet available. In addition

to the Serratia identified from dying insects, some of our isolates have been identified as species of Enterobacter, a bacterium commonly associated with insect intestinal tracts, Acinetobacter and Pseudomanas. Those 14 isolates characterized as being Serratia are being tested for pathogenicity against late 2nd and early 3rd instar Japanese beetle larvae in the laboratory. Bacteria have been added to nutrient broth, incubated for two days, and then mixed in sterile soil. Larvae placed in the soil are being examined for mortality and symptoms of amber disease. Results of these test are not available at this time.

During the past year, we have recovered additional isolates of bacteria from C. hirta in California, Japanese beetle in Ohio, soil in China, and a white grub in Japan.

Proposed Research

Nematodes - The primary emphasis during the coming year will be to establish the effectiveness of those nematodes already in culture against white grubs. Those nematodes which show the greatest activity in laboratory bioassays will be used in small plot field tests during the spring and fall of 1993. Efforts will continue to interest commercial nematode producers in our isolates. In addition, we will continue our isolation of new strains of both

heterorhabditids and steinernematids from soil and scarab larvae as outlined in our Research Proposal. We will pay particular attention to recovering nematodes from field infected Japanese beetles and masked chafers, and to isolate nematodes that may be active at cooler temperatures.

Bacteria - Emphasis during the next year will be placed on obtaining preliminary identifications of those bacteria collected in the past year, and on a more complete identification of those bacteria now identified as Setrratia. A combination of Serratia selective media, biochemical tests, and EM examinations will be used. In addition, heavy emphasis will be placed on establishing the pathogenicity of isolates identified as being close to Serratia entomophila or S. proteamaculans. Those isolates from masked chafers will be tested against either the northern masked chafer in Ohio, or C. hirta in California. Better bioassay methods will need to be developed for testing bacteria against Japanese beetle larvae. Standard techniques of feeding bacteria on carrot pieces have not been satisfactory. Isolation of new strains of bacteria from Japanese beetle and northern masked chafer in the Northeast, and masked chafer species in California will also continue.

1991-92 Expenditures

During the past year, Dr. M. G. Klein, Research Entomologist, USDA-ARS, Wooster, Ohio has devoted about 10% of his time to this project.

As of October 31, 1992 expenditures have been as follows:

Travel M. G. Klein to New Jersey	10/15/91	$ 205
Specific co-op agreement with Rutgers, University		
	9/24/91	5850
	2/15/92	5850
Specific co-op agreement with UC, Davis	9/26/91	4550
	4/07/92	5650
Rustrak Temperature Recorder	2/27/92	454
Dagger Scientific - Bacteriology media	2/10/92	471
Total Costs to Date		$24830

Biological Control

4/16/92
7/16/92

Accepted

LARGE-SCALE INOCULATIVE RELEASES OF THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA GLASERI*: ASSESSMENT 50 YEARS LATER

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RUNNING TITLE: ASSESSMENT OF S. GLASERI INOCULATIVE RELEASES

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ABSTRACT

Billions of nematodes were released from 1939-42 throughout the state of New Jersey (563 sites) in an effort to colonize the entomopathogenic species Steinernema glaseri (Steiner) for biological control of the Japanese beetle, Popillia japonica (Newman). Because of the onset of World War II and the post-war development of chlorinated hydrocarbon insecticides, little effort was expended to evaluate the outcome of these introductions. We evaluated this colonization program by collecting soil samples in 1991 from 304 geographically and ecologically diverse sites across New Jersey. The soil samples were assayed for entomopathogenic nematodes using the Galleria bait method. Overall, 66 (21.7%) soil samples were positive for entomopathogenic nematodes: 24 steinernematids and 42 heterorhabditids. The most common species isolated was H. bacteriophora (38 isolates), followed by S. glaseri (14), S. carpocapsae (4), S. feltiae (4), Heterorhabditis spp. (4), and Steinernema spp. (2). S. glaseri was recovered from only from the southernmost third of the state. We conclude that the colonization effort initiated more than 50 years ago was unsuccessful. The reasons remain uncertain, but intolerance of S. glaseri to temperate climates is one likely explanation. That

is, southern New Jersey appears to represent the northernmost range of this neotropical species. Moreover, early workers were unaware of the nematode's mutualistically associated bacterium, Xenorhabdus poinarii, which plays important roles in killing insect hosts and in nematode reproductive potential. We show that the bacterium is inhibited by antimicrobial compounds used by these workers during mass rearing, so it is probable that only the nematode portion of the nematode-bacterium complex was released.

KEY WORDS: Steinernema glaseri; entomopathogenic nematode; biological control; Heterorhabditis bacteriophora; Japanese beetle; Popillia japonica; Xenorhabdus poinarii.

Journal of Economic Entomology
Section: Biological and Microbial control

Date: 4/10/92
Accepted

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Comparative Evaluation of Entomopathogenic Nematode Strains Against Japanese Beetle, *Popillia japonica* (Coleoptera: Scarabaeidae)

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ABSTRACT Field evaluation of Heterorhabditis bacteriophora Poinar (HP88 strain & a New Jersey strain, NJ-2) and Steinernema glaseri Steiner (NC strain & a New Jersey strain, NJ-43) against Japanese beetle, Popillia japonica Newman larvae showed that all strains reduced grub populations at a level comparable with the chemical insecticide (bendiocarb). Although strains and species did not differ significantly in mean level of control(H. bacteriophora HP88 and NJ-2, 51.0% and 71.6%; S. glaseri NC and NJ-43, 50.4% and 70.1%), variation in the level of control provided by the New Jersey strain of S. glaseri NJ-43 was less than the NC strain. Persistence and dispersal in the field did not differ between species and strains. Laboratory bioassays showed that the New Jersey strains were more pathogenic compared with laboratory strains. A new technique was used to count the viable bacteria retained by individual infective juveniles and found that S. glaseri NJ-43 strain retained not only more bacteria (19.5 compared to 4.9 bacteria/infective juvenile) but also the proportion of nematodes retained bacteria was higher than the NC strain(77% compared to 55%). There was no significant difference either in the number or in the proportion of nematodes retaining bacteria among H. bacteriophora strains.

KEY WORDS Insecta, Popillia japonica, entomopathogenic nematodes, Xenorhabdus, persistence, biological control

Figure 1

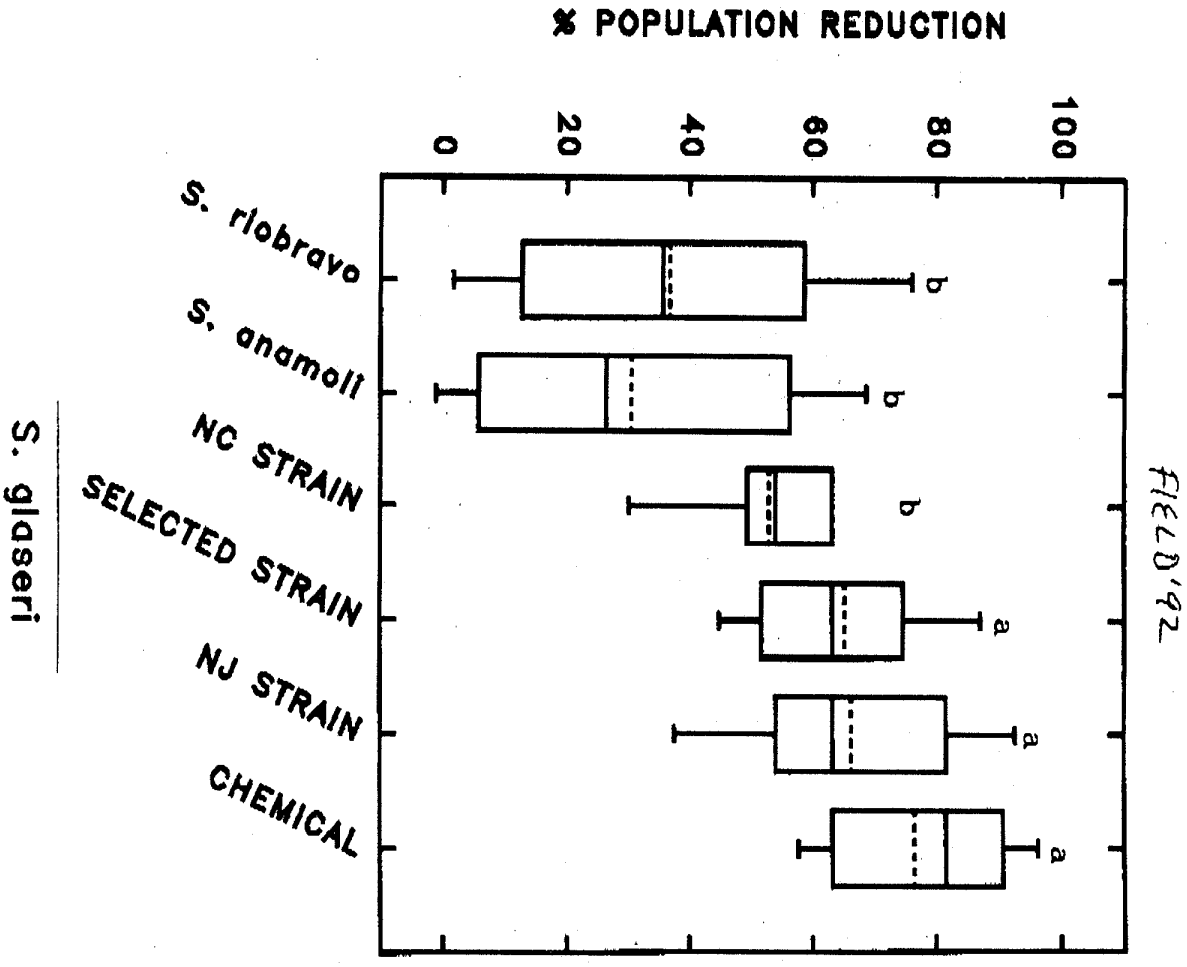
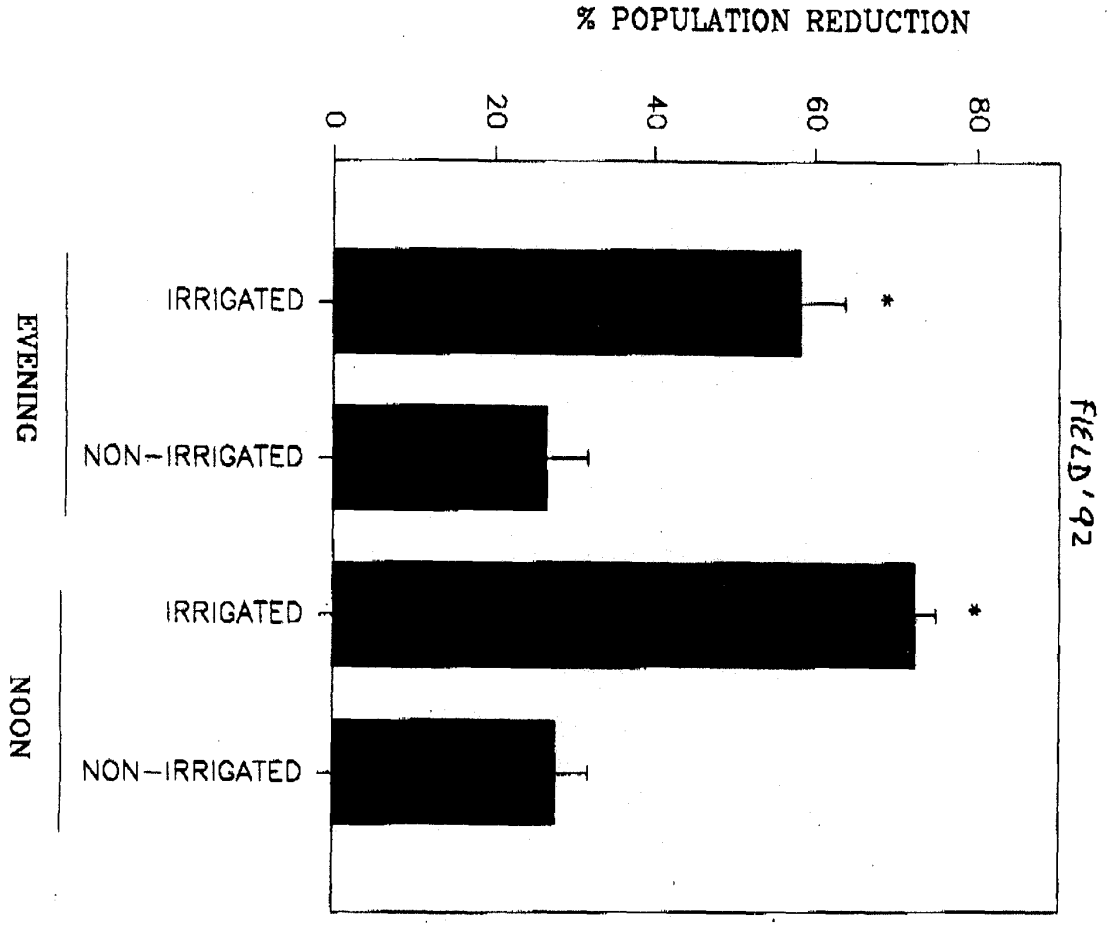

% POPULATION REDUCTION
0
20
40
60
80
100
S. riobravo
S. anamoli
NC STRAIN
SELECTED STRAIN
NJ STRAIN
CHEMICAL
b
b
b
a
a
a
S. glaseri
FEB 0, 92
Field trail

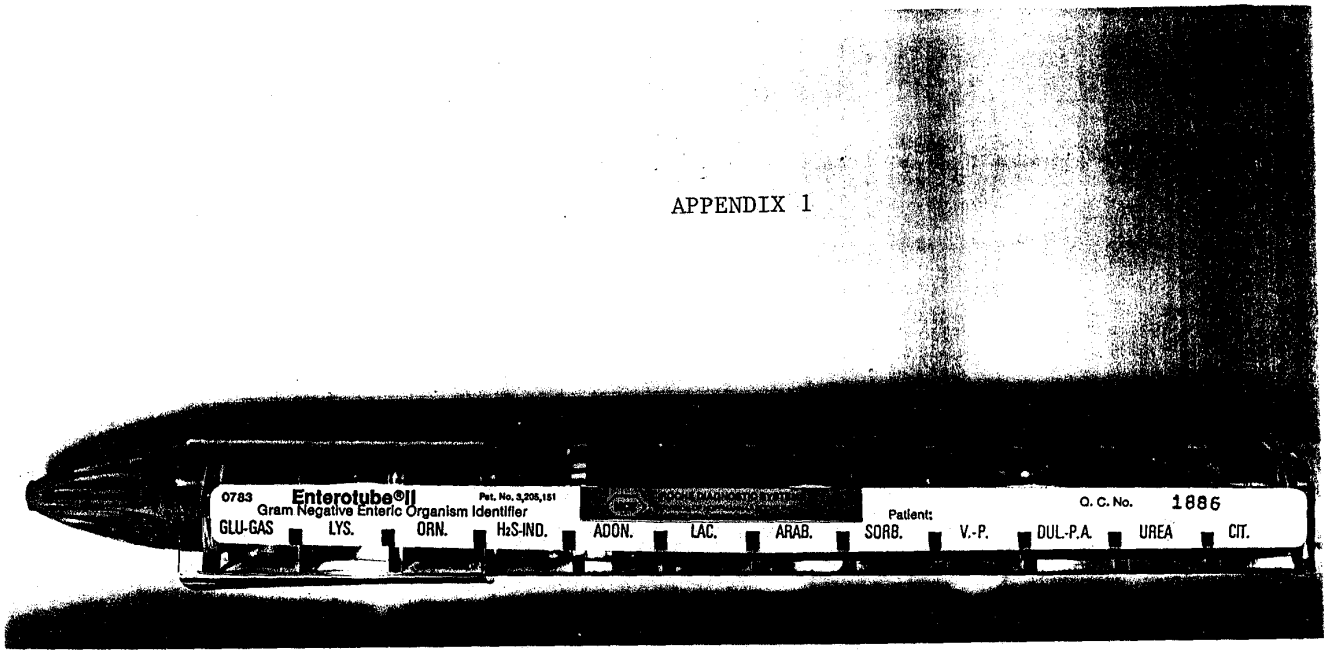
Figure 2

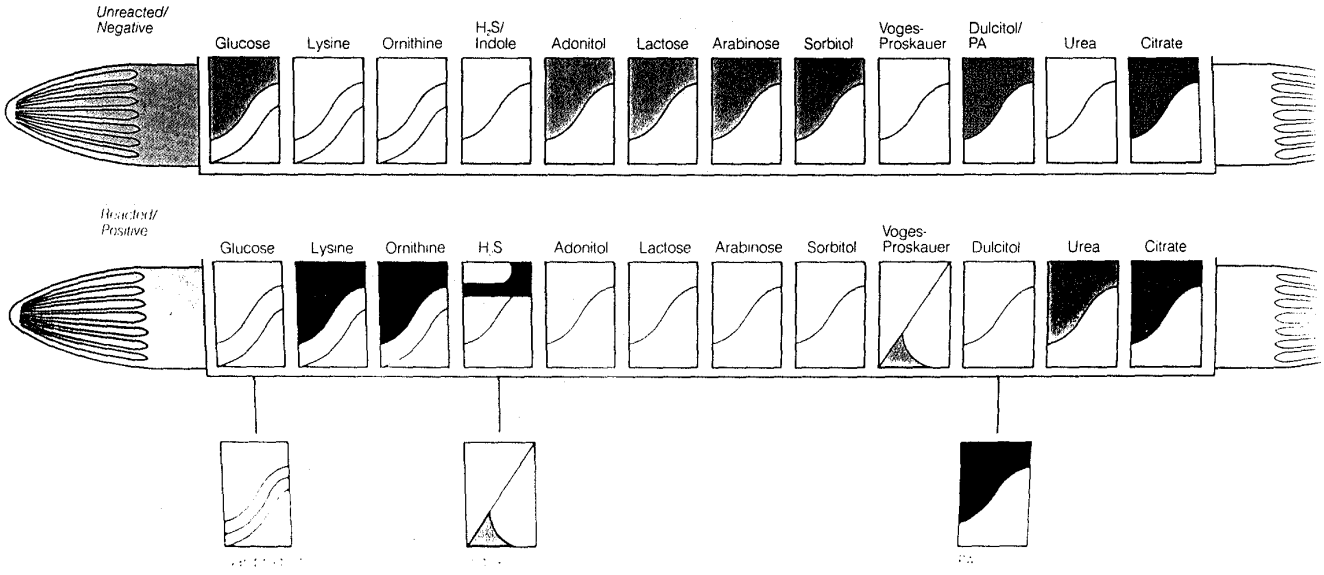


FIELD '92

Field Trial

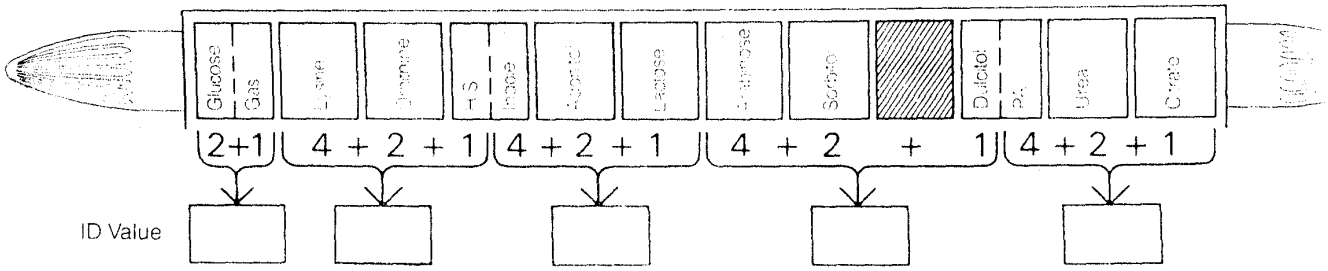
APPENDIX 1





Note: VP utilized as confirmatory test only

ENTEROTUBE® II



ID Value

Culture Number or Patient Name Date Organism Identified

00269