אפיון מהלך מחלת עיוות התפרחות והצימוח במנגו ויחסי הגומלין בין הפתוגן *Fusarium mangiferae* לאקרית הפקע של המנגו *Aceria mangiferae*

חיבור לשם קבלת תואר דוקטור לפילוסופיה

מאת אפרת גמליאל-אטינסקי

הוגש לסינט האוניברסיטה העברית בירושלים,

ינואר 2009

Characterization of mango malformation disease and the interaction between the pathogen *Fusarium mangiferae* and the mango bud mite *Aceria mangiferae*

Thesis submitted for the degree of

"Doctor of Philosophy"

By

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Submitted to the Senate of the Hebrew University

January, 2009

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ABSTRACT

Mango malformation is one of the most destructive diseases of this crop, occurring in most mango producing regions worldwide. The disease is characterized by malformation of vegetative growth and inflorescences, causing serious yield loss since malformed panicles do not bear fruit. *Fusarium mangiferae* Britz, Wingfield & Marasas, previously known as *F. moniliforme* Sheldon and later as *F. moniliforme* Sheldon var. *subglutinans* Wollenweber & Reinking, has been identified as the causal agent of mango malformation disease in Israel.

Inoculum availability and conidial dispersal patterns of *Fusarium mangiferae* were studied during 2006 and 2007 in an experimental orchard. The spatial pattern of primary infections in a heavily infected commercial mango orchard corresponded with a typical dispersal pattern caused by airborne propagules. Malformed inflorescences were first observed in mid-March, gradually increased, reaching a peak in May, and declined to negligible levels in August. The sporulation capacity of the malformed inflorescences was evaluated during 3 consecutive months. Significantly higher numbers of conidia per g malformed inflorescence were detected in May and June than in April. Annual conidial dissemination patterns were evaluated by active and passive trapping of conidia. A peak in trapped airborne conidia was detected in May and June for both years. The daily pattern of conidial dispersal was not associated with a specifically discernable time of day, and an exponential correlation was determined between mean relative humidity (RH) and mean number of trapped conidia. Higher numbers of conidia were trapped when RH values were low (< 55%).

Conditions affecting germination and growth of *F. mangiferae* were studied in vitro. Both conidial germination and colony growth required temperatures above 5°C, and reached a peak at 28°C and 25°C, respectively. A minimum of 2-h wetness-period was required for conidial germination, reaching a peak after 8 h of wetness.

In order to determine the pathogen penetration site, artificial inoculations of different plant organs were conducted. High incidence of fungal colonization in buds, predominantly the apical buds, was detected. When soil was infested, the pathogen was detected in the roots, 19 weeks post inoculation, but not in above-ground parts of the plants; symptoms were

also not observed. In order to test whether dry malformed inflorescence debris could serve as a source of inoculum, debris were placed over apical buds. Higher colonization of the infested buds was obtained compared to that of the untreated controls. The surface of mango leaves was inspected for the presence of *F. mangiferae* throughout the year. Incidence of sampled leaf-disks bearing conidia from an infected orchard peaked in June and July and decreased during the following months. Incidence of airborne infections in the orchard of 1-month-old branches was studied over a 3-y period. Incidence was the highest in May and June. Colonization pattern within the host tissue was determined in naturally infected vegetative and woody branches. Percent colonization of *F. mangiferae* was significantly higher in node sections than in the internode sections. Histopathological studies were also conducted in order to detect localization of the pathogen within bud tissue. Colonization was observed in trichomes, apical meristem and bract tissue, and was not associated with any particular location within the bud.

The role of the mango bud mite, *Aceria mangiferae*, in carrying conidia of *F. mangiferae*, vectoring them into potential infection sites and assisting fungal infection and dissemination was studied. Following exposure of the mite to a green fluorescent protein-marked isolate, conidia were observed clinging to the mite's body. Agar plugs bearing either bud mites and/or the pathogen were placed on leaves near the apical buds of potted mango plants. Conidia were found in bud bracts only when both mites and conidia were co-inoculated on the plant, demonstrating that the mite vectored the conidia into the apical bud. Potted mango plants were inoculated with conidia in the presence or absence of mites. Frequency and severity of infected buds were significantly higher in the presence of mites revealing its significant role in the fungal infection process. Mite presence was monitored over a period of 1 year with two traps, one located in a diseased orchard, and the other in a growth chamber. No windborne bud mites bearing conidia were found, however, high numbers of windborne conidia were detected in the spore traps. These results suggest that *A. mangiferae* can carry and vector the pathogen to the apical bud and assist in fungal penetration, but does not appear to play a role in the aerial dissemination of conidia.

This is the first detailed report on airborne dispersal of *F. mangiferae*, serving as the primary means of inoculum spread. This study has shed light on infection dynamics and colonization patters of *F. mangiferae*, and on the interaction with the mango bud mite *A.*

mangiferae. In summary, a possible cycle for mango malformation disease is proposed. Malformed inflorescences and malformed vegetative growth serve as a source of inoculum. Inoculum disseminates passively in the air as conidia or fall from dry malformed inflorescences as dry debris. Most of the conidia fall on the mango canopy and reach the infection site by at least three different routes: falling by chance on the apical bud; being vectored on the body of the bud mite *A. mangiferae*; and, via conidia in dry debris falling into the funnel-like structure of the apical buds. Infection of apical buds may occur if appropriate conditions are met: temperatures between 5°C and 37°C accompanied by at least 2 h of wetness. Moderate temperatures and longer duration of wetness may accelerate the infection process. After penetration, the pathogen colonizes the bud tissue but does not progress beyond this point. Apical buds could either differentiate into a reproductive inflorescence, or remain vegetative and develop into a young shoot. Inflorescences from a colonized bud may emerge malformed, probably due to a build-up of the pathogen until an infection threshold is met. Alternatively, when a young shoot emerges from an infected apical bud, the pathogen might colonize the apical and lateral buds of the young shoot, and remain localized and dormant in buds until bud break.

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6.1 Gamliel-Atinsky, E., Freeman, S., Sztejnberg, A., Maymon, M., Ochoa, R., Belausov, E.,and Palevsky, E. (2009). Interaction of *Aceria mangiferae* with *Fusarium mangiferae*, the causal agent of mango malformation disease. *Phytopathology* 99:152-159 (cover page issue).

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1. INTRODUCTION

Mango malformation is a severe disease of the crop which is widely spread in almost all mango-growing regions worldwide (Crookes & Rijkenberg 1985, Kumar et al. 1993, Kvas et al. 2008, Ploetz 2001, Ploetz et al. 2002). Since malformed inflorescences do not bear any fruit, malformation is a major constraint to mango production and high losses in yield are reported due to the disease from various mango producing countries (Kumar et al. 1993, Majumder & Sinha 1972, Noriega-Cantứ et al. 1999, Ploetz 2001, Ploetz et al. 2002, Sao Jose et al. 2000). In Israel the disease was detected approximately 35 years ago (Goldman et al. 1976). All growing areas in the south and in the center of the country are severely infected and most of the orchards were uprooted due to this disease. Today, the crop is mostly cultivated in the north.

Symptoms of disease are associated with hormonal imbalance in the host that results in misshapen growth of both vegetative and productive parts of the tree (Kumar & Beniwal 1992, Kumar et al. 1993, Majumder & Sinha 1972, Ploetz 2001, Ploetz 2003, Prasad et al. 1972). Vegetative malformation includes hypertrophy of young shoots, shorter internodes, dwarfed malformed leaves and an overall tightly bunched appearance of the shoot (Fig. 1). Inflorescence malformation includes short, thick and branched axes of the inflorescence, larger flowers while increased numbers of male and hermaphroditic flowers that are formed are either sterile or eventually abort. Malformed inflorescences do not bear any fruit, hence the great losses caused by this disease (Kumar et al. 1993, Majumder & Sinha 1972, Noriega- Cantú et al. 1999, Ploetz 2001, Ploetz et al. 2002, Sao Jose et al. 2000).

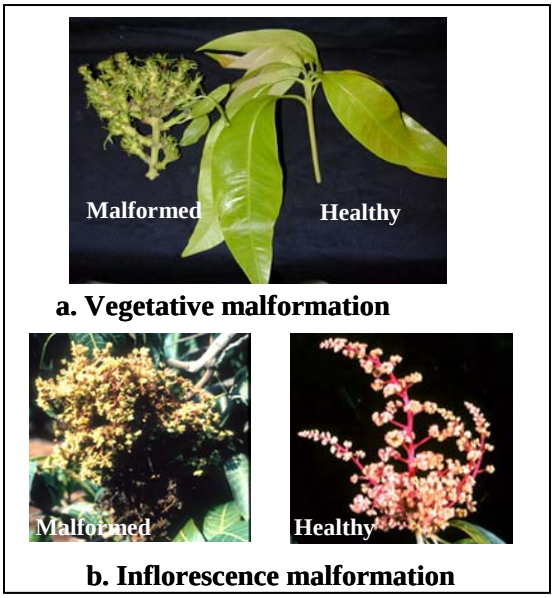

Fig. 1. Symptoms of mango malformation disease on (**a**) vegetative growth and (**b**) inflorescences.

Several causal agents, all members of the genus *Fusarium*, have been associated with this disease. *Fusarium mangiferae* Britz, Wingfield & Marasas (previously recognized as *F. moniliforme* Sheldon and later as *F. moniliforme* Sheldon var. *subglutinans* Wollenweber & Reinking) has the largest geographic distribution (Lima et al. 2008). Koch postulates were completed with this species for the first time in 1966 (Britz et al. 2002, Chakrabarti & Ghosal, 1989, Freeman et al. 1999, Kvas et al. 2008, Manicom 1989, Marasas et al. 2006, Noriega-Cantúr et al. 1999, Ploetz 2003, Ploetz & Gregory 1993, Summanwar et al. 1966, Varma et al.1974). Koch postulates were recently completed with *F. sterilihyphosum* Britz, Wingfield & Marasas (Lima et al. 2008) which is distributed in Brazil and South Africa (Britz et al. 2002, Marasas et al. 2006) and also with a new phylogenetic lineage that is closely related to *F. sterilihyphosum* and is known so far only from Brazil (Lima et al. 2008). Another recent report from Mexico completed Koch postulates with local strains of *Fusarium* sp., which were different from *F. mangiferae* and *F. sterilihyphosum* (Rodríguez-Alvarado et al. 2008). Koch postulates were not completed for two other taxa, *Fusarium* sp. nov. and *F. proliferatum* (teleomorph: *Gibberella intermedia*) Samuels, Nirenberg & Seifert distributed in Malaysia (Britz et al. 2002, Marasas et al. 2006).

Little is known about the epidemiology of the disease, dissemination of conidia, location of infection sites, modes of infection and colonization of plant tissue (Kumar et al. 1993, Ploetz 2001, Ploetz 2003), or whether wounding is an obligatory condition for infection (Ploetz & Gregory 1993). Most of the infection studies were performed by wounding the plant tissue, assuming that a wound is necessary for fungal penetration and infection (Manicom 1989, Ploetz 2001, Ploetz & Gregory 1993, Summanwar et al. 1966). Despite this, two studies reported development of malformation symptoms following inoculations conducted without wounding of the plant tissue (Chakrabarti & Ghosal, 1989, Freeman et al. 1999). The primary mechanism for long-distance dispersal of the pathogen is hypothesized to be via infected nursery stock or by the mango bud mite, *Aceria mangiferae*, vectoring fungal conidia. However, the method of pathogen dispersal within trees, or spread from tree to tree in an infected orchard is unknown, although it is well documented that the disease spreads slowly within infected orchards (Ploetz 2001, Ploetz 2003). Single- celled microconidia are produced in abundance and carried on sympodially branched conidiophores bearing mono- and polyphialides, while macroconidia are usually three to five cells, borne on a sporodochia. No sexual stage is known for this species (Britz et al. 2002, Leslie & Summerell 2006).

1.1 Dissemination of conidia

Ploetz (2001) indicated that aerial dissemination of conidia appears to be uncommon, based on a report that no conidia were caught using rotary spore traps placed in an infected orchard (Varma et al. 1971). However, a study from Mexico reported the trapping of macroconidia in an infected mango orchard, using a volumetric spore trap. In that study, identification of the pathogen was done microscopically, for the genus level *Fusarium* spp., and only for macroconidia (Noriega-Cantú et al. 1999). Another study suggested that the conidia are the main source of inoculum, and also that the fungus "being a weak pathogen" invades the host via soft organelles, *i.e.*, vegetative and floral buds, and flowers (Chakrabarti & Ghosal 1989). A study on the distribution of the pathogen in affected trees in Florida reported the highest colonization incidence in malformed vegetative and floral shoots, decreasing incidence in asymptomatic shoots and rare colonization in branch tissue, even

when the branch was supporting a malformed inflorescence (Ploetz 1994). When whole infected seedlings were sectioned, pathogen colonization descended from the top to the lower sections (Youssef et al. 2007). The latter study further demonstrated that survival of conidia declined very rapidly in soil and also that the pathogen was not detected in mango seeds, seed coat, or flesh, implying that the pathogen is not seedborne. Ploetz (1994) and Youssef et al. (2007) indicate that the pathogen is not a typical soilborne pathogen, nor does it move systemically from roots acropetally, and that mango buds are apparently the primary sites for infection. When malformation was managed in commercial orchards in Egypt by removing affected vegetative and floral terminals, the mean disease incidence were lower than in non-managed orchards (Ploetz et al. 2002). This management practice has also been used in India, Israel, and South Africa (Ploetz 2001).

Dispersal patterns of many foliar fungal pathogens are well documented, including several airborne Fusaria species, for example *F. circinatum*, the causal agent of pine pitch canker (Correll et al. 1991, Garbelotto et al. 2008), *F. graminearum*, causal agent of Fusarium head blight of wheat (Fernando et al. 2000, Inch et al. 2005), and *F. guttiforme* (syn. *F. subglutinans* f. sp. *ananas*), causal agent of fusariosis in pineapple (De Matos et al. 1997, Ploetz 2006). Detecting significant airborne conidia of *F. mangiferae* may support the hypothesis that conidia are the primary infection structures and shed light on better understanding the temporal and spatial patterns of *F. mangiferae*'s conidia dispersal and their involvement in the epidemiology of malformation.

1.2 Location of infection sites and colonization pattern

Since the pathogen was detected in malformed panicles but was rarely detected in branches (Ploetz 1994), it was postulated that vegetative, and floral buds are probably the primary sites for infection (Ploetz 2003), however, this needs further support. Root infection was reported to cause symptoms at the root collar and the canopy, but since those studies lacked appropriate controls their results are questionable (Ploetz 2001). Kumar et al. (1993) suggested that the localized pattern of disease development within a tree indicates systemic infection of a slow-moving soil-borne source of infection. Recently, Youssef et al. (2007) reported that inoculum can survive in soil although survival of bare conidia declined very

rapidly under summer conditions. Furthermore, naturally infected panicles that were buried 30 cm under the soil surface, resulted in a 20% inoculum survival after 24 weeks (Youssef et al. 2007).

Within-tree spread is poorly understood (Ploetz 2001). The pathogen was isolated frequently from malformed tissue, but infrequently from supporting branches (Crook & Rijkenberg 1985, Ploetz 1994, Ploetz & Gregory 1993), therefore it was concluded that systemic colonization of mango behind the apical parts of the plant might be rare. Ploetz (2001) suggested that the infrequent infections found in old branches could be remnant infections that were left behind as the shoot grew, and that basipetal colonization of mango is rare. When vegetative malformed mango seedlings, growing under infected trees were sampled for detection of the pathogen, colonization was concentrated mainly in the apical meristem sections (97%) and gradually declined to 5% colonization in the roots, indicating that infections are not systemic, with infections of apical meristems originating from inoculum from malformed panicles (Youssef et al. 2007).

1.3 Role of the bud mite *Aceria mangiferae* in epidemiology of the disease

The identity of the causal agent of mango malformation disease has been controversial for many years and other abiotic and biotic factors have been proposed as the primary causal agents of this disease (Denmark 1983, Narasimhan 1954, Nariani & Seth 1962, Ochoa et al. 1994, Zaher & Osman 1970). The putative role of the mango bud mite *Aceria* (=*Eriophyes*) *mangiferae* Sayed (Eriophyidae), was partly based on the fact that eriophyoid mites are known to cause bud proliferation, “witches broom” and gall symptoms of inflorescences in other plants (Westphal & Manson 1996). In addition, herbivores may facilitate fungal infection by two main mechanisms, either by vectoring pathogen propagules, and/or by creating wound sites for fungal penetration (Agrios 1980, Hatcher & Paul 2001), and a number of studies have reported association between herbivorous mites and fungal spores (Evans et al. 1993, Evans et al. 1998). For example, the mite *Brevipalpus phoenicis* (Tenuipalpidae) was found in association with the fungal pathogen *Elsinoe fawcettii* Bitancourt & Jenkins, the causal agent of citrus scab on sour orange (*Citrus aurantifolia*) in Honduras (Evans et al. 1993), but the significance of the mite in the epidemiology of the

disease was not investigated. More research however is needed in order to determine the role of herbivorous mites, in particular the eriophyoid mites as vectors of plant pathogens of fungal nature.

Aceria mangiferae, initially described in Egypt (Sayed 1946), is commonly found within closed generative and vegetative mango buds, in both malformed and healthy trees (Sternlicht & Goldenberg 1976). These mites disseminate by wind from opening buds, land passively on a random tree, and actively find their way into mango buds. Thereafter, the mite settles and begins feeding by penetrating its stylets into the epidermal cell wall, creating shallow wounds of approximately 2-5 µm in depth (Krantz & Lindquist 1979, Westphal & Manson 1996). *A. mangiferae* was identified in both healthy and diseased trees, and in the absence of a direct correlation between the mite and mango malformation, it was proposed that mango malformation might result from an interaction between the mite and *F. mangiferae* (Prasad et al. 1972, Sternlicht & Goldenberg 1976).

When attempts to trap airborne conidia failed (Ploetz 2001), a hypothesis emerged indicating that the bud mite serves as a vector for the fungal conidia (Ploetz 2001). Summanwar and Raychaudhuri (1968) recovered the pathogen from *A. mangiferae*'s body, when sampled from diseased trees, similar to another research reporting the isolation of *Fusarium* spp. from mites sampled from diseased and apparently healthy apical buds (Labuschagne et al. 1993). Several studies have reported the production of symptoms after inoculating mango seedlings with bud mites collected from diseased trees (Labuschagne et al. 1993, Manicom 1989, Nariani & Seth 1962). For example, Manicom (1989) indicated that spraying the pathogen's conidia on apical buds did not yield symptoms, but when mites were added, 8% of the apical buds were malformed, concluding that the presence of mites may have enhanced infection. An additional conclusion that can be drawn from this work is that the mites are capable of vectoring the fungal conidia into the apical buds. While the studies described above present interesting information it appears that the methods employed by these researchers could have lead them to unfounded conclusions. For example, when transferring mites from diseased trees, contamination by conidia associated with the mites may have occurred, moreover, several studies lacked numerical data, statistical analysis, and sufficient evidence (Nariani & Seth 1962, Summanwar & Raychaudhuri 1968). Therefore, from the present literature it is still not clear that the two organisms interact in the

epidemiology of this disease, partially due to a lack of suitable tools for tracking the fungal pathogen.

The objectives of this study were to:

1. Determine the annual inoculum availability in infected orchards
2. Study the annual and diurnal patterns of conidial dissemination
3. Evaluate conditions affecting germination and growth of *Fusarium mangiferae*
4. Determine the location of infection sites in the tree
5. Assess annual infection dynamics in the orchard by evaluating both the time when conidia land and infect trees and the pattern by which the pathogen colonizes tree tissue
6. Determine whether *Aceria mangiferae* can carry *F. mangiferae*'s conidia on the surface of its body, and assess its ability to vector the pathogen into the infection site
7. Evaluate *A. mangiferae*'s role in assisting the fungal infection process
8. Evaluate the role of *A. mangiferae* in the aerial dissemination of conidia

2. METHODOLOGY

2.1 Plants, fungus and bud mites

Two-year-old mango seedlings, susceptible to malformation (Maya cultivar grafted on rootstock 13/1), planted in 10 liter pots containing local red loam, were used in inoculation experiments. Two isolates were used throughout the experiments; (1) a local Israeli wild-type *F. mangiferae* isolate #34 (MRC 7560) (Steenkamp et al. 2000), and (2), a green fluorescent protein-marked isolate (gfp-1) (see sections 2.3 and 2.4). The monoconidial cultures were maintained on potato dextrose agar (PDA; Difco Laboratories, Detroit) at 25°C. All orchard-related experiments were conducted on naturally diseased cv. Haden trees in the Volcani experimental orchard in Bet Dagan. For all mite-related experiments, *A. mangiferae* was collected directly from infested apical buds sampled from the Volcani experimental orchard. To prevent contamination and permit quantitative inoculation, apical buds were separated into bracts, inspected under a stereomicroscope at x40 magnification (Wild, Switzerland), and the numbers of bud mites on each bract were counted while other arthropods were removed using a fine brush.

2.2 Inoculations, isolation from plant tissue and fungal identification

Conidial suspensions were obtained by adding sterile water to the cultured plates, mixing the suspension and filtering it through a four-layered gauze pad. Seedling inoculations were performed by placing 20 µl of conidial suspension (5×10^6 conidia per ml water agar 0.1%) on apical buds, and covering them overnight with plastic bags that were sprayed with water.

Isolation and identification of the fungus from stem and root tissues was performed as follows: plant material was sectioned into 5 mm pieces, surface sterilized for 10 s in 70% ethanol, then 3.5 min in 3% sodium hypochlorate and plated on NASH *Fusarium*-selective medium (Nash & Snyder 1962). After 6 days, fungal colonies that resembled *Fusarium* were transferred to PDA and identified by morphology, under a microscope, and verified by using a molecular polymerase chain reaction (PCR) method with specific primers (Zheng & Ploetz

2002). Isolation of the green fluorescent protein (gfp) isolate from plant tissue was conducted by plating material on PDA medium amended with 50 μg/ml hygromycin.

2.3 Gfp transformation

Plasmid pSK1019, was kindly provided by Dr. Seogchan Kang (Department of Plant Pathology, Pennsylvania State University). The plasmid contains the hygromycin B resistance (*hph*) gene under the *Aspergillus nidulans trpC* promoter and the EGFP gene in a 1.6kb fragment under a Ch GPD promoter, cloned between *EcoRI* and *HindIII* sites of a pBHt2 vector (Mullins et al. 2001). The vector was transformed into an *Agrobacterium tumefaciens* strain Eha105 by electroporation (1.5Kv, 200 ohms, 50μF). Transformation was carried out as previously described (Mullins et al. 2001) with the following modifications: dilution of *A. tumefaciens* was conducted in the presence of acetosyringone; 200 μl of co-inoculated bacteria cells and conidia were plated directly onto 10 ml cocultivation medium, and incubated at 25°C for 2 days. Then, 10 ml selection medium containing 100 μg/ml hygromycin B, without the presence of moxalactum, was overlaid on each plate and incubated at room temperature for 5 to 7 days. Gfp transformants were isolated on PDA supplemented with hygromycin B (50 μg/ml).

DNA extraction was carried out as previously described (Freeman et al. 1993). Southern hybridization was performed to determine T-DNA copy number of transformants. DNA was digested with *Hind*III and the products separated by electrophoresis on a 0.8% agarose gel in 0.5×TAE (20 mM Tris-acetate, pH 8.0 and 0.5 mM EDTA) buffer. Prehybridization, labeling, hybridization and high stringency washes of the membrane (Hybond-XL; Amersham Pharmacia, Buckinghamshire, UK) were performed as described according to the DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Diagnostics, Mannheim, Germany). A 850 bp fragment from the hygromycin *hph* gene was labelled and used as a probe. Mitotic stability of transformants was tested as previously described (Mullins et al. 2001). Transformants were cultured on PDA and transferred 6 times onto fresh PDA plates and also to PDA amended with 50 μg/ml hygromycin, and their resistance to hygromycin was tested to verify stability.

2.4 Gfp isolate pathogenicity

One of the six transformants (gfp-1), was used in two experiments to evaluate pathogenicity and symptom development on mango seedlings. For the first experiment, six potted plants were transferred to a growth chamber in September 2006, with 12 hrs fluorescent light supplied by four 40W/2300 lm daylight bulbs and constant temperature of 25±2°C. Three plants per isolate (bearing a total of 15 apical buds) were inoculated with either gfp-1 or wild-type isolates, as previously described. Two weeks post inoculation, buds were surface sterilized and plated for evaluation of fungal growth. For the second experiment, in March 2007, six potted plants per isolate were each inoculated with either the gfp-1 or the wild-type isolate. Half were incubated in a growth chamber under 29/21°C day/night temperature regime for 14/10 hrs, respectively, for the induction of vegetative growth, and half were incubated for 1 month under 17/12°C day/night, for 10/14 hrs, respectively, for the induction of flowering and symptom development. Three plants served as water-inoculated controls.

2.5 Dissemination of conidia

2.5.1 Spatial patterns of disease severity in a commercial orchard

Two commercial plots in kibbutz Cholit, Southern Israel, were assessed for disease severity during the 2005 flowering season. Plot 1 (C), consisting of forty eight 20-year-old Tommy Atkins trees, was located 15 m from and adjacent to a heavily diseased orchard consisting of 26-year-old Keitt mango trees (inoculum source plot- A). Plot 2 (B), consisting of forty eight 26-year-old mango trees cv. Keitt, was located further away from the diseased plot and continuous to plot 1 (Fig. 2).

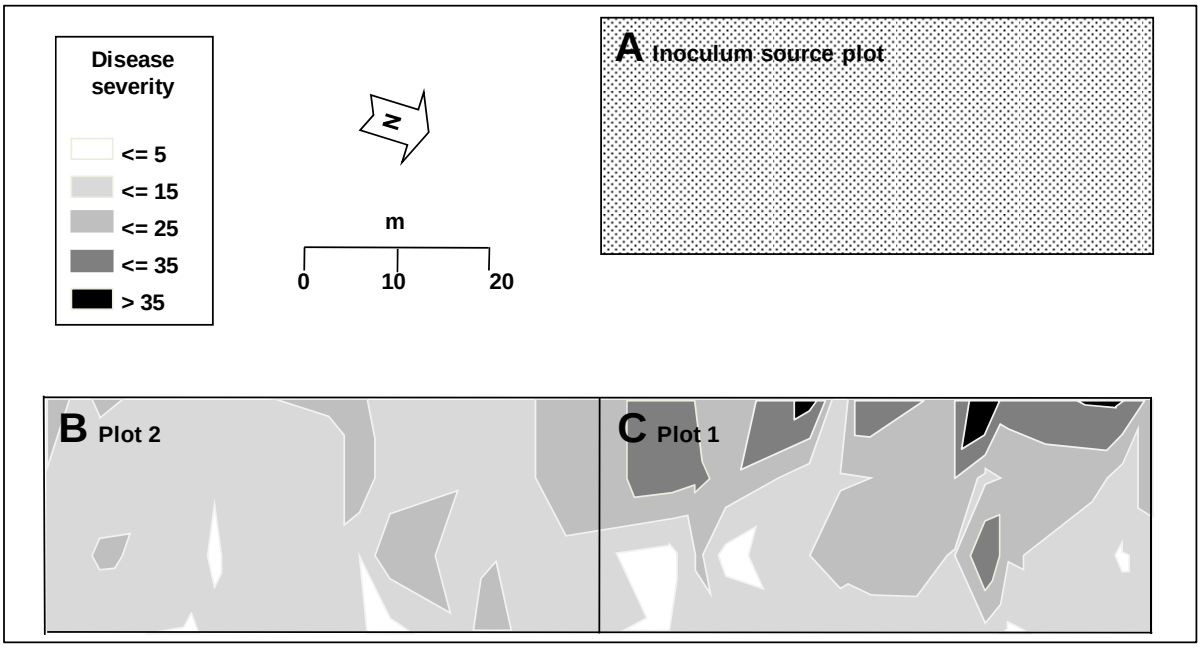

Fig. 2. Spatial pattern of mango malformation in two mango plots in Cholit commercial orchard, Southern Israel, during 2005. A, Inoculum source plot; B, Number of malformed inflorescences per tree in plot no. 2 (cv. Keitt); C, Number of malformed inflorescences per tree in plot no. 1 (cv. Tommy Atkins).

Both plots 1 and 2 were pruned heavily, 20 to 30 cm above the main trunks, using a chain saw in 2004. Malformed inflorescences were enumerated for each tree, three times during the season, on the 8th and 12th March and 6th June, and following each disease assessment, malformed inflorescences were removed. A cumulative total value of disease severity was determined according to a scale of less than 5, 5-15, 15-25, 25-35, and above 35 malformed inflorescences for each tree. A contour plot was drawn with the Contour Plot function in JMP 5.0.1 (SAS Institute Inc., Cary, NC) software, using the total malformed inflorescences per tree and the coordinates of each tree on the map. The cumulative values were also used to calculate the correlation of the distance from the inoculum source plot. Values were categorized to 3 m groups (the distance between neighboring trees) measured from the nearest point of the inoculum source plot. Averages and standard errors were calculated for each category, plotted, and analyzed using SigmaPlot 2001 software (SPSS, Chicago, IL, USA).

2.5.2 Annual inoculum availability

Inoculum presence was measured by counting the number of fresh malformed inflorescences from ten trees growing in three parallel rows in the middle of a diseased plot located in the Volcani experimental mango orchard, at Bet Dagan, consisting of fourteen rows of trees with ten trees per row. The trees consisted of a mixed cultivation of five mango cultivars, Keitt, Maya, Nimrod, Haden, and Palmer, all susceptible to *F. mangiferae*, representing the high disease severity of the orchard. Severity (i.e., the number of malformed inflorescences per tree) was determined starting from early March during both 2006 and 2007, and conducted at 3 to 4 weeks intervals until early September. Averages of malformed inflorescences per tree (± standard error) were calculated.

Sporulation capacity of the malformed inflorescences (i.e., the number of conidia produced per 1 g of malformed inflorescence) was evaluated during 3 consecutive months (April, May, and June) of the flowering seasons of 2006 and 2007. Six malformed inflorescences (8 to 18 cm in length) were randomly sampled at each period from the Haden cultivar in the Volcani experimental orchard. One gram per sample was placed in 50 ml sterilized distilled water in Erlenmeyer flasks, and shaken in a Lab-line orbit shaker (Lab Line Instruments Inc., Melrose Park, Illinois) for 1 h at 150 rpm. Inflorescence debris was filtered through a gauze pad and the liquid was centrifuged at 8000 rpm in a Hermle Z 400 K centrifuge (Hermle Labortechnik Wehingen, Germany) for 10 min. The pellet was suspended in 1 ml of sterilized distilled water, transferred to 1.5 ml Eppendorf vials, and diluted 10- and 100-fold, vortexed, and 0.1 ml from each dilution was plated on the NASH selective medium for *Fusarium* isolation. Two plates per dilution were assessed for *F. mangiferae* after 5 days of incubation at 25±2°C. The average number of conidia per g malformed inflorescence was calculated for each sampling date. Data were converted to log scale and analyzed using the Tukey-Kramer test. Data collected in each year were analyzed separately.

2.5.3 Annual pattern of conidial dissemination

The conidial dissemination pattern was evaluated by active and passive trapping of conidia in the Volcani experimental orchard during 2006 and 2007. Both active and passive traps were placed in the middle of the ten-tree plot described above. For the active trapping

method, airborne conidia were monitored by sucking in air continuously at a speed of 10 liter/min on Burkard adhesive 'Melinex' clear tape, for periods of 7 days, using a Burkard volumetric spore trap (Burkard Scientific Sales Ltd., Rickmansworth, United Kingdom) which was placed 1 m above ground level. The adhesive tape was then cut into seven equal 1-day pieces, washed with 5 ml sterile water, conidia were concentrated by centrifugation at 12,000 rpm in a Hermle Z 400 K centrifuge, and 1 ml was plated on five plates containing NASH *Fusarium*-selective medium. After washing, tapes were blotted on plates containing NASH *Fusarium*-selective medium and removed from the plates the following day. Fungal identification was preformed as previously described. Conidia were trapped in 2006 continuously from May until December, excluding 1 week in July, and the months of October through December, where only 1 week per month was sampled due to nil or very low presence of inoculum (airborne conidia). In 2007 the trap was operated 21 days in January, 6 days in February, and 14 days in March, then continuously from April through August, excluding 1 week in June and in July, and then 1 week in September, November and December.

For the passive trapping method, twenty 90 mm diameter plates containing NASH *Fusarium*-selective medium were exposed in the orchard adjacent to the Burkard volumetric spore trap, by placing them at a height of 1.5 m above ground level. During 2006, plates were exposed once a month, and twice in the months of July and August. In 2007, plates were exposed once a month from February through November, and twice a month in March, April, June, July, and September. The lids of plates were removed at 15:00 hour for each exposure period and replaced at 08:00 hour of the following morning. Subsequently, the plates were incubated in the laboratory and *F. mangiferae* was detected as previously described. Colony numbers representing numbers of trapped conidia of *F. mangiferae* per plate were calculated for each exposure period.

2.5.4 Diurnal patterns of conidial dissemination

In order to evaluate the daily pattern of conidial dispersal, the Burkard adhesive 'Melinex' clear tape was dissected into 3-h pieces during a 3-week period in June 2007, and each piece was placed in a 1.5 ml Eppendorf vial containing 1 ml sterilized water, vortexed for 20 s, and plated on NASH *Fusarium*-selective medium, as described. Hourly

measurements of relative humidity (RH) and temperature were recorded at a nearby (0.5 km) meteorological station by the Israeli meteorological service, at Bet Dagan. Three-hour averages were calculated for both RH and temperature. In order to evaluate possible coincidence between RH and trapped conidia, data of conidia trapped during 3-h intervals and RH was divided into nine 5-RH-unit groups from RH=50% to RH=95%, and one relatively dry group of 40%<RH<50%. For each group the average RH and the average trapped conidia were calculated. Data were analyzed using SigmaPlot 2001 software (SPSS, Chicago, IL, USA).

2.6 Location of infection sites and colonization pattern

2.6.1 Conditions affecting germination and growth of *F. mangiferae*

Conidial suspensions (5×10^4 conidia per ml) of isolate #34 were incubated for 16 h at seven temperatures from 5 to 35°C, at 5°C increments. Lactophenol Cotton Blue (Sigma-Aldrich, St. Louis, Missouri) stain was added to each conidial suspension at the end of the incubation period and after light microscopic observations (×20 magnification, Wild, Heerbrugg, Switzerland), percent conidial germination was calculated.

Measurements of colony growth were determined by placing fresh *F. mangiferae* fungal disks of 4.5 mm in the center of 90 mm PDA plates. Plates were incubated at each temperature as described. After 5 days, the new colony growth area was measured. Each treatment was conducted four times, and the mean growth area and standard errors were calculated.

Conidial germination of *F. mangiferae* was observed on GLASSTIC ® slides with grids (HYCOR, Biomedical Inc., Garden Grove, CA, USA). Twenty µl drops of 10^4 conidia per ml were placed into the slide cells and maintained inside a moist chamber on a moistened filter paper at 25°C. Every hour, between 0 to 8 h of wetness exposure, 3 drops were removed from the moist chamber, placed in front of a vent for 15 min to dry and then transferred to a dry chamber containing silica gel. After 8 h, when all drops were dry, a drop of Lactophenol Cotton Blue was placed in each slide cell and percent germination of conidia was calculated as previously described. In these experiments each treatment was conducted

three-four times and the experiment was conducted twice. Means and standard errors were calculated for each treatment using JMP 5.0.1 (SAS Institute Inc., Cary, NC) software and the data analyzed using SigmaPlot 2001 software (SPSS, Chicago, IL, USA).

2.6.2 Location of infection site

Potted plants were fumigated twice with Dichlorvos (Divipan, 1000 g/l Makhteshim-Agan, Omer, Israel), using a fumigator (Hagarin, Yavne, Israel) to ensure they were void of mites and insects. The base of the stem was ringed with a water-based adhesive (Rimifoot liquid, Rimi Chemical Co. Ltd., Petach Tikva, Israel) to prevent infestation by ambulant arthropods. Different plant organ (apical buds, lateral buds, branches and leaves) were inoculated with the gfp-1-marked isolate and with a water control as described. There were 20 replicates per organ, and the trial was performed twice. One month post inoculation, the plant organs were dissected, surface sterilized and plated on PDA medium amended with 50 μg/ml hygromycin. After 5 days, *F. mangiferae* transformed colonies that developed were enumerated and mean numbers of colonized plant organs were determined. A Chi-square test was used to determine levels of significance between the plant organs colonization ($P<0.05$).

Potted plants were grown in a chamber under constant temperature conditions of 25±3 °C for a period of 19 weeks. The plants were inoculated by pouring a 1 liter conidial suspension of isolate #34 (10^{10} conidia per ml) into the pots. Five plants treated with water served as negative controls. After 19 weeks, plants were uprooted and both roots and upper stems were sampled for detection of fungal colonization. For this purpose, five roots segments per pot, 2 cm in length, were sampled randomly. In addition, all upper stems were sectioned into 2 cm segments. Each section was then sub-sectioned and one, 5 mm piece, was sampled. All pieces were surface disinfected, plated on PDA medium and percent colonization of plants' roots and stems was determined per potted plant. The trial was conducted twice, each with ten replicates (plants), and means of *F. mangiferae* colonized pieces in the roots and stems were calculated per experiment. A Chi-square test was used to determine levels of significance between roots and stem colonization.

2.6.3 Colonization pattern within the host tissue

Pattern of colonization was determined in naturally infected trees. Two groups of branches were sampled from infected trees in the Volcani experimental orchard. The first group was sampled from young growth tissue of approximately 1-month-old, termed "vegetative", and the second contained branches from 3rd to 5th growth segments, approximately 2-year-old termed "woody". Branches were sectioned into 5 mm-long pieces from either the node areas containing lateral buds of the branch or from the internode areas. Pieces were surface sterilized, plated on *Fusarium*-selective medium, and assessed for fungal colonization, as described previously. The experiment was conducted twice, 32 branches were sampled in the first experiment, and 20 branches were sampled in the second experiment. Percent colonization of sections, out of total number of sections was calculated per branch for each of the node and internode tissues. Mean percentages and standard error of colonized sections were calculated. A *t*-test analysis was conducted to determine levels of significance between colonization incidences in the nodes *vs.* internode tissues. Data underwent arcsine and square root transformation before analysis.

For histopathological studies, microscopic observations were conducted on gfp-inoculated buds in order to determine the specific location of colonization within the bud. Inoculated apical buds were manually dissected lengthwise into thin sections. Images of gfp-marked conidia and hyphae were acquired using a confocal laser-scanning Olympus IX81microscope (Tokyo, Japan). Confocal images were obtained via a PLAPO X40 WLSM immersion objective lens at an excitation wavelength of 488 nm (Argon laser), a BA515-525 emission filter for gfp, and BA660IF emission filter for auto-fluorescence.

2.6.4 Infection dynamics

Dry malformed inflorescences ("dry debris") that were sampled from Volcani experimental orchard were used as a source of inoculum for infesting potted plants. Samples of the debris were surface sterilized, placed on PDA medium plates, and verified to contain viable conidia. Half of the buds in each trial were infested with the dry debris, and the other half served as a control and was not infested. Buds from the two groups (infested and not infested) were similar in size and location on the plant. Four-hundred mg of dry debris were moistened with 400 µl tap water and placed on top of each treated apical bud. After

infestation, buds were sprayed with tap water till run-off. Ten days later, before bud break, all apical buds were removed, surface disinfected and plated on *Fusarium*-selective medium. The trial was conducted three times on six potted plants bearing a total of 70, 76 and 96 buds per trial, for trial 1, trial 2 and trial 3, respectively. Percent of colonized buds for the infested and control treatments was calculated and a chi-square analysis was performed using JMP 5.0.1 (SAS Institute Inc., Cary, NC) software.

In order to estimate the seasonal dynamics of airborne conidia of *F. mangiferae* landing and infecting trees in the orchard, leaves of mango trees (cv. Haden) were sampled monthly during March through September in 2008. Nine 10 cm disks were sampled randomly from 30 leaves sampled from three trees. The leaf disks were plated on *Fusarium*-selective medium and removed 16 h later. This method of 16 h blotting of the disks ensured that conidia, if present on the surface of the leaf disk, germinated on the medium. Plates were incubated for 5 days at 25±3°C for development of typical *Fusarium* colonies. The incidence of leaf-disks bearing conidia on their surfaces was calculated, as well as the mean and standard error of the incidence of colonized disks per sampling date. A Tukey-Kramer analysis was used to determine differences in leaf-coverage between sampling dates ($P<0.05$).

The timing of airborne infections in the orchard was assessed monthly during 3 consecutive years (2005-2007) by dissecting the apical and lateral buds from each of sixty vegetative (1-month-old) branches sampled from the Volcani orchard. Five mm sections were disinfected, plated on Nash *Fusarium*-selective medium, and evaluated for fungal colonization. The branches were then grouped into four categories: (i) those that were not colonized by *F. mangiferae*; (ii) those that were colonized only in the apical buds; (iii) those that were colonized only in the lateral buds ; and (iv) those that were colonized in both the apical and lateral buds. For each month, percent branches which were colonized only in the apical buds was calculated. It was assumed that these buds were infected by airborne conidia originated from infected inflorescences. Monthly averages and standard error were determined.

2.7 Role of the bud mite *A. mangiferae* in epidemiology of the disease

2.7.1 Mites bearing fungal conidia

A. mangiferae collected from infested buds of various mango cultivars, were exposed to the gfp-1 isolate of *F. mangiferae* using two different methods. For the dipping method, 20 mango bud bracts, bearing approximately 100 bud mites per bract, were dipped for 5 s in the gfp-1 suspension of 10^6 conidia/ml. After allowing the bud bracts to dry, mites were removed with an ultra-fine paint brush and mounted on double-sided sticky tape for microscopic observation. In the second method, 30 mites were placed on a five mm^2 PDA plug which was inoculated 48 h beforehand with the gfp-1 isolate. After 24 h, mites were removed from plugs and inspected for conidia as described above.

Images of gfp-marked conidia were acquired using a confocal microscope as described in the previous section. Transmitted-light images were acquired using Nomarski differential interference contrast.

2.7.2 Mite and fungus share mutual habitat

Microscopic observations were conducted on apical buds inoculated with both bud mites and gfp-marked conidia, in order to detect the physical proximity of the two organisms sharing similar habitat within the apical buds. Confocal microscopy (described in the previous section) was used for images of inoculated bract sections. Scanning electron microscopy (SEM) was also used for observation where bracts were mounted on stubs without any fixation or drying and observed with JSM-5410LV scanning electron microscope (JEOL Ltd., Tokyo, Japan). This SEM has a “low vacuum mode” which enabled observation of the samples without any preparation.

2.7.3 Vectoring of the pathogen by mites into apical buds

Potted mango plants were placed in a growth chamber at a constant temperature of 25±2°C under diurnal 12 h light conditions. Two weeks before inoculation the plants were fumigated and clean of mites and insects as described. Each plant was placed in a disinfected plastic cage and treated with one of the following four treatments: 1. One hundred mites were placed on two 5 mm^2 agar (PDA) plugs colonized with 3-day-old gfp-1 isolate. The plugs were then transferred to a leaf, at a distance of approximately 5 cm from an apical bud;

2. One hundred mites were placed on two 5 mm^2 agar plugs (without the fungus) and then transferred to a distance of 5 cm from an apical bud, as described above; 3. Two 5 mm^2 agar plugs with the gfp-1 isolate were placed at a 5 cm distance from an apical bud; and 4. Untreated control, agar plugs without mites or fungus. Four apical buds were inoculated in each treatment and the experiment was repeated five times. Two days following inoculation the apical buds were inspected with a stereomicroscope and the bud mites were counted. Then, the gfp conidia (if present) were washed from the bud bracts, plated on PDA amended with 50 μg/ml hygromycin, and after 5 days, gfp colonies were enumerated. Conidia enumeration data underwent square root transformation, and data of bud mites was expressed as proportions of the original number of mites that were inoculated, and transformed by arcsine square root before analysis. A *t* test was used to determine significance of each mean from zero.

2.7.4 Bud mite assistance in fungal colonization

Potted mango plants were fumigated twice, and placed in plastic cages as described above. Three days post fumigation, plants were treated with one of the following treatments: A. Forty apical buds were inoculated with gfp-1 conidia, B. Forty apical buds were inoculated with gfp-1 conidia, then, after 4 days inoculated with 50 mites per bud. The experiment was repeated twice in consecutive years, during 2006 and 2007. Twenty-one days post inoculation, buds were harvested, separated into bracts, disinfected, plated on hygromycin-amended PDA medium, and fungal colonization was calculated. Two parameters were measured: the frequency of colonized buds, expressed as the ratio of colonized buds calculated from the total number of buds in the treatment; and severity of colonization, expressed as the average of colonized bracts per colonized bud. Statistical analysis of the first binary variable was performed using a chi-square test, and that of the second parameter, using a *t* test, $P<0.05$.

2.7.5 Role of bud mites in aerial dissemination of conidia

2.7.5.1 Mite-fungal phenology

The presence of the pathogen and bud mites, within apical buds, was monitored during a 4-year survey in the Volcani orchard. Sixty 1-week-old apical buds (Haden cultivar), were marked, and after 3 weeks removed and examined in the laboratory. Each apical bud was

dissected into bracts and inspected with a stereomicroscope for the presence of mites, then, surface sterilized and plated on a NASH *Fusarium*-selective medium for the detection of the pathogen. Percentages of buds populated with mites and the pathogen were calculated. Numbers of mites per apical bud were recorded for the period of May 2006 until December 2007. During December 2006, March, November and December 2007, there was no development of young branches in the orchard. For these samples, only the numbers of mites were enumerated. To determine whether presence of the fungus affected mite population density within the bud, mite population levels were compared in colonized and uncolonized buds by the fungus during May through October in 2006 and 2007. The effects of sampling date, presence of the fungus and their interaction were analyzed by two-way ANOVA test. As the interaction was not significant, the effect of the presence of the fungus on mite population density was determined by *t* test.

2.7.5.2 Trapping of airborne mites

Trapping of airborne mites was performed inside a growth chamber as follows: 12 branches from mite-infested trees in the orchard were pruned and their severed ends were washed in distilled sterilized water to prevent the milky sap from clogging the stem vessels. The branches were placed in water in 250 ml Erlenmeyer flasks, and their openings were plugged with cotton and sealed with Parafilm (American Can Co., Greenwich, CT, USA). Using this methodology, the shoots continued to grow and the buds opened up allowing the mites to migrate in the airflow. Branches were placed in front of a fan in a wind tunnel apparatus (Fig. 3).

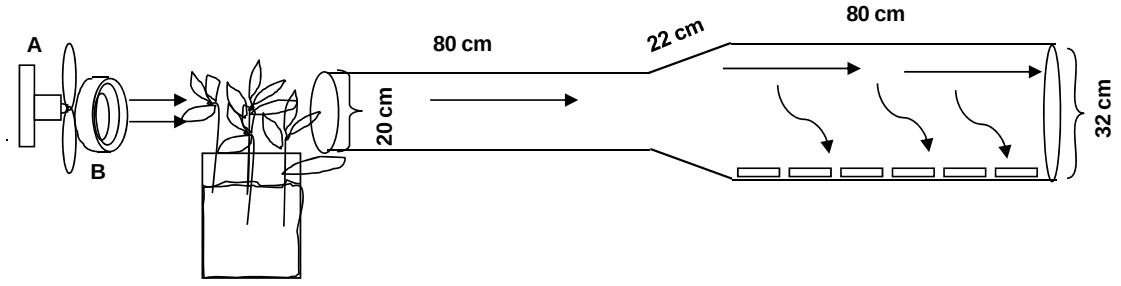

Fig. 3. Wind tunnel apparatus. Branches were placed in front of a fan (A) with 3 polyethylene rings (B) attached to it in order to prevent air turbulence. Two polyethylene cylinders were placed downwind from the branches. The first cylinder was connected to a cone that was then connected to

the second cylinder. Twenty-six Vaseline™ coated slides, were placed on the bottom of the wider cylinder. Straight arrows indicate airflow direction.

When the air current moved from the narrow cylinder to the wider one, the velocity was reduced, thereby allowing the windborne eriophyoid mites to drop from the airflow onto the Vaseline™ coated slides. To test the efficacy of this system we used four heavily infested tomato plants with the tomato russet mite (TRM), *Aculops lycopersici* Massee, which belongs to the same family (Eriophyidae) as *A. mangiferae*, and has similar body dimensions. The tomato plants were placed in the wind tunnel apparatus and after 2 days, slides were inspected under a stereomicroscope. An average of 25.9 ± 1.5 TRM were found on each slide indicating that this method could be successfully used for detection of airborne eriophyids. The experiment to detect *A. mangiferae* was repeated four times with branches sampled in: October 2005, and January, February and March 2006.

For monitoring windblown mites under field conditions, a freely rotatable wind trap, made of 200 × 20 cm PVC pipe mounted on a pole attached to a wind vane, was placed in the Volcani experimental mango orchard surrounded by heavily infected trees (spaced 3 × 4 m apart) (Duffner et al. 2001). A sheet of polycarbonate plastic (200 × 9 cm) covered with 70 Vaseline™-coated slides, was inserted into the PVC pipe. Slides were replaced once a month from September 2005 until September 2006. After each exposure, slides were collected and examined for the presence of the mite under a stereomicroscope. Mites found on the slides were enumerated and examined for the presence of conidia at X40 using transmitted Nomarski differential interference contrast and plated on NASH *Fusarium*-selective medium for fungal detection.

2. RESULTS

3.1 Gfp transformation and pathogenicity

Six stable gfp transformants were obtained, five containing a single integrative copy of the plasmid, and one (gfp-3) containing two integrative copies of the plasmid (Fig. 4). An assessment of the mitotic stability of transformants determined that all of them maintained their hygromycin B resistance after six successive transfers.

In the first pathogenicity experiments, both the wild-type and the gfp-1 isolates infected 27% of the buds. In the second experiment half of the seedlings inoculated with either the wild-type or gfp-1 isolates (cultivated at 29/21°C day/night conditions) started sprouting 3 months post inoculation, with vegetative malformation symptoms developing in all plants. The other half of the inoculated seedlings (exposed for 1 month to 17/12°C followed by 1 week at 29/21°C, day/night temperatures, respectively) started to bloom and typical disease symptoms were again observed in all the plants. Water-inoculated control plants remained healthy.

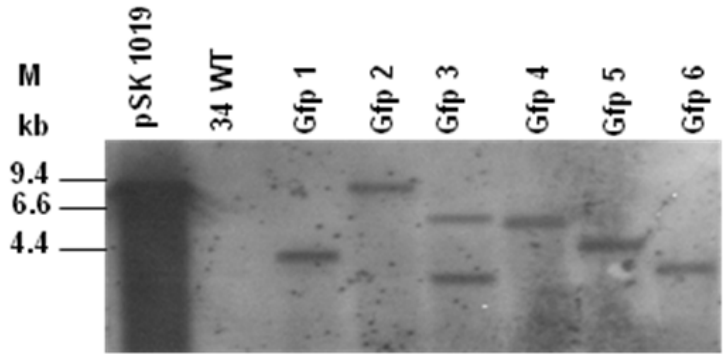

Fig. 4. Southern hybridization of *Hind*III-digested genomic DNA from the pSK 1019 plasmid, the wild-type isolate (34 WT) and six transformants (gfp 1-6) of *Fusarium mangiferae*. DNA size markers (M) in kilobase are included at the left.

3.2 Dissemination of conidia

3.2.1 Spatial patterns of disease severity in a commercial orchard

High numbers of infected inflorescences were detected in trees adjacent to the heavily malformed orchard in Cholit which presumably served as the inoculum source (Fig. 2A, page 20). Disease severity ranged from 0 to 25 malformed inflorescences per tree in plot 2 (cv. Keitt, Fig. 2B), which was lower than that in plot 1 (cv. Tommy Atkins, Fig. 2C). The latter was closer to the diseased orchard consisting of infected trees bearing over 60 malformed inflorescences per tree. Within plot 1, lower severity of the disease was detected in trees located further away from the diseased orchard. A decreasing exponential curve best described the coincidence between higher disease severity in trees and proximity to the inoculum source plot (Fig. 5).

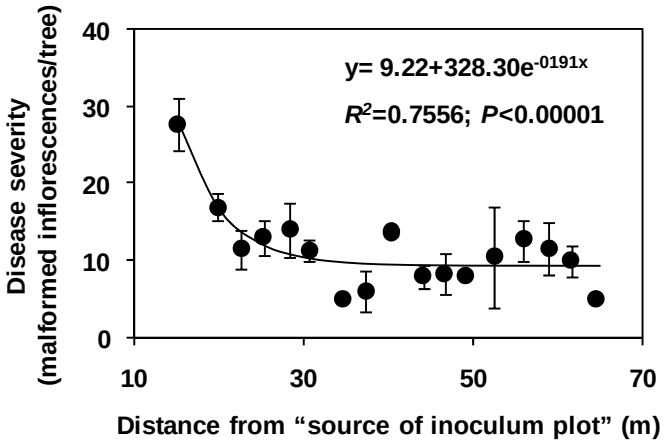

Fig. 5. Mango malformation severity calculated in respect to distance from an adjacent, heavily infected orchard (inoculum source plot). Vertical bars represent standard error of the mean (±SE).

3.2.2 Annual inoculum availability

Malformed inflorescences were first observed in the Volcani experimental orchard during mid-March in both 2006 and 2007. The number of malformed inflorescences per tree increased gradually thereafter reaching a peak in May, with an average of 30 and 84

malformed inflorescences per tree, in 2006 and 2007, respectively. Disease severity declined to negligible levels in August (Fig. 6).

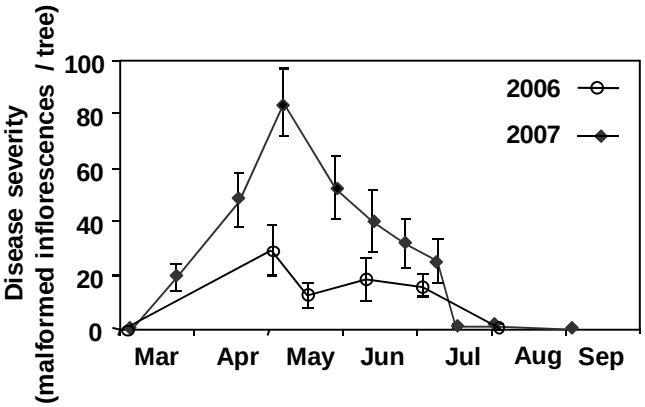

Fig. 6. Mango malformation severity in the Volcani experimental orchard during 2006 and 2007 at Bet Dagan, Israel. Data points represent averages for ten trees from different cultivars. Vertical bars represent the standard error of the mean (SE).

Overall, high numbers of conidia were obtained from malformed inflorescences, with sporulation capacity ranging from 5.9×10^3 conidia per g malformed tissue sampled in April 2007, to a peak of 1×10^6 conidia per g malformed tissue sampled in May 2006. Significantly ($P \leq 0.0003$) more conidia per g malformed inflorescence were detected in May and June than in April, during the 2 years of the survey (Fig. 7).

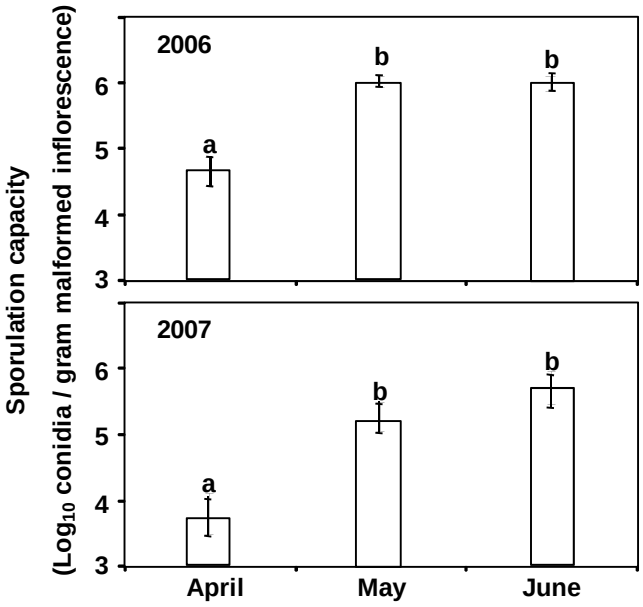

Fig. 7. Sporulation capacity of malformed inflorescences sampled from the Volcani experimental orchard over a 3-month period during 2006 and 2007. Vertical bars represent standard error of the mean (±SE). Values were compared using Tukey-Kramer significant difference test for each year separately (P<0.0001 for 2006; P=0.0003 for 2007). Means with a common letter are not significantly different.

3.2.3 Annual pattern of conidial dissemination

A peak in trapped airborne conidia was detected in the Volcani experimental orchard, using the Burkard volumetric trap in May and June for both 2006 and 2007 (Fig. 8A). During this period, values of trapped conidia varied markedly from days with zero captures (e.g., 8 and 21 May 2006, and 4 May and 17 June 2007) to days with high numbers of trapped airborne conidia (e.g., 215, 62, 159, and 167 conidia trapped on 26 May and 9 June 2006, and 13 and 14 May 2007, respectively). During July through December in both years, low levels of conidia were trapped; in most days no conidia were trapped, and for the remainder of the days, levels of 1 to 11 conidia were trapped per day.

In general, similar results were obtained using the selective medium for passive trapping of airborne conidia on plates in the orchard (Fig. 8B). Elevated levels of conidial

dissemination were detected during April through July in 2006 and during May through July in 2007, with 4.6±0.25 and 1.8±0.09 (average colonies per plate ± SE), respectively. During the months January to March and August to December, 2006 and February to April, and August to November, 2007, the number of conidia trapped in the Petri plates was low, averaging 0.7±0.09 colonies per plate in 2006 and 0.2±0.03 in 2007 (Fig. 8B).

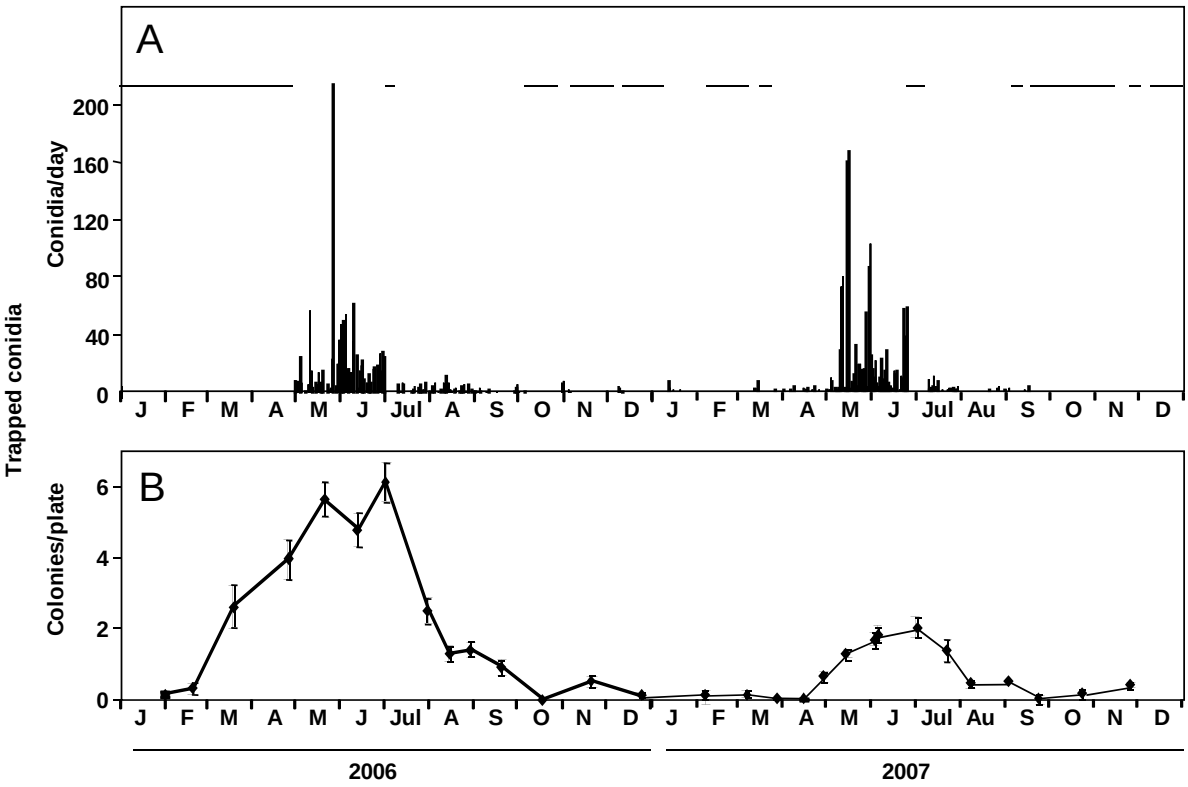

Fig. 8. Annual pattern of conidial trapping of *Fusarium mangiferae* in the Volcani experimental orchard during 2 consecutive years. A, Daily enumeration of conidia using the Burkard volumetric trap. The fragmented line represents non-trapping periods. B, Daily traps of conidia on plates containing *Fusarium*-selective medium exposed overnight from 15:00 to 8:00 hour under orchard conditions. Vertical bars represent standard error of the mean (±SE).

3.2.4 Diurnal patterns of conidial dissemination

The daily pattern of conidial dispersal did not relate to a specific discernable time of the day during the 21 days, 3-h-trapping period. For example, on 21 June, between 20:00 and

23:00 hour, there was a daily dispersal peak of 20 conidia, as opposed to a daily dispersal peak of 26 conidia on 23 June at the 14:00 to 17:00 hour (Fig. 9). Daily peaks of eight dispersed conidia were also detected between 11:00 to 14:00 hour on 9 June and between 05:00 to 08:00 hour on June 10th (data not shown).

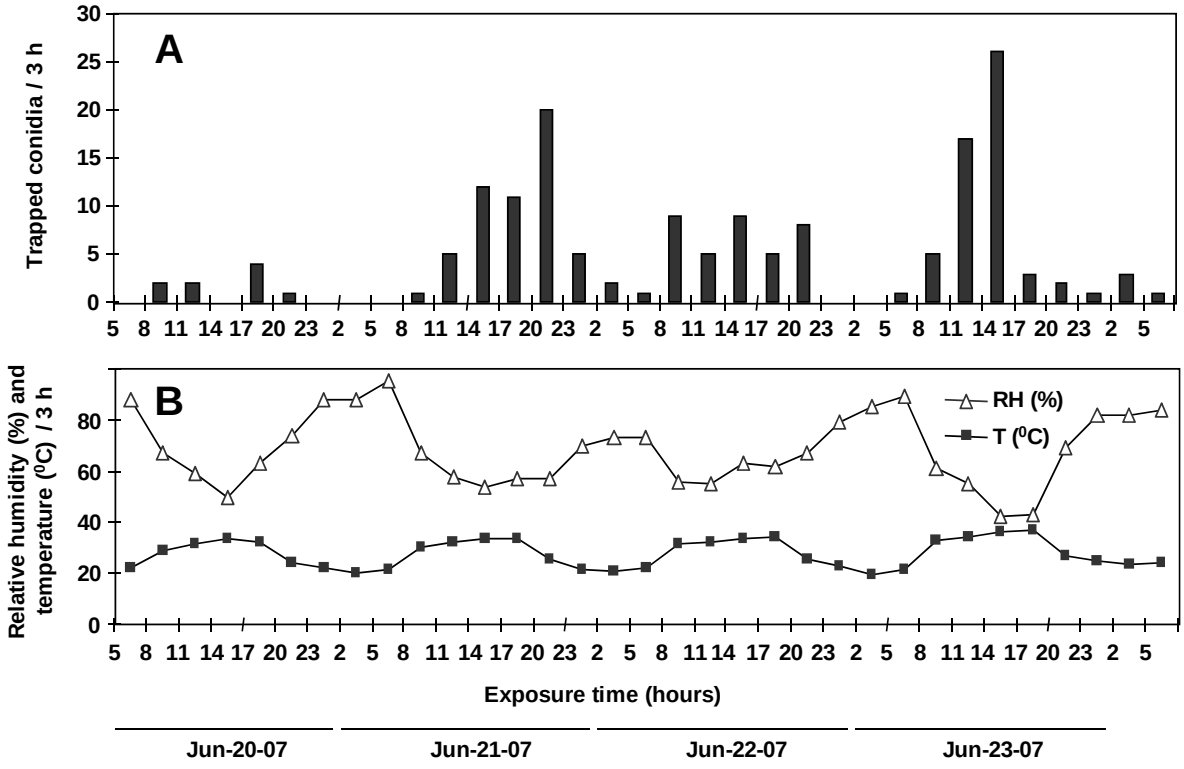

Fig. 9. Levels of diurnal trapped conidia of *Fusarium mangiferae* and relative humidity and temperature during the period of trapping. **A,** Diurnal pattern of trapped conidial during 4 consecutive days in June, 2007, using a Burkard volumetric spore trap. **B,** Relative humidity (%) and temperature (°C) data recorded during the trapping periods.

An exponential correlation was determined between mean RH and mean number of trapped conidia. Higher numbers of conidia were trapped when RH values were low, in particular below values of 55% (Fig. 10).

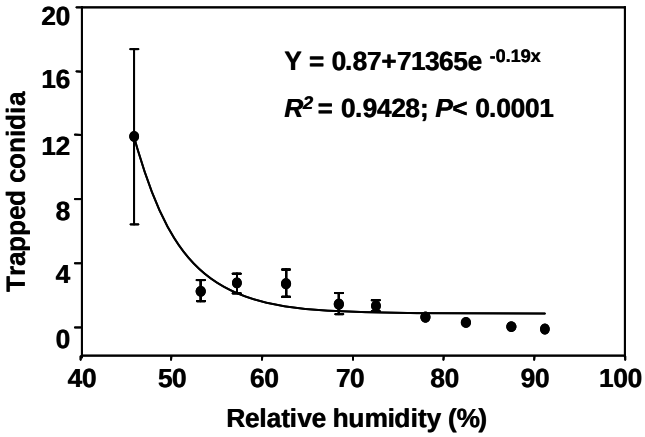

Fig. 10. Relationship between trapped conidia of *Fusarium mangiferae* and relative humidity (RH). Conidia were trapped in the Volcani experimental orchard during June 2007 over a period of 3 weeks for every 3 h. RH averages were calculated per each RH category of 5 units from 50 to 90%, excluding the first category that included 10 RH units from 40 to 50%. Vertical bars represent standard error of the mean (±SE).

3.3 Location of infection sites and colonization pattern

3.3.1 Conditions affecting germination and growth of *F. mangiferae*

Both conidial germination and colony growth required temperatures above 5°C in order to commence the germination and growth processes (Fig. 11). Moreover, both conidial germination (Fig. 11A) and colony growth (Fig. 11B) increased with a corresponding elevation in temperature and reached a peak at optimal temperatures of 28°C and 25°C, respectively.

A minimum of 2-h-wetness period was required for the beginning of the conidial germination process at 25°C, increased with increasing exposure to wetness, and reached a peak after 8 h of wetness (Fig. 12).

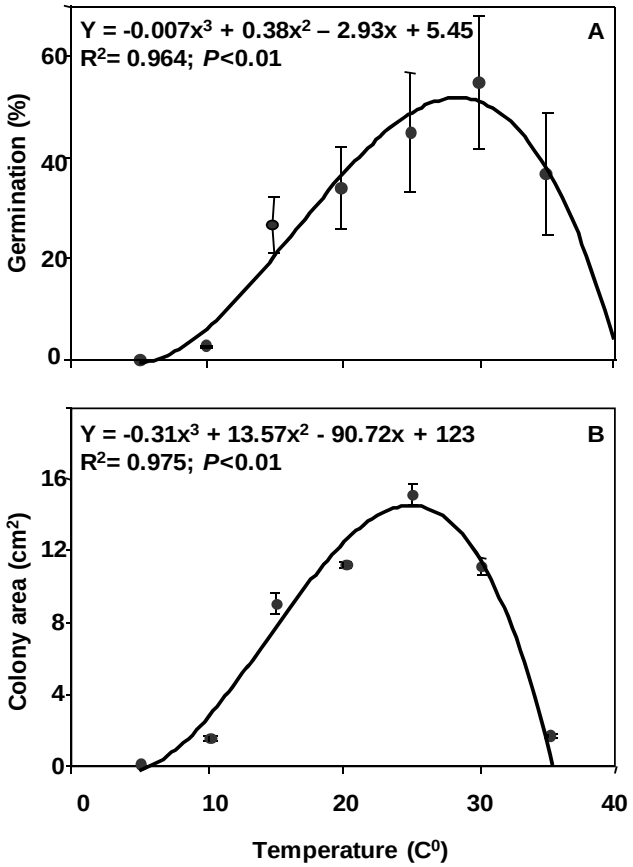

Fig. 11. Effect of temperature on **A** conidial germination and **B** fungal colony growth of *Fusarium mangiferae*. Vertical bars represent standard error of the mean (±SE).

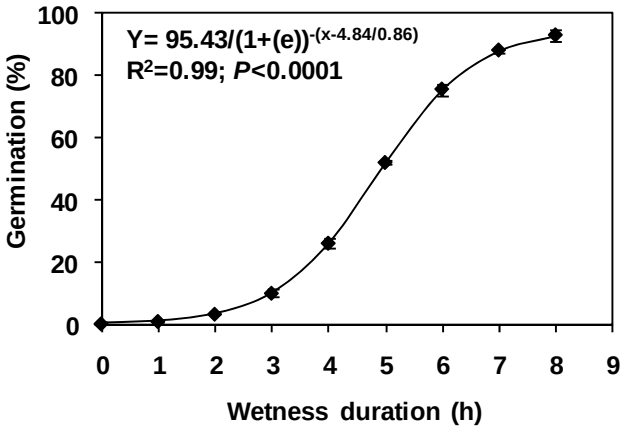

Fig. 12. Effect of wetness duration on germination of conidia of *Fusarium mangiferae*. Vertical bars represent standard error of the mean (±SE).

3.3.2 Location of infection site

Artificial inoculations of apical and lateral buds, branches, and leaves of potted plants resulted in high incidence of fungal colonization in buds, predominantly the apical buds (Table 1). In trial 1 all apical buds were colonized with the pathogen, significantly less in lateral buds, while very low and insignificant levels of colonization were detected in branches and leaves. Similar results were obtained in trial 2 but were characterized by less overall colonization incidence. The apical buds were the most colonized organ, followed by lateral buds, while branches and leaves remained uncolonized (Table 1).

Table 1: *Fusarium mangiferae* colonization (%) of plants after artificial inoculations of various organs or of soil.

Inoculation site	Isolation from	Trial 1[a]	Trial 2	Trial 3	Trial 4
Organ	Apical buds	100 a	20.0 a		
	Lateral buds	60.0 b	5.6 ab		
	Branches (internode sections)	5.0 c	0.0 b		
	Leaves	0.0 c	0.0 b		
Soil	Roots			60.2 a	37.8 a
	Stems (internode and node sections)			0.0 b	0.0 b

[a]Different letters within each trial column denote statistical significance ($P<0.05$) using Chi-square test.

In the soil inoculation trials, the pathogen was detected in the roots of infested soil. Nineteen weeks post inoculation, 60.2% and 37.8% of the root segments were colonized by *F. mangiferae* in trials 3 and 4, respectively, but not in above-ground parts of the plants (Table 1). In addition, disease symptoms were not observed in the above ground plant parts. All water-inoculated control plants were disease-free and the pathogen was not detected in

the roots or the upper parts of the plant. Furthermore, the pathogen was not detected in above parts of soil inoculated plants, 52 weeks after inoculation (data not shown).

3.3.3 Colonization pattern within the host tissue

Percent colonization of *F. mangiferae* within naturally infected branches was significantly higher in node sections containing lateral buds than in internode sections (between lateral buds) where colonization was negligible in both groups of sampled branches. Percent colonization within node sections of vegetative branches was 12.0±3.7%, significantly higher than 0.1±0.1% within internode sections. Similar results were observed for woody branches, where significantly higher percentage of colonization (6.8±2.3% of the sections) was detected in the node sections, as compared with that observed in the internode sections (1.3±0.7%).

Confocal microscope images of apical bud sections inoculated with gfp-marked isolate of *F. mangiferae* revealed fungal colonization patterns which were not specific to a particular site within the bud tissue (Fig. 13). Gfp-marked mycelia were detected within trichomes (Fig. 13A and B), in bract tissue (Fig. 13A, C, D and E), and within apical meristems (Fig. 13A and C).

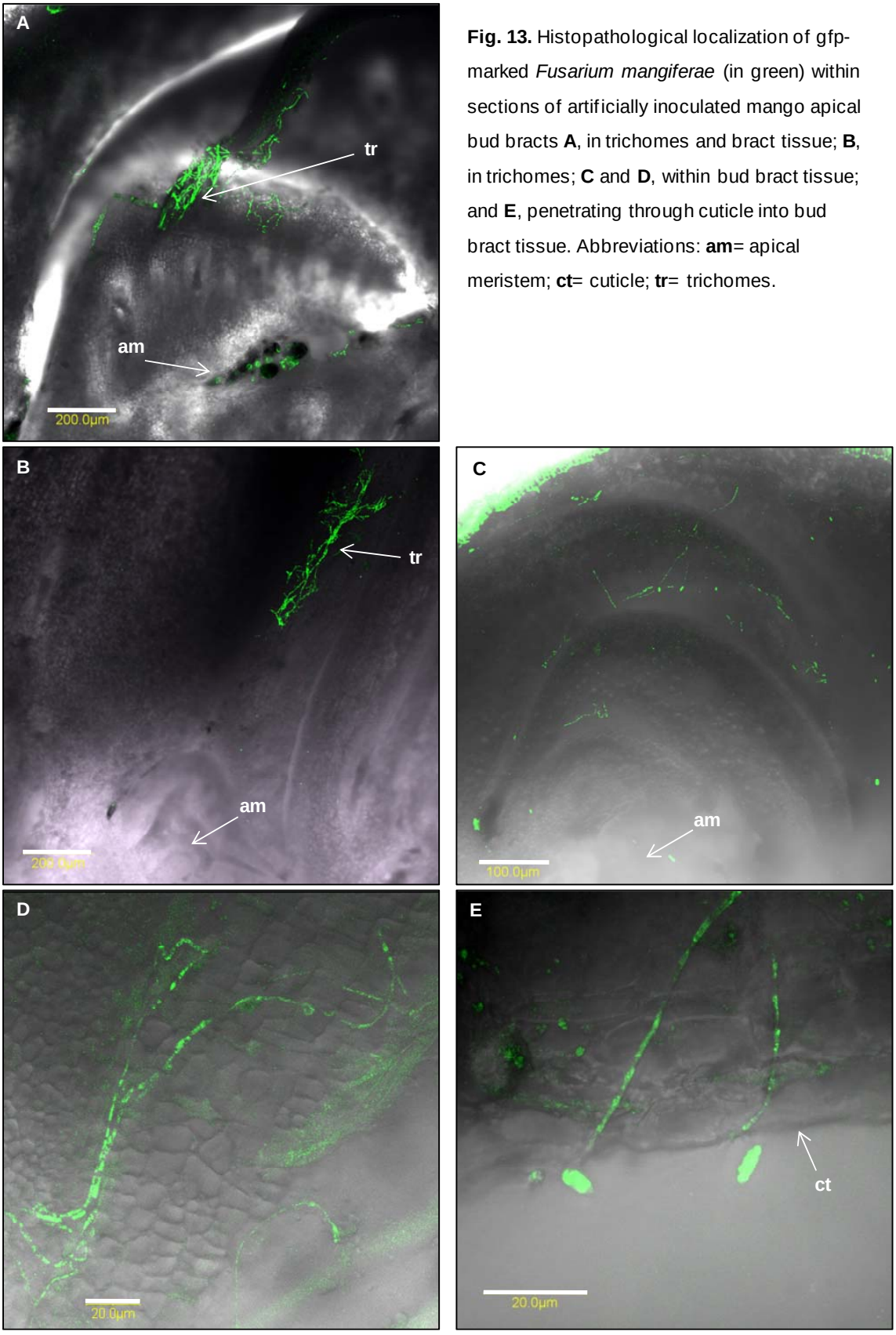

Fig. 13. Histopathological localization of gfp-marked *Fusarium mangiferae* (in green) within sections of artificially inoculated mango apical bud bracts **A**, in trichomes and bract tissue; **B**, in trichomes; **C** and **D**, within bud bract tissue; and **E**, penetrating through cuticle into bud bract tissue. Abbreviations: **am**= apical meristem; **ct**= cuticle; **tr**= trichomes.

3.3.4 Infection dynamics

When dry malformed inflorescences were used to infest apical buds of potted plants, the pathogen was detected in 52.6% and 20.1% of the infested buds of trials 1 and 3, which was significantly higher than that detected in the non-infested untreated controls (Table 2). In trial 2, 13.9 % colonization was detected in the infested buds, which was higher but not significantly different than that of 2.8% colonization detected in the untreated control.

Table 2: *Fusarium mangiferae* colonization (%) of apical buds of potted plants following artificial infestation with dry debris of naturally infected malformed inflorescences from Volcani experimental orchard.

Treatment	Trial 1[a]	Trial 2	Trial 3
Infested	52.6 a	13.9 a	20.1 a
Untreated control	0.0 b	2.8 a	0.0 b

[a]Different letters within each trial column denote statistical significance (P<0.05) using Chi-square test.

F. mangiferae conidia were detected on the surface of mango leaves, sampled from infected trees, throughout the sampling period. The incidence of leaf-disks bearing conidia on their surfaces varied over time, peaking in June and in July and decreasing in August and September 2008 (Fig. 14).

One-month-old branches started to develop in the orchard in the late spring (May), and continued to appear until early autumn (November). Incidence of 1-month-old branches colonized with *F. mangiferae* in the apical bud but not in the lateral buds during the 3-year period of the survey, was the highest in May and June reaching 8-10 % colonization of the total number of sampled branches (Fig. 15). Incidence of colonized branches decreased in the following months of July through September, where approximately 2% of all branches were colonized only in the apical bud tissue. During October and November only a very low incidence of colonized branches was detected (Fig. 15).

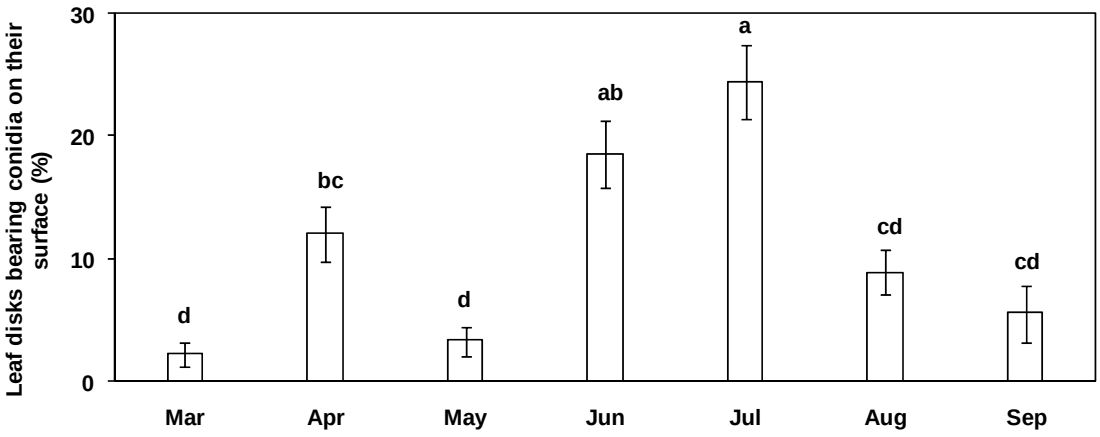

Fig. 14. Presence of conidia of *Fusarium mangiferae* on the surface of leaves sampled from the infected Volcani experimental orchard during 2008. Data underwent arcsine conversion and statistical significance was determined using ANOVA Tukey-Kramer analysis ($P<0.05$). Vertical bars represent standard error of the mean (±SE).

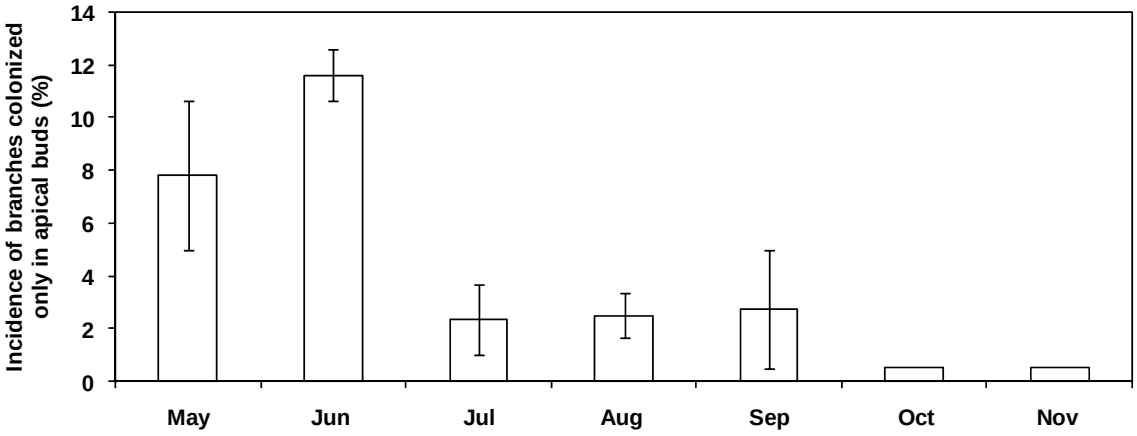

Fig. 15. Seasonal incidence of vegetative branches colonized with *Fusarium mangiferae* only in the apical buds but not in the lateral bud sections. Branches were sampled from Volcani experimental orchard during the years 2005-2007 and the mean average colonization per year was determined. Vertical bars represent standard error of the mean (±SE).

Percent colonization of *F. mangiferae* within naturally infected branches was significantly higher in node sections containing lateral buds than in internode sections

(between lateral buds) where colonization was negligible in both groups of sampled branches. Percent colonization within node sections of vegetative branches was 12.0±3.7%, significantly higher than 0.1±0.1% within internode sections. Similar results were observed for woody branches, where significantly higher percentage of colonization (6.8±2.3% of the sections) was detected in the node sections, as compared with that observed in the internode sections (1.3±0.7%).

3.4 Role of the bud mite *A. mangiferae* in epidemiology of the disease

3.4.1 Carrying of the pathogen and vectoring thereof into apical buds

Inoculation of bud mites with a conidial suspension using the dipping method was not successful. When using the second inoculation method (mites released on agar plugs colonized with the gfp-1 isolate), gfp fluorescing conidia were observed on the mites (Fig. 16). Conidia of the pathogen did not seem to cling to any particular part of the mites' bodies.

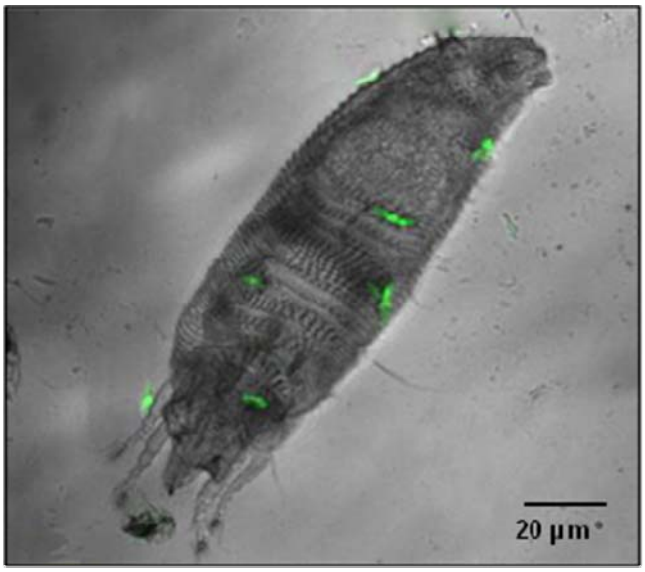

Fig. 16. Mango bud mite, *Aceria mangiferae*, bearing conidia of isolate gfp-1 of *Fusarium mangiferae* (shown in green), the causal agent of mango malformation disease.

Gfp-1 conidia were found in apical buds only when both bud mites and conidia were co-inoculated on the plant (Table 3). The numbers of gfp-1 conidia found in the apical buds in that treatment as well as the numbers of mango bud mites found in the apical buds in the two treatments where mites were inoculated, were significantly different from zero (Table 3).

Table 3. Average number of *Aceria mangiferae* and gfp-1 marked *Fusarium mangiferae* conidia / apical mango bud[a].

Inoculation with	**Bud mites**			**Gfp conidia**		
	Average	SE	*P* [b]	Average	SE	*P* [c]
Mites with gfp conidia	1.4	0.71	0.017	0.45	0.20	0.014
Mites alone	1.75	0.81	0.005	0		
Gfp alone	0			0		
Untreated control	0			0		

[a]Twenty buds / treatment were evaluated on potted mango plants.

[b]Prior to a *t* test analysis (to determine significance of each mean from zero) an arcsine and a square root transformation were performed on the proportions of mites (from the 100 mites that were inoculated).

[c]Prior to a *t* test analysis (to determine significance of each mean from zero) data underwent a square root transformation.

3.4.2 Mite and fungus share mutual habitat

Both the mango bud mite *A. mangiferae* and the pathogen *F. mangiferae* were observed within bracts of apical buds (Fig. 17). Hyphae of *F. mangiferae* were observed in close proximity with *A. mangiferae* (Fig. 17A). Hyphae and conidia of *F. mangiferae* were observed growing around trichome of the bud bracts; conidia were also detected on the mite's body (Fig. 17 B). One germinating conidia and fungal hyphae were observed upon the body of *A. mangiferae* (Fig. 17C).

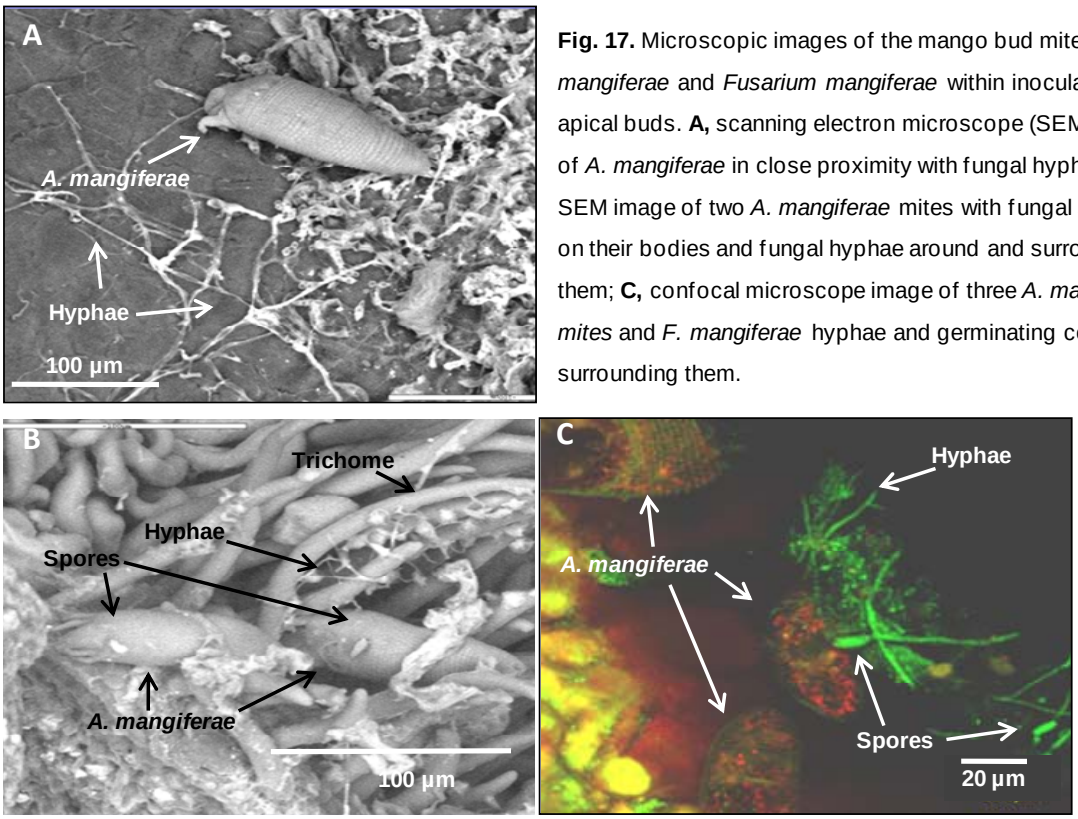

Fig. 17. Microscopic images of the mango bud mite *Aceria mangiferae* and *Fusarium mangiferae* within inoculated apical buds. **A,** scanning electron microscope (SEM) image of *A. mangiferae* in close proximity with fungal hyphae; B, SEM image of two *A. mangiferae* mites with fungal spores on their bodies and fungal hyphae around and surrounding them; **C,** confocal microscope image of three *A. mangiferae mites* and *F. mangiferae* hyphae and germinating conidia surrounding them.

3.4.3 Bud mite assistance in fungal colonization

Frequency and severity of fungal colonization was significantly higher ($P<0.05$) in buds inoculated with both bud mites and conidia, than in buds inoculated with conidia alone conducted in consecutive years during 2006 and 2007 (Fig. 18). In 2006, significantly higher colonization was recorded in the treatment with dual inoculations of both conidia and bud mites ($\chi^2_1=8.418$; $P=0.0037$) (Fig. 18A). Severity of colonization in 2006 was also significantly higher ($t_{48}=5.077$; $P<0.0001$) in the combined mite and conidia treatment (Fig. 18B). Similar results were obtained in 2007, where significantly higher colonization rates were detected in the combined conidia and mite inoculations ($\chi^2_1=4.082$; $P=0.043$), including more severe colonization rates ($t_{52}=2.684$; $P<0.009$).

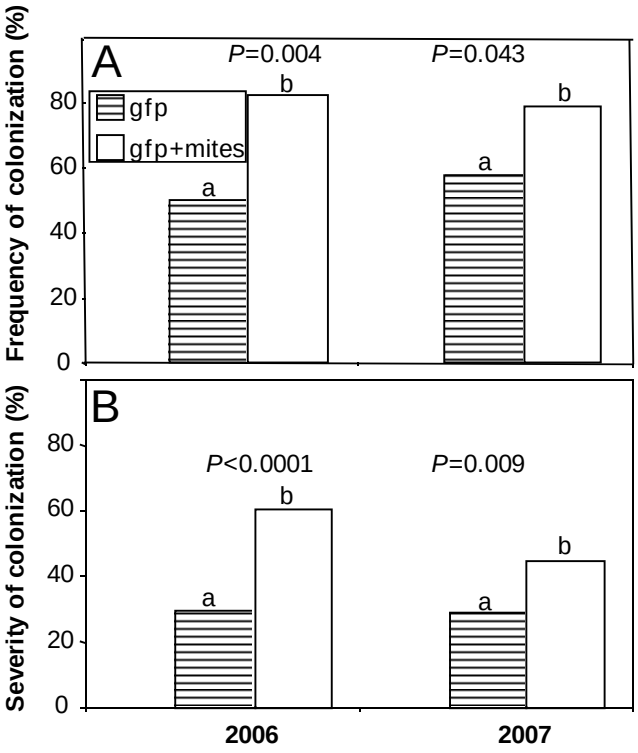

Figure 18. Frequency (A) and severity (B) of *Fusarium mangiferae* (isolate gfp-1) colonization in inoculated mango apical buds with and without the presence of bud mites, performed twice during 2006 and 2007. Severity of colonization was measured as the frequency of infected scales per infected bud. Statistical analysis of the binary variable in A was calculated using a chi-square test. Statistical analysis of the continuous variable in B was calculated using a *t* test. Significance refers to each pair of means per year separately. Treatments with different letters are significantly different ($P<0.05$).

3.4.4 Role of bud mites in aerial dissemination of conidia

A. mangiferae and *F. mangiferae* were present in apical buds throughout the year. More than 67% of all apical buds in each sample were populated with bud mites, whereas the frequencies of *F. mangiferae* were much lower (Fig. 19). Average numbers of *A. mangiferae* per apical bud varied from 18 mites per bud in May and December 2006 and in March 2007, to a peak of 62 and 56 mites per bud in July 2006 and October 2007, respectively. On average, for the 10 sampling periods in both years, 50 mites per bud were detected in buds colonized by the fungus, which was significantly higher than that of 33.6 detected in buds not colonized by the fungus ($t_{586}=3.731$; $P=0.0002$).

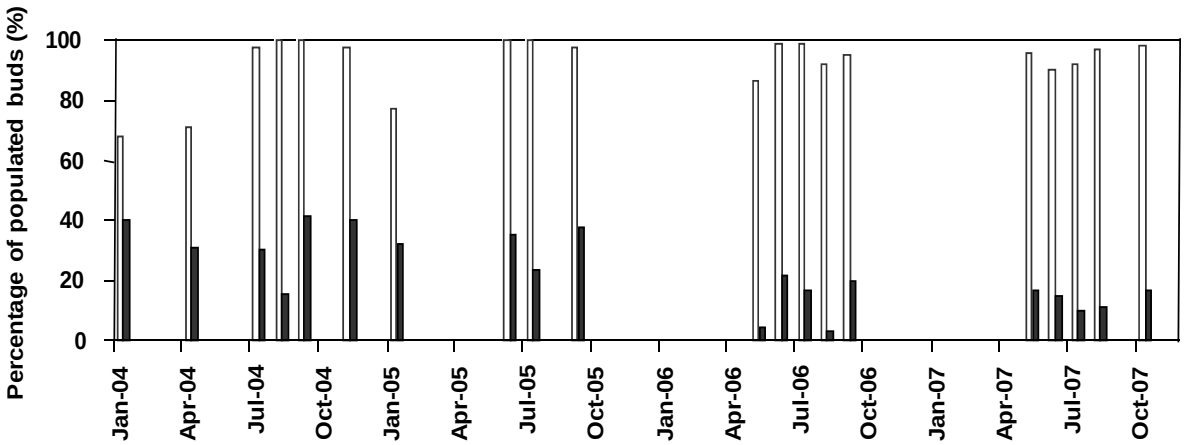

Fig. 19. Percentage of buds populated with *Aceria mangiferae* (open bar) and with *Fusarium mangiferae* (filled bar), sampled from 1-month-old mango branches of Haden cultivar from the Volcani experimental orchard, Bet Dagan during the years 2004-2007.

In the wind tunnel apparatus, 2, 12, 7, and 5 mango bud mites were detected in the growth chamber during the four trials. A number of viable *F. mangiferae* conidia were also recovered (1, 3, 1, and 1, respectively), but none were found on the trapped wind-borne mites. Thirteen bud mites were captured using the wind-mite trap in the Volcani orchard, from September 2005 to September 2006, none bearing *F. mangiferae* conidia on their bodies. In contrast, a high inoculum density of *F. mangiferae* conidia were trapped by the volumetric spore trap in the Volcani orchard, with a peak in airborne conidial numbers being recorded during May and June, in both 2006 and 2007 (Fig. 8A). The peak in aerial dissemination corresponded with the peak of malformed inflorescences in the orchard.

3. DISCUSSION AND CONCLUSIONS

4.1 Dissemination of conidia

This part of the thesis contains the first detailed report on aerial dispersal of conidia of *Fusarium mangiferae*. Abundant amounts of airborne conidia were detected in an infected orchard, suggesting that this is a significant means of spread of this fungus. Inoculum availability was estimated by monitoring both formation and maturation of malformed inflorescences in an infected orchard, considered to be the main inoculum source (Chakrabarti & Ghosal 1989, Ploetz et al. 2002). An annual peak in malformed inflorescences was detected in May during a 2-year survey. Following and parallel to this peak, elevated levels of conidial dispersal were detected in May and June using both the active Burkard and passive plate trapping methods during 2 consecutive years, apparently due to their release from mature malformed inflorescences.

Seasonal variations in conidial dispersal for other *Fusarium* spp. are a common phenomenon and were previously reported for several pathogens. Seasonal dynamics of airborne dispersal were demonstrated for *F. graminearum* macroconidia trapped in wheat plots using Burkard air samplers (Fernando et al. 2000, Inch et al. 2005), and also for *F. crookwellense*, *F. moniliforme*, *F. culmorum*, *F. sporotrichioides*, *F. equiseti*, and *F. subglutinans* trapped within the same wheat plot (Fernando et al. 2000). Seasonal fluctuation was also observed for *F. circinatum* airborne conidia trapped within forests in California (Correll et al. 1991, Garbelotto et al. 2008, Schweigkofler et al. 2004) and for *F. guttiforme*, causal agent of fusariosis in pineapple, showing a distinct high season for conidial dispersal starting in July through March and reaching a peak in January (De Matos et al. 1997). A previous attempt to trap airborne conidia of *Fusarium* in a heavily malformation infected mango orchard using glass slides in rotary spore traps failed (Varma et al. 1974), possibly due to the small size and lack of unique characteristics of conidia which made microscopic detection impossible. In this work, the traditional methodology of detecting *Fusarium* conidia using the Burkard sticky tape was modified. Instead of counting propagules on the sticky tape under the microscope, they were washed off the tape, plated on selective *Fusarium* medium, and colonies were identified using both microscopic observation and

specific PCR primer amplification. Results obtained from this thesis contradict those of Inch et al. (2005) who suggested that spores trapped in a Burkard 7-day spore trap cannot be plated and identified to the species level since they do not remain viable on the adhesive surface. Many studies have reported the active trapping of airborne *Fusarium* conidia, all of which utilized the technique of microscopic identification to detect pathogens from the trap. This technique, however, facilitated detection of conidia and larger sized macroconidia, only to the genus level (Fernando et al. 2000, Inch et al. 2005, Noriega-Cantú et al. 1999, Rossi et al. 2002a). Diurnal dispersal of conidia was not associated with a specific time of the day, similar to results from a previous study reporting sporadic conidial release of six *Fusarium* species, sampled during 2 years in infected wheat plots in Canada (Fernando et al. 2000). During June 2007, diurnal dispersal patterns of conidia of *F. mangiferae* correlated with lower levels of relative humidity, in particular, when relative humidity decreased below 55% (Fig. 10). Similarly, Noriega-Cantú et al. (1999) reported that a peak in trapped airborne macroconidia of *Fusarium* spp. was detected in infected mango orchards when relative humidity was low (55%). This is also in agreement with the previously reported pattern of conidial dispersal of *F. guttiforme*, in pineapple, which coincided with relative humidity levels lower than 55% (De Matos et al. 1997). Low relative humidity appears to be a major factor associated with the diurnal conidial dispersal of many airborne pathogens such as powdery mildews in vineyards (Willocquet & Clerjeau 1998), sweet cherry (Grove 1998), apple (Sutton & Jones 1979), and other dry-dispersed fungal pathogens (Fitt et al. 1989).

Three commercial mango plots were surveyed for disease development during 2005. Since two of the plots were heavily pruned in the previous year, it was assumed that primary infections in those plots originated from the adjacent, highly infected, plot which was considered the inoculum source plot. Indeed, a distinct pattern was detected which resembled a classic primary disease gradient where all infections are due to spores originating from an inoculum source (Madden et al. 2007). The disease gradient in both plots (Fig. 5) corresponded well with the disease gradient characteristics described by Madden et al. (2007): “The disease shape is characterized by high disease intensity at and near the inoculum source, and generally declines with increasing distance from the source. The rate of the decline is usually large at small distances from the source, and small at large distances from the source.” Plots 1 and 2 (Fig. 2) were located north-east and east from the inoculum

source plot, respectively, which coincides with the common direction of the wind in this area at the time when relative humidity is low. Conidial dispersal by the wind appeared to cover a distance of up to 35 m. Better understanding of plant pathogen dispersal characteristics may contribute to the ability to forecast disease development from early infections, assist in determining the optimal timing and means of control and improve decision making for an optimal disease management program (Jeger 1999).

4.2 Location of infection sites and colonization pattern

Very little or no information has been published regarding conditions affecting germination and growth of *F. mangiferae*, and regarding the infection patterns of this pathogen. Since conidia of *F. mangiferae* are likely to be the most prevalent propagules resulting in spread of the pathogen, evaluating conditions affecting their germination and growth may contribute to a better understanding of the infection process. Low temperatures (<5°C) did not permit conidial germination and colony growth. Optimal temperatures for growth and germination vary between different *Fusarium* species (Leslie & Summerell 2006, Rossi et al. 2002b), while the optimal temperature for germination and growth of *F. mangiferae* conidia was 28°C and 25°C, respectively. Therefore, this temperature range does not appear to be a limiting factor in seasonal disease development of mango malformation in mango cultivation areas worldwide. In most fungal pathogens, successful infection depends on a minimal duration of wetness (Huber & Gillespie 1992) provided in the form of rain or dew (Carisse et al. 2000, Luo & Michailides 2001, Rotem 1994, Webb & Nutter 1997). A minimum of 2 h of wetness was required before conidial germination, and an optimum of 8 h wetness was recorded. This was similar for macroconidia of *F. graminearum* requiring a minimum of 2-6 h of RH 100% for germ tube emergence (Beyer et al. 2004). Therefore, moisture is also not a restricting factor for infection under field conditions where these requirements are routinely obtained during the rainy season (October through March) or during heavy dew events commonly occurring during the dry season from May through August (Berkowicz et al. 2004, Goldreich 2003), the periods when infection may occur in the orchard.

Location of infection sites is assumed to be the apical buds, but this lacked substantial evidence (Ploetz 2003). Results presented here support this assumption and demonstrate how foliar colonization by the pathogen was successful following inoculation of the buds, but failed when the soil, branches or leaves were inoculated. Data in this thesis support those of Youssef et al. (2007), that infections in young seedlings growing under malformed mature trees were affected via the apical meristem. Since *F. mangiferae* does not form chlamydospores (Leslie & Summerell 2006), inoculum survival declined rapidly in the soil (Youssef et al. 2007), and the pathogen was not detected beyond the root tissue of soil-inoculated-plants, it appears therefore that infections through the roots are not plausible, indicating that buds, predominantly apical buds, are the main sites of infection.

Most of the surface area in mango orchards is occupied by leaves and since conidia disseminate passively in the air and arbitrarily land on trees, it is likely that most of the leaves are covered with conidia. How then, do conidia reach their infection site, the apical buds? One possible way is by landing randomly on apical buds, although, the probability for this event to take place is rather small. Another route is via transport of conidia on the body of *A. mangiferae*, the mango bud mite, from their arbitrary landing sites on leaves into buds, the exclusive habitat of the mite. A third possible route, which is presented in the current study, is via dry malformed inflorescence debris, falling on top of apical buds that form, together with surrounding leaves, a funnel-like structure. Dry-malformed-inflorescence debris falling on top of apical buds is a common phenomenon in mango orchards. When dry debris were placed over apical buds of potted plants and moistened (mimicking rain events), the pathogen was capable of infecting bud tissue, demonstrating that debris containing conidia may constitute a source of inoculum.

In order to determine the seasonal pattern of aerial infections, leaves and branches were sampled monthly from the orchard and monitored for the presence of the pathogen. The incidence of leaf-disks bearing conidia on their surface was the highest during June and July, where 20-25% of the disks bore conidia on their surface. In addition, colonization of the pathogen was detected in apical but not lateral buds of 1-month-old branches, further indicating the mode of aerial infection. Incidence of branches colonized in the apical but not in the lateral buds was highest in May and June, which coincides with a peak of malformed inflorescences and conidial dissemination detected in an infected mango orchard. Another

study of seasonal infection dynamics in mango orchards in Mexico detected three annual peaks of disease incidence, which occurred during and immediately following the flowering season (Noriega-Cantú et al. 1999).

Colonization of the pathogen was observed in node sections of both vegetative and woody branches. Several studies previously reported isolation of the pathogen from malformed tissue, but not from supporting branches (Crookes & Rijkenberg 1985, Ploetz 1994, Ploetz & Gregory 1993), concluding that systemic colonization of mango behind apical portions of the plant might be rare. This conclusion might be deceiving since the pathogen appears to colonize the node sections of the sampled branches, but the internode sections of the branches which do not contain the pathogen, may have been sampled instead. Colonization of the node sections (lateral buds) in branches can also explain the "infrequent infections found in old branches" (Ploetz 2001), which suggests a non-continuous colonization pattern within the tree. Histopathological studies within inoculated apical bud tissues demonstrated random pattern of fungal colonization, not associated with a particular location.

4.3 Role of the bud mite *A. mangiferae* in epidemiology of the disease

Research was conducted to determine the involvement and role of *A. mangiferae* in mango malformation disease, an issue of controversy for many years (Ploetz 2001). Three stages of the disease cycle were studied: reaching the infection site, colonization, and aerial dissemination. For each stage the question whether the mite assists the fungal pathogen was addressed. A gfp transformed isolate of *F. mangiferae* was utilized as a tool which distinguished this work from previous studies. Using this marked strain allowed definite identification of the pathogen, preventing confusion with other natural infections. The gfp-1 isolate was stable and infective, causing typical disease symptoms in inoculated plants.

Eriophyoid mites effectively transmit plant viruses by ingesting the plant pathogen into their gut (Jones et al. 2004, Slykhuis 2006). However, with the *A. mangiferae* and *F. mangiferae* interaction, morphological measurements suggest that conidia are too large to be ingested, thereby allowing only external bearing by the mite. A similar conclusion was reached by Oldfield and Proeseler (1996), who indicated that the minute diameter of

eriophyoid mouthparts may preclude ingestion of larger plant pathogens and that some viruses are too long for the mite to ingest, suggesting that a specific orientation of the virus is required for it to be passed through the oral opening of its vector. Eliminating the possibility of the mite carrying the conidia within its body reinforces the importance of our results demonstrating how *A. mangiferae* can, under laboratory conditions, bear conidia on its body. By using gfp-labeled conidia it was shown unequivocally that *A. mangiferae* can carry *F. mangiferae*, unlike previous studies of *A. mangiferae* sampled from diseased apical buds (Labuschagne et al. 1993, Summanwar & Raychaudhuri 1968) which did not specifically identify the fungus as *F. mangiferae* and could not exclude contamination of mites by unidentified *Fusarium* spp. during transfer from malformed buds to PDA plates and to microscope slides.

While both the fungus and the mite are disseminated aerially and randomly land on the tree, only the mite has the capacity to actively seek out, discover and successfully colonize the apical bud. Therefore, the feasibility of conidial transport on the body of the mite from their arbitrary landing sites on leaves into buds, apparently their exclusive infection sites was studied. As conidia were observed within apical buds only when an adjacent leaf was inoculated with both mites and conidia, and since buds of the other control treatments were devoid of conidia (Table 3), it was concluded that the only way for the conidia to reach the apical bud in these controlled seedling inoculation experiments is via the mite as a vector. These results are in agreement with those of Manicom (1989), who obtained enhanced malformation symptoms on seedlings by attaching malformed buds containing mites and fungus as opposed to a conidial spray alone

After reaching the infection site, conidia penetrate the host. This process takes place within the apical bud — a common habitat for both the mite and the fungus (Abou Awad 1981, Sternlicht & Goldenberg 1976). Microscopic observations of inoculated buds demonstrate the close proximity of these two organisms, sharing the same habitat- the apical bud (Fig. 17). As in previous studies, high frequencies of apical buds populated with *A. mangiferae* were observed throughout the year (Pena et al. 2005, Prasad et al. 1972, Zheng & Ploetz 2002), and higher numbers of mites were found in buds colonized by the fungus compared to non-colonized ones (Labuschagne et al. 1993). This positive correlation

between the two organisms could be explained, among other possible explanations, by either the fungus providing a better habitat for the mite or the mite providing more attractive infection sites for the fungus. Positive interactions between mites and disease are also suggested by a positive correlation between numbers of mites in malformed tissue and severity of disease in some previous studies (Sao Jose et al. 2000), although this correlation is not present in some other studies (Prasad et al. 1972).

One possible mechanism for positive interactions between *A. mangiferae* and *F. mangiferae* is that mites increase in number due to nutrients becoming available following fungal infection; however, the results of previous studies regarding this mechanism have been inconclusive (Labuschagne et al. 1993, Manicom 1989), perhaps because humidity was insufficient to support effective infection by *F. mangiferae* (Nariani & Seth 1962). It was found that in potted plants the presence of *A. mangiferae* inside the buds increased frequency and severity of bud colonization by the pathogen (Fig. 18). However, while mite feeding sites may facilitate fungal germ tube penetration into the bud tissue, they are not necessary for infection, since high frequencies of infected buds resulted from inoculations made in the absence of the mites or artificial wounds.

Since attempts to trap windborne conidia failed (Ploetz 2001, Ploetz 2003), a hypothesis emerged suggesting that *A. mangiferae* may act as a vector for long-range dissemination of fungal conidia (Ploetz 2001). Unlike the methodology in previous studies, a modified technique (washing and plating the Burkard rotary trap band, over selective medium plates) was used to successfully determine the conidial dispersal pattern under field conditions over a 2-year period. Attempts to capture airborne mites in the orchard over a 1-year period were also partially successful, despite the limits imposed by the biology of the mite. Unlike eriophyid vagrant mites that inhabit leaves and disseminate in the air in high numbers (Childers & Achor 1999), airborne population levels of *A. mangiferae* are relatively low as they inhabit closed apical buds and disperse from them when bud break commences, each bud opening independently (Whiley 1993). None of the airborne *A. mangiferae* that were trapped in the orchard and in the growth chamber bore conidia on their bodies. In addition, aerial conidia were abundant in the absence of mites. Thus, it appears that the mites do not contribute substantially to the conidial dissemination process, and that conidia can be

passively dispersed through the air from malformed panicles. The mite and the conidia apparently “meet” after landing on the tree canopy, when the mite starts its journey towards the apical bud.

4.4 Summary

Results obtained from this thesis shed light on the epidemiology of mango malformation disease and may assist in developing an improved control program. Abundant amounts of *F. mangiferae* airborne conidia are dispersed in infected orchards and may serve as a significant source of primary inoculum. Therefore, it may be plausible to direct control efforts towards either reducing inoculum load and/or protecting apical buds from airborne infections, during the dissemination period. Maintaining strict sanitation in the orchard by immediate removal of malformed tissues may serve as an efficient, simple, and available management strategy for mango malformation disease.

The interaction between *A. mangiferae* and *F. mangiferae*, the causal agent of mango malformation disease, has been suggested for many years, but never thoroughly studied. In this research a better understanding of the nature of the interaction and the potential for *A. mangiferae* to enhance disease frequency and severity in the orchard has been provided. It has been demonstrated how *A. mangiferae* can bear fungal conidia on its body, vector it into the apical bud and facilitate fungal colonization.

In summary, a potential cycle for mango malformation disease is proposed (Fig. 20). Malformed inflorescences and malformed vegetative growth serve as a source of inoculum. Inoculum from infected panicles and malformed vegetative tissue disseminate passively in the air as conidia or fall from dry malformed inflorescences as dry debris. Most of the conidia fall on the mango canopy and reach the infection site by at least three different routes: falling by chance on the apical bud; being vectored on the body of the bud mite *A. mangiferae*; and/or, via conidia in dry debris falling into the funnel-like structure of the apical buds. Conidial germination and infection of apical buds may occur if appropriate conditions are met: temperatures between 5°C and 37°C accompanied by at least 2 h of wetness. Moderate temperatures and longer duration of wetness may accelerate the infection process. Presence of *A. mangiferae* inside the buds assists fungal penetration and increases

frequency and severity of infection. After penetration, the pathogen colonizes the bud tissue but does not progress beyond this point. Apical buds could either differentiate into a reproductive inflorescence following appropriate exposure to cold temperatures, or remain vegetative and develop into a young shoot (Nunez-Elisea et al. 1996). Inflorescences from a colonized bud may emerge malformed, probably due to a build-up of the pathogen until an infection threshold is met (Ploetz 2001, Ploetz 2003). Alternatively, when a young shoot emerges from an infected apical bud, the pathogen may colonize the apical and/or lateral buds of the young shoot, remain localized and dormant in buds until bud break. This young shoot may show symptoms of vegetative malformation or bare the pathogen within bud tissue without showing typical disease symptoms.

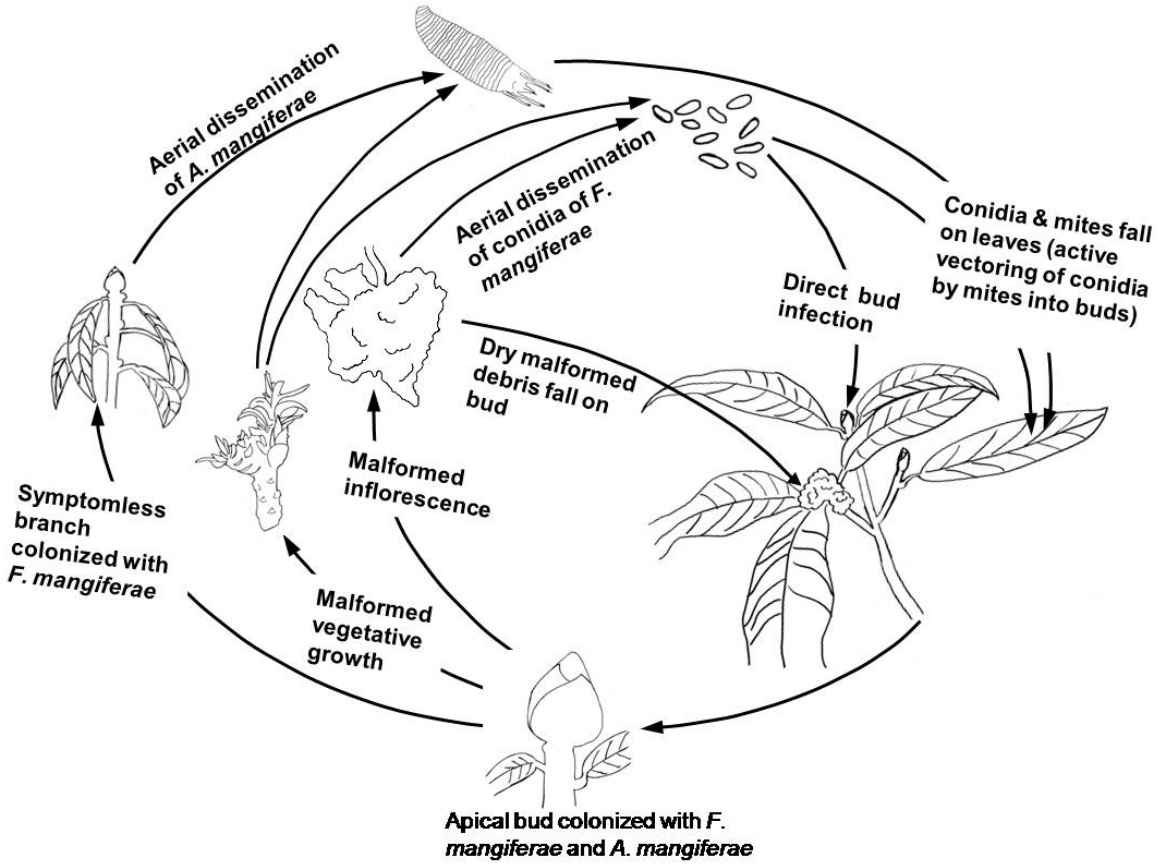

Fig. 20. Proposed cycle of mango malformation disease caused by the pathogen *Fusarium mangiferae*.

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6. APPENDICES

Articles published in the Journal *Phytopathology*

Ecology and Epidemiology

Interaction of the Mite *Aceria mangiferae* with *Fusarium mangiferae*, the Causal Agent of Mango Malformation Disease

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Accepted for publication 1 October 2008.

ABSTRACT

Gamliel-Atinsky, E., Freeman, S., Sztejnberg, A., Maymon, M., Ochoa, R., Belausov, E., and Palevsky, E. 2009. Interaction of the mite *Aceria mangiferae* with *Fusarium mangiferae*, the causal agent of mango malformation disease. Phytopathology 99:152-159.

The role of the mango bud mite, *Aceria mangiferae*, in carrying conidia of *Fusarium mangiferae*, vectoring them into potential infection sites, and assisting fungal infection and dissemination was studied. Following the mite's exposure to a green fluorescent protein-marked isolate, conidia were observed clinging to the mite's body. Agar plugs bearing either bud mites or the pathogen were placed on leaves near the apical buds of potted mango plants. Conidia were found in bud bracts only when both mites and conidia were co-inoculated on the plant, demonstrating that the mite vectored the conidia into the apical bud. Potted mango plants were inoculated with conidia in the presence or absence of mites. Frequency and severity of infected buds were significantly higher in the presence of mites, revealing their significant role in the fungal infection process. Conidia and mite presence were monitored with traps in a diseased orchard over a 2-year period. No windborne bud mites bearing conidia were found; however, high numbers of windborne conidia were detected in the traps. These results suggest that *A. mangiferae* can carry and vector conidia between buds and assist in fungal penetration but does not play a role in the aerial dissemination of conidia between trees.

Additional keyword: Eriophyidae, mite–fungal interactions.

Mango malformation is one of the most destructive diseases of this crop, occurring in most mango-producing regions worldwide (7,17,30,32,33). The disease is characterized by malformation of vegetative growth and inflorescences, causing serious yield loss because malformed panicles do not bear fruit (17,19). *Fusarium mangiferae* Britz, M. J. Wingf. & Marasas, previously known as *F. moniliforme* J. Sheld. and later as *F. moniliforme* J. Sheld. var. *subglutinans* Wollenw. & Reinking, has been identified as the causal agent of mango malformation disease (4,5,12,20,21,26, 31,41).

Little is known about the epidemiology of the disease, dissemination of conidia, location of infection sites, modes of infection, and colonization of plant tissue (30), or whether wounding is an obligatory condition for infection (32). Most of the infection studies were performed by wounding the plant tissue, assuming that a wound is necessary for fungal infection (20,30,32,41). Despite this, two studies reported development of malformation symptoms following inoculations conducted without wounding of the plant tissue (5,12).

The identity of the causal agent has been controversial for many years and other abiotic and biotic factors have been proposed as the primary causal agents of this disease (8,23,24,27,44). The putative role of the mango bud mite *Aceria* (=*Eriophyes*) *mangiferae* Sayed (Eriophyidae) was partly based on the fact that eriophyoid mites are known to cause bud proliferation, "witches broom", and gall symptoms of inflorescences in other plants (42).

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doi:10.1094/PHYTO-99-2-0152

In addition, herbivores may facilitate fungal infection by two main mechanisms: either by vectoring pathogen propagules or by creating wound sites for fungal penetration (3,14), and a number of studies have reported association between herbivorous mites and fungal spores (10,11). For example, the mite *Brevipalpus phoenicis* (Tenuipalpidae) was found in association with the fungal pathogen *Elsinoe fawcettii* Bitancourt & Jenkins, the causal agent of citrus scab on sour orange (*Citrus aurantifolia*) in Honduras (10), but the significance of the mite in the epidemiology of the disease was not investigated. More research is needed in order to determine the role of herbivorous mites and, in particular, the eriophyoid mites as vectors of plant pathogens of a fungal nature.

A. mangiferae, initially described in Egypt (36), is commonly found within closed generative and vegetative mango buds in both malformed and healthy trees (39). These mites disseminate by wind from opening buds, land passively on a random tree, and actively find their way to mango buds. Thereafter, the mite settles and begins feeding by penetrating its stylets into the epidermal cell wall, creating shallow wounds of approximately 2 to 5 μm in depth (16,42). *A. mangiferae* was identified in both healthy and diseased trees and, in the absence of a direct correlation between the mite and mango malformation, it was proposed that mango malformation might result from an interaction between the mite and *F. mangiferae* (34,39). When attempts to trap airborne conidia failed (30), a hypothesis emerged indicating that the bud mite serves as a vector for the fungal conidia (30). Summanwar and Raychaudhuri (40) recovered the pathogen from *A. mangiferae*'s body, when sampled from diseased trees, and other research reported the isolation of *Fusarium* spp. from mites sampled from diseased and apparently healthy apical buds (18). Several studies

Ecology and Epidemiology

Inoculum Availability and Conidial Dispersal Patterns of *Fusarium mangiferae*, the Causal Agent of Mango Malformation Disease

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Accepted for publication 27 September 2008.

ABSTRACT

Gamliel-Atinsky, E., Sztejnberg, A., Maymon, M., Shtienberg, D., and Freeman, S. 2009. Inoculum availability and conidial dispersal patterns of *Fusarium mangiferae*, the causal agent of mango malformation disease. Phytopathology 99:160-166.

Inoculum availability and conidial dispersal patterns of *Fusarium mangiferae*, causal agent of mango malformation disease, were studied during 2006 and 2007 in an experimental orchard. The spatial pattern of primary infections in a heavily infected commercial mango orchard corresponded with a typical dispersal pattern caused by airborne propagules. Malformed inflorescences were first observed in mid-March, gradually increased, reaching a peak in May, and declined to negligible levels in August. The sporulation capacity of the malformed inflorescences was evaluated during three consecutive months. Significantly higher numbers of conidia per gram of malformed inflorescence were detected in May and June than in April. Annual conidial dissemination patterns were evaluated by active and passive trapping of conidia. A peak in trapped airborne conidia was detected in May and June for both years. The daily pattern of conidial dispersal was not associated with a specifically discernable time of day, and an exponential correlation was determined between mean relative humidity (RH) and mean number of trapped conidia. Higher numbers of conidia were trapped when RH values were low (<55%). This is the first detailed report on airborne dispersal of *F. mangiferae*, serving as the primary means of inoculum spread.

Fusarium mangiferae Britz, M. J. Wingf. & Marasas (previously recognized as *F. moniliforme* J. Sheld. and later as *F. moniliforme* J. Sheld. var. *subglutinans* Wollenw. & Reinking), is the causal agent of mango malformation disease (1,2,8,18, 19,21,24,26,32,34). At least three additional taxa have been associated with the disease: *F. sterilihyphosum* Britz, Wingfield, & Marasas from Brazil and South Africa, and *Fusarium* sp. nov. and *F. proliferatum* Samuels, Nirenberg & Seifert (teleomorph: *Gibberella intermedia*) from Malaysia (1,19). Mango malformation is prevalent in most mango-producing areas worldwide (4,14,19,23). Because malformed inflorescences do not bear any fruit, malformation is a major constraint to mango production and high losses in yield are reported due to the disease from various mango-producing countries (14,17,21,23,27,30). Symptoms of this disease are associated with hormonal imbalance in the host that results in tightly bunched, misshapen growth of vegetative leaves, shoots, and productive parts. Both vegetative shoots and axes of inflorescences are shorter, thicker, and highly branched. Inflorescences are compact and larger than normal, possess more male flowers, and hermaphroditic flowers that are formed are either sterile or eventually abort (13,14,17,23,24,28).

The epidemiology of this disease is poorly understood (14,23, 24). The primary mechanism for long-distance dispersal of the pathogen is hypothesized to be via infected nursery stock or by the mango bud mite, *Aceria mangiferae*, vectoring fungal conidia. However, the method of pathogen dispersal within trees or spread from tree to tree in an infected orchard is unknown, although it is well documented that the disease spreads slowly within infected orchards (23,24). Single-celled microconidia are produced in abundance and carried on sympodially branched conidiophores bearing mono- and polyphialides, while macroconidia are usually three to five cells, borne on a sporodochia. No sexual stage is known for this species (1,15). Ploetz (23) indicated that aerial dissemination of conidia appears to be uncommon, based on a report that no conidia were trapped using rotary spore traps placed in an infected orchard (35). However, a study from Mexico reported the trapping of macroconidia in an infected mango orchard using a volumetric spore trap. In that study, identification of the pathogen was done microscopically for the genus level *Fusarium* spp. and only for macroconidia (21). Another study suggested that the conidia are the main source of inoculum, and also that the fungus "being a weak pathogen" invades the host via soft organelles (i.e., vegetative and floral buds, and flowers) (2). A study on the distribution of the pathogen in affected trees in Florida reported the highest colonization incidence in malformed vegetative and floral shoots, decreasing incidence in asymptomatic shoots, and rare colonization in branch tissue, even when the branch was supporting a malformed inflorescence (22). When whole infected seedlings were sectioned, pathogen colonization descended from the top to the lower sections (37). The latter study further demonstrated that survival of conidia declined very rapidly in soil and also that the pathogen was not detected in mango seed, seed coat, or flesh, implying that the pathogen is not seedborne. Ploetz (22) and Youssef (37) indicated that the pathogen is not a typical soilborne pathogen, nor does it move systemically from roots acropetally, and that mango buds are apparently the primary sites for infection. When malformation was managed in commercial orchards in Egypt by removing affected vegetative and floral terminals, the mean disease incidence was lower than in nonmanaged orchards (27). This management practice has also been used in India, Israel, and South Africa (23).

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doi:10.1094/PHYTO-99-2-0160

Ecology and Epidemiology

Infection Dynamics of *Fusarium mangiferae*, Causal Agent of Mango Malformation Disease

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Accepted for publication 9 February 2009.

ABSTRACT

Gamliel-Atinsky, E., Sztejnberg, A., Maymon, M., Vintal, H., Shtienberg, D., and Freeman, S. 2009. Infection dynamics of *Fusarium mangiferae*, causal agent of mango malformation disease. Phytopathology 99:775-781.

Conditions affecting germination and growth of *Fusarium mangiferae*, causal agent of mango malformation disease, were studied in vitro. Both conidial germination and colony growth required temperatures >5°C and reached a peak at 28 and 25°C, respectively. A minimum 2-h wetness period was required for conidial germination, reaching a peak after 8 h of wetness. High incidence of fungal colonization in buds, predominantly the apical buds, was detected compared with inoculated leaves. The pathogen was detected in the roots of inoculated soil 19 weeks postinoculation but not in aboveground parts of the plants, and symptoms of the disease were not observed, either. Dry, malformed inflorescence debris serving as a source of inoculum caused significantly higher colonization (52 and 20%) of inoculated buds, compared with that (0%) of the untreated controls. Incidence of sampled leaf disks bearing propagules of *F. mangiferae* from an infected orchard peaked in June and July and decreased during the following months, whereas airborne infections on 1-month-old branches was the highest in May and June, corresponding with inoculum availability released from infected inflorescences. Colonization pattern, determined in naturally infected vegetative and woody branches, was significantly higher in node sections than in the internode sections. This study sheds light on infection dynamics, colonization pattern, and the disease cycle of *F. mangiferae* in mango.

Additional keywords: *Aceria mangiferae*.

Mango malformation is a severe disease of the crop which is widely spread in almost all mango-growing regions worldwide (6,13,14,25,28). Symptoms of disease include misshapen growth of both vegetative and productive parts of the tree (12,13,18, 25,26,29). Vegetative malformation includes hypertrophy of young shoots, shorter internodes, dwarfed malformed leaves, and an overall tightly bunched appearance of infected shoots. Inflorescence malformation includes short, thick, and branched axes of the inflorescence, larger flowers, and increased number of male flowers. Malformed inflorescences do not bear any fruit, hence the great yield losses caused by this disease (13,18,22,25,28,33).

Several causal agents, all members of the genus *Fusarium*, have been associated with this disease. *Fusarium mangiferae* Britz, M. J. Wingf. & Marasas (previously recognized as *F. moniliforme* J. Sheld. and later as *F. moniliforme* var. *subglutinans* Wollenw. & Reinking) has the largest geographic distribution (16). Koch postulates were completed with this species for the first time in 1966 (3,5,7,14,19,20,22,26,27,35,36), and also recently with *F. sterilihyphosum* Britz, Marasas & M. J. Wingf. (16), which is distributed in Brazil and South Africa (3,20). Likewise, pathogenicity tests were also conducted with a new phylogenetic lineage that is closely related to *F. sterilihyphosum* and has been isolated to date only from Brazil (16). Another recent study from Mexico reported the successful completion of Koch postulates with local strains of *Fusarium* spp., which were different from *F. mangiferae* and *F. oxysporum*; however, further work is required to compare this species to *F. sterilihyphosum* (30). Pathogenicity tests were not conducted for two other taxa, *Fusarium* sp. nov. and *F. proliferatum* Samuels, Nirenberg & Seifert (teleomorph: *Gibberella intermedia*), reported to affect mango in Malaysia (3,20).

Epidemiology of mango malformation is not well understood (13,25,26). A recent study on dispersal patterns of conidia of *F. mangiferae* suggests aerial dispersal as the primary mechanism for fungal dissemination (9) but further information regarding location of infection sites, infection processes, and colonization within the tree is still unknown. A previous study reported that the presence of *Aceria mangiferae*, the mango bud mite, within buds increased frequency and severity of bud colonization by the pathogen, indicating that the bud mites may enhance fungal infection (8). Because the pathogen was detected in malformed panicles and vegetative shoots but rarely detected in branches (24), it was postulated that vegetative and floral buds are probably the primary sites for infection (26); however, this needs further support. Root infection was reported to cause symptoms at the root collar and the canopy but, because those studies lacked appropriate controls, the results are questionable (25). Recently, Youssef et al. (38) reported that inoculum can survive in soil although survival of bare conidia declined very rapidly under summer conditions. Furthermore, naturally infected panicles that were buried 30 cm under the soil surface resulted in a decline of 80% in inoculum survival after 24 weeks (38), further indicating that the pathogen is not a typical soilborne fungus.

Within-tree spread of the pathogen is poorly understood (25). The pathogen was isolated frequently from malformed tissue but infrequently from supporting branches (6,24,27); therefore, it was concluded that systemic colonization of mango behind the apical parts of the plant might be rare. Ploetz (25) suggested that the infrequent infections found in old branches could be remnant

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doi:10.1094/PHYTO-99-6-0775

אפיון מהלך מחלת עיוות התפרחות והצימוח במנגו ויחסי הגומלין בין הפתוגן *Fusarium mangiferae* לאקריית הפקע של המנגו *Aceria mangiferae*

חיבור לשם קבלת תואר דוקטור לפילוסופיה

מאת אפרת גמליאל-אטינסקי

הוגש לסינט האוניברסיטה העברית בירושלים,

ינואר 2009

תקציר

מחלת עיוות התפרחות והצימוח במנגו קיימת ברוב אזורי הגידול בעולם ונחשבת לאחת המחלות הקשות ביותר בענף. המחלה מאופיינת בצימוח וגטטיבי מעוות ובתפרחות מעוותות שאינן מניבות פרי, מצב הגורם לאבדן יבול גבוה. הפטרייה *Fusarium mangiferae* Britz, Wingfield & Marasas שכונתה בעבר *F. moniliforme* Sheldon וגם- *F. moniliforme* Sheldon var. *subglutinans* Wollenweber & Reinking זוהתה כמחוללת המחלה בארץ.

זמינות המידבק ותבנית הפצת הנבגים של *Fusarium mangiferae*, נחקרו במהלך השנים 2006 ו-2007 במטע ניסיוני במכון וולקני. תבנית ההפצה המרחבית במטע מנגו מסחרי נמצאה דומה לתבנית טיפוסית של הפצת נבגים באוויר. תפרחות מעוותות נצפו לראשונה באמצע חודש מרץ, כמות התפרחות המעוותות עלתה בהדרגה עד לשיא במאי וירדה עד לרמות זניחות בחודש אוגוסט. כמות המידבק בתפרחות מעוותות נבדקה במהלך שלושה חודשים עוקבים. בחודשים מאי ויוני נמצאו יותר נבגים/גר׳ תפרחת מעוותת במובהק ($P \leq 0.0003$) מאשר באפריל, במהלך שתי שנות הדגימה. תבנית הפצת הנבגים השנתית נבחנה בעזרת שתי שיטות לכידת נבגים. שיא בלכידות נבגים נמצא במטע וולקני בחודשים מאי ויוני בשתי שיטות הלכידה. תבנית ההפצה היומית לא נמצאה קשורה לשעה מסויימת ביום ונמצא קשר אקספוננציאלי בין לחות יחסית ממוצעת (RH) ומספר הנבגים שנלכדו. כמות גבוהה יותר של נבגים נלכדו בתנאי לחות יחסית נמוכים מ- 55%.

התנאים המשפיעים על נביטת הנבגים וצימוח התפטיר נחקרו in vitro. הן נביטת הנבגים והן צימוח התפטיר התרחשו בטמפרטורות גבוהות מ- 5 מ״צ, והגיעו לשיא ב- 28 מ״צ ו- 25 מ״צ, בהתאמה. תהליך נביטת הנבגים החל לאחר מינימום של שתי שעות הרטבה והגיע לשיאו לאחר שמונה שעות הרטבה. על מנת לקבוע את זהות אתר ההדבקה בוצעו ניסויי אילוח של אברי צמח שונים בצמחי עציץ. הפקעים, ובייחוד הפקעים האמיריים נמצאו מאוכלסים עם הפתוגן בשכיחות הגבוהה ביותר. כאשר אולח מצע הגידול, נמצא הפתוגן לאחר 19 שבועות בריקמת השורשים אך לא בחלקים העל-קרקעיים של הצמח ולא ניצפו תסמיני מחלה בצמחים. על מנת לבחון אם חלקי תפרחת יבשים ומעוותים עשויים לשמש כמקור מידבק, הונחו חלקי התפרחת על גבי פקעים אמיריים ונמצא איכלוס גבוה במובהק בפקעים מאולחים מאשר בפקעים לא מאולחים. שטח הפנים של עלי מנגו ממטע וולקני נבחן במהלך השנה לנוכחות *F. mangiferae*. שכיחות דיסקיות העלים מעצים נגועים הנושאות נבגים על פניהן הגיעה לשיא בחודשים יוני ויולי וירדה בהדרגה במהלך החודשים העוקבים. מועדי ההדבקות נקבעו בסקר תלת-שנתי במטע וולקני בענפים צעירים בני כחודש. הרמה הגבוהה ביותר של ענפים צעירים נגועים שהודבקו מהאוויר התגלתה בחודשים מאי ויוני. האיכלוס בריקמת העץ, הן בענפים צעירים והן בענפים מעוצים התרכז באזורי הפקעים ולא בינהם, והפטרייה לא נמצאה בצורה רציפה ברקמות הצמחיות. על מנת

לקבוע את המיקום המדויק של הפתוגן בתוך ריקמת פקעי מנגו, נחתכו פקעי מנגו לפרוסות דקות ונילקחו להסתכלות מיקרוסקופית. האיכלוס בפתוגן נראה בריקמת הטריכומות, המריסטמה האמירית ובריקמת חפי הפקע ללא אסוציאציה למיקום מסויים בתוך ריקמת הפקע.

הקשר בין אקרית הפקע, *Aceria mangiferae*, לפתוגן *F. mangiferae* נחקר בהיבטים הבאים: נשיאת נבגי הפתוגן, הסעתם לאתרי החדירה ועידוד תהליך ההדבקה. לאחר חשיפת אקריות לנבגי פטרייה מסומנים בחלבון פלואורוסנטי ירוק, נראו נבגים צמודים ונישאים על גבי גוף האקרית. קוביות אגר הנושאות את האקרית ו/או נבגי הפתוגן בשילובי טיפולים שונים הונחו על גבי עלי מנגו בצמחים שגודלו בעציצים. נבגי הפתוגן נמצאו בתוך חפי פקעים רק כאשר הצמחים אולחו הן עם הפתוגן והן עם האקריות, ממצא המצביע על יכולת האקרית להוביל את הנבגים אל תוך פקעי המנגו. צמחי מנגו בעציצים אולחו עם נבגי הפטרייה בנוכחות או בהיעדר אקריות. הן שכיחות הפקעים המודבקים והן חומרת ההדבקה היו גבוהים במובהק בנוכחות אקריות, עובדה החושפת את התפקיד החשוב של אקריות הפקע בעידוד תהליך ההדבקה. נוכחות אקריות הפקע באוויר נאמדה במהלך שנת לכידה באמצעות שתי מלכודות אקריות, האחת הוצבה במטע וולקני והשניה בחדר גידול. לא נמצאו אקריות פקע הנושאות נבגים על גופן, זאת בניגוד לכמויות גדולות של נבגים שנלכדו במלכודות הנבגים. תוצאות אלו מצביעות על תפקיד אקרית הפקע של המנגו בנשיאה והסעת נבגי הפתוגן אל עבר אתרי החדירה, ועידוד תהליך ההדבקה. לאקרית הפקע אין ככל הנראה תפקיד בהפצת הנבגים באוויר.

זהו הדיווח המפורט הראשון אודות הפצת נבגי *F. mangiferae* באוויר ותפקידם כמקור המידבק העיקרי במטע, אודות התפתחות המחלה בעצי מנגו נגועים ואודות יחסי הגומלין בין אקרית הפקע של המנגו לפתוגן מחולל המחלה. לסיכום, מוצע מעגל מחלה חדשני ושונה ממעגל המחלה המקובל. תפרחות מעוותות וצימוח וגטטיבי מעוות משמשים כמקור המידבק. המידבק נפוץ באוויר בצורה פסיבית כנבגים או נופל מתפרחות מעוותות בחלקיקים יבשים. רוב הנבגים נופלים על עלוות עצי המנגו ומגיעים אל תוך אתר החדירה לפחות בשלושה מסלולים אפשריים: בנפילה אקראית על פקעים אמיריים, בהסעה על גוף אקרית הפקע, ובתוך חלקי תפרחת מעוותת יבשים שנופלים על הפקע האמירי שמצוי במרכז מבנה שנראה כמשפך ניקוז. הדבקת הפקעים האמיריים מתרחשת רק בהימצאות התנאים הבאים: טמפרטורות בין 5 מ״צ ו- 37 מ״צ שמלוות בלפחות שעתיים של רטיבות. טמפרטורות נוחות ותקופת רטיבות ארוכה יחסית יאיצו את תהליך ההדבקה. לאחר החדירה מאכלס הפתוגן את רקמות הפקעים אך לא מתקדם מעבר להם. פקעים אמיריים יתמיינו לתפרחות או ישארו וגטטיביים ויתמיינו לענף צמיחה. תפרחות שמקורן בפקע המאוכלס עם הפתוגן עלולות לצמוח מעוותות, כנראה עקב הצטברות של הפתוגן בריקמה. לחילופין, כאשר ענף צעיר צומח מפקע נגוע, הפתוגן עלול לאכלס את הפקעים הצידיים ואת הפקע האמירי בענף ולהשאר מקומי ורדום עד לגל הצימוח הבא.