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INDUCE RESISTANCE IN SUBTROPICAL FRUITS FOR THE PREVENTION OF DECAY INITIATED FROM QUIESCENT INFECTING PATHOGENS

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Executive Summary-

The work that was done in the Fourth year included testing of the non-pathogenic mutants that can be used for the control of postharvest diseases in subtropical fruits. In Philippines the natural non-pathogenic mutant of *C. gloeosporioides* was tested in the field. In Israel the work was directed to test the effect of the Philippino isolates in the field and no results are available till now.

Section I

A. Research objectives

The intention of this research is to develop new technology for control quiescent infections of subtropical fruits by enhancing mechanism of resistance that have been already elucidated by utilizing the non or reduced pathogenesis mutants or direct elicitor of the resistance mechanism. The kind of non pathogenic *Colletotrichum* strain are similar to the Path-1 that infects avocado fruit and does not cause significant disease symptoms but appears to stimulate host defense responses. Host organisms to be studied include avocado and mango fruits. The pathogens will be *Colletotrichum gloeosporioides* in avocado and mango fruit, and selected Colletotrichum strains.

1. Selection and development of non-pathogenic *Colletotrichum* mutant.

It is imperative for the fungus in order to induce resistance to penetrate the fruit cuticle. Based in our early results *Colletotrichum* is the best organism that can penetrate the fruit cuticle and enhance the host defense response. Several steps for the development of non-pathogenic strains will be done parallel.

Natural selection: non-pathogenic strain will be isolated from small limited lesion in decaying fruit. We will test whether several *Colletotrichum* sp. from host other than avocado and mango, can enhance resistance to *Colletotrichum*. We expect to detect other non-pathogenic strains that breached the peel, remain as endophytes, but are not between those known pathogen attacking mango or avocado.

UV treatments: UV treatment will be used for the development of new mutants and selection similarly as described previously (Freeman and Rodriguez, 1992). This method has been very efficient for obtaining the Path-1 mutant.

REMI- Restriction enzyme mediated integration technique (Redman and Rodriguez, 1994) will be used for obtaining mutant of *C. gloeosporioides*. This method was used for tagging and cloning of genes required for pathogenicity by Nir Yakoby in Prusky's lab. An efficiency of 80 transformants per microgram of DNA was obtained by this method in the Israeli Lab.

B. Research accomplishments

Phillipines

The main achievement of the this year was the use of the reduced pathogencity strain ***Colletotrichum capsici*** for the control of anthracnose in leaves and fruits applied at different phenological periods of the mango growth.

1. Israel

In the last year of work our research was concentrated in the use of the Philippino strain to prevent decay development in Israel. Results are still not available. Most of the work in the project was done in the Philippino part of the project.

2. Philippines

Induced Resistance in Subtropical Fruits for the Prevention of Decay Initiated from Quiescent Infecting Pathogens

First year of experiments

I. Effect of Weekly and Fortnightly Sprayings of *Colletotrichum capsici* Mutant at Different Stages of Mango Development on Fruit Anthracnose Infection

Methodology

The ability of *Colletotrichum capsici* mutant (Isolate $013M_4$) to effectively control mango anthracnose in the field at the different stages of mango development was determined. The effect of the frequency of $013M_4$ application on the development of fruit anthracnose was also tested. Application of $013M_4$ at the different stages of growth was done weekly and fortnightly with the following treatments: a) weekly spraying starting at pre-bloom; b) weekly spraying at mango flowering; c) weekly spraying at fruit set; d) fortnightly (once in 2 weeks) spraying starting at pre-bloom; e) fortnightly spraying starting at mango flowering and f) fortnightly spraying starting at fruit set.

A standardized inoculum suspension of 10^6 spores/ml from 7-day old cultures of $013M_4$ was used and 48 hours later, the test organism was challenge inoculated with the pathogen, *Colletotrichum gloeosporioides*, using the same inoculum concentration (10^6 spores/ml).

Periodic spraying of the experimental units was then done following the given spray schedule until the fruits were at the green nature stage when they were ready for harvest. Three control set-ups for each growth stage of mango were used: a) untreated; b) inoculated with the pathogen only; and c) application with $013M_4$ only.

Results

The *Colletotrichum capsici* mutant (Isolate $013M_4$) was highly effective in reducing the incidence of fruit anthracnose infection irrespective of the stage of mango development when treatment with the mutant was initiated. When spraying with the mutant was started at the pre-bloom stage, weekly or fortnightly (once in two weeks) treatments were equally effective in reducing fruit anthracnose (Table 1). The same trend as above was observed when treatment with the mutant was started at the flowering stage (Table 2) as well as at fruit set (Table 3).

These results indicate that treatment with the mutant once in two weeks (fortnightly) is just as effective as weekly treatment. Thus, to save time, money and effort, fortnightly spraying is preferred.

Table 1. Effect of weekly and fortnightly application of *Colletotrichum capsici* mutant ($013M_4$) on anthracnose infection at the pre-bloom stage of mango development

Treatment	Average Percentage Infection (%)	
	Schedule of Application	
	Weekly	Fortnightly
Pathogen only	25.30a	23.66a
$013M_4$+Pathogen	8.53b	7.65b
$013M_4$ only	8.20b	9.76b
Control	8.02b	8.02b

[1] Figures with the same letter (s) are not significantly different from each other at $LSD_{.05}$.
[2] Average % infection based on the total surface area of the fruit infected.
[3] Inoculations were done weekly and fortnightly at the pre-bloom stage of mango development.
[4] Fruits were harvested at 120 days after flower induction and data were gathered 10 days after harvest when the fruits turned yellow.

Table 2. Effect of weekly and fortnightly application of *Colletotrichum capsici* mutant ($013M_4$) on anthracnose infection at the flowering stage of mango development

Treatment	Average Percentage Infection (%)	
	Schedule of application	
	Weekly	Fortnightly
Pathogen only	19.02a	18.65a
$013M_4$+Pathogen	6.36bc	8.50c
$013M_4$ only	3.95b	4.58bc
Control	3.23b	3.23b

[1] Figures with the same letter (s) are not significantly different from each other at $LSD_{.05}$.
[2] Average % infection based on the total surface area of the fruit infected.
[3] Inoculations were done weekly and fortnightly at the flowering stage of mangc development.
[4] Fruits were harvested at 120 days after flower induction and data were gathered 10 days after harvest when the fruits turned yellow.

Table 3. Effect of weekly and fortnightly application of *Colletotrichum capsici* mutant ($013M_4$) on anthracnose infection at the fruit set stage of mango development

Treatment	Average Percentage Infection (%)	
	Schedule of application	
	Weekly	Fortnightly
Pathogen only	19.57a	14.92b
$013M_4$+Pathogen	2.63c	2.27c
$013M_4$ only	1.51c	1.38c
Control	1.75c	1.75c

[1] Figures with the same letter (s) are not significantly different from each other at $LSD_{.05}$.
[2] Average % infection based on the total surface area of the fruit infected.
[3] Inoculations were done weekly and fortnightly at the fruit set stage of mango development.
[4] Fruits were harvested at 120 days after flower induction and data were gathered 10 days after harvest when the fruits turned yellow.

II. Effect of Inoculation with the Pathogen, *Colletotrichum gloeosporioides* on Anthracnose Development in Fungicide Treated Trees

Methodology

The effect of inoculation with the pathogen, in fungicide treated plants was determined. The pathogen, *Colletotrichum gloeosporioides,* was inoculated weekly and fortnightly at the pre-bloom, flowering, and fruit set stages of mango development. An inoculum concentration of 10^6 spores/ml from 7-day old cultures of *C. gloeosporioides* was prepared and inoculated weekly and fortnightly at the different growth stages of mango mentioned earlier. Fungicide was applied twice during the entire mango season along with the pesticides and fertilizers as well as other routinary farm practices.

A control set-up with untreated or uninoculated experimental units was provided for each growth stage of mango. Mango fruits were harvested at the green mature stage (120 days after flower induction) and incubated at the laboratory for 10 days. Symptoms of anthracnose infection were observed and noted.

Results

Inoculation of mango with the pathogen, *Colletotrichum gloeosporioides* at the pre-bloom stage of mango development appeared to be the most devastating among the other growth stages. Application of the pathogen at this stage, whether inoculation was done weekly or fortnightly, resulted to zero harvest as the experimental units had already

fallen off before the green mature stage has been reached. This goes to show that the pre-bloom stage is the most susceptible stage to mango anthracnose infection (Table 4).

Between the flowering and the fruit set stages, the fruit set stage seemed to be less susceptible to mango anthracnose as 14.36 and 16.36 average % infections which were not significantly different from one another were noted when inoculated weekly and fortnightly, respectively, at the fruit set stage. These were significantly different from the weekly and fortnightly average % infections at the fruit set stage at 45.0 and 28.82% infections, respectively.

These results show that the usual farm practice of fungicide application was not able to control anthracnose infection with the inoculation of *C. gloeosporioides* at the different growth stages of mango. High average % infection based on disease severity was observed which were significantly different from that of the control which was not inoculated with the pathogen.

Table 4. Effect of Inoculation with the Pathogen, *Colletotrichum gloeosporioides* on Anthracnose Development in Fungicide Treated Trees

Treatment	Average Percentage Infection (%)		
	Schedule of application		
	Weekly	Fortnightly	Control
Pre-bloom	No fruits harvested	No fruits harvested	5.07a
Flowering	45.0b	28.82c	4.62a
Fruit Set	14.36d	16.36d	3.61a

[1] Figures with the same letter (s) from a to c are not significantly different at $LSD_{.05}$ in comparing treatments in a row and w to y in comparing treatments in a column.
[2] Average % infection based on the total surface area of the fruit infected.
[3] Inoculations were done weekly and fortnightly at the fruit set stage of mango development.
[4] Fruits were harvested at 120 days after flower induction and data were gathered 10 days after harvest when the fruits turned yellow.

III. Stability and Efficacy of *Colletotrichum capsici* Mutant ($013M_4$) and the Pathogen, *Colletotrichum gloeosporioides* After Storage for Various Lengths of Time at 10°C

Methodology

The test isolate from *Colletotrichum capsici* which had been altered through UV radiation showing reduced pathogenicity ($013M_4$) and the pathogen, *Colletotrichum gloeospioides* were grown in prune agar medium and incubated for 10 days in the case of *C. capsici* and 7 days in the case of *C. gloeosporioides* to allow sufficient sporulation of the organisms.

Cultures of $013M_4$ and *C. gloeosporioides* were stored for different lengths of time (0, 1, 2, 3, 4,5, 6, 8, 10, and 12 months) at 10°C. The stability of both organisms after storage for each length of time at 10°C was tested. The effectiveness of 013 M_4 in inducing resistance to anthracnose in mango

and the virulence of *C. gloeosporioides* were tested after storage at 10°C for various storage periods mentioned earlier.

Pathogenicity tests were conducted and the efficicacy of both $013M_4$ and *C. gloeosporioides* was evaluated.

Results

The effectiveness of *Colletotrichum capsici* mutant, $013M_4$ reduce the incidence of anthracnose in mango and the virulence of the pathogen, *Colletotrichum gloeosporioides* after storage of the organisms for different periods were determined. Prolonged storage of the organisms at 10°C did not seem to affect the efficiency of either $013M_4$ or *C. gloeosporioides* (Table 5).

Results showed that the average percentage infection of $013M_4$ on the first month was not significantly different from that on the 2nd, 3rd, 4th, 5th, 6th, 8th, 10th, and 12th months. This showed that the reduced pathogenicity characteristic of $013M_4$ was maintained. The same was true for *C. gloeosporioides* as the average percentage infections were not significantly different from one another when the pathogen was stored for 1 month at 10°C until 12 months, suggesting the stability of the organism in the growth medium. In the same manner, challenge inoculation of the pathogen, *C. gloeosporioides*, with $013M_4$ did not show any variability as the average percentage infections upon storage for 1, 2, 3, 4, 5, 6, 8, 10, and 12 months at 10°C were not significantly different from one another. This only implied that the effectiveness of $013M_4$ to reduce anthracnose infection was not affected by the length of time the organism was stored in prune agar medium.

Table 5. Efficacy of *Colletotrichum capsici* Mutant ($013M_4$) and the Pathogen, *Colletotrichum gloeosporioides* After Storage for Various Lengths of Time at 10°C.

Length of Storage	Average Percentage Infection (%)		
	C. gloeosporioides only	$013M_4$ + *C. gloeosporioides*	$013M_4$ only
1 month	88.25a	35.00b	3.75c
2 months	78.75a	26.25bd	6.75ce

3 months	75.00a	22.50be	4.50cf
4 months	72.50a	36.25b	13.75cde
5 months	62.25a	26.50bd	5.50ce
6 months	77.75a	35.00b	8.00ce
8 months	74.00a	33.75b	5.5ce
10 months	85.25a	27.5bd	8.00ce
12 months	68.75a	21.25bdef	3.75c

[1] Data based on the average of four replications.
[2] Average % infection based on the total leaf surface area infected.
[3] Figures with the same letter(s) are not significantly different at $LSD_{.05}$.

IV. Test on the Stability and Efficacy of *Colletotrichum capsici* Mutant ($013M_4$) and the Pathogen, *Colletotrichum gloeosporioides* in Filter Paper Disks After Storage for Various Lengths of Time

Methodology

Cultures of *Colletotrichum capsici* mutant, $013M_4$ and the pathogen, *Colletotrichum gloeosporioides* were maintained in sterilized filter paper disks placed in a desiccator with silica gel at 0°C. For *C. gloeosporioides,* sterile paper disks were placed on the surface of prune agar plates containing the 4-day-old growth of the organism while for $013M_4$, disks were placed in potato agar plates. The organisms were incubated for 10 days at room temperature and allowed to grow until the disks were covered with the fungal growth. The disks were then kept in sterile coin envelopes which were stored in a desiccator containing silica gel at 0°C.

The efficacy of $013M_4$ and *C. gloeosporioides* were tested through periodic inoculation and challenge inoculation. Filter paper disks stored for a

particular length of time were transferred to a fresh suitable medium and allowed to grow and sporulate in the said medium. A spore load of 10^6 spores/ml was used in the succeeding inoculations in testing the efficiency of the organisms. The effectiveness of $013M_4$ to reduce anthracnose infection and the virulence of the pathogen after storage for various lengths of time was evaluated.

Results

The effect of storage of *Colletotrichum capsici* mutant, $013M_4$ and the pathogen, *Colletotrichum gloeosporioides* grown and maintained in filter paper disks for various lengths of time was determined. The efficiency of $013M_4$ in reducing anthracnose infection and the virulence of the pathogen appeared not to be affected after 5 months of being maintained in filter paper disks (Table 6). However, slower growth rate and sporulation of both $013M_4$ and *C. gloeosporioides* were observed as storage in filter paper disks of the organisms was prolonged. More slants which contained the fungal growth with spores were needed to produce the required inoculum concentration of 10°C spores/ml.

Both $013M_4$ and *C. gloeosporioides* failed to grow after 6 months of incubation, when the filter paper disks containing the fungal growth with spores were transferred to potato agar slants and prune agar slants which would serve as the growth media of the said organisms. Maintenance of $013M_4$ and *C. gloeosporioides* in filter paper disks as stock cultures appeared not to be an effective preservation technique since loss of viability of the organisms was observed when subjected to such storage condition.

Table 6. Efficacy of *Colletotrichum capsici* Mutant ($013M_4$) and the Pathogen, *Colletotrichum gloeosporioides* in Filter Paper Disks After Storage for Various Lengths of Time

Length of Storage	Average Percentage Infection (%)		
	C. gloeosporioides only	$013M_4$ + *C. gloeosporioides*	$013M_4$ only
0 month storage	96.50a	30.00b	5.00c
1 month storage	90.75ad	37.50b	3.25c
2 months storage	87.25 ad	35.00b	4.25c
3 months storage	83.25 ad	43.75b	6.50c

4 months storage	80.00ad	41.25b	8.5c
5 months storage	75.75d	35.00b	6.5c

[1] Data based on the average of four replications.
[2] Average % infection based on the total leaf surface area infected.
[3] Figures with the same letter(s) are not significantly different at $LSD_{.05}$.

V. Mass Production of *Colletotrichum capsici* Mutant and Pathogen, *Colletotrichum gloeosporioides* Inocula

Methodology

A suitable growth medium which would allow proliferation of the spores of *Colletotrichum capsici* mutant, $013M_4$ and the pathogen, *Colletotrichum gloeosporioides*, was determined. The organisms were grown in various media: Potato Dextrose Agar, Potato Agar, Prune Agar, Mango Decoction Agar, Pepper Decoction Agar. After sufficient growth of the organisms covered the agar or after 7 days of incubation, a thin cellophane and disinfected leaves of mango and pepper were placed over the surface of the agar containing the fungal growth. The cellophane and the mango and pepper leaves were disinfected in 95% ethanol for 10 minutes. The organisms were allowed to grow over the surface of the cellophane and the mango and pepper leaves. After 14 days of incubation, the cellophane and the leaves were removed from the medium and were suspended in 10 ml water. Spores were dislodged from the plastic film and the leaves using a sterile wire loop.

Several broth media which would allow profuse sporulation of $013M_4$ and *C. gloeosporioides* were prepared in 250 ml Erlenmeyer flasks. Using a cork borer, disks from 7-day old cultures of 013 M_4 and *C. gloeosporioides* were inoculated in different broth media: Sweet Potato Broth, Mango Decoction Broth, Pepper Decoction Broth, Potato Broth and, Prune Broth. The flasks containing the different broth media were agitated using a rotary shaker to facilitate aeration. The spore load in each of the broth media was counted after 14 days of incubation at room temperature with constant shaking of the culture. The number of spores observed in broth media, leaves, and plastic film were compared.

Results

In mass production of *Colletotrichum capsici* mutant, $013M_4$, Potato Dextrose Agar and Potato Agar appeared to be the most suitable medium as highest number of spores was counted when grown in these media (Table 7). Sporulation was further enhanced when pepper leaves which were previously disinfected in 95% ethanol were placed over the surface of the medium with the fungal growth. An average spore concentration of 3.86×10^6 in 10 ml

water was counted when Potato Agar medium was amended by adding pepper leaves over the surface of the medium after 7 days of incubation when the organism has already sufficiently grown in the medium. Using Potato Dextrose Agar medium, 2.68 x 10^6 spores were counted when the fungal spores were dislodged from the pepper leaves in a 10 ml water. The average number of spores counted using the two media were not significantly different from one another.

Less sporulation, however, was observed when a cellophane was placed over the surface of the agar and when the organism was grown in liquid shake culture medium. The average spore concentrations when plastic film and when liquid shake culture medium were used, were significantly different from that of the average spore concentration when leaves were placed over the surface of the media.

Table 7. Mass Production of *Colletotrichum capsici* mutant, $013M_4$ in Different Growth Culture Media

Growth Culture Media	Average Spore Concentration
Mango Leaves	
Potato Dextrose Agar	2.68 x 10^6 ab
Potato Agar	3.86 x 10^6 a
Prune Agar	2.00 x 10^5 c
Cellophane	
Potato Dextrose Agar	1.05 x 10^6 bc
Mango Decoction Agar	0 c
Pepper Decoction Agar	3.75 x 10^4 c
Prune Agar	8.75 x 10^4 c
Shake Culture	
Camote Broth	2.50 x 10^4 c
Mango Decoction Broth	1.25 x 10^4 c
Potato Dextrose Broth	0 c
Pepper Decoction Broth	1.25 x 10^4 c
Potato Broth	1.25 x 10^4 c

[1] Data based on the average of four replications.
[2] Average spore concentration based on the total number of spores counted.
[3] Figures with the same letter(s) are not significantly different at $LSD_{.05}$.

Sporulation of the pathogen, *Colletotrichum gloeosporioides* appeared to be favored when grown in Prune Agar and Potato Dextrose Agar which was amended with mango leaves disinfected in 95% ethanol, showing the highest number of spores with 5.09 x 10^6 spores and 5.64 x 10^6 spores, respectively (Table 8). Less number of spores were counted when cellophane was placed over the surface of the media and when liquid shake culture media was used. The average number of spores from broth media and from the media amended with cellophane were significantly different from

the average number of spores from that of the media amended with the mango leaves.

Table 8. Mass Production of the Pathogen, *Colletotrichum gloeosporioides* in Different Growth Culture Media

Growth Culture Media	Average Spore Concentration
Mango Leaves	
Potato Dextrose Agar	5.64×10^6 a
Potato Agar	8.00×10^5 bc
Prune Agar	5.09×10^6 a
Cellophane	
Potato Dextrose Agar	3.75×10^4 b
Mango Decoction Agar	1.25×10^5 b
Pepper Decoction Agar	2.00×10^5 b
Prune Agar	3.88×10^5 b
Shake Culture	
Sweet Potato Broth	1.58×10^6 cd
Mango Decoction Broth	5.00×10^5 b
Potato Dextrose Broth	1.94×10^6 d
Pepper Decoction Broth	5.25×10^5 b
Prune Broth	1.63×10^5 b

[1] Data based on the average of four replications.
[2] Average spore concentration based on the total number of spores counted.
[3] Figures with the same letter(s) are not significantly different at $LSD_{.05}$.

Second year of experiments.

Induced Resistance in Subtropical Fruits for the Prevention of Decay Initiated from Quiescent Infecting Pathogens

I. Mass Production of *Colletotrichum capsici* Mutant ($013M_4$) and *Colletotrichum gloeosporioides* Inocula

Several methods for easy production of numerous conidia of *Colletotrichum capsici* mutant ($013M_4$) and *Colletotrichum gloeosporioides* in the shortest possible time had been tested. We previously showed that growing $013M_4$ and *C. gloeosporioides* in pepper

and mango leaves, which are the host plants of the respective organisms harvested sufficient inocula. However, contamination by the faster growing organisms was encountered, as prolonged incubation is needed in this method. This method is also tedious and time-consuming such that mass production of conidia using this method is not recommended.

This study aims to develop a more suitable and easier technique to produce high amounts of inocula.

Methodology

Colletotrichum capsici mutant ($013M_4$) and *Colletotrichum gloeosporioides* were inoculated in flat bottles (350 ml capacity) containing the agar medium, in the same manner as inoculation in test tube slants. From the stock culture, a loopful of the agar containing the fungal growth was obtained and transferred to sterile agar media (30 ml medium per flat bottle). Potato agar and prune agar were used as growth and sporulation media for $013M_4$ and *C. gloeosporioides*, respectively. This is because our previous study showed that sporulation of the organisms was favored in these media. The organisms were incubated for 7 days.

A suspension of the culture was made by adding 10 ml of sterile distilled water and the spore concentration was determined using a haemacytometer. The number of spores/ml harvested was compared with the number of spores/ml grown in test tube slants.

Results

Using flat bottles to grow the organisms in artificial solid culture media yielded higher number of spores than the traditional method of culturing in test tube slants. A spore concentration of 3.86×10^6 spores/ml of $013M_4$ was noted when grown in potato agar flat bottles after four days of incubation as compared to 2.64×10^6 spores/ml in test tube slants using the same media when 10 ml of sterile distilled water was added to each. For *C. gloeosporioides*, 2.0×10^6 spores/ml was noted when grown in potato dextrose agar flat bottles while growing in potato dextrose agar slants showed a spore concentration of 1.65×10^6 spores/ml after four days of incubation.

This is attributed to the fact that the flat bottles have larger surface area as compared to test tube slants. This allowed more space for the organism to grow and sporulate, hence, is the most effective in mass production of the inocula among the methods tested.

II. Determination of the Mechanism of Action of *Colletotrichum capsici* Mutant ($013M_4$) in Reducing Anthracnose Infection

A. Test for volatile compounds

Methodology

From the stock culture, a loopful of the agar containing *Colletotrichum capsici* mutant ($013M_4$) was transferred to potato dextrose agar plates. After four days of incubation, when $013M_4$ had sufficiently grown and was already established in the agar media, the petri dish cover was removed and was then replaced by another dish containing a newly transferred *C. gloeosporioides*, also grown in potato dextrose agar. The two organisms contained in separate plates were incubated on top of the other. The plates were secured using a parafilm and were then observed for any inhibitory effect to *C. gloeosporioides* due to possible release of volatile compounds by $013M_4$.

Result

The mode of action of *Colletotrichum capsici* mutant ($013M_4$) in reducing anthracnose infection caused by *Colletotrichum gloeosporioides* appeared to be due to factors other than production of volatile compounds. This was based on the observation that uninhibited growth of the pathogen, *C. gloeosporioides* still occurred.

B. Effect of *Colletotrichum capsici* Mutant ($013M_4$) culture filtrate

Methodology

The effect of the cultrate filtrate from *Colletotrichum capsici* mutant ($013M_4$) on growth, sporulation and spore germination of the pathogen, *Colletotrichum gloeosporioides*, was determined.

A spore suspension was made from 7-day old cultures of $013M_4$ and was passed through sterilized Seitz filter with several layers of filter papers to extract the filtrate excluding the spores. After the filtrate had been collected, a loopful was obtained and checked if spores are still present. The filtrate should be free of any spores or mycelia.

To test the effect of the filtrate on growth of the pathogen, the growth media was amended with the incorporation of the filtrate. Ten milliliters of the filtrate was added to a flask containing 250 ml of melted potato dextrose agar when the media had already cooled just before pouring into petri plates. A loopful of the agar from the stock culture containing growth of *C. gloeosporioides* was then inoculated to the amended petri plates. The plates were incubated for 7 days and were observed for any inhibition of growth when grown in media amended with the culture filtrate of $013M_4$. The number of spores was also noted to determine if the culture filtrate would affect sporulation of *C. gloeosporioides.*

The effect of the filtrate on germination of the spores of *C. gloeosporioides* was also studied. Different proportions of the culture filtrate were added to the spore suspension of *C. gloeosporioides* in sterile glass slides to make 1:1, 1:2, 1:3 and 1:10 dilutions. The slides were incubated in a moist chamber and the percentage germination after 24, 48, 64, and 72 hours were noted. Data were based on the first 200 spores that were counted.

Culture filtrate was also applied to mango leaves to determine its ability to reduce anthracnose infection. The filtrate was spray inoculated at different time intervals: simultaneous inoculation to *C. gloeosporioides* and 24, 48, and 72 hours prior to *C. gloeosporioides* inoculation.

Drop inoculation of the filtrate was also done by placing 5µl of the filtrate to the same spot in the leaf where 5µl of *C. gloeosporioides* spore suspension had already been inoculated. Inoculated leaves were incubated and were observed for symptom development.

Results

The filtrate used was extracted from 7-day old cultures of *Colletotrichum capsici* mutant ($013M_4$). The acidity of the filtrate was determined and was noted to be at pH 6.

Amending the growth media of 013M4 by the addition of 10 ml of the culture filtrate to 250 ml melted potato dextrose agar did not affect the growth of *Colletotrichum gloeosporioides*. Growth of the pathogen was not in any way inhibited and after 7 days of incubation, was not observed to be different from the control. Sporulation was also not affected when culture filtrate was incorporated to the growth medium.

Germination was likewise not affected when the spores were incubated with the culture filtrate from $013M_4$. Spores treated with the culture filtrate had about the same percentage germination as the control, if not higher. Normal elongation of germ tube was also observed even with the application of the filtrate.

Neither spray application of the culture filtrate to the mango leaves prior or simultaneous to inoculation of the pathogen, *C. gloeosporioides*, inhibited or reduced anthracnose infection in mango leaves. Infection was still prevalent even upon treatment with the $013M_4$ culture filtrate regardless of the stage the filtrate was applied. Drop inoculation of the filtrate at the same spot the pathogen was inoculated, likewise developed anthracnose infection at the inoculated area.

C. Test for antagonistic activity using dual culture

Methodology

This test was done by growing *Colletotrichum capsici* mutant ($013M_4$) and *Colletotrichum gloeosporioides* in the same petri plates containing the agar medium. Both organisms were seeded on the agar medium on the same day and this was done by placing small agar disks containing growth of the organism at the opposite ends of the plates.

The plates were incubated at room temperature for 7 days and were observed for any inhibition of growth.

Results

Dual culture tests showed that growth of the pathogen, *C. gloeosporioides* and *Colletotrichum capsici* mutant ($013M_4$) was somewhat inhibited. The hyphae of $013M_4$ and *C. gloeosporioides* could not be easily differentiated when viewed under a microscope at the junction where the two cultures met. Further tests are still needed to determine any direct or specific action of 013M4 against *C. gloeosporioides* to see if competition for space and/or nutrients or parasitism may be factors that

can be considered in determining the effect of $013M_4$ on *C. gloeosporioides*. Spore suspension of $013M_4$ can also be tested for antagonistic effect that may cause abnormalities in spore germination of the pathogen.

III. Determination of the Mechanism of Action of *Colletotrichum capsici* Mutant ($013M_4$) in Reducing Anthracnose Infection

Methodology

A second trial of the field experiments conducted during the previous mango season on the effect of *Colletotrichum capsici* mutant ($013M_4$) applied at different frequency and at different stages of mango development on mango anthracnose in the field, is currently in progress.

In one study, application of $013M_4$ is done earlier than the previous experiment as $013M_4$ is sprayed in the foliage prior to flower induction and will be continued through the flowering stage up until the green mature stage of the fruits is reached. Application of $013M_4$ at the flowering and fruit set stages will also be conducted as in the past experiments.

Treatment with $013M_4$ will be done weekly and fortnightly (once in two weeks) as in the past. Fruits will be harvested at the green mature stage, at 100-120 days after flower induction.

SUMMARY OF RESULTS

General

The identification and filed test of natural mutants obtained in Philippines I was further developed. Significant amount work has been concentrated in the development of the natural Philippino mutant and how to understand the mechanism of induce resistance.

Philippines

Experiments where a natural selective strain of *C. capsicci* was used in Philippines for the control of the wild type anthracnose succeeded and further experiments will be done.

C.FUTURE WORK

PHILIPPINES

Plans for other experiments:

1. Determine the effectiveness of mutant $013M_4$ in reducing anthracnose infection in the leaves and inflorescence of mango. This is necessary to evaluate the overall efficacy of this organism in controlling anthracnose.

2. Determine the mechanism by which mutant $013M_4$ reduces anthracnose infection in mango

3. Conduct another trial on the efficacy and stability of mutant $013M_4$ in reducing anthracnose infection

4. Determine an effective formulation for field application of $013M_4$

5. Determine the effect of fungal (*Colletotrichum gloeosporioides*) components and culture filtrates of *C. gloeosporioides* in inducing resistance to mango anthracnose.

ISRAEL

The PI will be involved in next year experiments for field treatments with the biological control in Philipines. This will be done using the visit of a Philippino student in Israel.

Section II

Managerial Issues

Based in the present results and the development of our research the Israel and Philippines laboratories, we have presented a summary of our entire findings in the next International Mango Symposium that toke place in Brazil in September 2002. We hope that a joint manuscript will result from that meeting.

Beside that the development of the Philippininan strain and its possible use in a field experiments already during the year 2002, the use of this mutant will be tested in a joint field experiment during September of 2003. For this specific consultation, a student of Dr. Ilag will spend 2-3 months in the Israel where the experiment will take place. For this visit, the budget allocated for visit of students will be used. We request permition for this use of the travel money. Dr. Ilag has already accepted the changes and a letter was forwarded to Mr. Boaz Ayalon.

Collaboration.

Since the last report there have not been any traveling to each other laboratories. We have presented a joint publication in the next "Mango Symposium" that took place in Brazil where both representatives were present. We expect as well that in the late 2003 we will be a joint field

experiment that will be a conclusion of the experiments that have been done till now in Philippines.

Citations of Publications that have appeared or were accepted for publications

1. Prusky, E. Ilag, L. Ackerman, M., Kobiler . I. Fuchs, I. 2003. New Approaches for the control of postharvest diseases of mango fruits. Acta Horticulture: In press.
2. Prusky, D., Shalom, Y., Kobiler, I. Akerman, M. and Fuchs, Y. 2003. Postharvest fungicide treatments in mango fruits according to the level of quiescent infection of pathogens at harvest. Acta Horticulturae: In press.

Request for American Embassy Tel Aviv

To promote productivity of this project I am requesting, the transfer of 3.000$ from the money allocated for the Philippino Budget for Training to cover the supplies during the stay of the Philippino student in Israel. A letter of D.r Ilag was forwarded to the Dr. Boaz Ayalon following his request.