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PROGRESS REPORT No. 5

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Title: Genetic Improvement of Natural Enemies for Biological Pest Control: Selection for Resistance to Pesticides in Species of Aphytis.

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Background

The main purpose of this research program is to select for pesticide resistance in effective biological control agents. Species of Aphytis Howard (Hymenoptera: Aphelinidae), important parasites of armored scale insects (Homoptera: Diaspididae), have been chosen as the target organisms. Collaboration with the Philippines includes the transfer of native Aphytis stocks to Israel, and transfer of the relevant technology to the Philippines.

As explained in previous Progress Reports, the subcontract with the Cooperating Institution, University of the Philippines at Los Banos, was signed only towards the end of June, 1987. The Principal Investigator visited the Philippines for the first time in October, 1987, and a trainee from the Philippines visited Israel from March to September, 1988. A second visit to the Philippines will be made in 1989, and the research in Israel will be terminated in that year, but research in the Philippines will have to be continued until 1990. It is therefore requested that the project be extended until the end of 1990, that the final visit to the Philippines be made during the summer or fall of that year, and that the final report be prepared and submitted at that time.

Accomplishments Since Progress Report No. 4

I. Selection for Azinphosmethyl Resistance

(1) Aphytis holoxanthus

In an effort to increase genetic variability, the population of this species selected for azinphosmethyl resistance was exposed to the chemical mutagen EMS (ethyl methyl sulfonate).

In preliminary experiments, batches of adult wasps were exposed to various concentrations of EMS and allowed to oviposit in host scale insects. A marked reduction in fecundity, due to dominant lethal mutations, was noted. At 0.1% EMS, ca. one offspring was recovered per exposed parent. Assuming the overall mutation rate to be proportional to that of lethal dominants, this concentration was chosen in an attempt to attain an optimal rate of viable mutations at an acceptable loss of net progeny production.

Some 3200 wasps were exposed to solutions containing 0.1% EMS, 10% sucrose and 2.5% carmosine food dye, using the same equipment and procedures as used in exposure to pesticides (see our original Research Proposal, page 17). The resultant F_1 population was expanded, and has since been subjected to two selection cycles for

azinphosmethyl resistance, in alternate generations, at the LC_{90} level.

(2) Aphytis lingnanensis

The 'Ra'anana' population previously referred to as Aphytis ?coheni (see our Progress Report No. 4, page 3) has now been determined through biosystematic research to be Aphytis lingnanensis Compere (these two species are very closely related and may be regarded as siblings). A laboratory stock of A. lingnanensis was obtained from the Department of Entomology, University of California, Riverside, and reciprocal mating experiments between the two populations have established their conspecificity.

The last (17th) selection cycle of this line was exposed to 3200 ppm azinphosmethyl, and this resulted in 6% survival. The population was then expanded and exposed to graded concentrations of azinphosmethyl, to obtain dosage-response lines (see our original Research Proposal, pages 16, 18). Analysis of the results showed but a small increase of the LC_{50}, from 71 to 100 ppm. However, 25% survived at concentrations of 400, 800 and 1600 ppm, indicating a selection response of increased heterogeneity: this population by now includes a considerable proportion of highly resistant individuals, and could be further selected to eliminate susceptible genotypes.

Inasmuch as the male-selected line of this population was found to be superior (see below), further selection efforts have now concentrated on that line.

The imported laboratory strain of A. lingnanensis, assumed to be susceptible to pesticides, was utilized to establish the basic tolerance of this species to azinphosmethyl. Indeed, dosage-mortality tests with this population have shown it to be highly susceptible, with an LC_{50} value of 8.9 ppm and considerable homogeneity.

(3) Male Selection

The male-selection program for azinphosmethyl resistance in A. lingnanensis (see our Progress Report No. 4, page 4) was further pursued in our laboratory by our Philippine collaborator, Mr. Pio Javier. Within 7 selection cycles, the discriminating dose (LC_{80}) was increased from 400 to 1600 ppm. The resultant population was then expanded and tested as above. Analysis of dosage-response lines showed two inflection points near the LC_{50} level, again pointing to greatly increased heterogeneity and indicating that ca. 50% of the population were resistant to 200, 400 and 600 ppm, 27% to 1600 ppm and 13% to 3200 ppm of azinphosmethyl. This population is currently being selected at LC_{95} at alternating generations.

II. Selection for Permethrin Resistance

Selection of Aphytis lingnanensis for resistance to permethrin has been discontinued after 14 selection cycles, mainly because of manpower shortage. The selection response of this line has been similar to that of the mass selection program for azinphosmethyl resistance. Exposure levels were increased from 200 to 1600 ppm, but had to be reduced intermittently. At the last test, LC_{50} was found to be 267 ppm, as compared to 184 ppm in the original, field-collected population. However, the population now included ca. 25% resistant to 400, 800 and 1600 ppm.

III. Release of Selected Strain

Preliminary releases of Aphytis lingnanensis selected for resistance to azinphosmethyl have been carried out in Israel. An orange grove infested with California red scale (Aonidiella aurantii) parasitized by Aphytis spp. was located near Be'er Ya'acov (Coastal Plain). Scale samples were collected, from which a population of A. lingnanensis was obtained. This will be expanded and tested for pesticide tolerance. The sampled trees have been marked and used as release trees, and ca. 4000 and 2000 1-4 day old adult wasps of both azinphosmethyl-selected stocks were released on two consecutive weeks in November, 1988. These trees will be sampled to determine whether A. lingnanensis has become established and, if so, it will be recovered, tested and compared to the original population from that grove.

IV. Exchange of Parasites with the Philippines

From the latest consignment of Florida red scale material (Chrysomphalus aonidum) received from the Philippines, 27 live Aphytis, apparently holoxanthus, emerged in quarantine. These have been exposed to suitable hosts and are currently being reared for testing of pesticide tolerance.

The male-selected A. lingnanensis and the S_{25} A. holoxanthus population were carried by Mr. P. Javier to the collaborating laboratory at the University of the Philippines, Los Banos, for the purpose of establishing parallel lines of research.

V. Work in the Philippines

A report from the Philippine Collaborator, Professor B. Morallo-Rejesus, is attached herewith.

Immediate Work Plans

Selection of Aphytis holoxanthus for azinphosmethyl resistance will becontinued at the LC_{90} level in alternating generations, in order to assess the effects of the EMS treatment.

The A. holoxanthus colony reared from the last shipment of Florida red scale material from the Philippines will be expanded and tested for azinphosmethyl tolerance. Results will be compared to those of our previously tested populations, as well as to those obtained by Philippine collaborators.

The male-selected line of Aphytis lingnanensis will be maintained for additional releases during the coming spring/summer season. Every 2 or 3 generations, a selection cycle at LC_{90-95} will be carried out in order to maintain and possibly increase the level of resistance.

Several further releases are contemplated, according to procedures described in Part III of this Report (page 4), and will be accompanied by pre- and post-release tests for tolerance or resistance.

A visit to the University of the Philippines at Los Banos will be undertaken by Dr. A. Havron during January, 1989, with the following main objectives: (1) To finalize laboratory arrangements for receiving field-collected scale-insect and Aphytis material, and procedures for testing and selection experiments by local personnel. (2) To visit citrus groves in order to collect Aphytis species and their hosts, and to evaluate the suitability of sites for future releases. (3) To hand-carry additional stocks of selected A. holoxanthus and A. lingnanensis from Israel to the Philippines, in order to reinforce the colonies established at Los Banos from material brought by Mr. P. Javier upon his return in September, 1988.