

# Field evaluation of Mediterranean fruit fly mass trapping with Tripack® as alternative to malathion bait-spraying in citrus orchards

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## Abstract

The mass trapping technique based on the use of the female-targeted attractant lure Tri-pack® as an alternative to malathion bait-spraying (control treatment) was tested in two citrus orchards in the North of Tunisia against the Mediterranean fruit fly *Ceratitis capitata* during 2006 and 2007. Results of mass trapping trials in 2006 and 2007 indicated that adult males Medfly captures showed reductions respect to control of 37.62% and 40.2% respectively in mandarin orange variety (*Citrus reticulata*) orchard compared to 36.48% and 47.29% in Washington navel orange variety (*Citrus sinensis*) field. Fruit damage assessment showed significant differences between the mass trapping with Tripack® and malathion bait-spraying techniques in the reduction of the percentage of fruit punctures. The percentage of punctured fruit at harvest was significantly different between the treated and the control field in 2006 and in 2007 in the mandarin orange orchard. Nevertheless, in the Washington navel orange orchard, the percentage of punctured fruit at harvest was significantly different between the treated and the control field only in 2006. Thus, results obtained from this study showed that the mass trapping technique based on the use of the female-targeted lure Tri-pack® could be involved as an appropriate strategy for the control of the Medfly and is as effective as malathion bait spraying treatment without leaving pesticide residues on fruit.

**Additional key words:** *Ceratitis capitata*, insecticide, IPM, Medfly.

## Resumen

### Evaluación en campo del trampeo masivo de la mosca mediterránea de la fruta con Tripack® como alternativa a los tratamientos cebo con malatión en huertos de cítricos

Se probó en dos huertos de cítricos, en el norte de Túnez en 2006 y 2007, la técnica del trampeo masivo utilizando el atrayente de hembras Tri-Pack® contra la mosca mediterránea de la fruta, *Ceratitis capitata*, como alternativa a los tratamientos cebo con malatión, que constituye la forma convencional de control utilizada por los agricultores y que se utilizó como tratamiento control. En 2006 y 2007, en los ensayos de trampeo masivo, las capturas de machos adultos del insecto se redujeron respecto el control, respectivamente, un 37,62% y un 40,2% en la variedad de mandarina (*Citrus reticulata*) en comparación con un 36,48% y un 47,29% en la variedad de naranja Washington navel (*Citrus sinensis*). Al evaluar los daños en los frutos se observaron diferencias significativas entre el trampeo masivo con Tripack® y los tratamientos cebo con malatión con una reducción de las picaduras en los frutos El porcentaje de frutos con picaduras en el momento de la cosecha fue significativamente diferente en las parcelas control y tratada en el huerto de mandarina tanto en 2006 como en 2007. Sin embargo, en el huerto de naranja Washington navel, el porcentaje de frutos con picaduras recolectados fue significativamente diferente entre el tratado y el control sólo en 2006. Por tanto, los resultados obtenidos en este estudio muestran que la técnica de trampeo masivo utilizando el atrayente de hembras Tri-Pack® podría ser una estrategia adecuada para el control de la mosca mediterránea de la fruta y es tan efectiva como el tratamiento cebo con malatión, pero sin dejar residuos de plaguicidas en la fruta.

**Palabras clave adicionales:** *Ceratitis capitata*, insecticida, IPM.

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## Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedeman), is one of the most devastating pests of fruits and vegetables worldwide (Liquido *et al.*, 1997; Chueca *et al.*, 2007) and it is considered the most invasive of all members of the Tephritidae (Zucchi, 2001).

In Tunisia, citrus are among the basic sector of economy. Indeed, it represents 9.45% of fruit production value. The production has increased from 16,321 tonnes in 2007 to 23,217 tonnes in 2009 (GIFruits, 2009). The Tunisian citrus orchards show several varieties with considerable value such as Maltais, Clementine, Navels Oranges, Tangerines, Lemons, Sweet Oranges, Oranges Valencia, etc. (GIFruits, 2009). Medfly is one of the key pests on citrus. Current Tunisian control program is mainly based on applications of organophosphate insecticides, especially malathion mixed with protein baits (Bachrouch, 2003). However, the intensity of insecticide treatments with malathion has resulted in the development of resistant populations (Gahbiche, 1993; Fellah, 1996; Bachrouch, 2003). Moreover, the use of malathion is controversial worldwide because of human health concerns (Flessel *et al.*, 1993; Marty *et al.*, 1994) and the harmful effects on beneficial insects, activity and survival of natural enemies and non-target organisms (Troetschler, 1983; Daane *et al.*, 1990; Hoelmer and Dahlsten, 1993; Urbaneja *et al.*, 2004) consequently it has been banned from annex I of the European Union (EU) directive 91/414/EEC (MAPA, 2009). Therefore, research and development of effective control methods as alternative to chemical control are needed especially because of Tunisian oranges are mainly exported to EU market (GIFruits, 2006). These methods could include the use of traps baited with the female-targeted and male-targeted lures (Papadopoulos *et al.*, 2001; Broughton and Francis de Lima, 2002; Heath *et al.*, 2004; Tóth *et al.*, 2004). For Medfly, *C. capitata*, ammonium carbonate has long been known to attract females (Gothilf and Levin, 1989; Rynolds and Prokopy, 1997). Later on, an effective female-targeted trapping system consisting of a McPhail trap baited with three food-based, synergistically acting attractants (ammonium acetate AA, putrescine PT and trimethylamine TMA) was developed (Heath *et al.*, 1997; Katsouyannis *et al.*, 1999a,b). This synthetic food lure is more specific than the liquid protein baits, able to detect female Medflies at a lower level and is being used in early detection trapping networks (Anonymous, 2003). Moreover, several studies have demonstrated and con-

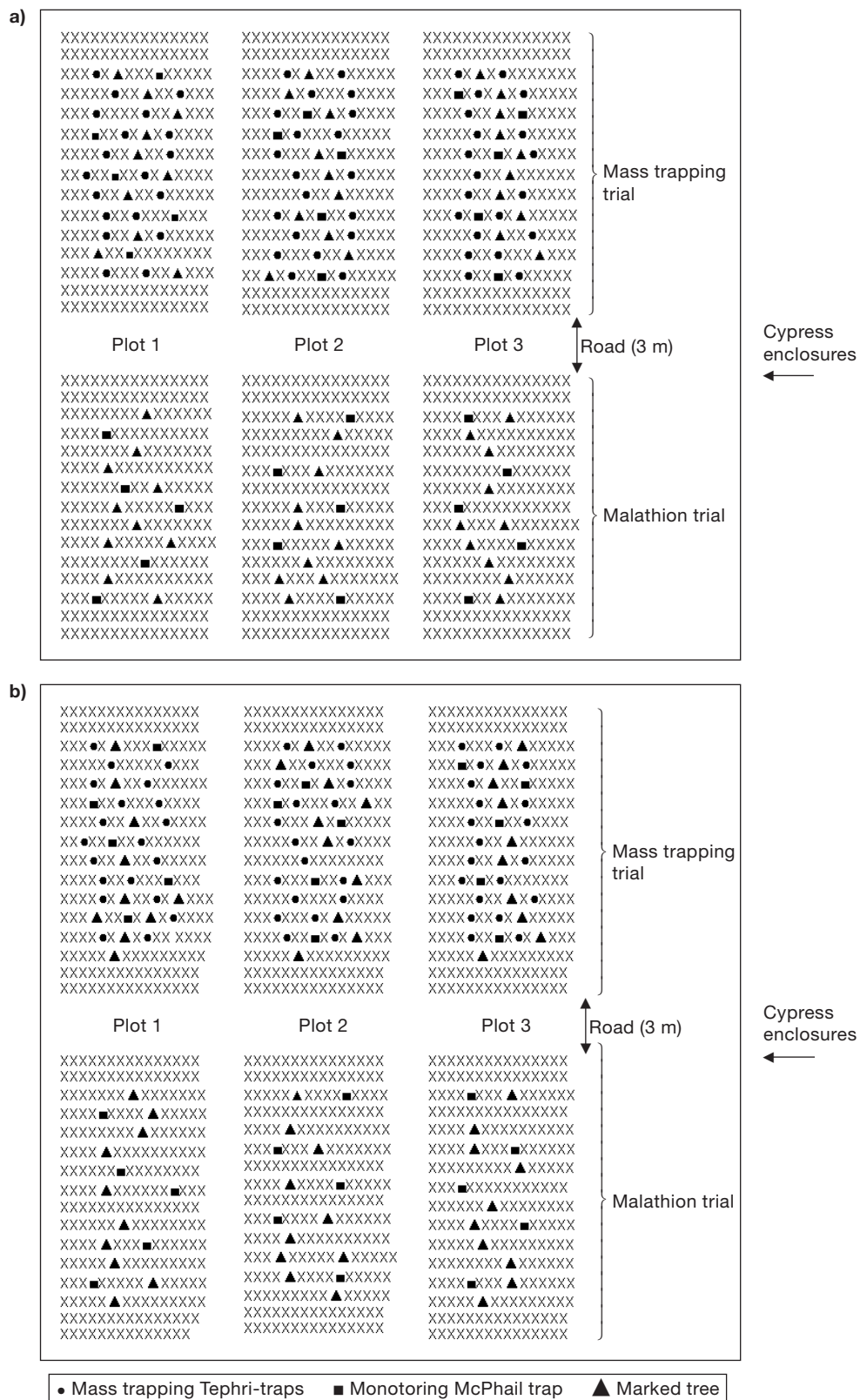
firmed the highly selectiveness and effectiveness of the combinations of several synthetic food attractants based on AA, PT and TMA for Medfly females capture (Heath *et al.*, 1997; Epsky *et al.*, 1999; Miranda *et al.*, 2001; Alemany *et al.*, 2004). Above the three compounds cited, several others were used in female attractant: cadaverine and n-methyl pyrrolidine (Navarro-Llopis *et al.*, 2008). Moreover, attractants of female Medfly were marketed under different trade names (Biolure, Biolure Medfly 100, TMA, SEDQ, Trypack and Tri-pack) (Navarro-Llopis *et al.*, 2008). Thus, female-targeted and male-targeted lures could be included as a component of an integrated pest management program (IPM) using the mass trapping technique. Indeed, the mass trapping technique has proven to be a powerful weapon in the control of *C. capitata*, and its application in Mediterranean countries has currently increased notably as a control method (Navarro-Llopis *et al.*, 2008).

The present work aims to assess the mass trapping technique based on the use of female food-attractant lure Tri-pack<sup>®</sup> as alternative to chemical control with malathion bait spraying in mandarin and Washington navel orange orchards.

## Material and methods

### Experimental fields

Trials were led in two citrus fields located in the north of Tunisia. The first experimental field located in Cap Bon area (Lat 36°45'18.45" N; Long 10°33'37.67"E; altitude 50 m) had 6 ha area planted with mandarin orange variety (*Citrus reticulata*). Trees spacing was 7 × 7 m with a density of 225 trees h<sup>-1</sup> (Fig. 1a). The field was divided in two parts. Each part contained three plots of 1 ha presenting three replicates. For mass trapping trial, within each plot Tephri-traps were separated by almost 25 m. Tree plots were separated by almost 10 m to obtain three independent replications (Fig. 1a). The second citrus field was located in the same area and had also 6 ha of area planted with Washington navel orange variety (*Citrus sinensis*). Tree spacing was 6 × 6 m with a density of 256 trees ha<sup>-1</sup> (Fig. 1b). This field was divided in two parts separated by cypress enclosures. The mass trapping trial area was divided on three plots of 1 ha each separated by almost 20 m. Tephri-traps were separated by almost 20 m to avoid interference. The two fields were separated by almost 5 km. Experimental tests were performed from



**Figure 1.** Design of the trial: location of mass trapping Tephri-traps, monitoring McPhail traps and marked trees for puncture survey in a) mandarin orange orchard and b) Washington navel orange orchard

September 20 to November 13, 2006 and from September 18 to November 20, 2007 for mandarin orange orchard whereas for Washington navel orange orchard, trials were performed from October 16 to December 13, 2006 and from October 16 to December 5, 2007. For both fields, malathion was used at the dose of 500 mL ha<sup>-1</sup> each 10 days.

### Traps, attractant and insect monitoring

*C. capitata* males were monitored by five McPhail traps baited with the parapheromone Trimedlure (TML, Agrisens-BCS Ltd) and malathion on moistened rolls of cotton wool as insecticide.

The mass trapping experiments were performed using Tephri-traps<sup>®</sup> (Sorygar, Madrid, Spain) baited with synthetic female-targeted food attractant lure marketed as Tripack<sup>®</sup> (5 mg a.i. ammonium acetate, 50 mg a.i. putrescine, and 2.50 mg a.i. trimethylamine; Kenogard SA, Barcelona, Spain) together with a tablet of dichlorvos (0.5 mg a.i. dimethyl 2,2-dichlorovinyl phosphate DDVP (Biagro, SLL, Valencia, Spain) as insecticide. Dichlorvos was the commonly used pesticide in Medfly traps due to its volatility, but nowadays other alternatives must be implemented because it has been withdrawn from annex I of the EU directive 91/414/EEC (MAPA, 2009).

For the mass trapping trial, a trap density-setting experiment was performed in 2005 in order to compare the performance of three trap grid densities (10, 20, 30 traps ha<sup>-1</sup>) in capturing adult males and females. Trials were conducted from September 11 to December 13, 2005 for mandarin orange orchard and from October 10 to December 5, 2005 for Washington navel orange orchard. Traps were hung in the trees facing south at a height of 1.5 m and the caught insects were counted weekly.

**Table 1.** Effects of different trap densities (trap ha<sup>-1</sup>) on the number of *C. capitata* captures (mean ± SE) per trap and per week in two different orchards in 2005

	Mandarin orange			Washington navel orange		
	10	20	30	10	20	30
Females	14.06 ± 1.45 <sup>c</sup>	44.87 ± 3.05 <sup>a</sup>	32.14 ± 1.81 <sup>b</sup>	41.10 ± 4.71 <sup>c</sup>	69.03 ± 5.72 <sup>a</sup>	63.41 ± 5.99 <sup>b</sup>
Males	5.95 ± 0.77 <sup>c</sup>	18.99 ± 0.98 <sup>a</sup>	13.86 ± 0.60 <sup>b</sup>	17.66 ± 1.78 <sup>c</sup>	29.62 ± 2.65 <sup>a</sup>	21.74 ± 2.39 <sup>b</sup>
Total	20.01 ± 2.5 <sup>c</sup>	63.86 ± 1.9 <sup>a</sup>	46.00 ± 1.50 <sup>b</sup>	58.77 ± 1.50 <sup>c</sup>	98.65 ± 2.20 <sup>a</sup>	85.15 ± 3.10 <sup>b</sup>

For each orange variety within columns comparisons were made between means of females, males and total captures for the three trap densities. Values followed by the same letter are not significantly different at  $P = 0.05$  by ANOVA).

### Fruit damage assessment

In order to determine the percentage of fruit damage due to the Medfly, 10 trees were randomly selected per mass trapping and malathion treated orchards. On each tree every fruit was weekly checked for Medfly punctures and the infested ones were marketed to be recognized in the subsequent examination.

### Statistical analyses

Reductions in the mean number of flies per trap at the different densities, the weekly male and female captures in the two orchards in the different years and the percentage of punctured fruits were analyzed by one-way analysis of variance using Statistica (Statsoft, 1998). A Duncan test was applied to the means to detect significant differences at the 0.05 percent level. Data are presented in tables as means with standard errors.

## Results

### Mass trapping grid densities setting

Results related to mass trapping grid densities setting are shown in Table 1. Significant differences between different trap densities were detected in both orchards. Mean numbers of total insects captured at 10, 20 and 30 traps ha<sup>-1</sup> were lower in the mandarin orange orchard (20.01, 63.86 and 46.00) than in the Washington navel one (58.77, 98.65 and 85.15). The highest captures not only in total number of insects but also in numbers caught of the different sexes, were always recorded at 20 traps ha<sup>-1</sup>. Consequently, this density was selected for mass trapping trials in 2006 and 2007.

### Efficacy of Tri-pack® in attracting Medfly females

Table 2 shows the captures of *C. capitata* males and females in the two orchards, using Tephri-traps® baited with Tripack®.

The total number of insects caught in the mandarin orange orchard (63.68 and 67.77) was lower the two years than those of the Washington one (87.66 and 89.43). In both orchards, the percentage of females caught was significantly higher than that of males (73.70 and 68.79% in the mandarin orchard; 72.26 and 72.30% in the Washington orchard, for 2006 and 2007 respectively). Consequently, the lure Tripack® could be used for mass trapping programs.

### Tri-pack® effect on Medfly population

The weekly Medfly male captures using McPhail traps are given in Figure 2. Significant differences were achieved between Medfly captures of mass trapping and malathion bait spraying plots in the two orchards for both years. Reduction rates of 37.62% and 40.2% were obtained in 2006 and 2007 respectively in mandarin orange field (Fig. 2a). In Washington navel orange orchard, a reduction rate of 36.48% was obtained in 2006. For the year 2007, a reduction rate of 47.29% was recorded (Fig. 2b).

### Tri-pack® effect on Medfly population at harvest

Table 3 shows the effect of mass trapping with Tripack® and malathion spray on fruit damage at harvest.

Results revealed that the percentage of punctured fruit at harvest is significantly different between mass trapping and malathion bait spraying treatments in both fields. Mean percentages of punctured fruit were lower in the mass trapping plot (23.32% and 19.68%; 31.99% and 25.01%) than in malathion bait spraying one (31.39% and 28.11%; 37.00% and 27.01) in mandarin and Washington orchards for 2006 and 2007 respectively.

## Discussion

Mass trapping is currently being used over larger areas in Mediterranean regions to control Mediterranean fruit fly, *Ceratitis capitata* (Wiedmann), and olive fruit fly, *Bactrocera olea* (Gmelin) (Delrio, 1989; Broumas *et al.*, 2002). Cohen and Yuval (2000) pointed out that the perimeter trapping strategy has obtained satisfactory results to avoid fruit fly intrusions in medium-to-large orchards, and this strategy depends on the efficacy of traps and lures.

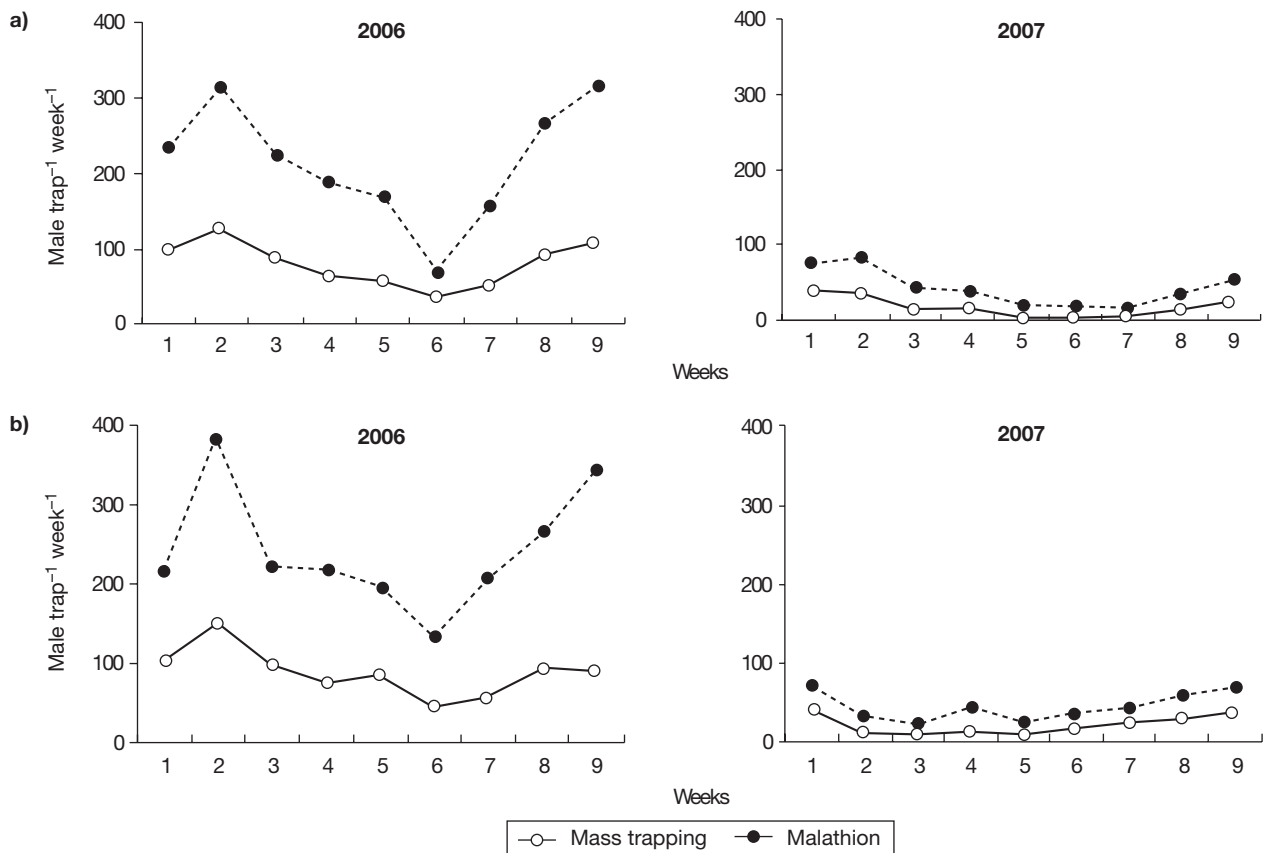
The results of this research underline the crucial role of the mass trapping technique on the reduction of Mediterranean fruit fly populations in citrus orchards as reported by Cunningham *et al.* (1978), Agunloye (1987) and McQuate *et al.* (2005).

Field trials using Tephri-traps® baited with the three food lures Tri-pack® (ammonium acetate, putrescine and trimethylamine) at the density of 20 trap ha<sup>-1</sup> outperformed the malathion bait spraying in reduction of insect population and decrease of fruit damage. Moreover, the mass trapping technique obtained high female captures in mandarin orange (73.70%, 68.79% respectively in 2006 and 2007) and Washington navel orange orchards (72.26%, 72.30% respectively in 2006 and 2007). These data confirm the reduction of fruit punc-

**Table 2.** Number of flies (mean ± SE) caught per week in two orchards using Tephri-traps® baited with Tripack® in 2006 and 2007

	Mandarin orange		Washington navel orange	
	2006	2007	2006	2007
Males	18.98 ± 1.09 <sup>a</sup>	20.46 ± 0.83 <sup>a</sup>	26.28 ± 0.74 <sup>b</sup>	26.77 ± 0.69 <sup>b</sup>
Females	44.78 ± 1.77 <sup>b</sup>	47.32 ± 1.56 <sup>b</sup>	61.38 ± 1.64 <sup>a</sup>	62.66 ± 1.7 <sup>a</sup>
Total	63.68 ± 2.31	67.77 ± 2.30	87.66 ± 2.31	89.43 ± 2.32
% females	73.70 ± 1.9	68.79 ± 1.28	72.26 ± 0.48	72.30 ± 0.5

Within rows, comparisons were made between respectively mean numbers of males and females found in traps. Values followed by the same letter are not significantly different at  $P = 0.05$  by ANOVA.



**Figure 2.** Weekly captures of male *Ceratitis capitata* in traps baited with Trimedlure in mass trapping and malathion bait spraying treatments in a) mandarin orange orchard and b) Washington navel orange orchard.

tures. Similar field trials conducted in Western Australia using female-targeted attractant BioLure showed that more females were caught in traps irrespective of trap type, climate, host tree, or population level (Broughton and De Lima, 2002). Besides, Miranda *et al.* (2001) reported that the use of Tephri-trap baited with Biolure for female mass trapping is recommended to control *C. capitata* in Spain.

Many studies demonstrated that the efficacy of trapping using female targeted attractant depends on trap types. Indeed, Gazit *et al.* (1998) indicated that trap type modifies the proportion of females caught. Navarro-Llopis *et al.* (2008) reported that it is better to use trap that achieves higher captures and better female proportions. Thus, the proportion of female catches is obviously influenced by attractant type, and it has been

**Table 3.** Effect of the pest control technique (mass-trapping and malathion spray) on the percentage of damaged fruits (mean ± SE) at harvesting in two orchards in 2006 and 2007

	Mandarin orange		Washington navel orange	
	2006	2007	2006	2007
Mass trapping	23.32 <sup>b</sup>	19.68 <sup>b</sup>	31.99 <sup>b</sup>	25.01 <sup>a</sup>
%	(104 ± 2.91 <sup>b</sup> )	(80.6 ± 2.07 <sup>b</sup> )	(181.4 ± 9.6 <sup>b</sup> )	(116.8 ± 1.64 <sup>a</sup> )
Malathion treated	31.89 <sup>a</sup>	28.11 <sup>a</sup>	37 <sup>a</sup>	27.01 <sup>a</sup>
%	(174.8 ± 2.39 <sup>a</sup> )	(141.2 ± 1.92 <sup>a</sup> )	(207.2 ± 8.7 <sup>a</sup> )	(129.4 ± 2.3 <sup>a</sup> )

Within rows, comparisons were made between respectively percentages and mean number punctured fruits in mass trapping and malathion treated for each variety. Values followed by the same letter are not significantly different at  $P=0.05$  by ANOVA.

shown that trimethylamine was the best attractant when used with ammonium acetate with or without putrescine (Heath *et al.*, 2004). The dispenser emitting the most ammonium acetate and trimethylamine is the best for insect catches (Navarro-Llopis *et al.*, 2008). This appears to support results of Tóth *et al.* (2007), who also reported that good *C. capitata* catches were achieved with baits lacking putrescine suggesting that putrescine can be left out from female-targeted lure combinations without dramatic change in activity.

In Greece, Katsoyannos and Papadopoulos (2004) demonstrated that yellow spheres traps baited internally or externally with female food attractant «Biolure» were 30 and 12 times more attractive for females and males respectively than unbaited spheres. Moreover, field trials conducted in several countries showed that traps baited with the three component attractants captured equal or greater numbers of females than the McPhail-type traps baited with an aqueous solution of protein hydrolysate NuLure and borax as preservative (Epsky *et al.*, 1999; Katsoyannos *et al.*, 1999a,b).

In addition, Broughton and De Lima (2002) reported that the synthetic female attractant is recommended for replacement of protein hydrolysate lures and may be used in either Tephri or McPhail traps in population monitoring and detection of *C. capitata*. Besides, Tóth *et al.* (2004) suggested that it is advisable to use both male- and female-targeted baits in separate and distant traps and not jointly in the same trap because the efficacy of detection or monitoring trials will be compromised.

Regarding trap types, our results are in accordance with those obtained by Miranda *et al.* (2001) who indicated that Tephri-traps could be used in *C. capitata* mass trapping. In contrast, Navarro-Llopis *et al.* (2008) reported that Tephri-traps should be avoided for female mass trapping because it captured significant fewer females than other traps, and in this technique females are the main objective. This difference could be attributed to the attractants compositions of the lures.

In conclusion, since citrus fruits are an important and valuable export commodity for Tunisia, the use of female Medfly food-bait lure is of particular interest in monitoring, detecting and control programs where high infestation rates with this pest are causing significant economic losses presenting 30 to 35% of the total quantity of fruit received in packaging units (Driouchi, 1990).

Results obtained from this study showed that the mass trapping technique based on the use of the female-

targeted lure Tri-pack® could be involved as an appropriate strategy for the control of the Medfly in Tunisia. It could be integrated with some of these existing methods in an IPM approach because it is as effective as malathion bait spraying treatment without leaving pesticide residues on fruit. Nevertheless, an economic evaluation of the cost of this technique should be undertaken especially when such method will be introduced at farmer's scale.

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