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Determination of host status of citrus fruits against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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Short title: Attractiveness of citrus to Medfly.

Abstract

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) is a pest of citrus in parts of Western Australia. Three citrus cultivars: Valencia oranges, Eureka lemons and Imperial mandarins, as well as non-citrus control fruits were examined for attractiveness and suitability to Medfly in the field and in the laboratory using choice and no-choice experiments. Oranges were more susceptible to Medfly than mandarins and lemons. Punctures in the skin had a significant impact on the degree of infestation in both citrus and non-citrus control fruit. Artificial infestation and larval survivorship was used to investigate the suitability of each cultivar to Medfly under laboratory conditions. Oranges and mandarins were suitable for the development of Medfly but lemons were a poor host. When each cultivar was in season, field cage trials demonstrated that infestation occurred in oranges and mandarins but not in lemons.

Key words *Ceratitis capitata*, citrus, host status, quarantine risk.

INTRODUCTION

Studies of Medfly larval development and survivorsip in a variety of fruits showed that it is a successful generalist (Krainacker *et al.* 1987). However, not all fruits are equally attractive to Medfly or suitable for its development (Jenkins & Shedley 1956).

After studying ovipositional preferences in Medfly using plastic domes, Freeman and Carey (1990) concluded that female Medfly was more likely to oviposit into fruits that are larger, spherical in shape and have a colour of a short wavelength. Tephritid fruit flies were unable to pierce fruits with tough skin, but oviposited freely into fruits with favourable skin penetrability and also tended to lay into pre-existing punctures (Papaj 1993). Balagawi *et al*. (2005) found ovipositional preferences in the Queensland fruit fly, *Bactrocera tryoni* (Frogatt) in three tomato cultivars by performing choice and no–choice experiments under laboratory conditions. The three cultivars were unequally attractive; there was a relationship between high skin penetrability and high oviposition preference; and an attraction to specific volatile compounds differentially present in tomato varieties.

Differences in susceptibility between citrus cultivars to Medfly was shown by Spitler *et al.* (1984), where grapefruits were highly favoured but lemons were almost immune to attack and unsuitable for development. Susceptibility of citrus to the Caribbean fruit fly, *Anastrepha suspensa* (Loew) was found to vary according to senescence factors, such as peel colour, resistance to punctures, and oil and limonin content (Greany *et al.* 1983).

Research on host suitability or offspring performance has shown that most fruit fly species, including Medfly, suffer high egg mortality when attacking citrus (Greany *et al.* 1983). It has been reported that in the wild, Eureka lemons cannot sustain Medfly egg development unless the fruit has been physically damaged while still on the tree (Back & Pemberton 1915).

Although differences in the host status of citrus cultivars to Medfly have been acknowledged, data on the level of attractiveness and susceptibilty of specific crops are not available. Such data would enable more accurate quarantine risk assessments to be made. The current study is a comparative assessment of host status in three citrus cultivars to provide data to reduce the severity of quarantine treatments in poor hosts of Medfly to enhance the fruit export market in Western Australia.

MATERIALS AND METHODS

Experimental conditions

The experiments involved the Mediterranean fruit fly (*Ceratitis capitata*) and three citrus cultivars, Valencia oranges, Eureka lemons and Imperial mandarins. Golden Delicious apples and Tegan plums, both well-known to be highly attractive to Medfly, were used as control fruits as a measure of comparison between citrus and non-citrus fruits. The flies were laboratory-reared and obtained from the Medfly colony (renewed annually with wild flies) at the West Australian Department of Agriculture & Food in South Perth. Test fruits of export quality were obtained from organic growers located in the nearby Gingin District. Field and laboratory trials were conducted between February and September 2005. Trials were conducted in a controlled environment laboratory at 26 ± 1°C; 60-65% relative humidity in a dark: light cycle of 12:12 hours.

Fruit characteristics

Physical and chemical parameters of the fruit were measured to provide an assessment of suitability for survival of immature Medfly stages at the time of infestation and for comparison of cultivars of infestation rates. Sugar content, pH, fruit weight and skin thickness were measured. A Brix meter was used to measure sugar content in the fruit. An Electronic Pressure Tester fitted with a plunger of 11mm diameter (EPT-1, Lake City Technical Product Inc., Kelwona, BC, Canada) was used to measure pericarp firmness in kg.

Trial 1: Host attractiveness

Treatments involved exposing fruit to 30 gravid females in laboratory cages (50 x 50 x 50 cm) for 24 hours. The fruit was either punctured (by piercing 50 holes, 4-5 mm deep in the peel with an entomological pin) or unpunctured. Fresh fruit was supplied at the end of each 24 hour period and the trial concluded after 4 days. The number of eggs laid into the fruit each day was obtained by dissecting the fruit under the microscope. The punctured and choice condition was set up in such a way that a punctured fruit from each citrus cultivar, as well as a punctured control fruit were exposed to the flies in the same cage. The punctured and no-choice condition consisted of two punctured fruits of only one cultivar per cage. These two procedures were then repeated with unpunctured fruits.

Trial 2: Host suitability

To determine the course of development of the immature stages of Medfly in the three citrus cultivars, 150 fruits of each variety were injected with 0.5 ml of 6 hour-old eggs in agar medium, using a hypodermic syringe (De Lima *et al.* 2007). An average of 350 eggs were injected into the fruit, after which the wound at the injection site was sealed with a drop of polyvinyl acetate adhesive and the fruit placed in a controlled temperature room at 26 ± 1°C and 60-65% RH. Thereafter, at 24 hr intervals, a sample of five fruits were dissected, the number of live and dead individuals in each stage counted, and the proportion present at each stage determined. The number of pupae and adults obtained per fruit were recorded in order to quantify a complete life history for each fruit variety.

Trial 3: Field trials

Field trials were conducted in a mixed fruit orchard in Gingin district and repeated over 3 days. Each day, four replicates of approximately 500 g of each test citrus fruit species and a control were selected randomly from the canopy and caged for 24 hours with 50 gravid females in mesh bags (240 mm long x 180 mm wide). The bags were securely fastened to the branches to prevent escape of flies and the entry of predators. Four replicates of the control fruit (a known host) were run concurrently in each trial. After 24 hours, the bagged fruits were removed from the tree and taken to the laboratory where each replicate was held separately in a ventilated plastic 'tote' box (400 mm long x 300 mm wide x 120 mm deep) over sand for pupation. After 14 days, the sand was sieved daily to extract pupae which were held in labeled petri dishes to record emergence of adults.

Data analyses

Host attractiveness

Data were transformed using natural log to normalize variance, and the sample means were compared by a number of paired sample t-tests. One-way ANOVA and LSD multiple comparisons were then performed on the data.

Host suitability

Host suitability was assessed by the development of Medfly life stages in each fruit type using life table analysis (Carey 1982) to estimate expectation of life for each stage ($\mathbf{e_x}$), the net reproductive rate ($\mathbf{R_o}$) and the capacity for increase ($\mathbf{r_c}$).

Field trials

Citrus varieties mature at different times – Valencia oranges in summer; Imperial mandarins and Eureka lemons in winter. Field trials were set up to compare the difference in infestation between oranges and plums in summer. In winter comparisons were: mandarins and oranges; and lemons and oranges. The number of pupae obtained from each variety in each comparison was assessed.

RESULTS

Fruit characteristics (average of 10 fruits)

Oranges were sweetest (9.3 % sugar), followed by mandarins (6.0 %) and lemons (7.4 %). Oranges were heaviest (293.8 g), followed by lemons (130.8 g) and mandarins (94.4 g). pH was highest in mandarins (3.72) followed by oranges (3.54) and lowest in lemons (1.96). Skin firmness was highest in lemons (13.3 kg), followed by oranges (9.4 kg) and lowest in mandarins (4.1 kg). In the control fruit (Golden Delicious apples) the characteristics were (10.1 % sugar), average weight (193.8 g), pH (3.71) and skin firmness (5.4 kg).

Trial 1: Host attractiveness

The highest number of eggs (Table 1) were recorded in the control (Golden Delicious apples), followed by oranges, mandarins and lemons. The largest difference in infestation levels occurred between lemons and other fruits, while the smallest difference was between oranges and apples. Punctures had a significant impact on infestation in oranges irrespective of choice ($P = 0.003$ choice; 0.012 no choice, α: 0.05), while in lemons punctures made a significant difference only when Medfly was given no choice of fruit ($P = 0.002$, α: 0.05).

Trial 2: Host suitability

The stage with the highest expectation of life ($\mathbf{e_x}$) was the early instar in all fruits (Table 2). The net reproductive rate ($\mathbf{R_o}$) was highest in apples (324) followed by oranges (160), mandarins (70) and lemons (1). The capacity for increase ($\mathbf{r_c}$) followed a similar trend being highest in apples and zero in lemons. These experimental results demonstrate that in lemons, where the capacity for increase was near 0 and the net reproductive rate was 1, the survival of a Medfly population in lemon orchards would be very low. In contrast, the other varieties of fruit would sustain viable populations.

Trial 3: Field trials

Diurnal temperatures varied from 14 – 29°C in summer and from 3 – 21°C in winter. The numbers of pupae obtained in both treatment and control replicates were very low. The total number of pupae obtained in 12 paired comparisons were: oranges (9) and plums (14); mandarins (9) and oranges (0); lemons (0) and oranges (0). The data were inconclusive for further analysis.

DISCUSSION

The difference in the total number of eggs laid in each fruit over the 4 day trial clearly indicated that the four fruits were not equally attractive to Medfly (Jenkins & Shedley 1956; Krainacker *et al.* 1987). Spitler *et al.* (1984) observed that most lemon varieties appeared to be almost immune to attack, unless overripe or suffering from physical damage. This observation was also acknowledged by Papaj (1993) and Katsoyannos *et al.* (1997). The current experiment showed that lemons were least attractive to Medfly out of the four test fruits, and there were significantly lower infestation levels in lemons when compared to the control, regardless of puncture and choice conditions.

A likely reason for the difference in infestation between fruits is skin thickness and penetrability. Rull and Prokopy (2004) demonstrated that tephritid fruit flies are sometimes unable to pierce through tough skin. Skin penetrability was lowest in lemons, which may explain why females did not favour them as a target for infestation. The sugar content, which was lowest in lemons and highest in oranges, may also account for the difference in

infestation levels obtained in lemons and oranges. Bodenheimer (1951) showed that fruits with a high sugar content are more susceptible to Medfly attack.

Oranges were found to be the most suitable citrus host out of the three test cultivars in terms of the numbers of female offspring produced at the end of the cycle. The net reproductive rate (Ro) was highest in oranges, followed by mandarins. Lemons had a value of 1, indicating a static size of each generation of a Medfly population developing in lemons. Furthermore, oranges had the highest value of $\mathbf{r_c}$ followed by mandarins and lemons were zero, showing that oranges are the most preferred and lemons the least preferred citrus host.

This study demonstrated that lemons are a very poor Medfly host, and provides a good explanation for reducing the length of cold-treatment exposure periods required to disinfest lemons (De Lima *et al.* 2007). Research on Medfly developmental biology in different fruit species may be used to optimise control measures and overcome quarantine barriers, thus enhancing the fruit export market. Further research using the intrinsic rate of natural increase in Medfly populations with respect to known limiting factors such as temperature and moisture (Eskafi & Fernandez 1990), may facilitate the determination of the limits of Medfly's ecological range and thereby help towards controlling its spread in the wild (Carey 1982, Krebs 1994).

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Table 1: Total number of eggs laid by 30 females in test and control fruit over four days.

Test fruit	Mean Weight (g)	Total number of eggs laid by 30 females	Proportion of total eggs laid
Eureka lemons	130.8	226	0.031
Imperial mandarins	94.4	1060	0.146
Valencia oranges	293.8	2172	0.298
Golden Delicious apples (control)	193.8	3827	0.525
Total		**7285**	**1**

Table 2: Estimated expectation of life for each stage ($\mathbf{e_x}$), the net reproductive rate ($\mathbf{R_o}$) and the capacity for increase ($\mathbf{r_c}$) of Medfly in test citrus fruits and control (apples).

Medfly life stage	**Test citrus fruit and control**			
	mandarins ($\mathbf{e_x}$)	**oranges** ($\mathbf{e_x}$)	**lemons** ($\mathbf{e_x}$)	**apples** ($\mathbf{e_x}$)
Eggs	0.86	0.78	0.96	0.88
Early larvae	2.27	2.22	1.06	1.75
Late larvae	1.55	0.74	0.85	0.8
Pupae	0.9	1.5	0.75	1.2
Adults	0.5	0.5	0.5	0.5
$\mathbf{R_o}$	70	160	1	324
$\mathbf{r_c}$	0.123	0.147	0	0.167