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PROGRESS REPORTS

Dr. Robert M. Altman
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Bureau of Science and Technology
Agency for International Development
Washington, D.C. 20523

Dear Dr. Altman:

I would like to begin by apologizing for the delay in providing this report of progress on U.S.A.I.D. grant 4.470. Identifying and Monitoring insecticide resistance in Brown Planthopper (BPH). As I hope you will agree we have made significant strides in this work.

This past growing season was a banner one for BPH in Southeast Asia. Resurgence after insecticidal treatment was especially problematic in Indonesia where they experienced outbreak populations of BPH even in rice varieties selected for BPH resistance which had not previously been overcome by BPH biotypes. It is still premature to have heard from Kasumbogo Untung as to the status of the third crop, but the outlook was not good. Taiwan had severe problems from second generation BPH, but mixing of insecticidal modes of action helped manage the populations. This strategy is certain, however, to produce cross resistant strains of BPH within a couple of years.

We have received excellent cooperation from our contacts in Indonesia and Taiwan, but little support from the Philippines. I have not heard from Ricardo Beang for almost one year, and I fear that something has happened to his status as BPH research leader at the National Crop Protection Center in Los Banos. Nevertheless, we have pursued our research on the available samples.

Enclosed please find our recent manuscript which has been accepted for publication in Pesticide Biochemistry and Physiology. The manuscript summarizes about half of the research we have accomplished. Let me encapsulate the major results from this work: 1) we derived a suitable in vitro methodology for assessing substrate specificities in whole body homogenates of BPH 2) we evaluated Indonesian, Taiwan and Philippine strains of BPH using and naphthyl acetate, cis and trans-permethrin, malathion and fenvalerate as substrates. 3) we identified eight different and active BPH esterases hydrolyzing the various substrates, 4) fenvalerate, a synthetic pyrethroid, required the highest level of enzyme to saturate hydrolysis and 5) permethrin, also a synthetic pyrethroid, hydrolysis occurred at a 5-10 fold greater rate than malathion or fenvalerate. These results demonstrate a variable picture of evolving resistance to organophosphate (OP) and synthetic pyrethroid (SP) insecticides by the BPH strains we tested. The high hydrolysis rate with permethrin confirms the field failure of this compound to control these strains in Indonesia. Further work is needed to clearly elucidate the complex multiple resistance picture in the Philippine and Taiwan strains.

Our next objective was to purify and separate individual esterases from susceptible and resistant strains of BPH from Indonesia. Our Indonesian cooperators provided us with the largest samples, therefore we had the most material to work with in these strains. Further collaborative work is underway as selection of isogenic OP and SP resistant strains of BPH are progressing under Dr. Kasumbogo Untung's able direction in Indonesia right now.

Our procedures were briefly as outlined below. BPH were homogenized in 20ml of 0.1 M Tris HCl pH 8.0. The homogenates were centrifuged at 10,000g for 10 minutes, and the supernatants used as enzyme sources. Supernatant was concentrated to 10 ml and applied on a chromatofocusing column and eluted with 400 ml of polybuffer (pH 4.0) which produced a pH gradient from 7 to 4. Esterases with different pI values were collected, concentrated with Amico 30 and applied to a Bio-HTP column pre-equilibrated with 5mM potassium phosphate buffer pH 7.0. After applying the enzyme preparation, 3 bed volumes of the starting buffer was passed through the column before a 5 20 mM phosphate buffer gradient was passed through the column. The esterase peaks were collected and concentrated to 5ml before being reappplied to a 2 X 10 cm sephadex G-100 column equilibrated with 50 0.1 mM Tris HCl buffer pH 8.0. The esterases separated and purified by the above column chromatography were used for enzyme kinetics experiments using malathion, cis-permethrin, trans-permethrin and fenvalerate as substrates. The kinetic assays enabled us to deduce the K_m and V_{max} values of the different insecticides for each isoenzyme. This enabled us to compare the hydrolysis rates of insecticides by individual isoenzymes and to determine the isozymes responsible for insecticide resistance.

From this work we learned **that the esterases in BPH were very active enzymatically, but were in lower quantity than we anticipated from previous work (Chang and Whalon 1986) on related arthropods.** We calculated the amount of BPH enzyme required to initiate hybridoma production and screening to be at least 3-4 mg and preferably 30 mg. Since an average adult BPH weighs approximately 7 mg wet weight and the amount of target esterases represent approximately 0.001 to 0.007 % of the wet weight, then **we would require between 4-30 million BPH or approximately 30,000 g!** When we started the project we estimated that the quantity of enzyme would be much higher in an individual BPH; approximately 0.1% which would have been in the same order of magnitude for the work we had done previously with other resistant arthropods (Chang and Whalon 1986). Currently we do not have sufficient sample material from Indonesia (or any other site) to try isolation of BPH esterases for monoclonal production and it is unrealistic to ask a cooperator to rear so many BPH.

At this point we have spent our grant allocation and still are not at our objective - that of producing a usable esterase monitoring system for BPH. I feel that we have made major strides in our understanding of the resistant mechanisms in this pest. Further, we could not have guessed this outcome a priori. However, there is another alternative to developing a detection assay for resistance in BPH.

In another related project using techniques from molecular biology we have generated a cDNA probe for the detection of mycoplasma-like organisms (MLO) transmitted by arthropod vectors like

BPH. From our experience in this effort, I now believe that development of a ~~cdna probe is~~ a better approach than monoclonal antibodies, especially given the difficulty in generating a sufficient quantity of purified enzyme from BPH for monoclonal antibody production and screening.

I would like to explore the renewal of this grant with the same resistance management rationale, but an updated methodology. Since the cooperators are in place, and we have already worked with both the BPH resistance mechanisms and molecular cloning in related insects the lag time in initiating a gene probe project would be minimal. Do you think that a renewal is feasible under these circumstances?

Sincerely,

Mark E. Whalon