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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 the **first** semi-annual report for this 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, 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 to the first objective:

(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.

This coincides with items cited in the **time-chart** of the 1st year (i.e., the effects of physical and nutritional factors on fungal development, and host-pathogen interactions):

Time Chart

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

At present, there are six isolates in our culture collection, one originating from Israel and five from the entomopathogenic fungal collection (ARSEF), of the USDA-ARS. Ithaca, NY, USA.

I. B) Research Accomplishments:

Preliminary tests to evaluate pathogenicity and virulence of the six available isolates of *N. rileyi* were performed with larvae of *Helicoverpa armigera* and *Spodoptera littoralis*. Conidia were obtained from multi-spored cultures of the 6 isolates, and inoculated onto 4th instar larvae of both species. The number of inoculation tests with each isolate are as appear in Table 1, using three inoculation methods:

(a) Dipping in an aqueous conidial solution containing 0.1% Tween 20;
(b) Physical contact of larvae with dry conidia from an *in vitro* culture or from an infected larva with external sporulation.
(c) Inoculation with aliquots of conidia suspended in 1-3 µl of soybean oil.

In experiments with both oil or aqueous preparations, the concentration of conidia was from 5×10^4 to 5.6×10^7 conidia /ml. Larvae were fed on either tomato leaflets, cabbage leaves or Castor Bean leaflets during the experiments and were held at 24 –

28° C under natural light conditions. Castor Bean is less appropriate as a sole source of food, and was substituted at later stages with a semi-synthetic diet.

Four isolates were pathogenic to the test insects:
ARSEF 539, from *Spodoptera exigua*, ex. Thailand, 1980;
ARSEF 323, from a *Spodoptera* sp., ex. Queensland, Australia, 1979;
ARSEF 1972, from *Spodoptera frugipedra*, ex. Brazil, 1985;
3431, from *S. littoralis*, Ramot Menashe, ex. Israel, 1986;

Isolates ARSEF 380 and 4094 did not infect the test insect species, which might be attributed in part to their poor sporulation.

Infection of *S. littoralis* and *H. armigera* by isolates ARSEF 539, ARSEF 323, ARSEF 1972 and 3431 ranged from 11 to 94% (Table 1). The different inoculation methods did not affect the level of infection. However, whenever infection was not achieved (in experiments not presented in Table 1), inoculation was predominantly by contact with dry conidia or application of conidia in soybean oil. Nevertheless, attempts will be made to improve the method of inoculation with soybean oil as carrier, in anticipation of field tests. Inoculation with an aqueous solution of 0.1% Tween 20 appears to be superior to other methods for routine use in laboratory tests. All the pathogenic isolates, except for isolate ARSEF 323, produced vast hyphal outgrowth and sporulation on the dead larvae following exposure to 100% RH for 24 h.

Table 1: Infectivity of *Nomuraea rileyi* isolates to two species of Noctuidae

No.	Fungal Source	Inoculation Method	Moth Species	% Mortality	Range of Death (days)
	ARSEF 539				
1.	SMA*	aqueous 6 x 10^6 /ml	*S. littoralis*	50 (n=10)	6-8
	ARSEF 323				
1.	YpSs**	dry conidia	*H. armigera*	85 (n=7)	10-?
2.	YpSs	soybean oil 4.5 x 10^7/ml	*H. armigera*	30 (n=7)	7-10
3.	YpSs	aqueous 9.2 x 10^6 /ml	*H. armigera*	85 (n=7)	6- 9
	Israeli 3431				
1.	YpSs	dry conidia	*S. littoralis*	33 (n=18)	8-11
2.	*H. armigera*	dry conidia	*H. armigera*	66 (n=9)	9-14
3.	YpSs	aqueous, 1.5 x 10^6 /ml	*H. armigera*	66 (n=9)	6-9
4.	YpSs	soybean oil 6.3 x 10^6/ml	*H. armigera*	20 (n=10)	8-9
5.	YpSs	soybean oil 5.6 x 10^7/ml	*H. armigera*	94 (n=18)	7-10
6.	*H.armigera*[3]	aqueous 1.5 x 10^7 /ml	*H. armigera*	20 (n=10)	6-6
7.	*H.armigera*[3]	aqueous 5 x 10^4 /ml	*H. armigera*	63 (n=11)	7-14
8.	*H. armigera*	dry conidia	*H. armigera*	70 (n=10)	5-8
9.	*H. armigera*	aqueous 9 x 10^5 /ml	*H. armigera*	66 (n=12)	5-14
10.	H. armigera	aqueous 6.5 x 10^5 /ml	*S. littoralis*	90 (n=10)	6-7
	ARSEF 1972				
1.	culture	dry conidia	*S. littoralis*	50 (n=8)	8-10
2.	larva	dry conidia	*H. armigera*	70 (n=10)	10-20
3.	*H. armigera*	dry conidia	*H. armigera*	88 (n=9)	6-11
4.	*H. armigera*	dry conidia	*H. armigera*	67 (n=9)	6-12
5.	*H. armigera*	soybean oil 3 x 10^7 /ml	*H. armigera*	60 (n=10)	8-12
6.	*H. armigera*	aqueous 4 x 10^7 /ml	*H. armigera*	72 (n=11)	6-12
7.	*H. armigera*	aqueous 3 x 10^6 /ml	*H. armigera*	90 (n=10)	7-10
8.	*H. armigera*	aqueous 7 x 10^5 /ml	*H. armigera*	73 (n=11)	6-8
9.	*H. armigera*	aqueous 7 x 10^5 /ml	*H. armigera*	45 (n=11)	8-11
10.	*H. armigera*	aqueous 3 x 10^5 /ml	*H. armigera*	50 (n=8)	9-11
11.	*H. armigera*	aqueous 3 x 10^5 /ml	*H. armigera*	40 (n=10)	8-9
12.	*H. armigera*	aqueous 5 x 10^4/ml	*H. armigera*	87 (n=8)	9-15
13.	*H armigera*[3]	aqueous 1.8 x 10^6 /ml	*H. armigera*	71 (n=7)	9-12

*SMA = Sabouraud's maltose-peptone agar; SMAY = SMA supplemented with 1% yeast extract. **The composition of YpSs is specified in: Tuite, J. 1969: Plant Pathological Methods. Fungi and Bacteria.

Tentatively, it seems that inoculation with 5 x 10^4 conidia/ml is as effective as higher conidial concentrations (e.g. tests with isolate 3431). Inoculation with conidia kept at 4 C for >4 months prior to use elicits lower infection levels. Therefore, conidia should be used as early as possible after formation. Initial mortality occurred within the first 6-10 days, with >50% of the cases occurring between 6-8 days.

2. Based on the above infectivity tests, single-spore cultures were prepared from isolates ARSEF1972 and ARSEF 539, originating from infected larvae. For the Israeli isolate, single spore cultures will be obtained from conidia originating from infected larvae from an experiment scheduled for the end of September.

3. The temperature range for germination was studied with single spore cultures of isolates ARSEF 1972 and ARSEF 539. Conidia (ca. 10^4 per dish) were seeded on SMAYT in small Petri dishes. The dishes were incubated in the dark at 5-35°C for up to 6 days and scored for germination every 24 h. For both isolates, highest germination and fastest germ-tube elongation was obtained at 25 and 30 °C. However, the isolates greatly differed with respect to the speed of germination at these temperatures. For ARSEF 539, maximal germination was achieved within 24 h, whereas for isolate ARSEF 1972 this occurred at 48 h. Comparison of germination of isolate ARSEF 539 and ARSEF 1972 at 25 and 30 °C after 14 h and 29 h, respectively, revealed no significant differences (arcsine transformed; *t*- test; α=0.05) with respect to percentage of germination and germ-tube length. At present, germination of both isolates at 25 and 30°C is examined after up to14 h of incubation, in order to compare the timing of germ tube emergence of each isolate at the two temperatures, and to determine the optimal temperature for germination of each isolate. In addition, hyphal growth and sporulation of these isolates at the same temperature range is currently being studied.

4. In order to obtain additional local isolates, we contacted agricultural extension personnel from private and governmental organizations in Israel and the PA, and we will be provided by them with any noctuid larvae the suspect of being infected by entomopathogenic fungi.

There is no published information on differential media for isolating *N. rileyi* from soil, although soil might be a presumptive reservoir for this fungal species. We will first exploit existing media for fungal isolation from soil (e.g., Martin's Rose Bengal medium and semi-selective media for isolation of *Metarhizium anisopliae* from soil). If necessary, we will incorporate selective fungicides (e.g., Dodine) and/or antiobiotics (e.g., Chloramphenicol) into SMAYT (Sabouraud's maltose agar plus yeast extract supplemented with 0.1% w/v Tween 80).

I. C) Scientific Impact of Cooperation:

Shortly after the project commenced, Dr. Uziel established the methodology in Rehovot to be used for assessing the efficacy of the different *Nomuraea rileyi* isolates. Professor Barakat came to Rehovot in order to coordinate these experimental procedures with those to be used in his laboratory in Hebron. Professor Barakat is presently being assisted in his studies by his technicians in Hebron. We have not yet succeeded in recruiting a Palestinian doctoral candidate with suitable qualifications for this study. One potentially good candidate, with an M.Sc.degree from Birzeit University in Clinical Microbiology, decided not to switch from medical microbiology to agricultural microbiology. This was disappointing, as Palestinian doctoral candidates are in short supply. We are looking for additional candidates. Meanwhile, we are also in the process of recruiting Palestinian M.Sc. candidates. At least one of them will be registered at Hebron University, and jointly supervised by Professor Barakat of Hebron and Professor Applebaum of the Hebrew University. Although the logistics of this is presently difficult, we intend to persevere and initiate this basis for cooperation and training of students.

I. D) Description of Project Impact:

Any impact can be expected only when this project is field-tested and students are trained in these aspects of insect pathology.

I. E) Strengthening of Middle Eastern Institutions:

The facilities for such studies in plant protection were established in Hebron University from previous cooperation between the two PIs. Specific equipment will be purchased during the second half of the first year and training will commence, beyond the level of cooperation between the senior scientists in this project, as soon as we recruit the graduate students.

Section II: Project Management and Cooperation

II. A) Managerial Issues:

A major budgetary concern for the Palestinian group is that they have not yet received the initial financial transfer of funds. A bank check was mailed directly from

RAMC/Paris to Hebron, but the bank in Hebron has not received any funds, and this proves difficult to cope with. The Palestinian PI, Professor Barakat, requests that this issue be resolved as soon as possible, in order that when we have the students in place, funds would be available for their support and for research expenditures.

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

Cooperation has been detailed above.

Professor Barakat, a Phytopathologist by training, attended the 7th International Mycological Congress in Oslo (August 11-17, 2002) in order to become acquainted with the current trends in studies on entomopathogenic fungi as biocontrol agents, and to meet scientists from the international community engaged in this subsection of Mycological research.

No publications can be expected during such an early stage of the project.

II. D) Request for USAID Actions:

In order to resolve the budgetary concern as detailed in **II. A)** Managerial Issues.