

SEMI-ANNUAL REPORT

Covering period: December 1, 2003 to May 31, 2004

**Submitted to the U.S. Agency for International Development; Bureau for
Economic Growth, Agriculture and Trade**

TITLE OF PROJECT: Epidemiology and control of mango malformation disease

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Section I: Technical Progress

Due the nature of possible spread of the pathogen via seedlings in Egypt a special trip was conducted by Freeman in April 2004 to set up experiments to evaluate this hypothesis (Appendix)

I. A) Research Objectives for this period

1. Determine source of inoculum, survival and epidemiology of mango malformation disease

- (i) Timing of sporulation and conidial dispersal. (Completed)
- (ii) Infection of bark, fruit and seed. (Completed)
- (iii) Isolation of the pathogen from different growth tissue. (Completed)
- (iv) Transfection via grafting. (Completed)
- (v) Root infections and survival of the pathogen in soil and panicles. (Completed)
- (vi) Role of *Aceria mangiferae* in vectoring *F. mangiferae*. (In progress)

2. Evaluate chemical control methods, pathogen interaction with mites, and screening of susceptible/resistant mango varieties to the disease

- (i) Investigate controlling malformation with combinations of miticides and different protectant and systemic fungicides. (In progress)
- (ii) Determine the relative resistance/susceptibility of diverse mango genotypes to malformation: (In progress)

3. Investigate the population biology and genetic diversity of the pathogenic *Fusarium* species (In progress)

4. Develop a reliable molecular diagnostic method for detection of the pathogen in infected mango tissue (In progress)

I. B) Research Accomplishments:

1. Determine source of inoculum, survival and epidemiology of mango malformation disease

(i) Timing of sporulation and conidial dispersal (Egypt). (Completed)

The time of the conidial release of *Fusarium mangiferae* was determined by sampling infected (phonological stages) panicles of mango at four stages of development i.e. fully swollen buds, bud inception, fully grown panicles prior to full bloom and panicles at full bloom, three times a month from the beginning of flowering (end of Feb to Mar) till the end of flowering (May). Infected panicles were collected from different mango orchards in the main growing governorates i.e. Ismailia, Sharkia, Kalubia, Giza, Fayoum and Nobaryia (Behera Governorate). Flowers from malformed panicles were surface sterilized with 5% sodium hypochlorite solution for 3 minutes, followed by successive washings with sterile distilled water and dried on sterilized filter paper and plated on PDA medium. Counts of fungal colonies were recorded by quantitative determination of spores in the same previously phonological stages of bud growth at the same time. One gram bud tissues or flowers, was shaken in 5 ml distilled sterile water for 30 min at 120 rpm and diluted serially for conidia counts (spores/gram) at various periods. Uninfected panicles from healthy trees were used as control. Data presented (Table 1) indicates that average number of conidia increased in malformed panicles in Egypt (similar to the previous report #2, conducted in Israel), increasing with maturity of the panicles, during April and May as compared with March in all cultivars. On the other hand, the average of conidia decreased in Zebda (resistant cultivar) as compared with Ewais and Socchari (susceptible cultivars).

Table 1: Quantification of *F. mangiferae* spores in infected mango panicles at four stages of development (phonological stage) of three cultivars different in their susceptibility to malformation

Bud Phonology	Stage of panicle	Average of conidia per mm square of hemacytometer								
		Ewaise			Sacchari			Zebda		
		March	April	May	March	April	May	March	April	May
Fully swollen buds	Still unknown	13.5*	34.2	36.7	21.5	38.0	37.5	0.0	4.5	6.0
Bud inception	infected	42.5	58.2	56.1	39.5	46.0	48.0	3.7	6.2	14.0
	healthy	0.0	0.0	2.0	0.0	5.0	0.0	0.0	0.0	0.0
Fully grown panicle prior to full bloom	infected	62.6	79.5	82.2	58.5	69.0	77.5	13.5	27.0	23.0
	healthy	0.0	2.0	0.0	0.0	1.0	3.0	0.0	0.0	1.0
Panicle at full bloom	infected	69.3	80.0	99.0	63.9	75.5	82.8	19.7	22.0	21.0
	healthy	1.0	0.0	0.0	0.0	3.0	2.0	0.0	0.0	0.0

* Average of five replicates.

(ii) Infection of bark (Egypt). (Completed)

Pieces of bark were sampled from different infected orchards in Ismailia, Sharkia, Kalubia, Giza, Fayoum and Nobaryia (Behera Governorate) during December 2003 till May 2004. Tissues were removed, washed thoroughly in tap water, surface sterilized and dried on sterilized filter paper. Bark pieces were plated on PDA and incubated at 28C for 7 days and pure cultures obtained (Table 2).

Table 2: Percentage of fungi isolated from mango braks from different locations in Ismailia, Sharkia, Kalubia, Giza, Fayoum and Nobaryia – Behera Governorate from Dec.2003 to May 2004.

Governorate	% of <i>Fusarium</i> spp. isolated from Dec.2003 to May 2004						Mean
	Dec.	Jan.	Feb.	March	April	May	
Ismailia	1	0	3	5	6	10	4.1
Sharkia	1	3	2	5	6	9	4.1
Giza	1	2	3	4	5	9	4.0
Kalubia	1	3	3	5	6	8	4.3
Fayoum	0	2	1	2	4	6	2.5
Behera	4	3	1	2	2	0	2.0
Mean	1.33	2.2	2.2	3.8	4.8	7.0	3.55

Data in Table 2 indicates that the fungus *Fusarium* was detected in most inspected mango barks at low frequency. *Fusarium* presence increased during March, April, and May as compared with December, January and February (very cold climate months in Egypt).

(iii) Isolation of the pathogen from different growth tissue. (Egypt) (Completed)

Five infected and five healthy branches of three mango cultivars, company, Sacchari (susceptible) and Zebda (resistant) were pruned from the infected or healthy panicles to the trunk of each tree. Isolation of the fungus from malformed and healthy tissues were made at different distances from the malformed and healthy parts, 5, 10, 20, 30, 60, 90, 120 and 150 cm. away from the outer cortex and inner xylem tissues. Results are presented in Table 3.

Table (3): Percentage of *Fusarium mangiferae* in host tissues of diseased and apparently healthy trees. Samples were taken from internal cortex and xylem tissues at different distances from the malformed tissues of three cultivars of mango differing in their susceptibility to malformation.

Dist. cm	State	% of malformation in these cultivars																				
		Company, susceptible									Succhari, susceptible						Zebda, resistant					
		Dec	Jan	Feb	Mar	Apr	May	Jun	Dec	Jan	Feb	Mar	Apr	May	Jun	Dec	Jan	Feb	Mar			
0	D	5	5	40	85	65	70	10	20	15	20	75	55	25	20	15	10	20	75	50	55	20
	H	0	0	0	20	10	0	0	0	0	0	10	5	5	0	0	0	0	5	10	0	0
5	D	5	0	10	15	15	30	0	0	0	10	25	0	20	5	0	0	0	20	10	10	10
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	D	0	0	0	0	5	5	0	0	0	0	0	5	0	0	5	0	0	5	5	0	5
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	D	5	0	10	0	5	25	5	0	0	0	20	5	10	0	0	0	0	0	0	0	5
	H	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0
30	D	0	0	0	0	0	0	5	0	0	0	15	10	5	0	0	0	0	10	0	0	5
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60	D	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90	D	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0
120	D	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
150	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Results indicate that *F. mangiferae* was isolated from infected vegetative and inflorescence malformation of all cultivars under study (Table 3), similar to results reported in Israel (see report #2). The frequency of *Fusarium* increased during March, April and May as compared with December, January, February or June. The fungus was isolated with high frequency close to the malformed tissues but frequency decreased, 5 cm away from the malformation. In few cases, the fungus could be isolated from more than 30 cm away from malformation (similar to results reported from Israel). Infection may have occurred in lateral buds as a result of mechanical injury or wound caused by mites or insects and spread within. Results show that Company and Succhari (susceptible cultivars) were more colonized than Zebda (less susceptible cultivar). The apparent healthy panicles and branches showed very low presence of *F. mangiferae* as compared with the malformed tissues.

(iv) Transfection via grafting. (Egypt) (Completed)

Healthy mango transplants (2 yr-old), of Succhari and Ewaise (highly susceptible) and Zebda (less susceptible or resistant) were used for this purpose during September and October 2003.

Transmission of the malformation disease through grafting was determined as follows:

a-Grafting infected material (scions) on the top of healthy nursery plants (susceptible and resistant rootstock); b-Grafting healthy material (susceptible and resistant on malformed nursery plants; c-Grafting healthy material beside apical malformation. In summary, grafting healthy scion of Succhari (susceptible cultivar) on malformed rootstocks showed malformed growth on two transplants which represent 11% of disease transmission while 16 nursery plants showed healthy growth till the present date. However, grafting healthy scions of Zebda (resistant cultivar) beside apical malformation or in the same place after removing malformation recorded no disease transmission and all grafting transplants forming healthy growth till the present date. Therefore, it could be recommended that mango malformation disease can be transmitted through grafting materials. Also, grafting resistant and healthy material is necessary to avoid disease transmission. Similar results were observed in Israel (report #2).

(v) Root infections and survival of the pathogen in soil and panicles. (Israel) (Complete)

Infected inflorescences were buried in soil to a depth of 10 cm or left above the surface and sampled over a period of 180 days to determine survival percent (Table 4). Each inflorescence was divided into 10 parts, 3 replicates per treatment and plated on *Fusarium*-specific medium.

Table 4: Percent survival in time of *F. mangiferae* in inflorescences buried in soil (10 cm) or on the soil surface

Days	In soil (survival %)	Soil surface (survival %)
0	96	96
7	95	87
21	97	74
60	92	0
120	88	20
180	67.5	0

In summary, infected tissue buried in soil can harbor the pathogen much more effectively for greater lengths of time than inoculum in tissue on the surface which loses viability much more rapidly.

vi) Role of *A. mangiferae* in vectoring *F. mangiferae* (Israel) (In progress)

To determine whether the mango bud mite can carry *F. mangiferae* spores on its body, mites collected from infested buds in the orchard were exposed to a *gfp* marked isolate of *F. mangiferae* using two different methods. In the first method 20 mango bud bracts bearing approximately 100 mites were dipped in a *gfp*-marked *F. mangiferae* spore suspension of 10^6 spores per ml for 5 seconds. After letting the bud bracts dry, mites were removed from the bracts with an ultra fine paint brush and mounted on double-sided sticky tape. In the second method 30 mites were placed on an agar square (5mm²) which was inoculated 48 hours before with a *gfp* isolate of *F. mangiferae*. After 24 hours the mites were removed and mounted. Images were acquired using a confocal laser-scanning microscope system OLYMPUS IX81. Confocal images were obtained via a PLAPO 40X WLSM immersion objective lens at an excitation wavelength of 488nm (Argon laser), and a BA515-525 emission filter for *gfp*, and BA660IF emission filter for autofluorescence. Transmitted-light images were acquired using Nomarski differential interference contrast. Using the second inoculation method *gfp* glowing spores were observed on mites examined (figure 1). Spores did not seem to cling to any particular part of the mites' body. Mites removed from the spore suspension did not bear any spores at all. Our observations do suggest that mites can bear fungal spores. We are now using the agar squares infested with mites and the *gfp* strain to determine whether the mites move the fungal spores into the apical bud.

Potential infection sites of *Fusarium mangiferae*

Previous experiments have shown that apical buds are excellent sites for infection. The experiment described below was designed to determine whether leaves can serve as penetration sites as well.

Twelve stems bearing leaves were cut from young seedlings and placed in water containers. Three treatments were compared: 1) Fifty leaves were inoculated with a drop of spore suspension that was placed over a needle's prick; 2) fifty leaves were inoculated without a needle's prick; and 3) Twenty control leaves were inoculated with water. After ten days, the leaves were sampled and placed over selective media in Petri dishes. The fungus was found in 70 % of the pricked leaves, but only on the spot of infection and not around it. The other 30 % of the pricked leaves, as well as all of the non-pricked leaves, were not colonized by the fungus. These results suggest that the apical bud is serving as an exclusive site for primary infection and that the spores that reach leaves in the orchard can not penetrate the leaves, or, in case of an injury, are not able to expand within the plant tissue.

Phenology of *Fusarium mangiferae* and *Aceria mangiferae* on young shoots

The seasonal phenology of *F. mangiferae* and *A. mangiferae* on young shoots will be studied in order to gain knowledge on: 1) When and to what extent external primary infections occur throughout the year; 2) the possible correlation between external infected buds and the presence of

bud mites. In January 2004, 80 mango branches from the Volcani orchard were sampled and tested for the presence of bud mites and *F. mangiferae*. The branches were sampled from eight Hayden cultivar mango trees from two subgroups: four highly diseased trees (trees that in the 1999 estimation of disease had more than 55 malformed inflorescences per tree) and four slightly diseased trees. The stems bearing young apical buds were pruned for inspection under the previous season's bud. Apical buds were dissected into bud scales and carefully examined under a stereomicroscope for the presence of bud mites. 5mm pieces of dissected stems, as well as the bud scales, were surfaced sterilized 10 seconds in 70% ethanol and than 3.5 minutes in 33% sodium hypochlorate. Pieces were than laid over Nash medium Petri dishes. After six days, fungal colonies that resembled *Fusarium* morphology were transferred into PDA, then sorted again after a week and finally identified either by their morphology or by using a molecular PCR method.

The levels of incidence (%) of *Aceria mangiferae* and *F. mangiferae* in apical buds and stems were similar on trees that were lightly and heavily infected the year before. Forty % of all buds were infected. Half were infected in both bud and stem and half were infected only in the buds. The latter group, of which, 75% were inhabited with bud mites, were apparently inoculated externally with airborne or bud mite- carried spores. The spring sample was collected recently and is presently being processed. It is still premature to discuss the above results as two additional samples (summer and fall) must be conducted in order to receive a complete data set of the phenology of the mite-fungus interaction.

Morphological and genetic characteristics of regional strains of *Aceria mangiferae*

The association between *A. mangiferae* and *F. mangiferae* have been reported from North and South America, Africa and Asia. To test the hypothesis that different growing regions have different strains of this species or even different species of *Aceria*, mites were collected from: 1) Bet Dagan Orchard in Israel; 2) Homestead, Florida, USA and 3) Sharkiya, Ismaliya, Fayoun and Giza governates of Egypt. Slide preparations are now being prepared to compare morphological characteristics. To determine the possible genetic differences between mites from different regions, PCR methods developed for eriophyids will be used.



10 μ m

Fig 1. *Aceria mangiferae* carrying 3 *gfp* spores of *Fusarium mangiferae* on its body.

2. Chemical control methods and screening of susceptible/resistant mango varieties disease

(i) Investigate controlling malformation with fungicides. (Egypt and Israel) (In progress)

Preliminary *in vitro* testes were conducted to evaluate the efficacy of different fungicides group on mycelial growth of *Fusarium mangiferae*. The triazole group (Bromuconazole and Metaconazole) recorded high efficiency followed by Thiophanate-Methyl, Mancozeb, Cymoxanil and Chlorothalonil group. On the other hand, the Strobilin group (Pyraclostrobin + Nicobifen, Trifloxystrobin and Azoxystrobin) showed moderate efficiency while the Mono and Dibasic Sodium and Potassium Phosphate was less than others.

Field control of mango malformation on infected seedling and mature trees will begin during the next period.

New fungicides are being currently evaluated in Israel. The product Octave (active ingredient prochloraz) has proven to be effective in *in vitro* experiments. Field trials are currently running in Israel but evaluations of disease can only be evaluated in the summer once the malformed panicles have been removed during May to August, 2004.

(ii) Determine the relative resistance/susceptibility of diverse mango genotypes to malformation: (USA) (In progress)

The commercial nursery that has been hired to propagate Egyptian cultivars for use in these studies will, unfortunately, be unable to provide grafted plants before 2005. There remains a high demand for, and a limited amount of, scion material of these cultivars in Florida (plants are also needed by the USDA-ARS, Miami, Fairchild Tropical Garden, and the University of Florida's cultivar collection at TREC-Homestead). Due to this delay, a study has been initiated with cultivars that are available in Florida. Seven commercial cultivars have been used in the first of series of incubator studies. To the extent that was possible, they were chosen to cover a broad spectrum of genetic diversity in *Mangifera indica* (Fig. 1; Schnell et al., 1995). On 10 May 2004, a single plant each of 'Glenn', 'Haden', 'Irwin', 'Keitt', 'Tommy Atkins', 'Van Dyke' and 'Zill' was inoculated with isolates from two of the three VCGs of *F. mangiferae* that are present in Florida. Protocols developed previously in Israel were utilized to establish infection. After 2 weeks, plants will be transferred to a Conviron incubator set at temperatures that will induce flowering (15°C day/10°C night). Given the relatively small size of the incubator, experiments will replicated over time and with different isolates. By the time plants of Egyptian cultivars are available, resistance in Florida cultivars to three VCGs of *F. mangiferae* will be classified (Florida cultivars are among the most important worldwide), and conditions in Florida under which malformation can be initiated experimentally will be understood.

3. Investigate the population biology and genetic diversity of the pathogenic *Fusarium* species
DNA from all the isolates and additional ones collected since the last report is being extracted for the population genetic diversity studies. (In progress)

4. Development of a reliable molecular diagnostic method for detection of the pathogen in infected mango tissue

Species-specific primers from the lab of Ploetz are working well both in the lab of Freeman and Shalaby. These primers are being used to determine presence of the pathogen in infected tissue and to confirm the identity of the species *F. mangiferae*. (In progress)

I. C) Scientific Impact of Cooperation:

Two visits took place between the Egyptian and Israeli scientists.

Visit no. 1:

Freeman and Palevsky visited Shalaby in Giza, Cairo from 19th to 22nd February, 2004. The purpose of the visit was to evaluate the Egyptian experiments, collect mango bud mites and malformed tissue from various locations. Fortunately, our visit coincided with that of Dr. Adam Reinhart (AAAS Diplomacy Fellow) of the USAID-MERC Mission and that of Daniel O. Hastings (Second Secretary) US Embassy. During the first 2 days we traveled to Sharkia, Ismailiya, El-Fayoum and Giza Governates to conduct this work which was very fruitful. It was stressed that the propagation of seedlings within infected orchards should be investigated as these seedlings may be the cause of the spread of the disease in Egypt. Thus, it was decided that Freeman would plan a follow-up visit in April 2004 to tackle this problem. The third day was spent with all parties visiting infected orchards in the Ismailiya area.

Visit no. 2:

Freeman traveled to Egypt again from 16th to 23rd April, 2004 to conduct a field experiment in 4 plots in Sharkia and Ismailiya governates with Shalaby, to determine whether the source of distribution of malformed material is via seedlings. Seedlings were sampled from the 4 plots as well as malformed tissue from mature trees in the same orchards. Work is currently in progress in both labs to determine whether the same genotype is present in seedlings and mature trees and what parts of the seedlings are colonized by the pathogen.

Future visit:

The second annual scientific meeting is scheduled to take place end of November, 2004 in Sharm-el-Sheihk, Egypt, between the Israeli, Egyptian and US partners. The purpose of the meeting is to discuss progress according to the workplan, plan future experiments, sum up results and possibly write a draft of a joint publication.

I. D) Description of Project Impact:

As the project is only in its initial stages of progress this part of the project is still to be evaluated.

I. E) Strengthening of Middle Eastern Institutions:

The necessary equipment for the successful implementation of the research project is currently being purchased. In Israel, a compound phase DIC microscope has arrived and was installed. This piece of equipment is part of a pre-requisite for conducting the mite-associated work. In Egypt, all equipments needed for the project's plan have been purchased i.e., (Microscope with Camera, Growth Chamber, Image capturing system, Electrophoresis units and power supply, Oven, Centrifuge, cooling incubator, Laptop and PC, Digital Camera, and Data projector. A purchase order for PCR machine has been placed). In addition chemicals and supplies have been ordered according to the workplan.

I. F) Future Work: The project is commencing at a very good pace and following the workplan. Additional important work pertaining to distribution and spread of the pathogen via seedlings in Egypt is being evaluated.

Section II: Project Management and Cooperation

II. A) Managerial Issues:

The project is progressing to plan. No barriers or problems have arisen regarding the managerial issues pertaining to the project.

II. B) Special Concerns:

The special concerns protocols have not been changed and are current according to the project proposal.

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

Exceptional cooperation and collaboration has been achieved between the Israeli and Egyptian participants over the past 6 months. A second meeting was held to discuss progress according to the workplan, assess Egyptian studies in the lab, greenhouse, and field, and to collect mites and infected material, took place from 19 to 22nd February, 2004. Freeman traveled to Egypt again from 16th to 23rd April, 2004 to conduct a field experiments in Sharkia and Ismailiya with Shalaby, to determine whether the source of distribution of malformed material is via seedlings. Work in both labs currently in progress. The next coordination meeting with all participants is planned to take place in Egypt between all participants in Nov., 2004.

II. D) Request for USAID Actions:

There is no need for USAID intervention in any aspect of this project as the project is progressing according to plan and cooperation and collaboration is sound.

Field trip to infected mango plantations during visit in Egypt (22nd February, 2004): Including Daniel O. Hastings (Second Secretary) US Embassy; Dr. A. Reinhart (USAID-MERC); Project investigators: Dr. M. Sattar, Dr. A. Shalaby (Egypt) and Dr. S. Freeman, Dr. Palevsky (Israel)

Project Activities



Field trip collecting mites during visit in Egypt (20th February, 2004): (L-R) Dr. S. Freeman (PI); Dr. S. Youssef (Egyptian assistant); and (co-PI's) Dr. E. Palevsky, Dr. A. Shalaby and Dr. M. Sattar



APPENDIX

Seedling infection experiment:

Hypothesis:

Seedlings growing under mature trees are being infected from the pathogen produced and spread from flowers in canopies of the mature trees. Infection is NOT systemic and the pathogen is NOT located in lower stem sections and roots.

Workplan in general:

1. Collect infected seedlings from under mature trees possessing malformed panicles.
2. Section seedlings from the infected tips down to crowns and roots and plate on *Fusarium*-specific medium to determine the location of isolates (it is assumed that the infections are only in above ground sections from the infected tips to a short distance below).
3. Conduct species-specific PCR on isolates from seedlings and mature tree isolates to determine that they are *F. mangiferae*. Conduct ap-PCR on isolates from seedlings and mature tree isolates to compare with genotypes from mature panicles. It is presumed that the genotypes are the same which will prove the theory that the isolates in seedlings originated from the mature trees.
4. Any isolates from the below stem sections, crowns and roots will most likely NOT be *F. mangiferae* and these isolates will be amplified by species specific primers to prove this point.

Experimental design and specific workplan:

1. Collect 50 infected seedlings from Sharkia and 20 infected panicles from mature trees above the infected seedlings (Freeman, Shalaby and Sattar).
2. Collect 50 infected seedlings from Ismailia and 20 infected panicles from mature trees above the infected seedlings (Freeman, Shalaby and Sattar).
3. Isolates from infected samples (tips from 100 infected seedlings and tips of 40 infected panicles from mature trees) will be made on *Fusarium*-specific medium and DNA isolated from cultures – small infected samples will be collected in test tubes. *F. mangiferae*-specific primers will be used on all 140 cultures to verify that the pathogen is *F. mangiferae*. Arbitrarily-primed PCR will be performed to determine genetic diversity of the isolates – to show that the seedling isolates are the same as the mature tree ones (Freeman).
4. 100 seedlings will be sectioned, surface sterilized and plated on *Fusarium*-specific medium to determine location of infection of the pathogen in all the seedlings. Any cultures growing from the lower stems will be PCR-amplified with *F. mangiferae*-specific primers to verify that the pathogen is not in the lower stems (Shalaby and Sattar).
5. Cultures from roots (50 at random – 25 from each location) will be isolated to verify that *F. mangiferae* is not isolated from these parts – *F. mangiferae*-specific PCR will be conducted on these cultures to prove this (Shalaby and Sattar).

Outcome:

1. Joint publication (Freeman and team; Shalaby and team; Sattar and team)
2. Publish advisory bulletin to Egyptian farmers to begin cultivation of seedlings separate from mature plants to avoid spread of the pathogen and start production of disease-free seedlings