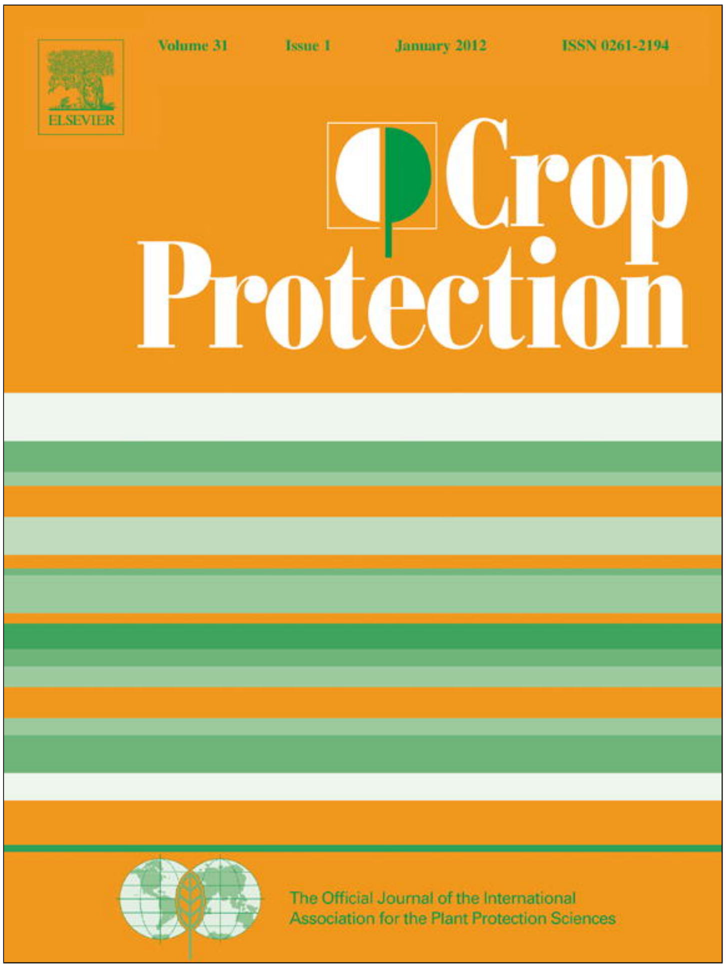
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Interaction between *Papaya meleira virus* (PMeV) infection of papaya plants and Mediterranean fruit fly infestation of fruits

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ABSTRACT

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wied.), is a pest of quarantine importance that usually only infests papaya fruits in advanced stages of maturity. However, some diseases, such as sticky disease of papaya plants, caused by the *Papaya meleira virus* (PMeV), break the natural resistance of fruits to fruit flies, enabling them to be infested while still unripe. Therefore, this study of the relationship of papaya sticky disease with *C. capitata* under field conditions was conducted to determine the period of security to guarantee the harvest of papaya fruits free of fruit flies in areas where sticky disease is endemic. Infestation of papaya fruits by *C. capitata* was evaluated from uninfected plants and from sticky disease infected plants, in seven stages of the disease and three stages of ripening of fruits. A direct relationship was observed between time of sticky disease symptoms and infestation of fruits by the Medfly, and it was determined that a period of four weeks after the first appearance of the symptoms of sticky disease in papaya plants was the period of security in which infestation of fruits by fruit flies did not occur. Infection of papaya plants with sticky disease was associated with a reduced level of benzyl-isothiocyanate (BITC), from 43.1 $\mu g\ ml^{-1}$ to 1.7 $\mu g\ ml^{-1}$, a natural chemical compound in the latex of papaya fruit, and considered to be associated with resistance to fruit flies.

1. Introduction

Fruit flies (Diptera: Tephritidae) are important pests of fruit production worldwide because of direct damage caused by larval feeding and because of quarantine restrictions of importing countries that impede the exportation of fresh fruits (Malavasi, 2000). Although papaya (*Carica papaya* L.) is considered a secondary host of tephritids, Brazil was unable to export papaya to the United States for 13 years with quarantine restrictions for the Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wied.), and *Anastrepha fraterculus* (Wied.), the two species of fruit flies that may infest papaya in the country (Martins and Alves, 1988; Martins et al., 1993). Brazil only resumed export of fresh papaya to the USA after development of the Program of Risk Reduction of Infestation by Tephritids (Systems Approach) (Anonymous, 2002). This pest risk reduction program has a strong biological and ecological base and reduces the risk of infestation by the pest to almost zero (Martins and Malavasi, 2003).

Sticky disease of papaya (commonly known as meleira in Brazil), caused by *Papaya meleira virus* (PMeV), is characterized by spontaneous fluid and latex exudation from fruits and leaves of papaya plants. After atmospheric exposure the latex oxidizes resulting in small necrotic lesions on the edges of young leaves (Ventura et al., 2003). Sticky disease is an important disease of papaya in Brazil, especially in northern Espírito Santo and southern Bahia States, the main areas of papaya production in this country, and if not adequately managed can cause the complete loss of productive plants. The disease was reported in the 1980s in orchards of papaya in the extreme South of Bahia and North of Espírito Santo is currently found disseminated in the principal production regions of the country (Rodrigues et al., 1989; Ventura et al., 2004). It is caused by a dsRNA virus that has isometric particles (40–50 nm in diameter), present in the latex vessels of the plants (Kitajima et al., 1993; Maciel-Zambolim et al., 2003; Tavares et al., 2004; Rodrigues et al., 2009a). Diagnosis of this disease is achieved mainly by observation of the symptoms on the young leaves and fruits, and the detection of dsRNA from leaves and latex by electrophoresis, or RT-PCR.

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The main symptom of papaya sticky disease is an intense and spontaneous exudation of latex that is more fluid than normal, and which oxidizes and darkens, forming dark streaks on the fruit and giving them a blackish appearance making them totally unviable for commercialization, as well as compromising their texture and flavor (Ventura et al., 2004). The removed of infected plants (roguing) at the beginning of the appearance of symptoms is the only way to control of this disease (Ventura et al., 2001, 2003, 2004).

Some diseases, such as sticky disease of the papaya plant (PMeV), break the natural resistance of the papaya fruits to fruit flies, probably by interfering in the physiology of the plant, enabling the fruit to be infested while still immature (Martins and Malavasi, 2003). This breaking of resistance was observed in commercial papaya orchards of Espírito Santo, and in southern Bahia (Martins and Malavasi, 2003). Nascimento et al. (2000) provided evidence under controlled conditions of a positive relationship between the fruit flies and papaya sticky disease, in which fruits from plants infected with the virus were infested with fruit flies while still in a green, immature stage.

In the systems approach, papaya fruits harvested in early stages of maturity (Stages 1 and 2 for exportation) have low risk of infestation by fruit flies because of the toxicity of the natural chemical compound benzyl-isothiocyanate (BITC), present in the latex of the fruit (Tang, 1971, 1973). BITC has ovicidal and oviposition inhibition activity against fruit flies, and its concentration decreases with the maturation of the fruit, making mature fruit more susceptible to infestation by these insects (Seo and Tang, 1982; Seo et al., 1983; Nascimento et al., 2003).

Green fruits of cv. Sunrise Solo from plants infected by papaya sticky disease were heavily infested by fruit flies, while fruits of healthy plants, with the same degree of maturity were not (Nascimento et al., 2000). This suggested that the virus of sticky disease of papaya has a direct effect on BITC levels, after observing a reduction of about nine fold in the amount of BITC in the latex of the fruit of papaya in the stage 1 of maturity 60 days after mechanical inoculation of plants with PMeV (Nascimento et al., 2003).

The interactions between fruit flies and papaya sticky disease are not completely understood, and the objective of this work was determine the period of security in which the fruits of diseased plants in the field are not infested by this pest to guarantee the quality and quarantine security of fruit for exportation.

2. Materials and methods

This study was conducted in an isolated papaya orchard, cv. Sunrise Solo (a commercial variety widely cultivated in Brazil), of 4.6 ha (6400 plants), in Linhares, State of Espírito Santo, with 640 randomly selected plants inoculated with the PMeV and monitored weekly for symptoms of papaya sticky disease for 52 weeks of the crop cycle. Presence of PMeV in papaya plants with symptoms of sticky disease was confirmed by molecular diagnosis of the virus in the latex of the plants by the use of PCR and RT-PCR (Rodrigues et al., 2005, 2009a, 2009b; Tavares et al., 2004). A high density of *C. capitata* in the study site was induced by the constant presence of mature fruits on the plants and adult fruit flies were monitored by Jackson traps with trimedlure.

Infestation of papaya fruits by fruit flies was evaluated from uninfected plants and from sticky disease infected plants based on the time after the appearance of the initial symptoms of sticky disease in plants because this is the point monitored for and observed by commercial producers for roguing of the plants infected by the virus of the disease. In these plants the experiment was done using a factorial design with 7 treatments (stages of the disease score) $\times$ 3 stages of fruit ripening, and 4 repetitions of 4 fruits for each stage, totaling 336 fruits evaluated. A combination of three fruit maturity stages and seven times of papaya sticky disease symptom treatments were evaluated. Fruit maturity stage treatments were: Maturity Stage 0 (fruits with surface of the skin completely green); maturity stage 1 (fruits with $<$15% of the surface of the skin yellow); and maturity stage 2 (fruits with the surface of the skin between 16 and 25% yellow), with maturity stages as defined by the Program of Exportation of Brazilian Papaya to the United States (Martins and Malavasi, 2003; Anonymous, 2008).

Fruits harvested and evaluated were: 1) fruits from healthy plants (uninoculated, uninfected) with no visual symptoms of sticky disease; 2) fruits from plants with initial visible symptoms of sticky disease (on the day symptoms were detected); 3) fruits from plants 4 weeks after symptoms of sticky disease detected; 4) fruits from plants eight weeks after symptoms; 5) fruits from plants 12 weeks after symptoms of sticky disease detected; 6) fruits from plants 26 weeks after symptoms of sticky disease detected; 7) fruits from plants 52 weeks after symptoms of sticky disease had been detected.

Four fruits were utilized per repetition, and placed individually in plastic containers with screen covers and with a layer of 1.0 cm of washed sand in the bottom for determination of fruit fly infestation of the fruits (percentage of fruits infested and number of pupae/fruit), ten days after a harvest.

The level of BITC in fruits was evaluated by gas chromatography (GC) with a nitrogen and phosphorus detector (180 °C), using two fruits/treatment in maturity Stage 0 from healthy plants and from plants 12 weeks after the appearance of the symptoms of papaya sticky disease (sticky disease symptom treatments 1 and 5, respectively) in a laboratory accredited by the Brazilian Ministry of Agriculture (MAPA). The part of the fruit analyzed was the mesocarp from the skin to a depth of 1.0 cm, because this is the region of oviposition and initial infestation of the Medfly.

The experimental design was a completely random split plot, with data submitted to analysis of variance (ANOVA) to determine the significance of individual differences at $P < 0.01$ and 0.05 levels. Significant means were compared by Tukey's test ($P < 0.05$). All statistical analysis was conducted using the SAEG statistical package (Ribeiro Junior, 2001).

3. Results

The presence of PMeV virus was confirmed by molecular detection of dsRNA, PCR and RT-PCR in all plants with papaya sticky disease symptoms.

3.1. Medfly infestation

Papaya fruits from plants with no sticky disease symptoms and up to 4 weeks after the appearance of the symptoms of the disease on the plant were not infested by fruit flies in any stage of fruit maturity evaluated. Fruit fly infestation was only observed 8 weeks after the appearance of the symptoms of the disease in plants in fruits of maturity stages 1 and 2, and 26 weeks after the appearance of the symptoms of the disease in plants in fruits of the maturity stage 0 (Table 1).

3.2. Papaya sticky disease severity

The evolution of sticky disease in papaya plants and the virus of this disease requires two to three weeks after inoculation to be positively diagnosed by PCR in plants, and the initial visual symptoms of the disease are only observed about six weeks after initial infection with the virus. A period of four weeks constitutes the maximum period of security for fruits of plants infected and

Table 1
Infestation (%) and number of *Ceratitis capitata* (Diptera: Tephritidae) pupae in papaya fruits harvested from plants infected by PMeV.

Papaya sticky disease symptoms	Fruits infested (%)			Pupae/fruit (N°)		
	Stage of maturity[a]			Stage of maturity		
	0	1	2	0	1	2
Plants without symptoms (uninoculated and uninfected)	0 aA[b]	0 aA	0 aA	0 aA	0 aA	0 aA
Plants with initial symptoms (on day disease symptoms first detected)	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA
Plants with symptoms 4 weeks	0 aA	0 aA	0 aA	0 aA	0 aA	0 aA
Plants with symptoms 8 weeks	0 aA	12.5 aA	50.0 bB	0 aA	0.4 aA	13.4 bB
Plants with symptoms 12 weeks	0 aA	25.0 aB	62.5 bC	0 aA	2.4 aA	11.6 bB
Plants with symptoms 26 weeks	6.3 aA	68.8 bB	62.5 bB	0.3 aA	18.3 bB	14.6 bB
Plants with symptoms 52 weeks	25.0 aA	68.8 bB	75.0 bB	7.3 bA	12.9 bAB	16.9 bB

[a] Maturity stages: 0 = completely green; 1 = fruits with <15% of the skin yellow; 2 = fruits with 16–25% of the skin yellow.
[b] Means followed by the same lowercase letter in a column or uppercase in a line, do not differ by Tukey's test (P <0.05).

symptomatic with sticky disease in the field to remain uninfested by the fruit fly (Fig. 1).

Analyses of benzyl-isothiocyanate (BITC) in fruits in the stage 0 of maturity from uninfected papaya plants compared to plants 12 weeks after initial visual symptoms of sticky disease demonstrated a reduction of up to 96% in BITC, from 43.1 μg ml^{-1} to 1.7 μg ml^{-1}.

4. Discussion

Papaya fruits in the green stage and in initial stages of maturity from plants with no visible symptoms of sticky disease were not infested by fruit flies. These results are consistent with those of Martins et al. (2000), in which fruits of healthy plants in the stages of maturity 1, 2 and 3, were not infested by these insects and infestation was only observed in fruits with more advanced maturity (stage $\geq$ 5), which are too mature for commercial sale. Liquido et al. (1989) also observed that an increase in the degree of infestation by two species of fruit flies was proportional to the degree of maturity of the fruit. The lack of infestation of papaya fruits from healthy plants by fruit flies is attributed to the presence of the natural compound BITC in the fruits whose concentration decreases as the fruit ripen (Tang, 1971, 1973; Seo and Tang, 1982; Seo et al., 1983; Nascimento et al., 2003).

BITC is a volatile substance, formed after the rupture of cells of the tissues of fruit (Flath and Forrey, 1977) and its concentration is reduced during the process of ripening of fruit (Liquido et al., 1989). The natural mortality of eggs and larvae of first instar fruit flies by BITC was demonstrated experimentally by Seo and Tang (1982) for *C. capitata* (Wied.), *Bactrocera dorsalis* Hendel and *Bactrocera cucurbitae* Coquillett. These authors related that this compound is formed naturally by the mixture of the enzyme thioglucosidase with latex when the cells of the tissues of the fruit are damaged during oviposition and feeding by the larvae of the tephritids. The process of fruit maturity is initiated from the interior, and the area of major production of BITC occurs in the tissues near the fruit epidermis (Flath and Forrey, 1977), which suggests that species of fruit flies with short ovipositors, such as *C. capitata*, present low risk of infesting papaya in the field.

Infestation of papaya fruits by fruit flies in the stage 0 of maturity from diseased plants with symptoms only after 26 weeks indicates that the infestation by the fruit fly is influenced, not only by the maturity of the fruit, but also by the disease, depending on the amount of time infected plants remain in the field. These results demonstrate the breaking of the resistance of papaya fruit to the pest in plants infected by the PMeV, which can be attributed to the reduction in the levels of BITC in the fruits since analyses of the fruits in the stage 0 demonstrate the reduction in the levels of this compound with time of development of sticky disease.

The association of *C. capitata* with papaya sticky disease was observed under a high density of fruit flies induced by allowing

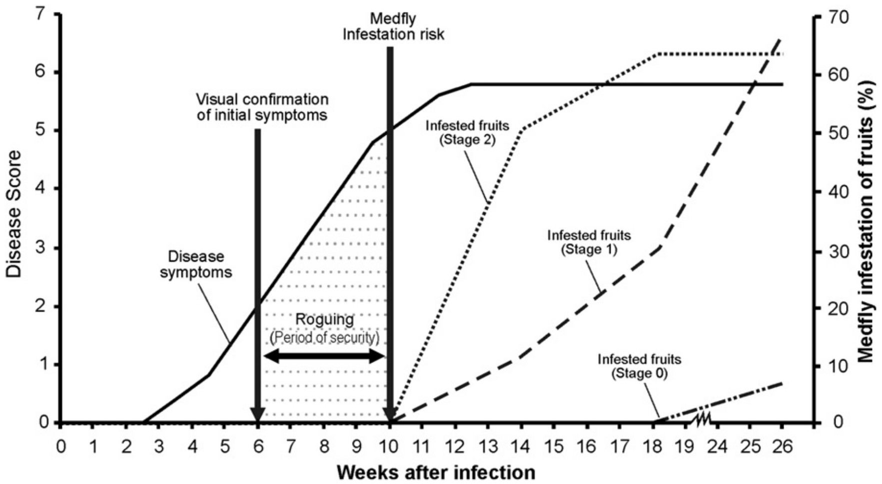

Fig. 1. Infestation of *Ceratitis capitata* (Diptera: Tephritidae) in papaya fruits in three stages of maturity (0, 1, 2) and graphical representation of the development of the symptoms of sticky disease in papaya plants showing the security of fruit free of infestation by Medfly at four weeks after infection and early symptoms, indicating the period of security for roguing of diseased plants which insures fruits free of infestation of this pest.

mature fruits to remain in the orchard and high incidence of the disease insured by mechanically inoculating plants with the virus. However, the probability of fruit fly infestations of papaya in commercial orchards is very low, especially in areas of fruit production for export to the United States, in which the application of the Systems Approach requires that the fruits for export cannot be past the stage 2 of maturity when harvested and the producing fields cannot contain fruits over stage 3 of maturity (Anonymous, 2008). All papaya orchards must be maintained in good phytosanitary condition in accordance with federal legislation (Anonymous, 2002), which requires systematic roguing of plants with initial symptoms of viruses. Beyond this, papaya is a non-preferred host of fruit flies and in its production there is a low prevalence of the fruit flies, such that since the beginning of the Program of Exportation, in September 1998, the index of capture of this insect in monitored orchards of 1 fly per trap/day (FTD $=$ 1), was never reached (Martins et al., 2005).

The efficiency monitoring of papaya plants, by producers of State of Espírito Santo to of one to two times per week, to detect and to eradicate the plants with sticky disease reinforces the security to prevent infestation of the papaya fruits by fruit flies. Evaluations of the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper) with the Associação Brasileira dos Exportadores de Papaya (BRAPEX), demonstrated that the probability of an infected plant escaping roguing, when done weekly, is 3.4^{-5} (Mizubuti and Maffia, 2003). In addition, the period of security of 4 weeks further reduces the probability that infected plants will not be detected. Fruits of plants infected and symptomatic in the field are not infested by the fruit fly, during which four to eight inspections in the field are realized for roguing, which reduces even more the probability of that a plant with sticky disease would not be detected.

Therefore, roguing at the beginning of the visual appearance of the symptoms of sticky disease of papaya plants and the practice of not leaving fruits beyond the stage 3 of maturity in the field, as required by the Systems Approach, are important measures to guarantee the phytosanitary quality of the field and quarantine security for the fruit fly in papaya fruits.

In conclusion, *Papaya meleira virus* breaks down the resistance of papaya fruits to fruit fly infestation, and there is a direct relationship between the time of plant infection with the virus and infestation of papaya fruits by the fruit fly *C. capitata* depending on the stage of fruit maturity. The possibility of infestation by fruit fly increases with papaya sticky disease development in the plant and the stage of maturity of fruits. The maximum period of security to avoid the infestation of papaya fruits by the fruit fly is four weeks after the appearance of the visual symptoms of sticky disease in the plant, and the infection of papaya plants by the virus was associated with reduced level of benzyl-isothiocyanate (BITC) in papaya fruit that reduced resistance to the fruit fly. The use of the Systems Approach as currently recommended guarantees the phytosanitary quality and the quarantine security to the fruit fly on papaya produced in the State of Espírito Santo, Brazil.

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