PN-ABC-871

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D C 20523

DATE: 11/9/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose VO

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. C5-127

PR#2 6/30/87

PR#4 6/30/88

Attachment

June 30, 1988

PROGRESS REPORT No. 4

Submitted to the U.S.-Israel Cooperative Development Research Program
U.S. Agency for International Development

Project No. C5-127

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

Principal Investigator: Professor David Rosen
Department of Entomology
Faculty of Agriculture
The Hebrew University of Jerusalem
P. O. Box 12, Rehovot 76100, Israel

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 project became operational in Israel on December 3, 1986. 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 has come to Israel in March, 1988, for a six-month visit.

Accomplishments Since Progress Report No. 3

The "Ami'ad" line of Aphytis holoxanthus has been further selected for azinphosmethyl resistance. Selection intensity was increased and relaxed periodically, using the modified exposure method described in Progress Report No. 3. LC_{80} is currently 1600 - 3200 ppm.

Unfortunately, this population was contaminated with Aphytis ?coheni, which appears to be a better competitor on ivy scale under laboratory conditions. We have since succeeded in establishing a pure culture of A. holoxanthus by passage through a selective host, Florida red scale, which is not attacked by A. ?coheni. This has entailed some loss of population and of selection potential.

Since there have been some indications that this A. holoxanthus population may have reached a selection plateau, an attempt will be made to increase its genetic variability through utilization of a chemical mutagen.

The Aphytis ?coheni line which is being selected for resistance to azinphosmethyl has now undergone 15 selection cycles. For the last two generations, the LC_{80} has been doubled twice and is now 1600 ppm. Sensitivity tests of this population have indicated the possible development of incipient resistance: Dosage-mortality lines show two inflection points, a situation typical of populations consisting of both susceptible and resistant individuals.

A separate line of the A. ?coheni population has been under selection for resistance to permethrin for 12 generations. The original LC_{80} of 350 ppm has been increased to 800 - 1600 ppm. After the 10th selection cycle, repellency to permethrin became apparent and the exposure method was modified as described for A. holoxanthus in our Progress Report No. 3.

The A. ?coheni population, which was originally obtained from a very heavily treated citron orchard, has yet to be compared to a more typical field population. Such a population has now been secured, but its conspecificity has to be established by reciprocal crossing tests. Inasmuch as A. coheni is for all practical purposes a sibling species of A. lingnanensis, a culture of the latter will also be obtained for crossing tests with our population.

A male selection project was initiated in an effort to increase the selection response of the A. ?coheni - azinphosmethyl line. Pupae of the S_{12} generation, 11-13 days of age, were sexed and the emergent virgin females were then exposed to suitable scale insect hosts. The all-male offspring of these females were selected with 400 ppm azinphosmethyl, resulting in ca 25 percent survival. Survivors were mated with virgin females, which were then exposed with them to hosts.

Another batch of virgin females was separately exposed to other hosts to produce males for the next selection. Male survivors were again mated to virgin females. Henceforth, only the offspring of matings of selected males and virgin, unselected females were used. All available pupae were collected from under scale covers and sexed; resultant virgin females were allocated for the production of males or as mates for survivors, whereas the males were included in the selection procedure.

In preliminary experiments, a suitable mating ratio was established for A. ?coheni. When the natural sex ratio of this species (ca 2.3 females per male) was exceeded, very few female progeny were obtained. Under our experimental conditions and projected selection intensity, a ratio of 1.5 females per male was found to be optimal for efficient utilization of virgin female material.

Mr. Pio Javier of the Department of Entomology, University of the Philippines at Los Baños, has arrived in Israel on March 6, 1988, to take part in this project. The male selection program has been his main responsibility, but he is also actively participating in all other aspects of the project.

Additional shipments of Florida red scale material have been received from the Philippines. Unfortunately, only one of these has yielded some 15 live Aphytis specimens, and preliminary examination has indicated that they are not A. holoxanthus. Efforts will be made to obtain further shipments.

Immediate Work Plans

Additional shipments of Florida red scale are anticipated to yield live Aphytis, which will be propagated and tested for pesticide tolerance.

The A. holoxanthus - azinphosmethyl selection line will be evaluated for the degree of resistance attained. In an attempt to increase genetic variability in this population, the chemical mutagen ethyl methane sulfonate (EMS) will be incorporated in the dyed sucrose solution which normally serves as the carrier for pesticides in our experiments. Pre-starved insects will be exposed for appropriate feeding periods and will then be released onto suitable hosts. Following the EMS treatment, selection will continue.

A laboratory stock of A. lingnanensis will be obtained from the University of California, Riverside, and reciprocal crossing tests will be performed with our A. ?coheni population.

The A. ?coheni mass selection program for azinphosmethyl resistance will be concluded, to be followed up by a male selection program with the same stock. The A. ?coheni - permethrin program will be intensified and pursued for several additional generations, to follow up the recently observed response.

Upon his return to the Philippines in September, Mr. Javier will establish facilities and requisites for a parallel rearing and selection program. Dr. A. Havron will then visit the Philippines in November.

December 31, 1987

PROGRESS REPORT No. 3

Submitted to the U.S.-Israel Cooperative Development Research Program
U.S. Agency for International Development

Project No. C5-127

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

Principal Investigator: Professor David Rosen
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P. O. Box 12, Rehovot 76100, Israel

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. To assure a broad genetic spectrum, Aphytis stocks obtained both from Israel and from the Philippines, which is part of their native home, are to be included in this study.

As explained in Progress Reports No. 1 and 2, the project became operational in Israel on December 3, 1986. 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 in October, 1987.

Accomplishments to Date

The "Ami'ad" selection line of Aphytis holoxanthus has undergone 8 further selection cycles with azinphosmethyl, at 2,000 - 16,000 individuals per generation. This population is currently under selection at 800 - 1600 ppm of azinphosmethyl, which is roughly equivalent to the recommended field dosage on citrus.

The exposure method for this population was revised after the 13th selection cycle, when a degree of repellency to the poison, as offered, became apparent at the high concentrations required for the predetermined mortality levels. Using the same equipment and handling methods as before (see the original Research Proposal, page 17), the wasps were henceforth confined in the cells with azinphosmethyl/ sucrose solutions for 20 hours, without prior starvation (replacing one hour of exposure after 3 hours of starvation). Better uniformity of exposure was thus obtained.

Several additional field populations were tested, and among them one stock of Aphytis holoxanthus, collected at Ma'ale Gamla, showed high tolerance to azinphosmethyl (LC85 at 800 ppm), close to the value originally found for the "Ami'ad" population currently under selection. A laboratory colony of this population was started and exposed at the current LC80 level of the selection line (800 ppm). The survivors were incorporated in the selection line, with the dual purpose of introducing possibly different, or superior, genes for azinphosmethyl resistance, and counteracting possible deterioration owing to prolonged selection to laboratory conditions.

The colony of Aphytis coheni, described in Progress Report No. 2, is currently being selected for azinphosmethyl resistance for the 9th generation. No repellency has been encountered with this stock, and selection is proceeding at 800 ppm.

A separate line of this population has been undergoing selection for 3 generations to permethrin, at 200 - 400 ppm and 20 - 35 percent survival.

In the Philippines, extensive surveys for armored scale insects and their parasites were initiated in 1987 prior to the Principal Investigator's visit, and adequate rearing facilities were established at the University of the Philippines, Los Banos. During October, 1987, the Principal Investigator joined the Cooperating Investigator and her associates in surveys of the Florida red scale and other potential hosts of Aphytis on citrus, coconut, etc., on the islands of Luzon, Mindoro and Mindanao. Numerous samples were collected and hand-carried to Israel, where they are being processed in quarantine. Further surveys have been made since then, and several laboratory cultures of armored scale insects have been started.

Immediate Work Plans

Further shipments of Aphytis will be made from the Philippines to Israel. As soon as adequate scale-insect cultures are established in the Philippines, Mr. Pio Javier of the University of the Philippines, Los Banos, will come to Israel for a six-month training visit. He is expected to arrive in March and will participate in all aspects of the project.

Any imported Aphytis species will be tested for their pesticide tolerance. The three selection programs will be pursued until a selection "plateau" or a prolonged "no response" situation is encountered. If the levels of resistance attained by then are deemed unsatisfactory, mutagenesis by irradiation or by chemical mutagens will be attempted. When a considerable resistance ratio is established by LC50 tests, genetic analysis will be undertaken as described in the original Research Proposal (page 19).

June 30, 1987

PROGRESS REPORT No. 2

Submitted to the U.S.-Israel Cooperative Development Research Program
U.S. Agency for International Development

Project No. C5-127

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Principal Investigator: Professor David Rosen
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P. O. Box 12, Rehovot 76100, Israel

Background

The main purpose of this research program is to select for pesticide resistance in effective natural enemies. Species of Aphytis Howard (Hymenoptera: Aphelinidae), important parasites of armored scale insects (Homoptera: Diaspididae), have been chosen as the target organisms. To assure a broad genetic spectrum, Aphytis stocks obtained both from Israel and from the Philippines, which is part of their native home, are to be included in this study.

As explained in Progress Report No. 1, the project became operational in Israel on December 3, 1986. The subcontract with the Cooperating Institution, University of the Philippines at Los Banos, was signed only towards the end of June, 1987. Funds are currently being transferred to the Philippines.

Accomplishments to Date

Base lines of tolerance to azinphosmethyl (an organophosphorus insecticide), carbaryl (a carbamate) and permethrin (a synthetic pyrethroid) were derived from tests carried out on a susceptible laboratory culture of Aphytis holoxanthus DeBach, according to the methodology described in the original research proposal (for further details, see Harron et al., 1987). From the resulting dosage-mortality lines (Fig. 1), the relevant LC_{50} and LC_{80} values were obtained (Table 1).

Table 1. Tolerance of a laboratory culture of Aphytis holoxanthus to three pesticides.

Pesticide	LC_{50} (ppm)	LC_{80} (ppm)	Slope (1/variance)	N (No. tested)
Azinphosmethyl	7.9	21.1	1.97	3258
Carbaryl	56.2	175.8	1.72	1062
Permethrin	97.7	182.0	3.12	1739

Ten stocks of Aphytis holoxanthus were collected from Florida red scale [Chrysomphalus aonidum (L.)] populations on citrus in various parts of Israel and tested for their tolerance to azinphosmethyl, carbaryl and permethrin. On comparison with the susceptible laboratory culture, the "Ami'ad" stock was found to be 3.8 times more tolerant to azinphosmethyl, and the slope of the dosage-mortality line was 0.47 as compared to 1.97, indicating a X4.2 higher variation in tolerance. The "Nir Israel" stock was X3.6 more tolerant to carbaryl than the laboratory culture. All other stocks were found to be inferior to these. Laboratory colonies have been established from the Ami'ad and Nir Israel stocks.

The Ami'ad colony has to date been subjected to 11 selection cycles, exposing 3500-6500 individuals per generation to solutions of azinphosmethyl. Selection intensity was maintained at 40% survival. The initial concentration of the azinphosmethyl solution (50 ppm = LC_{60}) was increased after 4 cycles to 100 ppm and after the 8^{th} cycle to 200 ppm, indicating a 4-fold increase in tolerance. Selection is presently proceeding at 400 ppm.

A colony of Aphytis coheni DeBach was obtained from the California red scale [Aonidiella aurantii (Maskell)] collected in a citron grove that had been subjected to a particularly intense

pesticide regime. Preliminary tests indicated a 10-12 times higher tolerance to azinphosmethyl and twice the tolerance to permethrin as compared to the laboratory culture of A. holoxanthus (no susceptible A. coheni population was available).

Prior to selection with A. coheni, laboratory procedures were adapted to accommodate work with this species. Relevant biological characteristics such as developmental period, longevity, fecundity and rate of increase were studied under laboratory conditions of 26±1°C, 60±5% R.H. and L:D = 16:8. Whereas the rate of development was similar to that of the A. holoxanthus cultures (minimum 14 days), the rate of increase per generation was considerably higher in A. coheni (X25 - X35 as against X2 - X6 in A. holoxanthus). This higher rate may sustain a considerably higher selection intensity. Similar numbers of all-male offspring were obtained from unmated females.

A selection program for azinphosmethyl resistance in A. coheni has been initiated at 400 ppm (LC_{75}), and is currently proceeding at 800 ppm after 3 generations.

Immediate Work Plans

As soon as the Philippine part of the project becomes operative, shipments of Aphytis will be made from the Philippines to Israel on a regular basis. The Principal Investigator will visit the Philippines in October, 1987, to coordinate research efforts. Extensive collection of Aphytis will be made at that time, in collaboration with the Cooperating Investigator.

Selection for azinphosmethyl resistance in A. holoxanthus will be pursued at increased concentrations and selection intensities, until a selection plateau is encountered.

Genetic analyses (by back-crossing) will then be undertaken in order to establish the nature of the resistance trait(s) (mono- or polygenic) and their dominance characteristics. Tests with other pesticides will be carried out in order to establish possible cross-resistances.

If imported stocks of A. holoxanthus are available, they will be used for the introduction of new genetic variability by hybridization and further selection. Alternatively (or concurrently) mutagenesis, preferably by γ irradiation, will be attempted for the same purpose. If other Aphytis species are imported, they will be tested for their tolerance to the three pesticides.

Selection for azinphosmethyl resistance in A. coheni will proceed at increasing selection intensities. A selection plateau will be followed by the same procedures as outlined for A. holoxanthus.

Reference Cited

Havron, A., D. Rosen and Y. Rössler. 1987. A test method for pesticide tolerance in minute parasitic Hymenoptera. Entomophaga 32: 83-95.

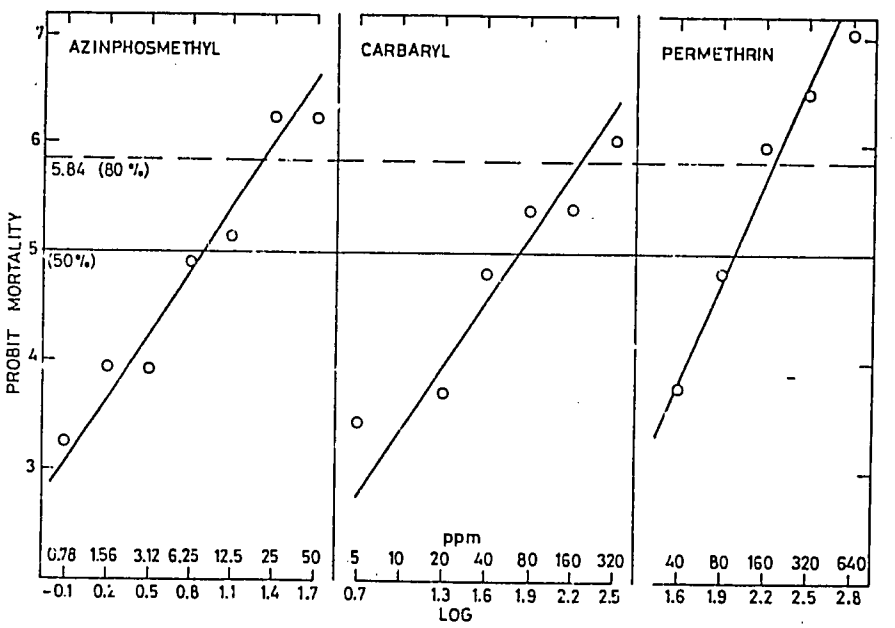

Fig. 1. Tolerance of a laboratory culture of Aphytis holoxanthus to three pesticides.