Mango Malformation Disease (MMD) and Witches Broom (WB); Comparison of Etiology, Biology, Symptomology and Management

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Executive summary

Mango malformation disease (MMD) is caused by species of the fungal pathogen *Fusarium*. Airborne conidia of the pathogen are the infection structures by which the pathogen causes disease. Conidia penetrate the plant tissue via apical and lateral buds and remain dormant until bud break. No systemic infection takes place, only local colonization of the bud tissues. When infected buds break, malformed vegetative and inflorescences are produced. A strategy was developed for management of MMD by elimination of the major inocula (conidia) sources of infection, i.e. malformed panicles, by pruning and removal of the diseased tissues. Thereafter, subsequent fungicidal sprays are applied to protect and cure affected buds from infection via airborne conidia.

Although the mango bud mite, *Aceria mangiferae*, has been suspected as a causal agent of MMD, different symptoms are caused by this pest in mango, termed "witches broom". However, exacerbation of MMD symptoms may occur following wounding of bud tissues by the bud mite, allowing penetration of the fungus at these locations.

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Mango malformation disease (MMD) caused by fungal pathogens belonging to *Fusarium* species

Introduction: Mango malformation disease (MMD).

Mango (*Mangifera indica* L.), which is considered the 'king of fruits' in India, has been cultivated for at least 4,000 years and possesses significant religious and cultural importance (Popenoe, 1932; Purseglove, 1972). Mango is an important commercial crop (Purseglove, 1972) that currently ranks fifth among the major fruits cultivated worldwide (FAOSTAT, 2023). The crop has thrived during recent years due to: (i) cultivation of high-quality clonal selections; (ii) rapid expansion into growing areas of China and parts of Africa; (iii) adoption of modern agricultural practices, including irrigation management, integrated disease and pest management, and the use of pesticides and other agrochemicals (Litz, 2009). Mango is grown commercially throughout the tropics and in many subtropical areas (Mukerjee and Litz, 2009). The flowering response of mango differs greatly in tropical as opposed to subtropical environments. In the tropics, flowering can be induced chemically while in the subtropics stimulation is unnecessary and is primarily dependent on chilling temperatures (Iyer and Schnell, 2009). Synchronization of vegetative growth to ensure that branch terminals are of the same physiological maturity is a prerequisite in flowering management programs (Davenport, 2003). Flowering in the tropics occurs once a year after tip pruning and chemical treatments, while under subtropical conditions flowering occurs in the spring after 5 weeks of exposure to day/night temperatures below 20°C/15°C, respectively (Davenport, 2003).

Mango malformation disease (MMD) is one of the most destructive diseases of this crop (Kumar et al. 1993; Ploetz, 2001). It affects both floral and vegetative structures of the plant. Although trees are not killed, effect of the disease on vegetative stages of the crop impedes canopy development, disrupts development of the inflorescence phase, and thus reduces fruit yield dramatically. Therefore, significant economic losses occur as malformed inflorescences do not bear fruit (Fig. 1).

Fig. 1. Mango trees severely infected with malformation disease.

In 1998, an estimated US$15 million of fruit were lost to the disease in Egypt (Ploetz, 2001; Ploetz et al. 2002), and losses in more important producing countries, e.g., India, would be significantly greater (Ploetz, 2001; Ploetz and Freeman, 2009). MMD was first recorded in India in 1891 (Kumar and Beniwal, 1991), and has subsequently been observed in Australia, Brazil, China, Egypt, El Salvador, India, Israel, Malaysia, Mexico, Myanmar, Nicaragua, Oman, Pakistan, Senegal, South Africa, Spain, Sri Lanka, Sudan, Swaziland, Uganda, the USA and elsewhere (Anonymous, 2013; Bastawros, 1996; Crespo et al. 2012; Crookes and Rijkenberg, 1985; Freeman et al. 1999; Freeman et al. 2014b; Goldman et al. 1976; Kumar and Beniwal, 1991; Kvas et al. 2007; Lim and Khoo, 1985; Lima et al. 2008; Marasas et al. 2006; Nor et al. 2013; Otero-Colina et al. 2010; Rodríguez-Alvarado et al. 2013; Senghor at al. 2012; Sinniah et al. 2013; Zhan et al. 2010). Since the pathogen is easily disseminated in infected budwood and exact knowledge of its geographic distribution is lacking, malformation may be even more widely distributed (Ploetz, 2001).

Disease symptoms.

MMD affects vegetative and floral meristematic tissues of the plant (Chakrabarti, 2001; Ploetz, 2001).

(i) Vegetative malformation.

Vegetative malformation seriously affects seedlings and young trees in nurseries, especially where seedlings are cultivated beneath the canopies of infected trees (Ploetz et al. 2002; Youssef et al. 2007), but also occurs on mature trees. Symptoms of vegetative malformation include hypertrophied, tightly bunched young shoots, with swollen apical and lateral buds (Fig. 2). These buds produce misshapen terminals with shortened internodes and dwarfed leaves that curve from the tip back towards the adaxial portion of the petiole (Figs. 2A and B). Shoot growth is arrested and shoots arising from the same bud produce the symptom of disease termed "bunchy-top" (Fig. 2C).

Fig. 2. Symptoms of vegetative malformation include hypertrophied, tightly bunched young shoots, with swollen apical and lateral buds (A); misshapen terminals with shortened internodes and dwarfed leaves, left and healthy shoots right (B); arrested growth from distorted stems from the same bud producing "bunchy-top" symptoms of the disease (C).

(ii) Floral malformation.

Floral malformation is most important economically since affected inflorescences do not set fruit (Kumar et al. 1993; Noriega-Cantú et al. 1999; Ploetz, 2001; Ploetz and Freeman, 2009; Youssef et al. 2007). Symptoms of floral malformation include primary or secondary axes on affected panicles that are shortened, thickened, and highly branched, eventually resembling a cauliflower when mature that may persist in the tree as a dry, black mass (Fig. 3). Malformed panicles produce up to three times the normal number of flowers, which range from one-half to two times the normal size and have an increased proportion of male to perfect flowers that are either sterile or eventually abort. Malformed panicles may also produce dwarfed and distorted leaves (phyllody). There are various types of malformed inflorescences (Fig. 3) comprising (i) a compact form containing a thick peduncle that remains green and fleshy; (ii) a loose form whereby the panicle is open in shape and larger than healthy inflorescences in size while the peduncle and main secondary branches are thick resembling that of a "witches' broom"; (iii) combined vegetative and floral malformation; and (iv) an intermediate form that includes various malformed shapes with varying degrees of compact and loose forms.

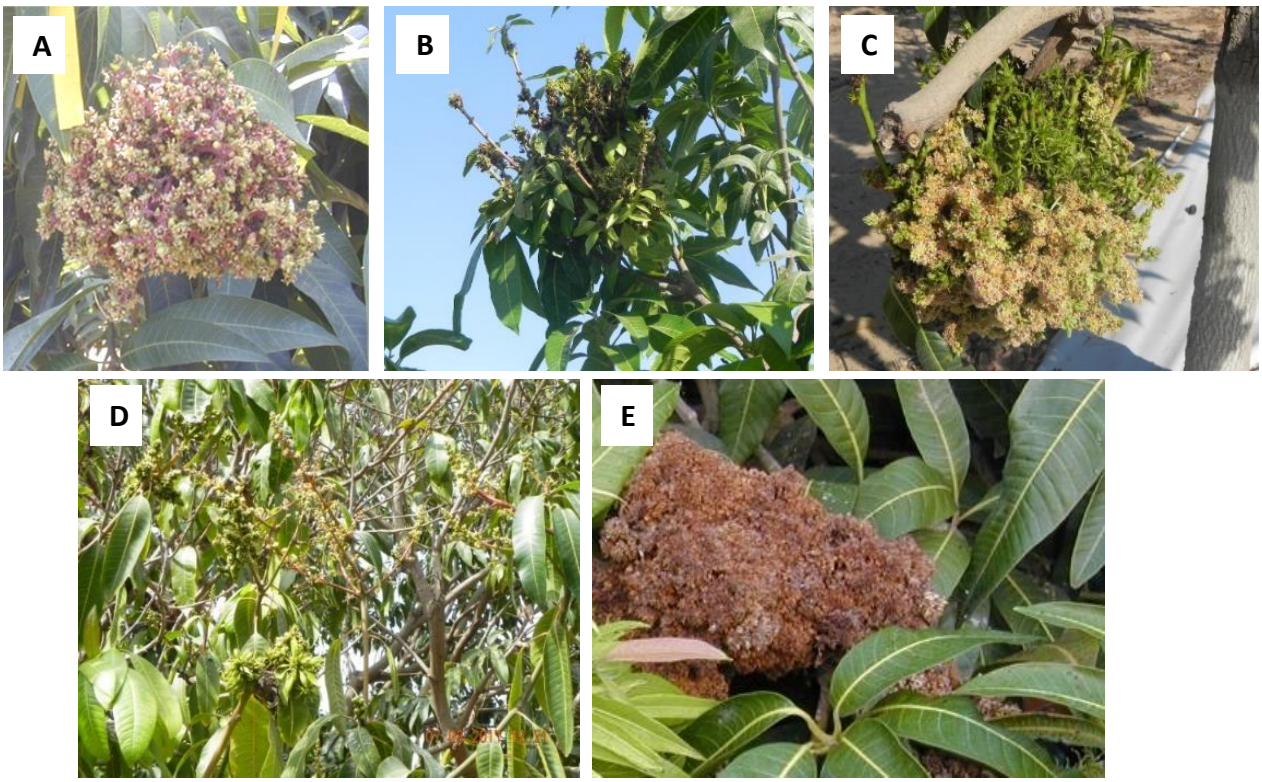

Fig. 3. Symptoms of malformed inflorescences include a compact form with thick, green/red fleshy panicles that resemble cauliflowers (A); a loose form with an open larger than normal inflorescences, but with thick secondary branches resembling a "witches broom" (B); a combination of various compact and loose forms of vegetative and floral symptoms (C and D); black masses of malformed panicles that can persist on the tree (E) (Gamliel-Atinsky at al. 2009c).

MMD Etiology.

Initially, Summanwar et al. (1996) indicated that a fungus in the *Gibberella fujikuroi* (Sawada) Wollenweb. species complex, identified originally as *Fusarium moniliforme* Sheld., was responsible for malformation of inflorescences. Varma et al. (1974) also demonstrated that *F. moniliforme* caused vegetative disease symptoms. Originally, *Fusarium subglutinans* (Wollenweb. and Reinking) Nelson, Toussoun and Marasas, was the official nameof the causal agent of disease, however, in recent years the pathogen has been renamed and is now termed *Fusarium mangiferae* Britz, Wingfield and Marasas, identified from Egypt, Florida (USA), Israel, Malaysia, and South Africa (Britz et al. 2002; Marasas et al. 2006; Ploetz et al. 2002; Steenkamp et al. 2000). *Fusarium mangiferae* was subsequently identified in India (O'Donnell et al. 1998; Zheng and Ploetz, 2002), Oman (Kvas et al. 2007), Spain (Crespo et al. 2012), Sri Lanka (Sinniah et al. 2013), China (Zhan et al. 2012) and elsewhere, and appears to be the most common causal agent of MMD worldwide (Fig. 4).

A second MMD causal agent, *F. sterilihyphosum* Britz, Wingfield and Marasas, was described originally for isolates from a limited area in South Africa (Britz et al. 2002). It was subsequently detected in Brazil (Lima et al. 2009), where it was shown to cause malformation after artificial inoculation (Lima et al. 2008).

Another MMD causal agent, *F. mexicanum* sp. nov., was described from Mexico (Otero-Colina et al. 2010). *F. mexicanum* was shown to differ significantly from other MMD taxa in the *G. fujikuroi* species complex according to multi-locus sequencing, (Otero-Colina et al. 2010; Rodríguez-Alvarado et al. 2007) (Fig. 4).

A fourth recently characterized species, *F. tupiense* sp. nov., resembles *F. sterilihyphosum* morphologically, is phylogenetically distinct from both *F. mangiferae* and *F. sterilihyphosum*, and produces a unique teleomorph in the *G. fujikuroi* complex. *F. tupiense* causes malformation in Brazil (Lima et al. 2012), Senegal (Senghor et al. 2012), and Spain (Crespo et al., 2016) (Fig. 4).

Additional species, *F. proliferatum* (Matsushima) Nirenberg (Lv et al. 2013; Zhan et al. 2010), *F. pseudocircinatum* (Freeman et al. 2014c), *F. neocosmosporiellum* (Molina-Cárdenas et al. 2021) and *F. decemcellulare* (Garcia-Lopeza et al. 2023) have also been reported as causal agents of MMD (Fig. 4). Although *F. mangiferae* predominates as the main MMD pathogen in the Eastern Hemisphere (old world), it has only been confirmed as a pathogen causing MMD in the Western Hemisphere (new world), in Florida (Britz et al. 2002; Zheng and Ploetz, 2002). Therefore, it is likely that *Fusarium* species reported from the new world may have adapted to mango, originating from alternative hosts (Otero-Colina et al. 2010).

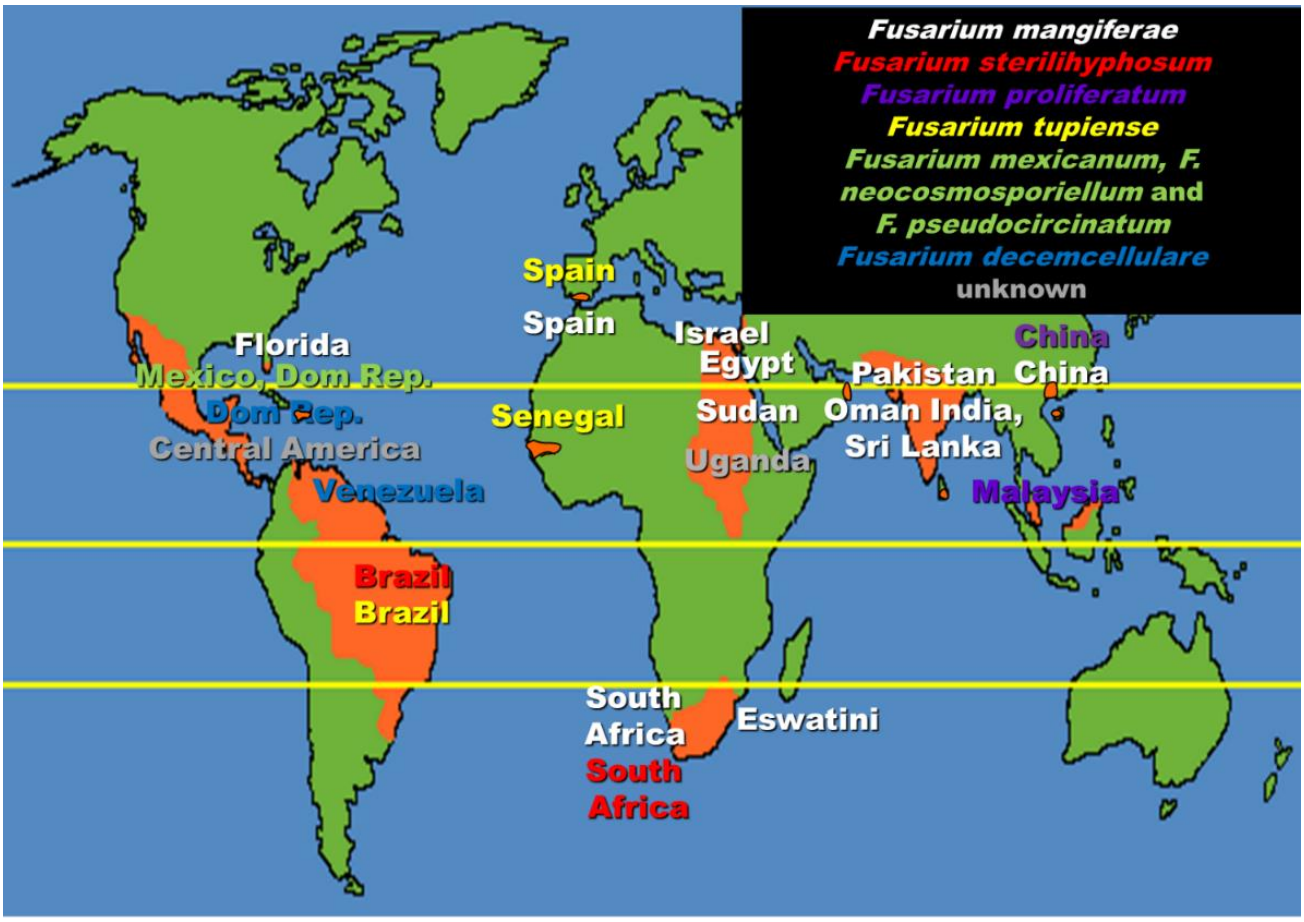

Fig. 4. Worldwide distribution of MMD *Fusarium*-related causal agent pathogens according to species, designated by color. Orange color indicates locations of MMD according to countries worldwide.

Identification of the MMD causal agents of *Fusarium* by molecular methods.

PCR primer pairs, used to amplify a specific DNA band for identification of a certain pathogen, have been used to diagnose some of the above taxa of *Fusaria*, causal agents of MMD. Zheng and Ploetz (2002) developed a primer pair, 1-3F/R, that amplifies a 608 bp DNA fragment for *F. mangiferae*, used for reliable diagnostic purposes of the pathogen (Youssef et al. 2007). Another pair, 61-2F/R, developed to diagnose *F. verticillioides* (published as *F. moniliforme*, in Müller et al. 1999), failed to amplify *F. mangiferae* DNA. However, when amplification protocols were modified, the primer pair amplified a 445 bp-fragment for strains of *F. sterilihyphosum* and *F. mexicanum* (Rodríguez-Alvarado et al. 2007). Additional primer pairs have been developed for *F. mangiferae* (Newman et al. 2012). In addition, species specific primers were developed for the identification of the MMD pathogen, *F. tupiense* (Lima et al. 2012)

Susceptibility of different mango cultivars to malformation.

Wide ranges in susceptibility/tolerance/resistance to MMD have been reported for different mango cultivars (Kumar and Beniwal, 1991). However, there are inconsistencies among these reports, and

none are based on experimental evidence, only on surveys (Ploetz, 2001). For example, the Egyptian cultivar ‘Eweis’ was reported to be moderately susceptible to MMD (Bastawros, 1996), but of low and high susceptibility in two additional references, respectively (Chakrabarti, 2011; Ploetz and Freeman, 2009). Likewise, ‘Kent’ and ‘Keitt’ cultivars, were reported to be immune to disease in Egypt (Bastawros, 1996), even though they were reported to be susceptible to natural infections and artificial inoculations in Israel (Freeman et al. 1999; Freeman et al. 2014b). These and other similar observations were recorded in naturally affected germplasm collections or commercial production orchards, in which no uniform infections were verified. Cultivars listed as “resistant” may have "escaped" infection when exposed to inoculum in naturally infected fields or were established from pathogen-free nursery stock (Ploetz, 2001). To date, experimental evidence for the resistance or susceptibility of commercial cultivars to MMD has not been scientifically confirmed or officially reported.

Previous approaches for managing MMD.

Diverse methods have been evaluated for the management of MMD, including the use of sanitation and application of fungicides and other chemicals. However, none of the above have provided adequate control of the disease.

Cultural control.

Sanitation including pruning of symptomatic tissues, removal and eradication of diseased material from affected trees, are the most common approaches for MMD management, once the disease is established in an orchard (Lahav et al. 2001; Manicom, 1989; Narasimhan, 1959; Noriega-Cantú et al. 1999; Singh et al. 1974). Sanitation, by removal of symptomatic panicles and pruning of the subtending three nodes has been recommended (Manicom, 1989), as it is assumed that this measure reduces MMD, presumably by reducing available inoculum in an orchard. Sanitation and removal of infected panicles from large trees may be difficult to implement as the affected plant material is not always easily accessible, and growers may be unwilling to devote the effort that is time consuming, laborious, and expensive. Regardless, sanitation is considered as an important and critical component of any integrated control strategy for MMD management.

Chemical control.

There have been many reports over the years involving fungicides and other chemicals for the management of MMD. In general, conflicting reports exist on the efficacy of fungicides for MMD

control. For example, benzimidazoles have been tested frequently against MMD, but their impact has been questionable, even when positive results have been reported. In addition, a single spray of Topsin-M applied at the bud differentiation stage reduced malformation to 19.8%, relative to 35.4% in the nontreated control, but data in this experiment were not evaluated statistically (Muhammad et al. 1999). In contrast, Ibrahim et al. (1975) indicated that benomyl inhibited the pathogen in Petri plates *in vitro*, but did not affect MMD development when sprayed on mango trees. Similarly, no effect of benomyl on MMD was observed in the field as reported by Chada et al. (1979) and Diekman et al. (1982). Furthermore, percent of malformed inflorescences was reduced significantly from 96% to 48% by injecting 'Keitt' tree trunks with fosetyl-Al, which is used primarily against diseases caused by oomycetes, however, no increase in fruit yield was reported (Darvas, 1987). Moderate control of MMD was reported with sulfates of cobalt (Co), cadmium (Cd) and nickel (Ni) in India, however, these compounds are not safe when applied to an edible crop for human consumption (Chen et al. 2023; Gupta et al. 2021; Singh et al. 1994; Ssempijja et al. 2020).

Combinations of methods for MMD management have also been tested (Covarrubias, 1980; Pinkas and Gazit, 1992). Iqbal et al. (2011) reported that pruning the terminal 45 cm of affected shoots in combination with a benomyl treatment resulted in a 70.4% decrease in malformation incidence compared to the nontreated control, although statistical significance was not demonstrated. In addition, Noriega-Cantú et al. (1999) reported that mango yields were significantly increased by an integrated management program that included sanitation by removal of the terminal 80 cm of symptomatic shoots, sprays of different fungicides and five applications of a sulfur acaricide.

The efficacy of fungicides for MMD management should be integrated with other alternatives, as they are potential management tools. Effective fungicides would need to be identified, as well as potential application intervals, to optimize their use. With most studies in which fungicides were used against MMD, spray applications were applied soon before or during bloom until fruit set, but not thereafter (Iqbal et al. 2011; Noriega-Cantú et al. 1999). Although the underlying assumption on which this application window was based and the rationale for the timing of spray treatments was not mentioned in these studies, two possibilities exist. It was hypothesized that fungicides would protect uninfected buds from inocula that are dispersed via malformed panicles that remain on trees. Alternatively, already affected buds may be "cured" by the application of fungicides, on condition that internal elimination of the pathogen would thus rely on thoroughly effective and systemic fungicides. In general, specific fungicides have not been used routinely to manage MMD worldwide. For example, in Israel no fungicide was registered for MMD control in mango until 2013, even though the disease was prevalent in the country since 1975 (Goldman et al. 1976). Since then, MMD has

prevented the establishment of new mango plantings in many affected areas and caused the removal of heavily infected orchards, especially in the Southern and Central regions of Israel (Freeman et al. *personal communication*).

New insights into MMD epidemiology and disease management.

(i) Inoculum availability, dissemination, and infection sites by the pathogen.

To date, significant progress has been made in discerning the etiology of MMD. However, advancement in the understanding of epidemiology of this disease is limited (Gamliel-Atinsky et al. 2009b; Kumar et al. 1993; Ploetz, 2001; Ploetz and Freeman, 2009). Studies that appear below refer to epidemiology of MMD caused by *Fusarium mangiferae*.

Malformed and healthy inflorescences develop simultaneously in infected orchards with an overlap of the formation of both types of inflorescences, the timing being dependent on cultivar type (Gamliel et al. 2009b). Microconidia are most likely the prevalent infective propagules of *F. mangiferae*. Numbers of conidia recovered from the surface of malformed panicles increased exponentially from bud break to panicle maturation, while only low numbers of conidia were trapped in affected orchards when panicles were young (Gamliel et al. 2009b). However, numbers increased significantly as panicles matured to the "cauliflower stage" of infection (Gamliel et al. 2009b). Conidia are the primary source of inocula produced by the fungus, forming profusely on live and dead malformed tissues (macroconidia are formed less commonly), while chlamydospores and ascospores are absent for this *Fusarium* fungal species (Leslie and Summerell, 2006). *F. mangiferae* conidial mortality of 100% was recorded with exposure of 3 min to UV radiation, and 2 to 4 hours exposure to direct sunlight; this may explain the slow rate of spread of MMD in orchards due to a high mortality rate of inocula (Freeman, unpublished; Klein-Gueta et al. 2004; Manicom, 1989).

The location of *F. mangiferae* in affected trees indicates that apical buds are the primary sites of infection, and that systemic colonization of branch tissues does not occur (Gamliel et al. 2009b; Gamliel et al. 2009c; Ploetz, 1994). As shown in Florida, *F. mangiferae* was restricted almost entirely to malformed floral and vegetative tissues (Ploetz, 1994). Infection was highest in malformed flowers and vegetative shoots, lower or nonexistent in asymptomatic tissues, and rare in branches even when they supported malformed panicles or shoots. Residual infections of *F. mangiferae* in scaffold branches and trunks were restricted exclusively to dormant lateral buds (Lahav et al. 2001; Youssef et al. 2007). It was confirmed that apical buds of mango were primary infection sites following isolation of the pathogen after artificial inoculations with green fluorescent-labelled (gfp)-labelled isolates of *F. mangiferae* and those transformed with the β-glucuronidase (GUS) reporter gene

(Cohen et al. 2017; Freeman et al. 1999). Injury of plant bud tissues may provide entry points for the pathogen; however, wounding is not a prerequisite for infection (Freeman et al. 1999).

It was shown that survival of conidia declined rapidly in soil during the summer months, under elevated soil temperatures, however, the pathogen may survive for longer periods in soil when protected within infected plant tissues (Youssef et al. 2007). For example, when naturally infected panicles were buried at a depth of 30 cm below the soil surface, survival was reduced to approximately 20%, after 6 months exposure (Gamliel-Atinsky et al. 2009c; Youssef et al. 2007). Some studies have reported that infection of mango roots by *F. oxysporum* (Kumar and Beniwal, 1991) or *F. mangiferae* (Abdel-Sattar, 1973; Kumar and Beniwal, 1991) could result in the development of malformation in seedlings, however, these findings were not experimentally supported. It was shown that infections of mango roots with *F. mangiferae*, did not result in systemic infections and did not result in symptom development (Youssef et al. 2007). Movement of the pathogen via seeds appears to be unlikely since seed infection has not been demonstrated (Saeed and Schlosser, 1972; Youssef et al. 2007). *F. mangiferae* can be distributed over long distances in infected budwood and plants (Prakash and Srivastava, 1987). Thus, latently infected plant material, that would not be evident or visible to production managers or quarantine officials, could move inconspicuously within and between countries. In addition, asymptomatic plants can be a source of spread of the pathogen, as well as propagation material such as budwood, if not accurately inspected using appropriate laboratory techniques (Freeman et al. 2014b).

In summary, published data indicate that *F. mangiferae* is restricted to apical, above-ground meristematic and lateral bud tissues of mango and that localized, but not systemic infections of these plant structures take place. Besides these susceptible infection sites, the pathogen is not present or survives poorly within plant organs, besides buds (Cohen et al. 2017). Thus, the restricted location of the pathogen to buds has significant implications for the management of MMD.

(ii) Spread of the disease via grafting material.

It has been well documented that MMD can be spread via grafting material containing infected buds (Freeman et al. 2014b; Youssef et al. 2007). Developing a protocol for the production of disease-free propagation material is essential in maintaining "clean" nursery stock and a "clean" breeding program. A protocol including heat treatments, combined with immersion of budwood in the fungicide prochloraz (at a concentration of 0.1% to 0.2% non-phytotoxic) was developed, in an attempt to cure highly infected MMD material of Keitt cultivar (Freeman et al. unpublished). Exposure of budwood to temperatures of 44 to 46 C for 30 min did not impair or reduce viability of bud break. Heat and

prochloraz combination treatments were most effective in reducing fungal survival in buds, of between 40 to 60%. However, during grafting of the treated budwood, a low grafting success rate was achieved (up to 10% success), which may be attributed to an inappropriate season of harvest of the plant material (during the fall), or phytotoxicity of the combined heating and prochloraz treatments.

(iii) Disease cycle.

The disease cycle of MMD, caused by *Fusarium mangiferae*, incorporating the mango bud mite *Aceria mangiferae* (Ploetz, 2001), (see in the following sections), was updated with data published from Israel (Freeman 2014b; Gamliel-Atinsky et al. 2009a; Fig. 5). In summary, malformed inflorescences and vegetative growth serve as sources of pathogen conidia that reach infection sites by at least three different ways: (i) aerial dissemination via wind; (ii) via contaminated *A. mangiferae* bud mites; and (iii) via infected host materials (e.g. malformed panicles) that may fall into the funnel-like structure of apical buds.

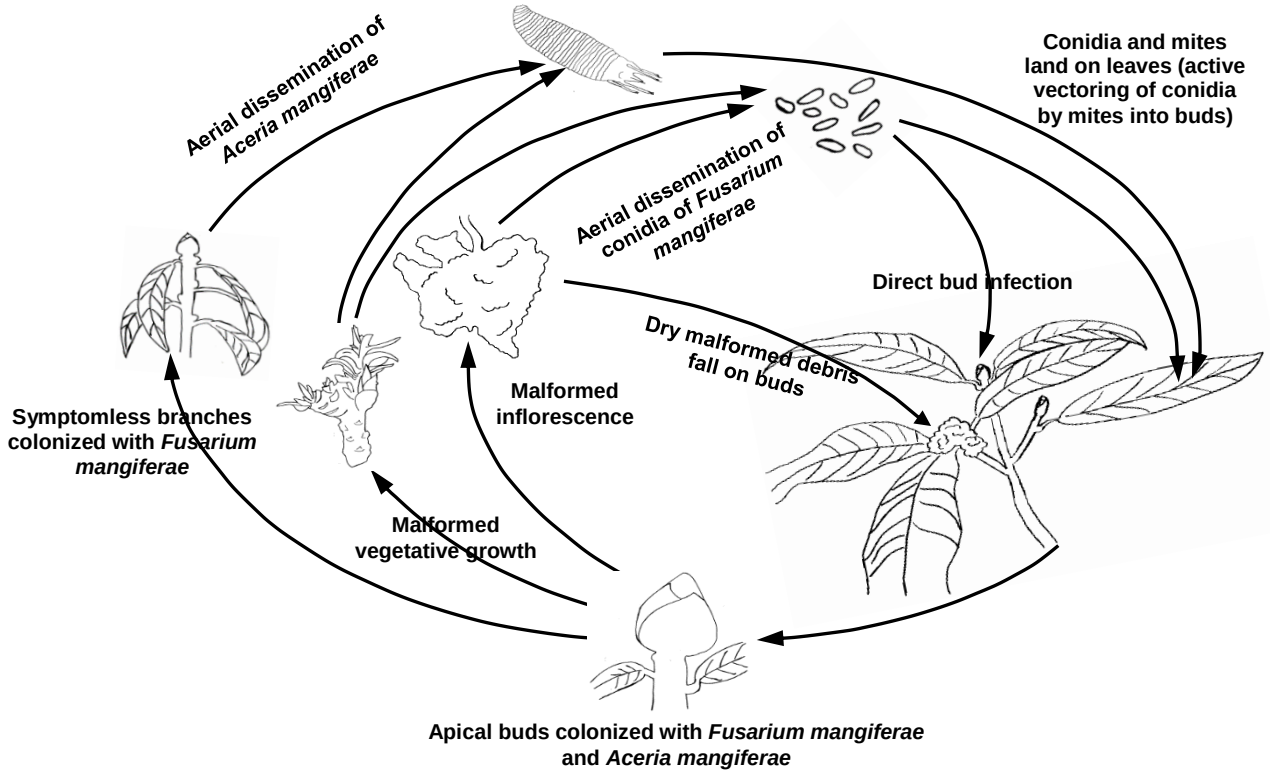

Fig. 5. Disease cycle of MMD caused by *Fusarium mangiferae*, modified from Freeman et al. 2014b.

Moisture-assisted dissemination of conidia might also take place (e.g., spread via dew droplets, rain or dispersal by splash irrigation), although this is unlikely to occur in arid mango production regions.

Conidial germination and infection may ensue with at least 2 h of wetness and temperatures from between 5 to 41°C but is accelerated between 15 and 30°C and wetness conditions above 3 h (Gamliel-Atinsky, 2009c). The presence of *A. mangiferae* in apical buds increases the frequency and severity of infection (Gamliel-Atinsky, 2009b), see below section on "Interaction between the mango bud mite and *Fusarium*". After host penetration, the pathogen colonizes buds, but not subtending branches and remains dormant within these tissues. Whether infected buds subsequently develop symptoms of MMD apparently depends on the extent to which they are colonized. It was reported that thresholds of infection, wherein panicles or vegetative shoots were symptomatic, were observed only when they were thoroughly colonized by the pathogen (Cohen et al. 2017; Freeman et al. 2014a); however, what are threshold levels and how can they be determined?

An integrated management strategy for MMD management.

(i) Development of an integrated management strategy.

Based on the disease cycle (Fig. 5), a spatial and time model was constructed for the management of MMD (Fig. 6).

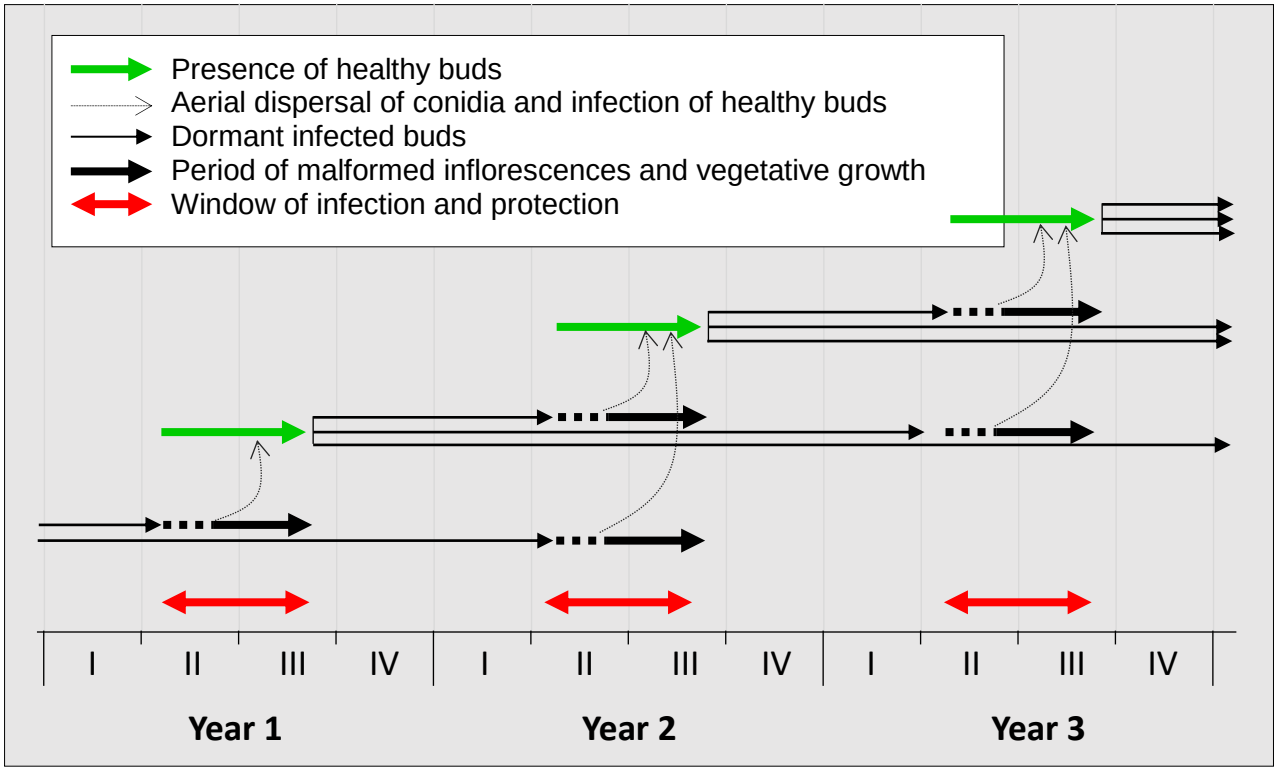

Fig. 6. Model of MMD epidemiology over a 3-year season in Israel. I = January to March; II = April to June; III = July to September; and IV October to December (Freeman et al. 2014b).

The model implies that MMD-affected tissues, release airborne conidia dispersed under field conditions or via the use of infected scion material (Ploetz, 2001), are sources of inocula for the

infection of new, pathogen-free plant material. Microconidia, that are the primary infective propagules of the pathogen, are produced on infected panicles and are dispersed aerially within and among orchards. Thus, inoculum dissemination and infection by the pathogen coincides with the presence of malformed tissues in the orchard. In the northern hemisphere, the first appearance of malformed panicles and vegetative growth begins in April and continues to emerge until late August. The precise dates may change from year to year depending on weather conditions and flowering dates for individual cultivars in each country. Conidia land on dormant buds on the same tree, or different trees in the same or nearby orchards. On occasions, new plantations are established adjacent to heavily infected orchards and the latter can serve as a source of inoculum for the newly planted trees (Fig. 7) (Gamliel-Atinsky, 2009c).

Fig. 7. Spatial spread of mango malformation disease (MMD). A mature infected mango orchard on the left serves as a source of conidia inoculum causing infection in an uninfected orchard on the right.

In practice, the 'window of infection' is also the 'window of protection' during which fungicides or other protectants should be applied to manage the disease (Fig. 6). The window of protection will vary in different areas, depending on the corresponding environmental and production conditions and when windows of infection occur, but the need to protect trees during the later periods is a prerequisite for disease control (Freeman et al. 2014a,b). Another element comprising the management strategy is the reduction of primary inoculum. To this end, malformed tissues are removed as soon as they appear, until the termination of flowering (termed "strict sanitation"). Removal should be limited to only the malformed panicles and not extending branches beyond the inflorescence sites. Infected buds can remain dormant for several months after which they may differentiate into malformed or non-malformed inflorescences, or vegetative shoots. A portion of the buds in mature sections of the tree may also remain dormant for extended periods of time. Should severe pruning (of major branches, limbs or trunks) be conducted during this period, latently infected buds located in mature parts of the tree, such as the trunks, may develop later into malformed tissues.

(ii) Evaluation of an integrated management strategy.

A prerequisite for implementing the integrated management strategy was to identify fungicides to be applied for the protection of buds from infecting conidia of *Fusarium mangiferae*. Initially, efficacy of various fungicides was evaluated in *in vitro* plate tests, against *F. mangiferae* (Freeman et al. 2014a). The ED_{50} value (*i.e.*, the concentration inhibiting 50% of the pathogen's radial growth) for procholoraz-Zn was 0.01 µg a.i./ml; carbendazim 0.7 µg a.i./ml; pyroclostrobin and boscalid 1.5 µg a.i./ml; and famoxadone, mancozeb, and azoxystrobin 10 µg a.i./ml. Additional fungicides that did not inhibit *in vitro* fungal growth of the pathogen compared to those listed above included bupirimate, flutolanil, mancozeb, tebuconazole, thiophanate methyl, triadimenol and triforine (Freeman et al. 2014a). Thus, the most promising fungicide, prochloraz, was further evaluated for the protection against bud infection by *F. mangiferae*. In greenhouse trials, it was found that infection was reduced by 90% when the fungicide was applied up to 14 days before inoculation (protective activity) and up to 14 days following inoculation (curative activity), spanning a 4-week period of infection, when applied at a concentration of 0.1 mg/ml (Freeman et al. 2014a). Numerous field experiments for the management of MMD were set up in naturally infected commercial orchards in Israel. In these experiments, it was shown that timely application(s) of prochloraz, at the time when inoculum is abundant in infected trees, were required to suppress MMD infections (Table 1) (Freeman et al. 2014a).

Table 1. The efficacy of prochloraz sprays in the suppression of MMD in six field experiments carried out in Israel (Freeman et al 2014a).

Exp. No.	Year[a]	Cultivar	Disease intensity in untreated plots[b]	Control efficacy (%) in treated plots[d]	Treatment[c]
1	2006	Tommy	23.3 (I)	-6.4 to 30.5	Untimely sprays
2	2006	Keitt	16.7 (I)	28.7 to 35.3	Untimely sprays
3	2007, 2008, 2009	Tommy	17.4, 26.3, 34.7 (I)	44.3*, 26.6*, 34.3*	Timely sprays
4	2007, 2008, 2009	Keitt	16.0, 24.6, 24.6 (I)	18.8, 28.0*, 28.0*	Timely sprays
5[e]	2009, 2011	Maya	9.6, 16.5 (I)	57.7*, 80.0*	Timely sprays
6[e]	2012, 2013	Keitt	37.5, 4.5 (S)	47.6, 48.9*	Timely sprays

[a]Assessments were performed in the years that follow the application of fungicides in the orchards. For example, in experiments nos. 1 and 2, the sprays were initially applied in 2005.
[b]MMD incidence (I): percentage of malformed panicles out of the total number of panicles that developed on the sampled trees; MMD severity (S): the number of malformed panicles that developed on the sampled trees.
[c]Untimely sprays: prochloraz sprays (6 to 12 in each experiment) were applied at periods that eventually were found to be beyond the window of protection; Timely sprays: prochloraz sprays (6 to 8 in each experiment) that were applied during the window of infection/protection.
[d]Control efficacy in treated plots represent disease incidence or severity (%) in the fungicide and control treatments, respectively. Numbers followed by an asterisk* denote a significant difference between treated and untreated plots, as determined by the Tukey-Kramer HSD test at $P = 0.05$.
[e]Strict sanitation, removal of malformed tissues as soon as they appeared until termination of flowering, was performed in these experiments.

When prochloraz was applied during an inappropriate timing event, either "pre-flowering" when inocula were not present in the orchard or "post-flowering", after inocula had already reached unprotected buds, MMD incidence was not reduced (Freeman et al. 2014a). However, when prochloraz sprays coincided with the production of inocula on infected panicles, MMD incidence declined significantly in treated compared to control plots (Table 1). Timely removal of primary inocula via strict sanitation is crucial for successful implementation of the integrated MMD management strategy. In an experiment conducted in Ma'agan, it was shown that over a 3-year period, when "strict sanitation" was combined with prochloraz treatment, a significant yield increase (39.3%) was achieved compared to the control unsprayed treatment (Freeman et al. 2014a). Strict sanitation or application of the fungicide alone resulted in insignificant increases in yields (17.8 and 14.4%, respectively). However, timely sprays in combination with strict sanitation achieved significant reduction of MMD incidence and increased yields (Table 1; Freeman et al. 2014a). Based on these studies, strict sanitation combined with prochloraz sprays during the window of infection/protection, is currently recommended in Israel (Freeman et al. 2014a). Prochloraz has been registered for the management of MMD in Israel while long-term implementation of the integrated management strategy provides cumulative reductions in disease annually, eventually achieving negligible levels of malformation in treated orchards over time. Alternative fungicides to prochloraz should be evaluated, as *F. mangiferae* may develop reduced

sensitivity and field resistance to this fungicide (Gea et al. 2005; Guarnaccia et al. 2014; Mavroeidi and Shaw, 2005).

(iii) MMD management practices worldwide compared with the integrated strategy.

MMD management practices vary in different mango-production countries worldwide. The Agricultural Research Council (ARC) in South Africa recommends removing the terminal three nodes of affected terminals, disposal in plastic bags followed by burning (De Villiers and Joubert, 2008). Furthermore, the ARC publication, states that if sanitation "is done every year the disease soon assumes insignificant proportions" and can be eliminated. Schoeman and Botha (2015) indicated that removal of malformed panicles should be conducted, including three additional nodes of the branch when malformed flowers are clearly visible. Furthermore, the authors recommended that removal of malformed branches followed by a spray of benomyl WP at 0.75 g/L led to a reduction of 44.3-80.2% in the prevalence of malformation in various experimental sites.

Likewise, removal of affected MMD inflorescences is recommended in Mexico. Disease in that country is reduced by application of copper sulfate pentahydrate and tribasic copper, after removal of terminal malformed shoots during the vegetative stage, or before sprays of potassium nitrate that promote flowering (Noriega-Cantú et al. 2012). However, the numbers of applications that are required for effective management of MMD in Mexico were not indicated. Thus, a combination of sanitation and sprays are suggested for MMD management in Mexico, but the timing and frequency of these treatments have not been specified.

In Brazil, no published MMD management protocols exist to date. However, a recent publication mentions that the spray of certain fungicides were effective for reduction of disease incidence (Da Silva et al. 2022). Accordingly, the commonly used measures included removal of lateral twigs or inflorescences bearing symptoms of malformation, and application of the most effective fungicides, methyl-thiophanate and fluxapyroxad mixed with pyraclostrobin (Da Silva et al. 2022).

To summarize, in the major MMD-affected countries of South Africa, Mexico and Brazil, the extent of recommended sanitation measures has not been elaborated. Besides the report from Noriega-Cantú et al. (1999), there are no data that demonstrate the efficacy of these treatments. Statistically significant reports would facilitate the adoption of these recommendations by producers who, in turn, could reduce the impact of this important disease. It is expected that long-term implementation of the management strategy will result in cumulative disease reductions. Presumably, elements of the integrated management strategy presented above, that is, sanitation and timely application of fungicides, were

included in management practices described from other countries. Nevertheless, the underlying rational concepts and the timing of implementation during application of the integrated management strategy differ from the other strategies. Sanitation within the integrated management strategy is aimed at removing the infected plant organs producing inocula (conidia) that infect healthy buds **externally**. Thus, sanitation has to be initiated as soon as malformed tissues are detected and continue as long as they are present. In most of the other countries, sanitation is aimed at removing the "systemically" infected branches bearing infected tissues, assuming that these branches are colonized, and they produce inocula (mycelia) that infect healthy buds systemically or **internally**. Thus, under this assumption, sanitation can be performed at any time; preferably after all malformed tissues are detected. However, fungicide sprays as part of the integrated management strategy are aimed at protecting the susceptible host tissues (buds) from infections by air-borne conidia. Therefore, spraying to protect non-infected buds from inoculum produced from malformed panicles has to be initiated as soon as they are detected, and continued until symptomatic tissues are no longer produced or removed. In Mexico, Brazil and South Africa, spraying was recommended at certain time periods but the rational for that timing was not specifically described.

"Witches broom" caused by the mango bud mite *Aceria mangiferae*

Mites affecting mango in general.

Most mite species feeding on plants, belong to super-families of the obligate plant parasitic Eriophyoidea (gall mites, bud mites, erinose mites, rust mites etc.) and Tetranychoidea (false spider mites, spider mites), while a number of additional species belong to other lineages of families, such as Eupodoidae and Tarsonemidae (Sarwar et al. 2015).

The Eriophyid and Tetranychid families of mites are predominant pests attacking mango. Representative eriophyids consist of *Aceria mangiferae, Cysaberops kenyae, Tegonotus mangiferae* and *Metaculus mangiferae* (Ayala-Ortega et al. 2019); while the tetranychids include *Oligonychus mangiferus*, *O. yothersi*, *O. punicae* and *Tetranychus cinnabarinus* (Jeppson et al. 1975; Keifer et al. 1982). There are other mite families and species affecting mango, such as the genus *Oulenziella* (Acari: Wenterschmitiidae) and *Brevipalpus oovatus* (Tenuipalpidae) originating from Africa, specifically Egypt. However, of all the mite fauna attacking mango, the mango bud mite, *Aceria mangiferae*, causes the most severe damage to this crop.

In Hawaii, *Tegonotus mangiferae* (Acari: Eriophyidae) feeds on the underside of leaves (Jeppson et al. 1975), while another species, *Metaculus mangiferae* causes russeting of terminal leaves, buds and inflorescences. The latter is an important pest in Egypt, India, Israel and Angola (Jeppson et al. 1975). The puncture wounds of several acarines (Acari: Tetranychidae) cause serious damage to leaves, which may dry and fall. Another major pest in Mauritius, India, Egypt, Israel and Peru is *Oligonychus mangiferus* (Rahman, 1940), while in Israel, the spider mite *Tetranychus cinnabarinus*, which inhabits on the underside of leaves, causes bronzing in the vicinity of the puncture wounds.

The mango bud mite, *Aceria mangiferae*, causal agent of "Witches broom" (WB).

The mango bud mite, *Aceria mangiferae* (Sayed) is considered one of the major pests of the crop worldwide. It was initially recorded in Egypt by Hassan (1944) and described at that time as *Eriophyes mangiferae* n. sp. but was later renamed *Aceria mangiferae* by Sayed (1946). The mango bud mite has also been reported in southern Asia, Brazil and seems to occur wherever mango is grown worldwide, affecting buds and inflorescences (Abou-Awad, 1981; Denmark, 1983; Doreste, 1984; Jeppson et al. 1975; Keifer et al. 1982; Ochoa et al. 1994). It was first recorded in Florida in 1959 and in Israel in 1976 (Sternlicht and Goldenberg, 1976).

Symptomology and damage.

There have been conflicting reports regarding resistance of mango cultivars to the mango bud mite. A study conducted by Peña et al. (2005) indicated that 22 mango cultivars had varying bud colonization densities of mites. Additional studies reported that no resistance existed among mango cultivars tested in India, although population densities varied according to location (Bindra and Bakhetia, 1970). However, in other studies, a distinct difference in susceptibility of various mango cultivars to mites was reported (Sternlicht and Goldenberg, 1976; Zaher and Osman, 1970). Certain characteristics of the cultivars' physical structure are in favor of colonization of buds by the mite. For example, in regard to the hazelnut bud mite it was suggested that mites first enter the tree between the lowest, or outermost stipules of the shoot tip, but that they can feed only on the youngest axillary buds, which have not yet developed protective scales. Thus, with plants of increasing susceptibility, it is easier for mites to penetrate in the vicinity of the apical region (Burgess and Thompson, 1985).

The mango bud mite attacks terminal buds of young and old mango trees, causing bud malformation and stunting of inflorescences (Abou-Awad, 1981). Typical "witches broom" symptoms of mango, caused by the mango bud mite, result in "atrophy" of apical leaves, where short internodes at the apex of seedlings develop, while associated leaves become atrophic, stop developing and dry out, and the epidermis of leaves, stems and petioles become brown and coarse (Ochoa et al. 1994). The various and typical mango bud damage symptoms caused by the mango bud mite *A. mangiferae* are summarized in Fig. 8.

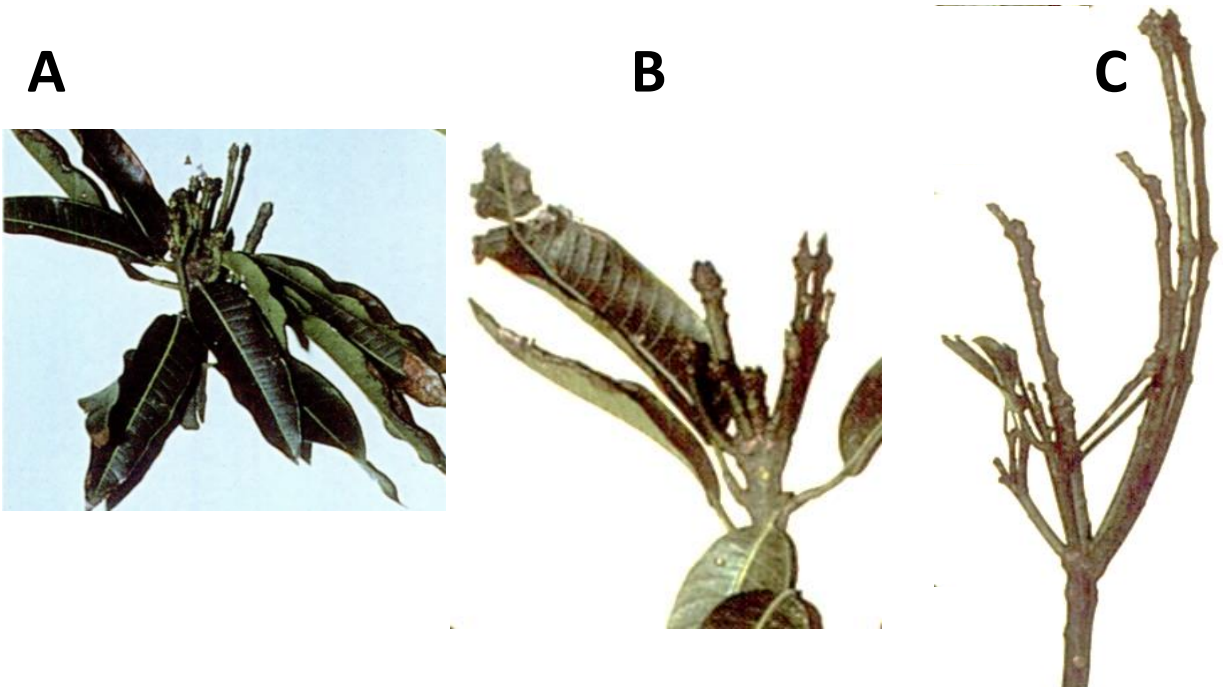

Fig. 8. Typical "witches broom" symptoms of mango, caused by the mango bud mite, *Aceria mangiferae*. **A**. Drying of terminal buds, stunting of vegetative growth, dieback of floral panicles with necrosis of bud tissues and gall formation; **B**. Distorted growth terminals with arrested growth, short stunted young stems close together at the terminal branch; **C**. Leaf fall resulting in sparse growth of twig-like branches including stubby, short vegetative branches with discolored buds.

Jeppson et al. (1975) reported that the mite stunts vegetative development of the bud and causes a phenomenon termed "witches broom". Dark brown staining of the bracts near the bottom of the buds indicates the presence of the mite, accompanied by the creation of galls resulting in injury to the buds (Abou-Awad, 1981; Peña et al. 2005). The mite causes necrosis of bud tissue cells, starting externally then progressing toward the center and internal parts of the bud, finally causing dieback of flower panicles, distortion of fresh plant growth, drying of terminal buds and vegetative growth, leading to branch dieback and mortality (Abou-Awad, 1981; Peña et al. 2005; Fig. 8). According to Keifer et al. (1982), *A. mangiferae* infestation of buds results in arrested growth and apical bud proliferation resulting in stunted, short, young stems grouped together at the terminal branch. Similar reports were published by Varma et al. (1974), indicating that the mango bud mite causes bud proliferation and appears to be responsible for necrosis of bud tissue cells. Upon leaf fall, the overall observation is sparse growth of twig-like branches, with stubby, short vegetative stems harboring discolored buds (Peña et al. 2005). Abou-Awad et al. (2011) reported that another eriophyiod, *Metaculus mangiferae* had a negative influence on the content of macro and micronutrients in mango under Egyptian conditions.

Biology and lifestyle.

Aceria mangiferae survives and proliferates within the terminal buds in large numbers throughout the year (Abou-Awad 1981; Sternlicht and Goldenberg 1976). Generally, eriophyoids are not spread evenly within all host plant structures, but a "clumped nature" of distribution has been described within a tree (Perring et al. 1996). Micro-environmental variation surrounding the plants causes this uneven distribution. In mango trees, mites were found within generative and vegetative closed buds (Sternlicht and Goldenberg 1976) and were released only when the scales of the buds became loose (Prasad et al. 1972). In inter-tree distribution, Eriophyoid mites are windborne and migrate from plant to plant via air currents. The mites "stand" erect with the aid of their anal suckers, face the wind and wave their legs, and are dispersed in this manner. Peña et al. (2005) reported on distribution and sampling techniques for the mango bud mite in Florida, USA and indicated that the bud mite exhibited aggregated patterns of spatial distribution. More mites were found on apical buds than on lateral-latent ones. During the winter season, mites prefer to infest buds located on small shoots while observations also showed that individuals appear in late spring or early summer between bases of the outer bud scales. In autumn and winter, mites were observed throughout the bud scales. According to seasonal activities, populations of active stages start to increase in April, reaching a peak in late

May, while populations fluctuate during June, July and August and then increase again, reaching a peak in late October (Peña et al. 2005).

Female adults measure approximately 220×48μm while the male has smaller dimensions of 170×45 μm and are white in color (Abou-Awad, 1981). They cannot be observed by the naked eye but can be visualized with a 10× hand-held lens (Abou-Awad, 1981). *Aceria mangiferae* life history has been described by Abou-Awad (1981); it is completed within approximately 15 days at 25–27 C. Females usually lay eggs among trichomes at the top of the bud, but also occasionally between base scales. Eggs are translucent white in color, measuring approximately 33×22 μm prior to hatching, followed by the larval, first instar nymph and adult stages. Egg population also follows the adult population colonization trend and reached a peak in late May, while a second smaller peak occurred in early winter months of November to December. During the winter months of February and March, infestation was mild. To determine the number of generations/year, relative percentages of eggs to other stages were estimated at weekly intervals indicating the occurrence of approximately eight generations. In a study conducted to determine the life cycle on inoculated seedlings (Abou-Awad, 1981), mites were active for two days after inoculation and then settled within terminal buds. On the third day they began to lay their eggs in groups, under the external bud leaf scales. Egg incubation period lasted from 4 to 7 days, with the completion of approximately eight generations per year, each lasting for about 15-19 days (Abou-Awad, 1981).

Biological control.

The biological control mite, phytoseiid *Amblyseius swirski* Athias Henriot, is associated with *A. mangiferae* (Abou-Awad, 1981). In Florida USA, several unidentified phytoseiid mites e.g. Tenuipalpid (*Brevipalpus phoenicis*), Tydeid and Tarsonemid (*Tarsonemus confusus* Ewing) also inhabit mango buds infested with the mango bud mite. Therefore, it is difficult to determine which mite species may serve as predator of the host *A. mangiferae* (Peña et al., 2005). Cabrera et al. (2008) indicated that the entomopathogenic fungus *Hirsutella thompsonii* is effective in management of *A. mangiferae*. Furthermore, Abou-Awad et al. (2011) reported the presence of *A. mangiferae* in mango buds that served as prey for two predatory mites, *Typhlodromus mangifer*us and *Typhlodromips swirskii*, in an abandoned mango orchard in Egypt, but they were unable to demonstrate a positive correlation between these predaceous mites and *A. mangiferae* populations. In addition, the cheletid mite, *Cheyletia wellsi*, was the only predator found in a phenology study in Egypt (Abou-Awad, 1981). In summary, specific interactions between potential biocontrol agents of the mango bud mite have only been partially observed and experimentally demonstrated.

Chemical control.

Chemical sprays have been reported as the most effective means for management and reduction of *A. mangiferae* pest populations in affected mango trees in orchards. Osman (1979) reported that applications of four full coverage sprays of dichlorvos were effective in controlling *A. mangiferae* in Egypt. However, Rai et al. (1966) cautioned that chemical control should be directed at apparently healthy and not malformed tissues. In Florida, agrimek (abamectin) plus citrus oil, fenproximate and fenpropathrin resulted in the lowest mite densities 12 days after application, and in time, agrimek plus citrus oil, and acequinocyl resulted in the lowest mite densities 26 days after treatment (Peña et al. 2005). In addition, aluminum phosphide (Phostoxin) completely controlled *A. mangiferae* when infested malformed shoots and saplings were exposed to two pellets of the compound within an iron bin, however, predatory mites were also killed (Bharadwaj and Banerjee, 1973). In another study, mite populations were reduced after application of eight different systemic insecticides, whereby two compounds, aldicard and phorate, where the most effective (Varma and Yadav, 1970). Abou-Awad (1981) reported that mite management required a strict pruning of infested material in the winter (January), followed by foliar applications of the miticides ethion 46.5 EC, kelthane 42 EC and wettable sulphur, at 2 week intervals for effective pest control. It should be noted that pruning of infested inflorescences and buds from the previous season decreased the mite population by approximately 30% during the following year, and increased yield by about 40% (Zaher and Osman, 1970).

Interaction between the mango bud mite and *Fusarium*.

Many plant feeding mites, representing different families such as Acaridae, Siteroptidae, Tydeidae, and Tarsonemidae interact with plant pathogenic fungi. Although species within the Eriophyoidea appear to be common phytophagous mites vectoring virus diseases, many plant pathogenic fungi interact with a considerable number of mites representing many families in different suborders (Krantz and Lindquist, 1979). Fungal infection may be facilitated by herbivores via two main mechanisms: (i) either by vectoring pathogen conidia, or (ii) by creating wound sites allowing fungal penetration and proliferation (Agrios, 1980; Hatcher and Paul, 2001). There has been controversy regarding a possible association between *Aceria mangiferae* and floral and foliar galls, i.e. mango malformation disease caused by *Fusarium* species (Cabrera et al. 2008; Denmark, 1983; Narasimhan, 1959; Ochoa et al. 1994; Sayed, 1946; Summanwar and Raychoudhury, 1968).

The association between *Aceria mangiferae* Sayed and the fungal pathogen *Fusarium mangiferae* Britz, Wingfield & Marasas, in mango, can be presented as a case study where the

underlying mechanisms clarifying the role of the mite in mango malformation epidemiology has been described (Gamliel-Atinsky et al. 2010). It should be reiterated that *A. mangiferae* is not directly involved in the appearance of MMD. For example, the mango bud mite *A. mangiferae* is present in Australia, however, MMD symptoms do not occur in that country (Ploetz and Prakash, 1997).

As demonstrated in the previous sections, MMD is a severe disease, widely distributed in almost all mango-growing regions worldwide (Freeman et al 2014b; Ploetz and Freeman, 2009). The mango bud mite was hypothesized as the causal agent of mango malformation for over 40 years, mainly due to high numbers of mites observed in malformed trees, and also because other members of the Eriophyoidea are known to cause symptoms termed "witches broom" and gall symptoms of inflorescences in other plants (Westphal and Manson, 1996). It is now clear that *A. mangiferae* is not the causal agent of MMD, however, various studies suggest that the mite interacts with the fungal pathogen resulting in increased severity of disease (Prasad et al. 1972; Sternlicht and Goldenberg, 1976; Gamliel-Atinsky et al. 2009a).

Role of the bud mite *A. mangiferae* in epidemiology of the MMD.

Research was conducted to determine the involvement and role of *A. mangiferae* in causing MMD, an issue of controversy for many years (Ploetz, 2001). Three stages of the disease cycle were studied: (i) reaching the infection site; (ii) colonization; and (iii) aerial dissemination. For each stage the question whether the mite assists the fungal pathogen was addressed (Gamliel-Atinsky et al. 2009a). A green fluorescent protein-transformed isolate of *Fusarium mangiferae* was utilized as a tool to allow definite identification of the pathogen, preventing confusion with other natural infections.

Possible associations/interactions between the mango bud mite and *Fusarium mangiferae* were investigated by Gamliel-Atinsky et al. (2010) to: (i) determine mutual habitat and site of interaction between the two organisms; (ii) determine whether *A. mangiferae* can carry conidia of *F. mangiferae* on or within its body; (iii) determine whether fungal conidia can be vectored into infection sites; (iv) to determine whether *F. mangiferae* can promote the fungal infection process; and (v) to determine whether *A. mangiferae* can disseminate fungal conidia aerially over long distances. For the purpose of some of these studies, a gfp-marked strain of *F. mangiferae* was used (Gamliel-Atinsky et al. 2009a), which distinguished it from that described in other previous studies, and helped distinguish the pathogen from opportunistic fungi and other contaminants.

(i) Mite and fungus share a mutual habitat.

Both the mango bud mite and the fungal pathogen were observed within bracts of apical buds (Gamliel-Atinsky et al. 2010; Fig. 9). Hyphae of *F. mangiferae* were observed in close proximity to *A. mangiferae* (Fig. 9A). Hyphae and conidia of *F. mangiferae* were observed growing around a trichome of the bud bracts, and conidia were also detected on the mite's body (Fig. 9B). Germinating conidia and fungal hyphae were observed upon the body of *A. mangiferae* (Fig. 9C).

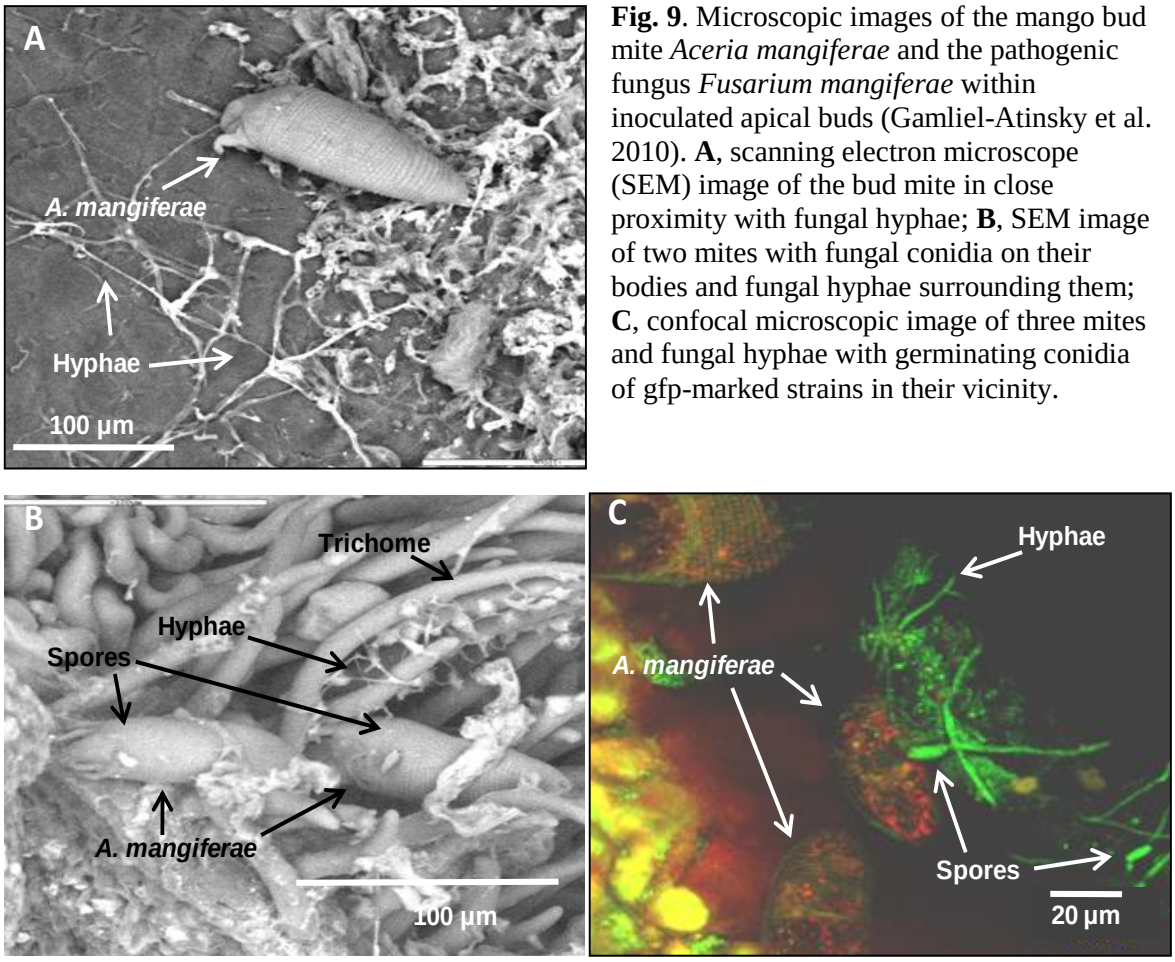

Fig. 9. Microscopic images of the mango bud mite *Aceria mangiferae* and the pathogenic fungus *Fusarium mangiferae* within inoculated apical buds (Gamliel-Atinsky et al. 2010). **A**, scanning electron microscope (SEM) image of the bud mite in close proximity with fungal hyphae; **B**, SEM image of two mites with fungal conidia on their bodies and fungal hyphae surrounding them; **C**, confocal microscopic image of three mites and fungal hyphae with germinating conidia of gfp-marked strains in their vicinity.

(ii) Carrying conidia of *F. mangiferae* on or within the mite's body.

It was observed that *A. mangiferae* mites that were exposed to the gfp-marked isolate of *F. mangiferae* and then inspected under a confocal microscope did not show any specific binding sites, however, conidia were found to cling to external parts of the bud mite (Fig. 10).

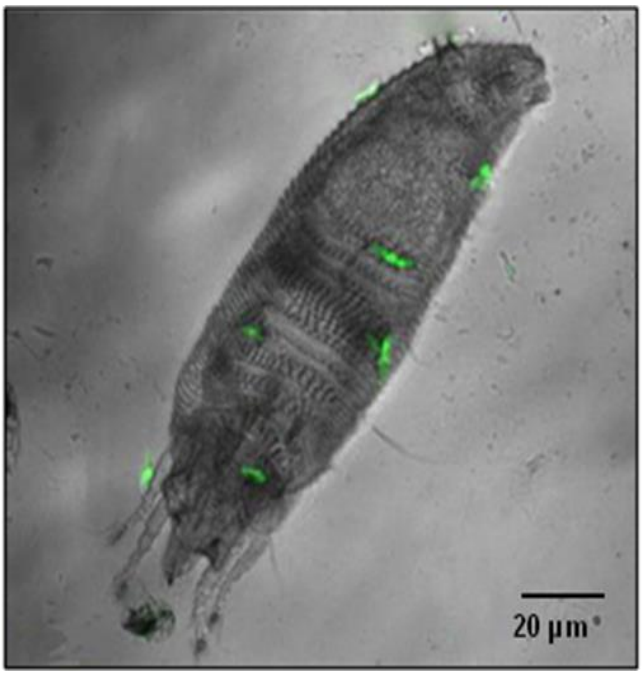

Fig. 10. Mango bud mite, *Aceria mangiferae*, bearing conidia of isolate gfp-1 of *Fusarium mangiferae* (shown in green), the causal agent of mango malformation disease (Gamliel-Atinsky et al. 2009a).

Measurements of both conidia and mite stylets indicated that the width of the mite mouthparts were substantially smaller than the diameter of the smallest *Fusarium* microspore (Fig. 11). In addition, a lack of continuity between the midgut and hindgut of eriophyoid mites (Nuzzaci and Alberti, 1996), further demonstrated that fungal conidia cannot be transferred and secreted in feces, as opposed to vectoring of viral pathogens, due to their minute size (Oldfield and Proeseler, 1996; Fig. 11).

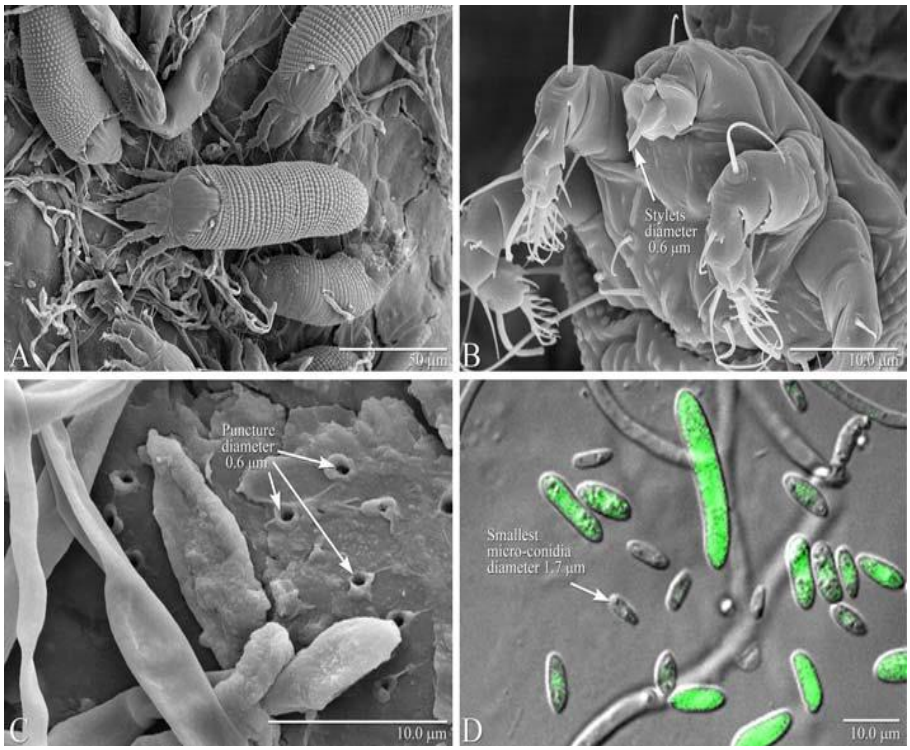

Fig. 11. A. Scanning electron microscope (SEM) images of mango bud mites, *Aceria mangiferae*, feeding on bud bracts; B. SEM image of mango bud mite anterior ventral view of stylet. C. SEM image of respective feeding puncture holes; D. confocal image of gfp-marked conidia and micro-conidia of *Fusarium mangiferae* (Gamliel-Atinsky et al. 2010).

Similarly, the minute diameter of eriophyoid mouthparts may preclude ingestion of larger plant pathogens. Thus, associations of the mango bud mite and *Fusarium* conidia is apparently only possible via external-body attachment, such as in the case of other acaropathogenic fungi (McCoy, 1996).

(iii) Mite vectoring conidia to penetration sites.

As demonstrated, apical buds are the exclusive penetration sites for conidia of the pathogen which also serve as the specific living habitat of *A. mangiferae* (Gamliel-Atinsky et al. 2009a,b). Most conidia of *F. mangiferae* disseminate in the air and randomly fall on the tree canopy which accommodates most of the orchards surface area. It was shown that the bud mite actively reaches the apical bud and in artificially inoculation experiments it was demonstrated that conidia reached the apical bud only in the treatment where both mites and conidia were co-inoculated onto leaves, indicating the potential of eriophyoid mites to serve as vectors of fungal pathogens (Gamliel-Atinsky et al. 2009a).

(iv) Mite promoting conidial penetration.

The possible role of *A. mangiferae*, involved in fungal penetration within the apical buds, was assessed. When apical buds of potted mango plants were inoculated with *F. mangiferae* in the presence and absence of bud mites, the frequency and severity of fungal colonization was significantly higher in buds co-inoculated with both fungus and mites as compared to each treatment alone. Thus, the presence of mites within the buds enhanced fungal colonization and disease incidence (Gamliel-Atinsky et al. 2009a).

(v) Role of bud mites in aerial dissemination of conidia.

It was proposed by Ploetz (2001) that the bud mite may be involved in aerial dissemination of conidia on its body. Thus, several trapping methods were used in an attempt to monitor both fungal conidia and mango bud mites in a heavily infected MMD orchard, over a 3-year period (Gamliel-Atinsky et al. 2009a). *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 percentage infection of buds by the pathogen was lower (Fig. 12). Average numbers of *A. mangiferae* per apical bud varied from 18 mites per bud in April and January, to a peak of 62 and 56 mites per bud during July and October, respectively. On average, for the 10 sampling periods over three 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 (Gamliel-Atinsky et al. 2009a).

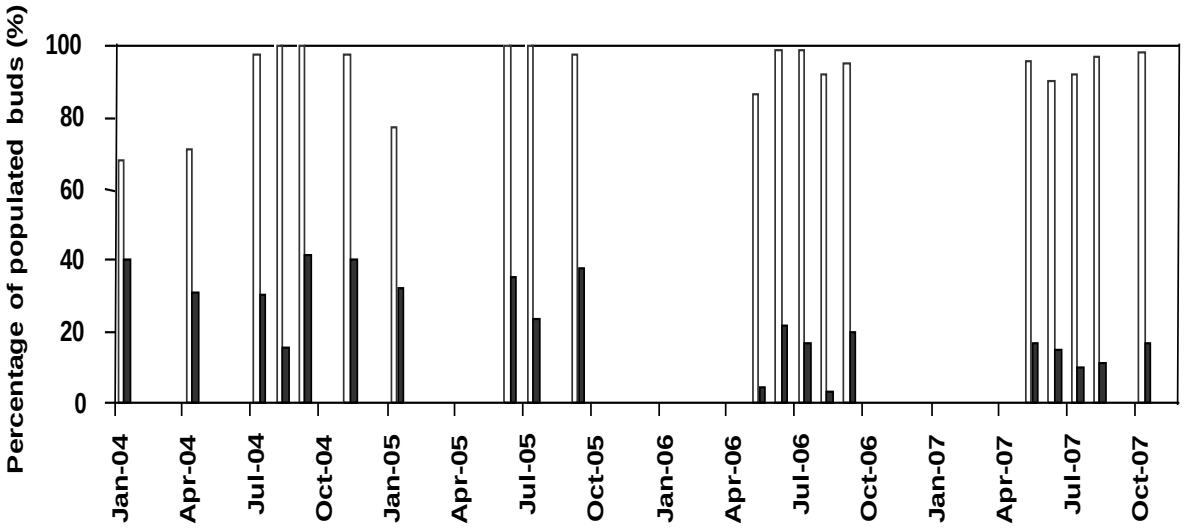

Fig. 12. Percentage of buds populated with *Aceria mangiferae* and *Fusarium mangiferae* (open bar) and infected with *F. mangiferae* alone (filled bar), sampled from one-month-old mango branches of Haden cultivar in a heavily infected orchard (Gamliel-Atinsky et al. 2009a).

Conidia of *F. mangiferae* were successfully trapped when placed in a heavily infected orchard using active volumetric spore traps as well as passive Petri dishes containing selective media, and an annual peak of dissemination was found in the spring/early summer months (Gamliel-Atinsky et al. 2009a; Fig. 12). The bud mite was trapped throughout the season, but presence of the fungus was not detected after placing these mites on selective media for detection of the fungus. In contrast, a high inoculum density of *F. mangiferae* conidia were collected from spore traps in a heavily infected orchard, with a peak in airborne conidial numbers being recorded during May and June, in two consecutive years (Gamliel-Atinsky et al. 2009b). The peak in aerial conidial dissemination corresponded to the peak of appearance of mature malformed inflorescences in the orchard. This indicates that conidia can reach the mango tree independently of the bud mite and that the latter does not seem to play a role in the windborne dissemination of the pathogen (Gamliel-Atinsky al. 2009a,b).

Co-infection of mango by *Aceria mangiferae* and *Fusarium mangiferae*.

It was reported that mango bud mites were found in trees affected by MMD as well as in non-malformed trees (Sternlicht and Goldenberg 1976). The mite's population remained active throughout the year in healthy as well as in malformed buds (Prasad et al. 1972). A correlation between the size of the necrotic area and the size of mite population was studied in Florida. A low

correlation was found between the two variables which was explained by the fact that other arthropod species may also cause necrosis of buds, such as *Tarsonemus confusus* and *Radionaspis indica* (scale aphid) (Peña et al. 2005). Another study that examined the mite's population in healthy and malformed buds found no difference in the population size (Prasad et al. 1972), in contrast to that describing healthy tissue being infested by much higher bud mite populations than that in malformed tissues (Labuschagne et al. 1993). Nevertheless, when populations of mites were compared between different orchards, a large difference in population size was detected in orchards with high severity of MMD (hundreds of mites found per 5g tissue), compared to orchards with low severity of MMD (ten or less mites found per 5g tissue) (Sao Jose et al. 2000). Thus, although contrasting data have been reported regarding population colonization of mites, it appears that both the mite and fungus are able to co-exist, while MMD symptoms were observed regardless of the levels of mite infestations, further attesting to the role of *Fusarium* species as the sole causal agent of disease.

Does the mango bud mite play a role in the development of MMD?

The mango bud mite, *Aceria mangiferae* Sayed (Eriophyidae), is often observed in high numbers in malformed trees. This association and the reported ability of the mite to cause hypertrophied buds on mango led some to consider it to be the cause of MMD (Narasimhan, 1954; Narasimhan, 1959; Nariani and Seth, 1962). However, this hypothesis was not supported as indicated by the following evidence:

(i) the mite is present in Australia, although rare outbreaks of MMD have been reported, only under quarantine conditions and after eradication, mite populations continued to thrive (Anonymous, 2013);

(ii) in other mango-producing areas, high populations of the mite can occur in trees that do not develop MMD symptoms (Sternlicht and Goldenberg, 1976);

(iii) acaricides did not reduce MMD incidence, although they dramatically reduced populations of the mite (Manicom, 1989).

(iv) spraying against the mite did not reduce the incidence of MMD in heavily infected orchards (Freeman et al. 2014a).

In an experiment to assess involvement of mite infestation on the levels of MMD it was shown that the application of an acaricide (EOS oil) alone did not reduce MMD infection levels, whereas the fungicide prochloraz alone and in combination with the oil reduced disease incidence (Freeman et al. 2014a; Fig. 13).

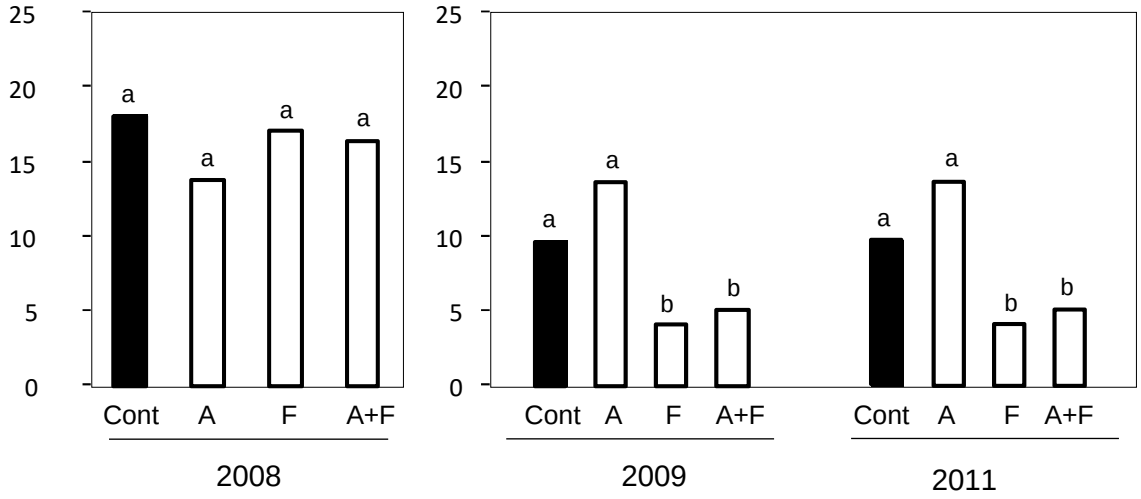

Fig. 13. Sole and combined effects of the fungicide prochloraz-Zn (F) and EOS acaricide (A) sprays, on suppression of MMD incidence (% infected trees) from 2008-2011. Sprays were not applied in the untreated control plots (black bars). In each year, bar values followed by a similar letter do not differ significantly, as determined by the Tukey-Kramer HSD test at $P = 0.05$ (Freeman et al. 2014a).

Nevertheless, the mite may play a role in the epidemiology of MMD. The pathogen was previously recovered from the mite on culture media (Crookes and Rijkenberg, 1985), and was also shown to adhere to its body (Gamliel-Atinsky et al. 2010) (Fig. 10). Gamliel-Atinsky et al. (2010) reported that the mite could not ingest the pathogen, due to its small mouth, but experimentally dispersed conidia of *F. mangiferae* to infection courts within mango buds, probably as a body adherent. Wounds caused by the mites' feeding could facilitate infection of buds by the pathogen (Crookes and Rijkenberg, 1985; Gamliel-Atinsky et al. 2010). Several studies mention that there is a high correlation between *A. mangiferae* populations in the buds and the incidence of MMD in mango trees, in addition to demonstrating that the application of acaricides can reduce the incidence of malformation symptoms (Ayala-Ortega et al. 2019).

However, in Israel, *A. mangiferae* did not appear to play a significant role in disseminating the pathogen among and between trees. Mites were not found in traps that were designed to monitor their movement in an MMD-affected orchard, although high numbers of *F. mangiferae* conidia were trapped (Gamliel-Atinsky et al. 2007). Whether, and under what circumstances, the mite plays a role in spreading MMD among trees and orchards in other mango-production areas should be investigated due to the potential impact these factors may have on MMD management strategies. Other arthropods that frequent infected panicles may serve as dispersal agents even though no conidia were detected on wind-borne mango bud mites originating from infected panicles (Gamliel-Atinsky et al. 2009a; Gamliel-Atinsky et al. 2010). Aerial dissemination of this pathogen by arthropods and other means should be re-examined.

Summary

In general, mango malformation disease (MMD) is caused by species of the fungal pathogen *Fusarium*. Airborne conidia of the pathogen are the infection structures by which the pathogen causes disease. Conidia penetrate the plant tissue via apical and lateral buds and remain dormant until bud break. No systemic infection takes place, only local colonization of the bud tissues. When infected buds break, malformed vegetative and inflorescences are produced. A strategy was developed for management of disease by elimination of the major inocula (conidia) sources of infection, i.e. malformed panicles, by pruning. Thereafter, subsequent fungicidal sprays are applied to protect and cure affected buds from infection via airborne conidia.

Although the mango bud mite, *Aceria mangiferae*, has been suspected as a causal agent of MMD, different symptoms are caused by this pest in mango, termed "witches broom". However, exacerbation of MMD symptoms can take place following wounding of bud tissues, allowing penetration of the fungus at these locations.

Future studies

(i) Etiology of the causal agents of disease.

Progress in several different areas of research may depend on identifying which *Fusarium* species cause MMD in different regions. The ability to identify different MMD agents would be needed to develop reliable disease diagnostics, as well as understand the diseases' etiology and epidemiology in different environments and improve MMD management strategies. The growing list of *Fusarium* spp. that cause this disease indicates that there is much to learn about MMD. How MMD pathogens other than *F. mangiferae* behave on mango is not known. For example, whether other species affect different mango cultivars to the same extent as *F. mangiferae* should be evaluated as it could generate additional management recommendations for MMD. For the same reason, it should be determined whether other *Fusarium* species interact with the mango bud mite, *Aceria mangiferae*, similar to *F. mangiferae*. And why/how several distinct species cause the same symptoms on this host should be examined as it could reveal much about the host/pathogen interaction and symptomology of disease.

(ii) Management of MMD.

Infection by *F. mangiferae* is not systemic (Gamliel-Atinsky et al. 2009a; Ploetz, 2001; Youssef et al. 2007), and the most susceptible organ of infection is the apical meristem (Gamliel-Atinsky et al. 2009c). These critical data have helped develop an integrated strategy to manage MMD, but the timing of fungicide applications has not yet been optimized. Additional data that are required for

optimization of the spray regime, (within the window of protection), should address weather conditions that impact dissemination and germination of conidia, and their infection of the host. In the tropics, where flowering is erratic and often synchronized chemically, integrated MMD management could be adapted. In Israel, implementation of strict sanitation, by continuously removing infected panicles, is aimed at reducing the primary inoculum in the orchard for minimizing the occurrence of new infections. Although malformed panicles are burned in some areas, this may not be possible where the practice is strictly regulated due to fire hazard and concerns about air pollution. Thus, alternative treatments, such as solarization, may be considered, whereby eradication of inocula from pruned infected panicles should be assessed.

(iii) New generation fungicides for MMD field control and infected budwood curing.

Nineteen "new generation" fungicides were used to recently screen *F. mangiferae* isolates in plates to determine efficacy in vitro. Currently, after six of these fungicides were shown to be very effective in vitro in plates, all of the compounds are being evaluated in two field experiments to determine efficacy for control of MMD. Two experiments using these fungicides were set up in 2023 and will continue for the next 2-3 years.

In addition, since latent infection of budwood is common, a treatment that may eliminate the pathogen from infected budwood would be invaluable. In this regard, these new fungicides will be examined for their curing ability of infected budwood, collected from a heavily affected orchard.

(iv) Screening for MMD resistance.

Rootstocks, wild mango species (for breeding purposes) and current cultivars are being screened for susceptibility/tolerance/resistance with representative isolates of *F. mangiferae*. The protocol entails artificial inoculation of buds before floral initiation and assessment of MMD incidence, vegetative and inflorescence, after bud break. Different cultivars are currently being screened using this technique.

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