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Management of mango malformation disease based on a novel strategy of timing of fungicide applications combined with sanitation

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ABSTRACT

Mango malformation disease (MMD) caused by *Fusarium mangiferae* severely affects the crop and is widely distributed in almost all mango-growing regions worldwide. Since malformed inflorescences do not bear fruit, MMD is a major constraint to crop production in affected areas. No effective management methods have been reported to date. The airborne nature of dissemination and infection of buds suggest that protection of buds from infection when inoculum prevails may be a plausible method for disease control from season to season. Various fungicides were assessed for their ability to control the pathogen under laboratory, greenhouse and field conditions. Prochloraz was the most effective fungicide in inhibiting *F. mangiferae in vitro* with a 0.01 μg mL^{-1} concentration required for 50% fungal growth inhibition. In greenhouse trials, protective and curative activity exceeding 90% was achieved when the fungicide was applied up to 14 days prior or post inoculation. Field experiments conducted over a number of seasons in different regions in Israel indicate that combined sanitation with timely applications of prochloraz resulted in a significant reduction in MMD disease severity and incidence, as well as a significant increase in yield in treated plots. It is assumed that long-term treatment by removal of infected panicles (the main source of inoculum) combined with timely sprays will result in disease reduction annually achieving negligible levels of malformation in treated orchards, in time.

1. Introduction

Mango (*Mangifera indica* L.) which is cultivated in many tropical and subtropical countries worldwide is seriously affected in most regions by mango malformation disease (MMD) (Kumar et al., 1993; Ploetz and Freeman, 2009). MMD affects floral meristematic and vegetative tissues (Chakrabarti, 2011; Ploetz, 2001). Floral malformation is economically important since affected inflorescences usually do not set fruit, resulting in significant loss of yield (Noriega-Cantú et al., 1999; Youssef et al., 2007). Malformed panicles produce dwarfed and distorted leaves, and primary or secondary axes on affected panicles are shortened, thickened and highly branched, and can result in a mass of tissue resembling that of a "cauliflower" in shape and size (Kumar et al., 1993; Ploetz and Freeman, 2009). Malformed panicles can persist in an affected tree until the following season as dry, black masses (Gamliel-Atinsky et al., 2009c). Vegetative malformation includes hypertrophy of young shoots, with swollen apical and lateral buds, which produce misshapen shortened internodes and dwarfed leaves. The growth of these shoots is arrested and subsequently several similar shoots arise from the same axillary bud resembling the "bunchy-top" symptom of disease (Chakrabarti, 2011; Ploetz and Freeman, 2009).

Mango malformation was first reported over a century ago in India (Kumar et al., 1993). Although conflicting reports regarding the causal agent existed, Koch's postulates were successfully performed with the fungal pathogen *Fusarium moniliforme* Sheldon (Summanwar et al., 1966), since renamed *Fusarium mangiferae* Britz, Wingfield & Marasas (Britz et al., 2002; Marasas et al., 2006). Subsequently, many studies worldwide have proven that the fungus is indeed the causal agent of MMD (Crookes and Rijkenberg, 1985; Freeman et al., 1999; Kumar et al., 1993; Varma et al., 1974). In recent years, additional *Fusarium* species such as *Fusarium sterilihyphosum* from Brazil and South Africa, *Fusarium mexicanum* from Mexico, *Fusarium proliferatum* from China and most recently, *Fusarium tupiense* from Brazil, have been implicated in MMD (Britz et al., 2002; Lima et al., 2008, 2012; Marasas et al., 2006; Newman et al., 2012; Otero-Colina et al., 2010).

In the past it was recognized that the pathogen spread gradually and unpredictably in infected orchards and in young seedlings

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(Schlosser, 1971; Singh et al., 1961). These observations regarding the pattern of slow disease development within only a few branches, lead the scientific community at that time to believe that the disease was spread systemically, within the vasculature of infected tissues (Kumar et al., 1993). Based on this presumption, the management strategies that were developed in different countries included sanitation and application of fungicides. Removal or sanitation of affected panicles and the subtending three nodes has been recommended, presumably for eliminating the inoculum persisting within the host tissue (Chakrabarti, 2011; De Villiers and Joubert, 2008; Lahav et al., 2001; Manicom, 1989; Narasimhan, 1959; Noriega-Cantú et al., 2012; Singh et al., 1974). Although some authors indicated that sanitation should be performed immediately after observing the diseased tissues, the timing of this action is not expected to be crucial for adequate disease suppression. Noriega-Cantú et al. (1999) reported that sanitation and the protection of shoots with systemic (benomyl) and contact (mancozeb) fungicides, and the control of mites and ants, contributed to a reduction of MMD. When positive results were reported for the benzamidazoles, it was not substantiated whether they had an actual effect since the fungicide was combined with pruning of branches 45 cm beyond infected panicles (Iqbal et al., 2011). In contrast, it was reported by others that benomyl inhibited the pathogen *in vitro*, but did not affect MMD when sprayed on trees (Chadha et al., 1979; Diekman et al., 1982; Ibrahim et al., 1975). Furthermore, most spray applications against MMD were applied soon before or during bloom until fruit set but not beyond (Iqbal et al., 2011; Noriega-Cantú et al., 1999), without a plausible rationale for the timing event. Nevertheless, the durability and long-term effectiveness of these measures are ambiguous (Chakrabarti, 2011; Covarrubias, 1980; De Villiers and Joubert, 2008; Pinkas and Gazit, 1992) and consequently, in many countries growers do not invest any efforts for suppressing MMD. Thus, developing effective management practices in MMD is still a challenge.

In recent years, studies on dispersal patterns of conidia of *F. mangiferae* suggest aerial dispersal as the primary mechanism for fungal dissemination (Gamliel-Atinsky et al., 2009a,c; Noriega-Cantú et al., 1999), and location of infection sites appears to be localized at the apical and lateral bud areas. Since the pathogen was detected in malformed panicles and vegetative shoots, but rarely detected in branches (Gamliel-Atinsky et al., 2009a), it was hypothesized that vegetative and floral buds are the primary sites of infection (Gamliel-Atinsky et al., 2009c). Youssef et al. (2007) showed that infections are not systemic, with infections of apical meristems most likely originating and disseminating via conidia from malformed panicles. Furthermore, airborne infections of the buds occurred predominantly in May and June in Israel, a period that corresponds with the timing of maturation and dispersal of inoculum from infected panicles in the orchard in Israel (Gamliel-Atinsky et al., 2009b).

Based on these observations, a plausible cycle for mango malformation disease caused by *F. mangiferae* in Israel was proposed by Gamliel-Atinsky et al. (2009c). Malformed inflorescences and malformed vegetative growth serve as sources of inoculum. Inoculum from diseased panicles and malformed vegetative tissue disseminate passively in the air as conidia, or fall from dry, malformed inflorescences as dry debris. Other possible means could also assist conidia in reaching the apical bud [(*e.g.,* transport of conidia in dew droplets or splash dispersal of conidia from leaves to buds, although the latter probably does not occur in Israel due to lack of rains in the early summer months when conidial dissemination takes place, and vectoring of conidia via the mango bud mite, *Aceria mangiferae* (Gamliel-Atinsky et al., 2009a))]. 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. Inflorescences from a colonized bud may emerge malformed, probably due to pathogen mediated effects until an infection threshold is met (Ploetz and Freeman, 2009). Alternatively, when a young shoot emerges from an infected apical bud, the pathogen may colonize the apical or lateral buds of the young shoot, then remain localized and dormant in buds until bud break (Youssef et al., 2007). This young shoot may show symptoms of vegetative malformation or harbor the pathogen within bud tissue without showing typical disease symptoms.

Based on the above mentioned scenario, we developed a conceptual model for MMD development in time and space. According to this model, the pathogen is windborne in nature, and conidia that are produced on malformed panicles or vegetative growth are dispersed in the air. Thus, inoculum dissemination and infection coincide with the existence of malformed tissues in the orchard. In Israel, the first malformed panicles and vegetative growth usually begin to appear in April and continue to emerge until late August (the exact date fluctuates slightly from year to year depending on weather conditions and is also dependent on cultivar flowering dates). Conidia are dispersed in the air, deposit on and infect dormant buds on the same tree, and trees in the vicinity. Occasionally, new plantings are located adjacent to heavily infected orchards and the former serve as a source of inoculum for the newly planted trees (Gamliel-Atinsky et al., 2009b). Infected buds remain dormant for several months and in the following season they differentiate into a malformed reproductive inflorescence or remain vegetative and develop into a malformed young shoot. A portion of the buds remain dormant for one or more years and may differentiate later. Malformed tissues serve as the source of inoculum in these seasons and contribute to buildup of the disease.

The practical implication of this model is that the 'window of infection' is also the 'window of protection'. Thus, protecting apical buds from airborne infections and maintaining strict sanitation in the orchard by immediate removal of malformed tissues may contribute to an improved management strategy for MMD. As it is assumed that the fungus infecting the panicles or vegetative tissues results from external inoculum, sanitation should be aimed at decreasing the inoculum sources and only malformed tissues need to be removed.

Having identified the 'window of protection', where application of prophylactic measures might be most effective, the study reported here has assessed fungicide control strategies in combination with other treatments. The main objectives of this study were to: (1) determine the efficacy of fungicides against *F. mangiferae* in *in vitro* assays and *in vivo*, in artificially inoculated mango seedlings under controlled conditions, and (2) evaluate whether chemical sprays, applied alone or in combination with other control measures during the 'window of protection', will provide adequate suppression of the disease in commercial orchards under outdoor conditions.

2. Materials and methods

2.1. Determining the efficacy of fungicides against F. mangiferae

2.1.1. Fungal cultures

Local wild-type *F. mangiferae* isolates from Israel, MRC 7559 (Fus 5) and MRC 7560 (Fus 34), were used throughout the experiments (Steenkamp et al., 2000). The monoconidial cultures were maintained on potato dextrose agar (PDA; Difco Laboratories, Detroit) at 25 ± 2 °C in a growth chamber in the dark. Conidial suspensions were obtained after 5 days incubation by adding sterile water to the