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FIRST ANNUAL REPORT

of

"DEVELOPMENT OF MICROBIAL CONTROL AGENTS
OF THE BROWN PLANTHOPPER"

a

Collaborative Research Project
Between the International Rice Research Institute (Philippines)
and the Boyce Thompson Institute (Cornell University,
Ithaca, NY)

Submitted by

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INTRODUCTION

The AID Office of Science and Technology-supported project on microbial control agents of brown planthopper (BPH) has completed the first of its scheduled three-year program. The project is a collaborative effort between the International Rice Research Institute (IRRI) in Los Banos, Laguna, the Philippines and the Boyce Thompson Institute (BTI) at Cornell University, Ithaca, New York. The BPH is generally acknowledged by rice entomologists in SE Asia to be the sirst or second most serious pest in this critically important subsistence crop. The project was fortunate to obtain the services of Dr. Michiel Rombach, an exceptionally enthusiastic, and capable person to serve as Research Associate living in the Philippines, and to have exceptional cooperation and support both intellectually and materially for the project in the Philippines by the International Rice Research Institute. It is the opinion of the PI that excellent progress was made during the first year.

The principal objectives of the project are as follows:

- Survey SE Asia for diseased BPH.
- Develop bioassay system(s) for fungi vs. BPH.
- Use bioassay to identify promising pathogens from existing fungus collection.
- Study growth of BPH pathogens in laboratory.
- Conduct small field trials for BPH control.
- Integrate pathogens with other control methods.

CALENDAR OF MAJOR EVENTS

September, 1983. D.W. Roberts travels to IRRI for organizational talks and collection of insect pathogens. M.C. Rombach joins the program in the Netherlands, where he collects literature and cultures from various culture collections in Europe of fungi with promise for brown planthopper control.

October, 1983. M.C. Rombach and wife come to the United States and conduct research on the culture of entomopathogenic fungi <u>in vitro</u>.

Late January, 1984. The Rombachs and D.W. Roberts journey to IRRI to establish the Rombachs there and to commence the research. Roberts conducts a survey trip to the south of the Philippines.

February, 1984. Roberts returns to the U.S. The Rombachs remain in the Philippines to conduct survey and laboratory experiments in the Philippines.

July, 1984. M.C. Rombach travels to Korea (on IRRI funds) to survey for insect pathogens with the IRRI biological control specialist. This is the only trip outside the Philippines for Dr. Rombach during the first year.

RESEARCH

In September, 1983, on a visit to IRRI the PI found that epizootics of Metarhizium sp. were occurring in BPH in the field and in the BPH greenhouse colonies. A large number of diseased insects were collected in fields and greenhouses and brought to Boyce Thompson Institute, where cultures were obtained. This lead to one of the most interesting discoveries of the first year's activity. Initially the fungi isolated were identified by a fungus taxonomist as Metarhizium anisopliae. However, isozyme analyses were conducted on these isolates and they proved to vary significantly from the M. anisopliae cultures in our fungus collection. On reexamination it was discovered that the fungus was a recently described species, M. flavoviride. This species had not been reported from Homopterous insects previously and it is extremely interesting that it was causing epizootics in brown planthopper populations. Fungicides are currently sprayed daily in the brown planthopper colony at IRRI in an attempt to control this and other fungi which are threatening the existence of the BPH-rearing program. Beauveria bassiana and some Entomophthorales species are also involved, and these fungi as well are being studied for potential field use for BPH control.

The new isolates of fungal pathogens of BPH were placed in liquid nitrogen in the fungus collections at IRRI and BTI. One of the activities of the past year has been to reorganize the IRRI fungal pathogen collection and to commence a catalog of this collection.

An epizootic of Hirsutella citriformis was encountered in southern Philippines (Mindanao) in early February, 1984. Such epizootics had been reported in the locality by IRRI employees in previous years, and they had been encountered by a BTI collaborator in Indonesia. Therefore, the genus Hirsutella was also singled out for study. In vitro culture studies were carried out both at BTI and at IRRI with H. citriformis and H. versicolor. Because of contamination and equipment problems in the tropical setting, the majority of the culture work was conducted in Ithaca. The second fungus, H. versicolor, has not been reported from BPH, but it is a common pathogen of other delphacid insects. Mass production of the fungi is needed to provide material to introduce into fields for BPH control. These fungi, particularly H. citriformis, are considered very difficult to culture in the laboratory. Nevertheless, we have obtained several isolates of this species in addition to some H. versicolor isolates from culture collections. Growth was studied at different temperatures and it was found that the optimum for both species was in the range of 25-30°C. The influence of 40 additives to a basal growth medium was tested. None of the additives enhanced the speed of growth significantly; however, sporulation of H. citriformis was stimulated by several compounds including sorbitol, proline, and ribonucleic acids. Growth on solid media was extremely

slow, but it was found that both species would adapt relatively rapidly to growth in shaken liquid media. In the case of H. citriformis this transformation occurred within three passages in liquid media, whereas the change of H. versicolor required was much more gradual, requiring approximately 7 transfers to reach peak performance. A number of liquid media were tested. It was found that a 4% oatmeal broth was an efficient and inexpensive substrate for H. citriformis. Also a new method developed at BTI wherein mycelium is dried in a carefully regulated way so that it is capable of producing spores in the field after long-term storage was tested with H. citriformis and H. versicolor with very promising results.

It is necessary to know the numbers of infective units present in suspensions used in the field and in bioassays. The standard methodologies are excessively labor intensive and slow, in some cases requiring up to 5 days to obtain answers. Accordingly, a technique to determine the number of conidia present in a suspension by measuring the turbidity with a spectrophotometer was developed. The method was found to be extremely useful for some of the pathogens of interest, particularly M. anisopliae and M. flavoviride.

Two BPH colonies were started and maintained at IRRI with the goal of producing approximately 10,000 adult hoppers per week. The colonies were situated in two separate locations in order to minimize the risk of losses due to epizootics of fungal diseases. As mentioned previously, fungicides are used in the greenhouses on a regular basis in an attempt to protect the colonies.

Bioassays with the BPH have proved very difficult and accordingly a number of systems have been developed and tested. A note on what is considered the best of these systems has been submitted for publication in the IRRI Newsletter. Bioassays have been conducted at the rate of about two per week. In spite of the fact that some tests were lost to high control mortality from other fungi, a number of fungal isolates have been tested for virulence to BPH. This work is extremely labor intensive and we are very fortunate to have the full cooperation of the IRRI entomology staff in carrying out the tests.

In looking forward to the time when entomopathogenic fungi can be integrated with chemical pesticides in BPH control, experiments are being conducted on the influence of pesticides commonly used in rice ecosystems on the germination, growth and sporulation of several of the entomopathogenic fungi. The experiments were designed to study the effects of the pesticides at different dosage levels on the three developmental stages of the fungi separately.

FUTURE ACTIVITIES

The activities for the second year of the project will include continuation of the research described above plus survey trips to the Indian subcontinent and perhaps to Australia and the Solomon Islands. Rombach will visit BTI for solving of technical and administrative problems at least once during the year. Dr. Roberts will join in at

least one of the survey trips to the Far East. It is anticipated that part of the funds for the surveys will be provided by the United Nations Development Program. More emphasis will be placed on experiments out of doors at IRRI in the next year. Small plot tests will replace a significant percentage of the laboratory bioassays, since we are assuming that this will more nearly approximate "real world" conditions. Preliminary tests in the field on different locations are being carried out at the time of writing.