ISPEC Tarım Bilimleri Dergisi
2020 : 4(3)

Araştırma Makalesi

E-ISSN:2717-7238
www.ispecjournal.com

ISPEC Journal of Agr. Sciences
2020 : 4(3)

Research Article

Effectiveness of Sterile Insect Technique for Medfly (*Ceratitis capitata*, Wiedemann, 1824) Control in Citrus Orchards of Moulouya Perimeter North East of Morocco

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DOI
https://doi.org/10.46291/ISPECJASvol4iss3pp405-421

Geliş Tarihi: 01/06/2020
Kabul Tarihi: 15/07/2020

Keywords
Integrated pest management, medfly, sterile ınsect technique, density, citrus orchards.

Abstract

The Sterile Insect Technique (SIT) is an effective pest control method developed worldwide against many species of Fruit Flies. It involves the release of male insects sterilized, at pupae instar, by exposure to ionizing radiation. The Area-wide control of medfly (*Ceratitis capitata*), with SIT based on sterile males release combined to other control techniques, was implemented since 2017 as a pilot area in Moulouya Perimeter in North-eastern Morocco. The main objective of this study is to evaluate the effectiveness of this tool in controlling Medfly and estimate sterile male population densities from data collected from trap catches after the release of a pre-determined number of sterile males in five Citrus orchards (0, 500, 1000 and 3000 sterile males per hectare) and the calculation of FTD (fly/trap/day) of sterile and wild males. The result showed that the percentage of recaptured males and FTD Sterile indices were related to release density by power function regression, while the sterile to wild ratio and release density were linear regression-adjusted. The finding confirmed the effectiveness of release in reducing the fly population in the TIS area by reducing the rate of citrus infestation in field and export shipments.

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INTRODUCTION

The Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann); Diptera: Tephritidae) (hereafter medfly) is one of the most destructive pest insects throughout the world. It causes a direct damage to a wide range of high-value fruit and vegetable crops, resulting in important yield losses and deterioration in the quality of the fruits. Also, countries with the presence of this pest are subject to quarantine restrictions on their exports to countries with "Medfly-free" status (Gutiérrez Samperio, 1976; Liquido et al., 2013). The SIT was first developed in the 1930s and implemented in the 1950s (Knipling, 1955). This control technique, that involves the use of radiation to sterilize insects, was first adopted in the United States and is now successfully applied worldwide (Spain, Australia, Tunisia, Argentina, Mauritius) against fruit flies and other insect pests (Klassen and Curtis, 2005; Vargas, 2008; FAO/IAEA, 2017). The SIT is adopted as a key component of area-wide integrated pest management (AW-IPM) programs for the eradication or suppression of fruit flies (Ortíz, 1999). Because of its autocidal action, reducing the use of insecticides, SIT is perceived as an environmentally friendly approach, which has led to its increased uptake worldwide (Shelly and Kennelly, 2002; Enkerlin, 2005; Shelly and McInnis, 2003; Shelly, 2005; Shelly, 2012). In order to set the release densities of sterile males, in a SIT programme, in areas with high Mediterranean fly infestation and to obtain a high level of efficiency, it is important, first of all, to determine the levels of the wild population through the use of a trapping system (FAO/IAEA, 2017) or through models (Barclay, 2005). The purpose of the SIT tool is to induce sterility in wild populations (Hendrichs et al., 2002). Its effectiveness is linked to the reaching of an optimal ratio between sterile males and wild males (Knipling, 1959; Shelly and McInnis, 2016; Zavala-Lopez and Enkerlin, 2016). The quality and ability of mass-reared and sterilized males to mate with wild females is a key factor in the effectiveness of SIT (Rull et al., 2005; Orozco-Davila et al., 2007). An accurate estimation of the density of wild populations is important as an indicator to determine the density of sterile insects required in the release (Rendón et al., 2004; Orozco et al., 2013; Flores et al., 2014). In 2016, the United States Phytosanitary Authorities, Animal and Plant Inspection Service (APHIS) banned the importation of Citrus fruits from the whole Morocco

followed by the ban of these commodities only from Berkane Area (APHIS, 2016). Moroccan Phytosanitary Authorities (ONSSA, National Office of Food Safety) implemented a phytosanitary management strategy and a work plan against medfly to mitigate the risk of re-interception in the USA of live medfly larvae in Citrus consignment from Berkane. The adoption of SIT as a technique to control medfly in Citrus orchards in the Moulouya Perimeter (Berkane) is the main element of this strategy (APHIS, 2016). The study aimed to estimate the variations in Medfly populations in traps after releasing fixed numbers of adult sterile males in citrus orchards. The main parameters to evaluate the performance of the SIT technique in the field and the effectiveness of control are the ratio of sterile males to wild males and fruit infestation in citrus orchards in the SIT area to decide on the recommended release densities.

MATERIAL AND METHODS

Study Site

The study was conducted in five Citrus orchards with Clementine variety, located in two protected areas as SIT-AW-IPM (34°59'22.833" N 2°23'16.5693" W) of Moulouya perimeter, near to Berkane city covering an area of 1293ha. The mean altitude of this area is 130 m above sea level with a typical Mediterranean climate and an annual rainfall of 300 mm. With an average of 26.5 C°, August is the hottest month and with an average of 11.3 C°, January is the coldest month of the year (BMH, 2018).

Biological Material

Sterile mass-reared adults of *Ceratitis capitata* were obtained, in the pupal stage, from the material imported into Morocco from Portugal and Argentina, where this species was mass-reared according to the procedures described by Domínguez et al., (2010). This method is totally based on irradiated pupae packed in paper bags placed in holding rooms in Agropolis of Berkane (35°1'57.713" N, 2°20'59.377" W). Sterile males are allowed to fully emerge and achieve sexual maturity, for approximately 6-7 days in climatic conditions that ensured 60±5% of Relative Humidity (RH) and Temperature of 24±2C° and were fed with a mixture of sugar and hydrolyzed yeast (4:1). As recommended by Shelly et al (2007), Soopaya, (2013), Juan-Blasco et al (2013) Pérez-Staples et al (2013), and Steiner et al (2013), 24 hours before the release, water and Ginger root oil (*Zingiber officinalis*) were supplied in bags with cotton wicks to improve mating propensity, encourage sexual activity and

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enhance flight ability of sterile males in the fields. After sexual maturity, the sterile male flies were released by a ground mechanism. Every two weeks, about 225000 sterilized emerged adults were released throughout the studied orchards. Sterile males were marked with a fluorescent dye spot to be distinguished from fertile males. Captured flies per each trap were placed in 100 cc plastic containers with 70% alcohol solution and transferred to the laboratory of Agropolis where they will be observed under binocular loupe and a UV lamp in a dark room. Captured flies were checked to separate marked sterile released from the wild flies and to provide information on the insect population levels in the studied area as described by Enkerlin et al., 1996.

Trapping system program

To recapture released sterile males, a trapping network was set in 2018. All traps were being geo-referenced, identified. On a two-weekly basis all traps were controlled over the experimental blocs. The type of trap used was the sexual trap "Maghreb med" (trimedlure parapheromoun as attractant and dichlorvos insecticide chip as toxicant). One trap, per plot, was hung in a tree at 10 m away from the release point. Traps were placed one day after the release of sterile insects and were inspected 14 days after each release. One day before the next release, the traps were removed from the orchard to avoid interference with the dispersal of the next group of released flies.

Relationship between fly densities and FTD of sterile males

Estimation of the relationship between sterile fly densities and trap catches was carried out based on the release-recapture methodology (Bloem et al., 1994, Hernández et al., 2007). The trial was carried out with four release densities, i.e. 0, 500, 1000 and 3000 sterile males per hectare ("0" density was discarded prior to data analysis). The five studied orchards were divided into four plots of 2.5 ha each. A 2 by 2 plot layout was set up for each orchard, with a distance of 100 m between plots to avoid any interference. Every plot was considered as an experimental unit, in which one of the four treatments (including "0" density release as a witness) was tested. Every orchard was a replicate. In the center of each plot, sterile flies were released every two weeks from early June to late October (after the fruiting and ripening season). Ten releases were carried out, rotating the different densities randomly among plots of each orchard. All orchards were managed in the same way (chemical sprays, sanitation,

fertilization, irrigation, etc.). For each release and modality of treatment, the number of recaptured flies has been transformed to FTD and the relationship between this index and the number of released sterile flies was analyzed. Trap efficiency was calculated as the proportion of adults recaptured from the number of sterile flies released in each plot. The trapping data were presented as the percentage of sterile males recaptured after release, flies per trap per day for wild males (FTD_{Wild}) and sterile males ($FTD_{Sterile}$) for each release density applied. As described by Jessup et al (2007), the indices were calculated as follows:

$$\text{FTD wild} = \frac{\text{Total captured wild flies}}{(\text{total number of traps}) * (\text{Number of days in the field})}$$

$$\text{FTD Sterile} = \frac{\text{Total recaptured sterile flies}}{(\text{total number of traps}) * (\text{Number of days in the field})}$$

Sterile to wild males' ratio

The index "sterile to wild medfly male's ratio" (Ratio (S:W) was calculated to determine the level of sterile males competing with the wild males for mating with wild females in the field. This ratio is given by dividing $FTD_{Sterile}$ by the FTD_{Wild}, obtained with the semi-weekly collecting data from trapping applied in the SIT (AW-IPM) to measure the evolution of over-flooding ratio (FAO/IAEA, 2018):

$$\text{Ratio (S:W)} = \frac{\text{FTD Sterile}}{\text{FTD wild}}$$

Control of export shipments and rate fruit infestation in orchards under SIT

In the field, a routine fruit observation was conducted in regular basis to evaluate the evolution of fruit infestation and the efficacy of the SIT methods by monitoring marked fruits for punctures (3 trees per plot and 80 fruits per tree). In the packinghouse, Citrus fruits from the SIT area were checked for medfly punctures presence in the export consignments. Approximately 20 samples were taken from the export shipments. The sample consists of 250 fruits that are individually placed in plastic containers with 1cm of sand in deep used as pupation media, for maturation and the infestation is monitored and assessed. All these operations were conducted in the fruit holding room of Agropolis Laboratory in Berkane city, with a controlled environment

to accelerate the development of the larvae in the fruit to get precise information in a relatively short period of time (Bjeliš, 2007; Bjeliš et al., 2013). At last, the relationship between release densities and fruit infestation was assessed.

Data and statistical analysis

Release densities were compared using General Linear Model Univariate (GLM) with post hoc HSD tukeyab test (Honestly significant difference) with $P \leq 0.05$. To assess the relationship between the percentage recapture and the release density and the relationship between FTD indices and release densities a multiple regression test with P = 0.01 was performed. All data statistical analyses were performed using the SPSS.25 software and Excel Microsoft.

RESULTS AND DISCUSSION

Relationship between the release density and FTD of sterile males

As expected, our results indicated significant differences between the three release densities (Tukey^{ab}'s HSD test, N= 50; α=0.05; Error=0.146, P< 0.01) with averages of 0.51, 1.64 and 2.54 $FTD_{Sterile}$ consecutively for release densities 500; 1000 and 3000 sterile males per hectare. Furthermore, the relationship between the FTD Sterile and the release density of sterile males was fit with a quadratic regression **($FTD_{Sterile}$ = -0.98+3.35E-3*(release density)-7.26E-7*(release density)^2** with (R^2 0.835; F 371.685 ; df 2, 147 ; P 0.05) **(Fig. 2)**. This relationship is specifically applied to Moulouya SIT area-specific experimental conditions. The same results, for other species of fruit flies, have been reported by Readshaw (1982) for *Lucilia cuprina* (Wiedemann) and Flores et al. (2014) for *Anastrepha ludens* (Loew). Several studies showed that to enhance captures, it is recommended to expose males in pre-release to some product (to methyl eugenol, ginger oil,) that increase the flight ability and the mating competitiveness of sterile males of fruit flies and the attractiveness of males to females (McInnis, 2011; Shelly 2016) .

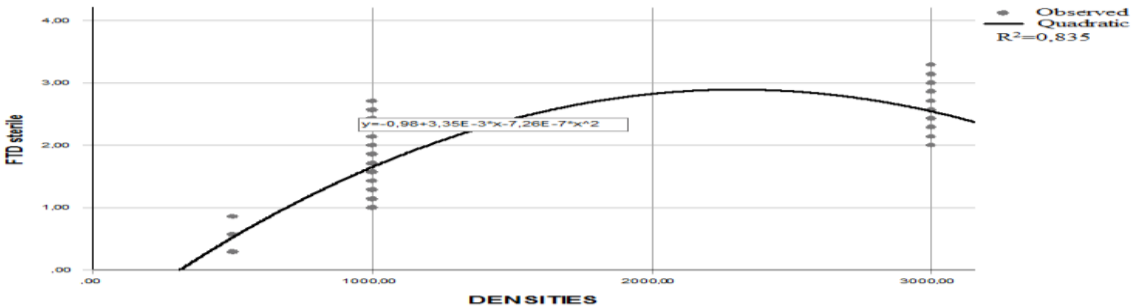

Figure2. Relationship between the release density and FTD of sterile males

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Relationship between the release density and the percentage recaptured of sterile males

The results obtained with Tukey[ab]'s HSD test (N= 50; α=0.05; Error=0.664; P=0.085 >0.05), concluded that the release density of 500 males/ha did not differ significantly from release density of 3000 males/ha, but the density of 1000 males/ha was significantly different from densities of 500 and 3000 males/ha (*p-Value* <0.05). The means of percentage recaptures with densities of 500; 1000 and 3000 males /ha were respectively 1.8379; 2.1840 and 3.4619%. The percentage of recaptured males was related to the release density by a power function quadratic regression (**Percentage of sterile males recaptured = 0.23+4.58E-3*(release density) -1.35E-6*(release density)^2** with (R^2 0.433; F 56.120; df 2,147 ; P 0.05)) **(Fig.3).** The percentage of recaptured males of fruit flies may be affected by other parameters as the trap efficiency, trap type, climatic conditions (Rainfall, RH, Wind, and Temperature) and IPM applied in orchards (Shelly, 2010).

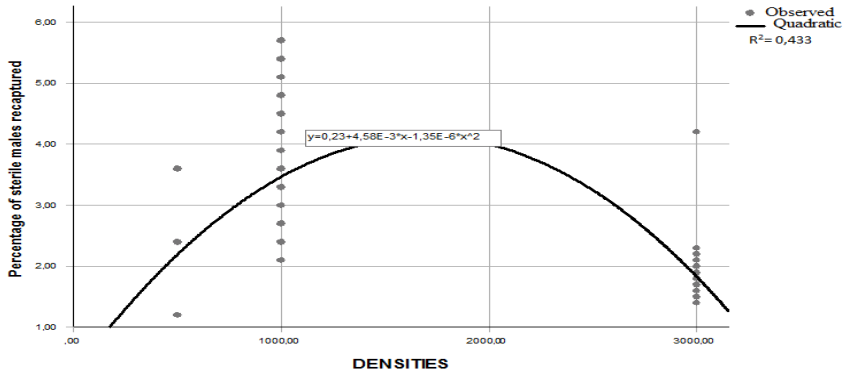

Figure3. Relationship between the release density and Percentage of sterile males recaptured

As expected the evolution of $FTD_{Sterile}$ along of ten releases showed that the release density of 3000 males/ha ensured an average (2.54 FTD) of $FTD_{Sterile}$ higher than those of release densities 1000 and 500 males/ha with consecutive averages of 1.64 and 0.51 FTD. Those FTDs showed small variations between releases and it didn't change along with ten releases **(Fig. 4-A)**. The index sterile to wild ratio of release density 3000 males/ha showed a significant increase between the first release and the last one, while, for the release densities 1000 and 500 males /ha, this index didn't significantly change between the start and the end of releases **(Fig. 4-B)**. This result is explained by the success of over-flooding by sterile males with the release density of

3000 males/ha. This evolution will contribute to protecting fruit at the ripening stage against medfly punctures that occurred to lay eggs in late earlier October. The trap efficiency is represented in the figure (4-C) which showed that the percentage of recaptured sterile males in the release density of 1000 males/ ha was higher than those of 3000 and 500 males/ha. The evolution of this percentage is slightly stable along with the ten releases.

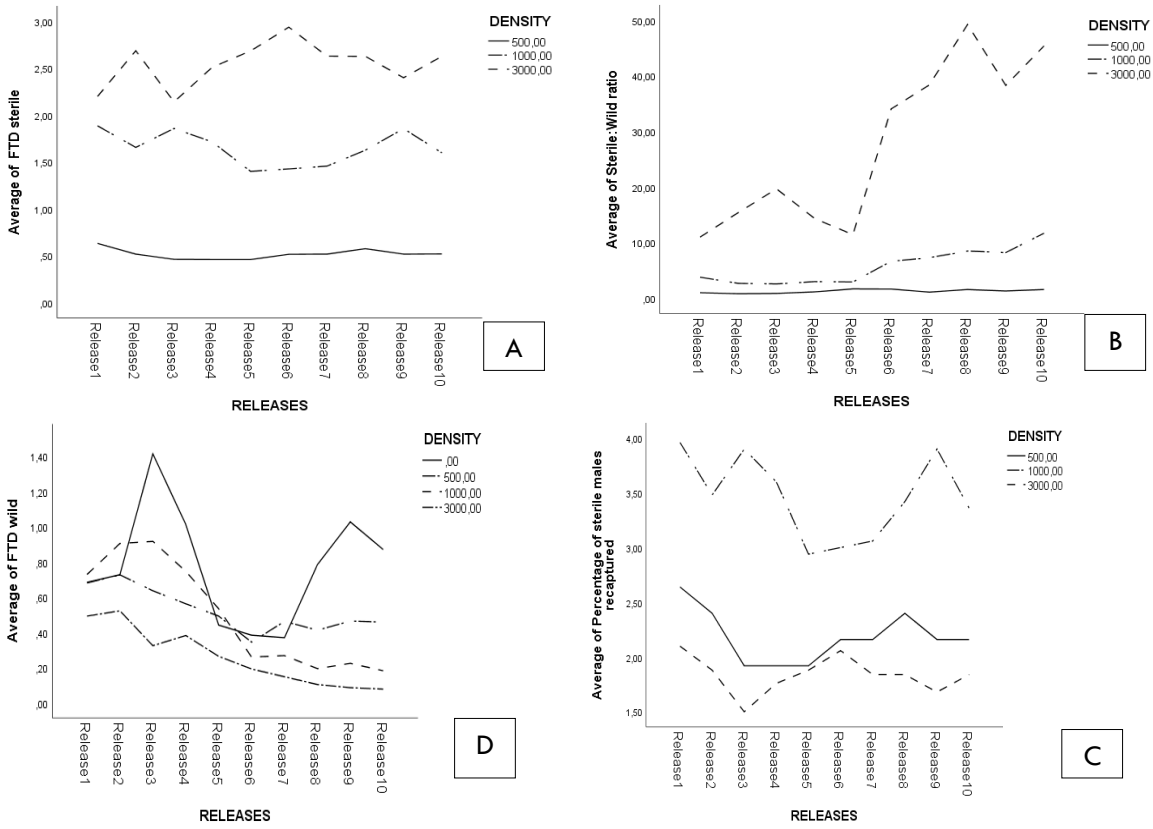

Fig.4: Evolution of averages of FTD $_{Sterile}$ (A), Sterile to wild ratio, and Percentage of sterile males recaptured (C) and FTD $_{Wild}$ (D) for each release density during ten releases.

Relationship between the release density and FTD$_{Wild}$

Figure (4-D) showed that in blocs with males' release (releases densities of 500, 1000 and 3000 males/ha) maintain FTD$_{Wild}$ at low levels at the end of releases with averages consecutively of 0.52, 0.49 and 0.26 FTD. However, in plots with the "0" release density which represents the witness, FTD$_{Wild}$ indices were higher than other plots with an average of 0.77 FTD. The results obtained with Tukey's HSD test

(N= 50; α=0.05; Error=0.07; P=0.000<0.05) showed that the witness with "0" release density is significantly different from all release densities and constituted a subset. The release density 3000 as subset while release densities 1000 and 500 are gathered in the same subset. A multiple regression test was applied on FTD_{Wild} in all release densities including "0" release density. The result showed that there was a negative correlation between the release density and FTD_{Wild} in all orchards under experimentation. Furthermore, the relationship between FTD_{Wild} and the release density was fit using a linear regression **(FTD_{Wild} = 0.68-1.49E-4*(release density)** with (R^2 0.22; F 56.006; df 1, 198; P=0.00< 0.05)) **(Fig. 5)**. This relationship has been specifically applied under Moulouya SIT area-specific experimental conditions.

Table 1. Multiple Comparisons between release densities for Independent variable FTD_{wild}

	(I) DENSITY	(J) DENSITY	Mean Difference (I-J)	Standard Error	Signifi cance	Confidence interval at 95 % Lower limit	Upper limit
Significant Differences of Tukey	0.00	500.00	0.2473*	0.05279	0.000	0.1104	0.3841
		1000.00	0.2753*	0.05279	0.000	0.1384	0.4121
		3000.00	0.5117*	0.05279	0.000	0.3748	0.6485

Calculated means based on observed means; Error = 0.070. And *. The difference is significant at α= 0. 05.

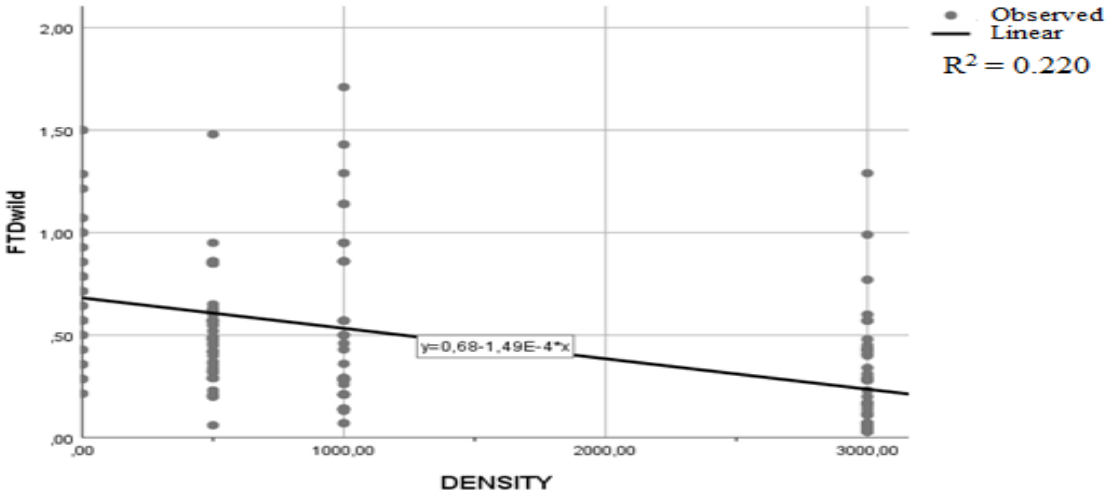

Figure 5. Relationship between the release density and FTD_{Wild}

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Relationship between the release density and Sterile to wild ratio

Result obtained from Tukey's HSD test (N= 50; α=0, 05; Error = 287,732; P<0,001) indicated that there is no significant difference between 500 and 1000 release densities (with means of Ratio $(S: W)_{500}$ = (1,2578 :1) and Ratio$(S:W)_{1000}$ = (5,72:1)) . However, these two densities showed significant differences with 3000 release density with a mean Ratio $(S: W)_{3000}$ = (27,72:1). There was a positive correlation between the release density and the Ratio (S: W) in all orchards of the SIT area. Furthermore, the relationship between the ratio (S: W) and the release density was fit using linear regression **(Ratio(S:W)=-4,49+0,01*(release density)** with (R^2 0,321; F 70, 115 ; df 1. 148 ; P 0.05)) **(Fig. 6)**. This relationship has been specifically applied under Moulouya SIT area-specific experimental conditions. A review of these ratios (Shelly and McInnis, 2016) indicates that tests tend to underestimate the required sterile-to-wild ratio in the field, and that, in most cases, ratios exceeding 50:1 are required to achieve some degree of suppression. Under open field conditions, these (S: W) ratios can also be affected by differences in the courtship performances of sterile and wild flies (Perez Staples et al., 2013), or by mismatching the dispersal of wild and sterile flies (Meats, 2007). Authors frequently mention an adequate ratio (S: W) as a requisite to a successful implementation of (AW-IPM) based on SIT. To cite but a few examples, Klassen (2005) lists "substantial sterile to wild ratios" and Lance and McInnis (2005) consider "a sufficiently high sterile to wild males ratio" critical for the success of SIT. In AW-IPM programs which include the SIT, optimal release densities constitute a key component, which has serious economic impacts and which often influences success or failure of this technique i.e. the number of sterile insects that need to be released to induce a downward trend in the target population, and the potential for dispersal of the released insects (Vreysen et al., 2007).

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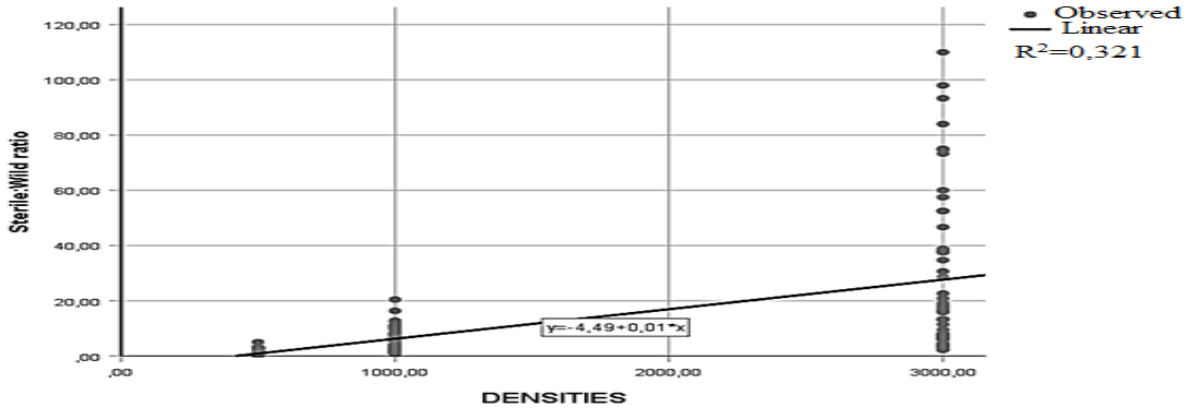

Figure 6: Relationship between the release density and Sterile to Wild ratio

Fruit infestation in SIT experimental area

In monitored trees, mean rates of fruit damage (fruits with punctures and/or larval fruit damage) in untreated (Witness) plots were consistently the highest (5%) at the monitored trees in the SIT area. Furthermore, the rate of fruit damage caused by Medfly was significantly higher in plots with a release density of 500 males/ha (3.4%) than in plots with the release densities of 1000 and 3000 males/ha with averages consecutive of 2.4 % and 1.6% **(Fig.7-A)**. Plots treated with a release density of 3000 males/ha showed the lowest level of fruit infestation rate with 1.6%.

In packinghouses, a significant reduction of the rate of fruit damage in export shipments brought from plots with high release density of sterile males has been noted. Fruits harvested from witness plots and treated with release density of 500 males/ha are more infested than fruits harvested from plots with a release density of 1000 and 3000 males/ha. The rate of fruit damaged at harvest was 2.08 % in plots with 3000 release density, 3.75 % in plots with 1000 release density. While in the witness and plots with a release density of 500 males/ha those rates were consecutively 5.57 and 5.86% **(Fig.7-B).**

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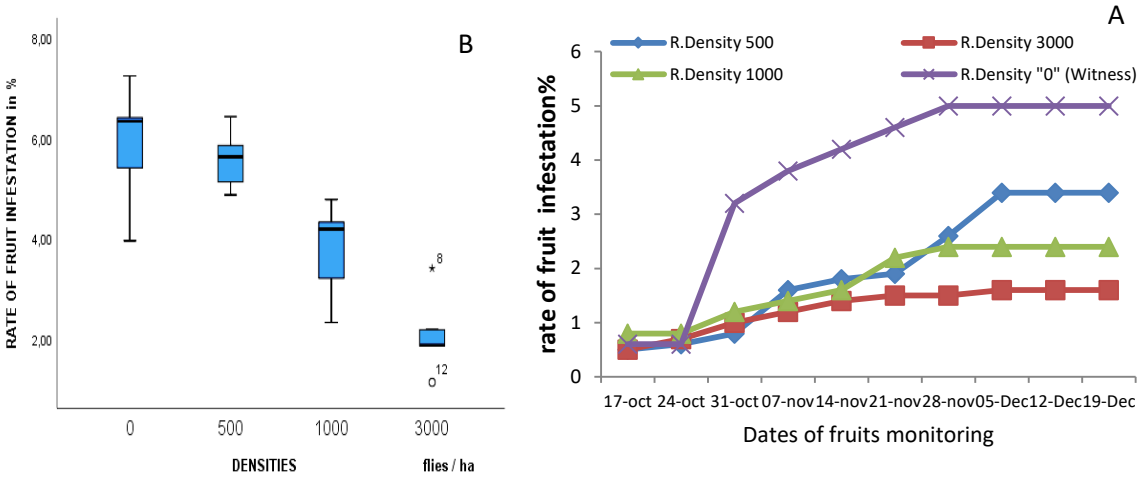

Fig. 7: Rate of fruit damaged by *Ceratitis capitata* females during 2018 in (A) Citrus trees monitored in plots treated with different release densities in SIT area and (B) in fruits of export shipment brought from SIT area to packinghouses

CONCLUSION

It is a challenge to bring together all relevant information about the sterile insect technique (SIT) and its application in area-wide integrated pest management (AW-IPM) programs mainly against Medfly. This technique combined with IPM control based on Mass trapping and orchard sanitation contributes to reducing the number of chemical cover sprays and mastering the population of adults of Medfly in Citrus Orchards of Moulouya Perimeter. Extending studies are required to establish a pattern of population dynamic and seasonal occurrence of medfly in the SIT area of Moulouya and master all technical parameters of sterile males' release, mainly densities of releases and sterile to wild males' ratio providing the increase of over-flooding ratio. The use of this technique to suppress medfly populations in Citrus orchards of Moulouya Perimeter is proven to be effective providing greater effectiveness with no negative environmental impact. Future efforts will be aimed at extending the SIT program to all Citrus production area in the Moulouya Perimeter. Incorporation collected data into the next SIT patterns may contribute to a better determining of the sterile fly density required per unit surface to effectively achieve the desired sterile to wild ratio, thus improving the effectiveness of the sterile release into AW-IPM programs for Medfly. The release-recapture method could be used to monitor population densities in the SIT area, but the way it is performed and the type of attractants used may lead to different population estimates that may be affected

by surrounding conditions. The results can be useful in extending the use of integrated pest management programs throughout the SIT area to suppress the Medfly.

Acknowledgements

Sincere thanks are devoted to ONSSA (National Office of food security) and Maroc Citrus (Citrus stakeholders Association in Morocco) staff in Berkane for assisting and helping in the field experiments and for collecting the data. Sincere gratitude is devoted to Abdelkhalek MORTADA engineer in Regional Office of Agriculture Investment of Moulouya for his expertise with analysis of the data and his many helpful comments on the manuscript.

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