

**THIRD SEMI-ANNUAL PERFORMANCE REPORT**

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**Title of Project**

**“Biocontrol of Armyworms with the Entomopathogenic Fungi  
*Nomuraea rileyi* and *Beauveria bassiana*”**

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## Section I: Technical Progress

### I. A) Research Objectives:

This is our third semi-annual report within the context of a research project whose aim is to contribute towards the reduction of chemical control presently in use against *Helicoverpa* (= *Heliothis*) *armigera*, *Spodoptera littoralis* and other major noctuid pests of crop production in the Middle East, this by utilizing the entomopathogenic fungus *Nomuraea rileyi* (a limited-range biocontrol agent).

Of the four full-program research objectives listed immediately below, progress reported herein relates mainly to objectives (a) and (d):

(a) To select strains of *N. rileyi* adapted to the environment in the Middle East and effective against local populations of *H. armigera* and *S. littoralis*.

(b) To devise a procedure for mass-propagation of *N. rileyi* in a cost-effective manner, and to formulate a preparation that is relatively stable to environmental factors.

(c) To perform pilot field-tests with *N. rileyi* and a commercial preparation of *B. bassiana*, in order to establish the efficacy of the preparations and formulations under local field conditions in the coastal plain, mountains and inner valleys in this region.

(d) To examine whether the presence of the fungus in early stages of germination and penetration, alters the composition of cuticular lipids, thereby affecting larval activity and feeding behavior of the larvae.

### I. B) Research Accomplishments:

These relate to the items cited in the **time-chart** of the 1<sup>st</sup> and 2<sup>nd</sup> years (i.e., the effects of physical and nutritional factors on fungal development, and host-pathogen interactions).

## Time Chart

Activity	1 <sup>st</sup> Year	2 <sup>nd</sup> Year	3 <sup>rd</sup> Year
<b>Fungal development:</b> Effect of physical factors			
Temperature			
Temperature- <i>humidity</i> -light			
<b>Fungal development:</b> Effect of nutritional factors			
Mass production and persistence			
Host- pathogen interactions			
Influence on larval behavior			
<b>Field experiments</b>			
<b>Training</b>			

### **Experimental and preliminary results**

(1) We are in the process of formulating a *N. rileyi* selective medium procedure from soil samples, in order to attempt to isolate further local isolates of this entomopathogenic fungus in agricultural fields in the West Bank and also from *H. armigera* larval cadavers collected in those same sites. The selective medium is under study in both Rehovot and Hebron.

Soil samples from tomato fields located near 4 Palestinian cities were collected (Hebron, 98 samples; Jericho, 15; Jenin, 20 and Tulkarem, 6). Samples were dispersed in 0.1% agar in water and shaken for 30 min. A 100 µl aliquot of the solution was seeded on plates containing a selective medium composed of SMAY + 0.025% chloramphenicol and various concentrations of Dodine (0, 20, 30, 40, 50, 60, 70, 80, 90, 100 mg/l) and screened for the fungus using the dilution plate technique. Plates were incubated in the dark for 4 weeks at 28°C. Initially, no colonies of *N. rileyi* were obtained from these samples, nor from samples spiked with *N. rileyi*. To check that the selectivity of the selective medium used in the process might be at fault, we designed an experiment in which we manipulated the concentrations of PCNB and Dodine in selective media. Preliminary results are encouraging since we obtained some growth of fungi when we lowered the concentration of these compounds in the media, but the overall picture is not fully clear yet because the experiment is not over yet. If this procedure is successful, soil samples will be screened again for the entomopathogen using an optimized formula of selective medium.

(2) We also collected dead larvae of *H. armigera* from the same agricultural fields in the West Bank, but so far have not been successful in identifying the target entomopathogenic fungus *N. rileyi*. However several other, as yet unidentified fungi were isolated in Hebron from the interior of some of the dead insects, which may be of some potential value. Meanwhile, we are trying to inoculate larval instars of *H. armigera* with these isolated fungi and any that seem important enough will be identified and exposed to further testing and trials.

(3) Several bioassay experiments, conducted in both Rehovot and Hebron, are currently in progress, to test the virulence of the two isolates 1972 and 539 against 3<sup>rd</sup> and 4<sup>th</sup> larval instars of *H. armigera*. Meanwhile, following are partial results:

Single-spore cultures from the two isolates ARSEF 1972 and ARSEF 539, and a single spore culture from the Israeli isolate (see first annual report), were prepared from the original cultures, after one passage through *H. armigera* larvae. The cultures were then sub-cultured for 3-4 months on the standard SMAY medium and stored at 4°C prior to use. Fungal pathogenicity was evaluated using 4<sup>th</sup> instar larvae of *H. armigera* and *Spodoptera littoralis*. Cultures were grown on SMAY (Sabouraud's Maltose Yeast extract Agar) for 2-3 weeks at 20-23°C in the dark and conidia were scraped from the surface, dispersed in soybean oil and filtered through gauze to remove hyphae. Serial dilutions of conidia were prepared and conidial viability determined.

Conidia were also prepared from laboratory-infected larvae. *H. armigera* and *S. littoralis* 4<sup>th</sup> instar larvae were topically treated with either soybean oil (control) or with conidial suspensions in soybean oil, then transferred individually to 50 mm diameter Petri dishes and fed with fresh cabbage leaves. The ambient temperature during these experiments was in the range of 24±2°C. Dead larvae were transferred to clean Petri dishes with moist filter paper, kept in the dark at about 25°C and examined daily for fungal growth for 3-4 weeks after inoculation. Larvae with no visible sporulation were dissected to examine the presence of hyphae within the host.

Isolate ARSEF 1972 was the most effective of the three isolates and larvae of *S. littoralis* were affected more than were larvae of *H. armigera* by several criteria:

A concentration of 10<sup>4</sup> conidia of the isolate ARSEF 1972 was sufficient to obtain maximal infection of *S. littoralis* larvae (69%); Mycosed larvae were first observed at all conidial concentrations about 6 days after infection. Infected larvae died within the

next 24 h when treated with a concentration of  $10^5$ , within 48 h when treated with a concentration of  $10^4$  and within 9 days when treated with a concentration of  $10^3$  conidia. ARSEF 1972 was less effective against *H. armigera* larvae: Conidial concentration to obtain maximal infection (36%) was about  $10^4$ ; Death of mycosed larvae was first observed at this conidial concentration 8 days after infection and occurred between 8-15 days. This could be expected, as this, and the other isolates tested, originated from field-infected *S. littoralis*

Does infectivity of *N. rileyi* isolates increase after serial passages through *H. armigera* larvae, as compared to cultures maintained on synthetic media and storage? Infection of *H. armigera* larvae with conidia of ARSEF 1972, obtained directly from freshly-sporulated *H. armigera* larvae, was no more effective than the standard preparation used above. This suggests that no benefit accrues from using preparations newly prepared from mycosed hosts, compared to preparations reared on the standard SMAY medium and stored under refrigeration. This is an encouraging result for subsequent mass propagation and formulation of fungal conidia of *N. rileyi*. It is contrary to common dogma, and this test will be repeated.

Isolate ARSEF 539 has been tested with *H. armigera* larvae:

Conidial concentration to obtain maximal infection (25%) was about  $10^4$ . Mortality of *H. armigera* larvae was less than that obtained with ARSEF 1972: Death of mycosed larvae was first observed at conidial concentrations of  $10^3$ - $10^5$  at 5 days after infection and occurred in the range of 5-16 days.

The Israeli isolate was also tested with *H. armigera* larvae at one concentration of  $0.8 \cdot 10^4$  conidia:

Maximal infection was about 33%, a percentage of mortality comparable to ARSEF 1972: Death of mycosed larvae was first observed by 7 days after infection and occurred in the range of 7-17 days.

If these preliminary results with single spore culture are corroborated in our ongoing experiments, this means that compared to the parent cultures, infectivity is reduced. If so, mass propagation and field tests, to be done later on, will be performed with preparations from heterogenous (multi-spore) populations.

More detailed results of all the above experiments will be documented in the second annual (fourth semi-annual) report.

### **I. C) Scientific Impact of Cooperation:**

The interaction of the two research groups from Rehovot and Hebron has been restricted hitherto to direct meetings in Rehovot and Jerusalem between the two cooperating investigators, due to circumstances beyond our control. **Dr. Aviva Uziel** was employed in Rehovot on a temporary basis during this reporting period and the selective procedure is to a large extent based on the studies she initiated and those continued in Hebron. She periodically contacted the Hebron laboratory by e-mail, in order to exchange details of experiments and results. The technicians in Hebron and a Palestinian M.Sc. student, **Mr. Izaldien Qtait**, who is now registered at Hebron University, have participated in this project during this reporting period. A graduate student (M.Sc. student), **Ms. Dana Yehieli**, is now working in Rehovot on elucidating the mechanism of differential infectivity of the different *N. rileyi* isolates to larvae of the two noctuids, *H. armigera* and *S. littoralis*, and perhaps to a third potential host species. The research topics of Ms. Yehieli and Mr. Qtait are on complementary aspects of host-pathogen interactions.

### **I. D) Description of Project Impact:**

Impact is anticipated after this project is field-tested and students are trained in these aspects of insect pathology.

### **I. E) Strengthening of Middle Eastern Institutions:**

As noted in our previous progress report, mobility of students and technical staff between the two locations is necessary for optimal training to take place. This cannot be realized presently, but we intend to request this as soon as possible.

## **Section II: Project Management and Cooperation**

### **II. A) Managerial Issues:**

A major ongoing concern for the Palestinian group has been the expedient transfer of funds requested. This has improved somewhat. Both cooperating investigators request attention to this issue.

### **II. C) Cooperation, Travel, Training and Publications:**

Cooperation has been detailed above.

We have decided to publish a joint paper on part of the research performed hitherto.

**II. D) Request for USAID Actions:**

As mentioned and understood in several sections, the lack of direct contact is hindering the *personal interaction, coordination* and ultimately, the optimal progress of this project. It is clear that in order to succeed more fully, we shall need an extension of the *tenure of this grant, for at least one year* (**no** additional budget allocation) and therefore request at this time such **extension for one year**. We hope that (earlier than later) *issues of security will be resolved and that restrictions on mobility in both directions eased*, so that we are able to realize fully our initial plans and intentions.