

Population monitoring of males steriles of Mediterranean fruit fly (*Ceratitis capitata* Wiedemann, 1824) in citrus orchards of the Moulouya region

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Abstract

The Mediterranean fruit fly is considered as the most dangerous insect on the world citrus fruits and exists in all citrus-growing areas of Morocco, with serious problems for Moroccan export. The management of this pest is mainly based on chemical control which leads to several issues, mainly the emergence of pest resistance, environmental pollution and negative impact on human health. The objective of this study is to assess the evolution of the sterile males of *C. capitata* during 2019 and 2020 in citrus orchards (Berkane Region) by a weekly monitoring system using two pheromone traps for males and two food attractant traps for females. The pupae of medfly's sterile males had been provided by a laboratory in Valencia (Spain), then were kept in a rearing room at INRA (Qualipole of Berkane) until the release dates in fields. The insect releases were done during 2017, 2018, and 2019. The adults sexing and the identification of sterile males were done in laboratory using a binocular magnifier and ultraviolet light. Our results indicated that the males were the most caught compared to the females for both years (2019 and 2020), with a total sum of 2607 males and 55 females caught during 2019. In 2020, 707 males and 29 females were recorded in traps. Furthermore, wild males were more caught than sterile ones during 2019, with a total sum of 2396 wild males compared to 211 sterile ones during 2019. However, no sterile male has been identified in 2020, as there was no releases done for that year.

Key words: *Ceratitidis capitata*, Mediterranean fruit fly, citrus, sterile males, pheromone traps.

Suivi des populations des mâles stériles de la mouche méditerranéenne des fruits (*Ceratitis capitata* Wiedemann, 1824) dans des vergers d'agrumes (Région de Moulouya)

Résumé

La mouche méditerranéenne des fruits est considérée comme l'insecte le plus dangereux sur agrumes dans le monde entier et elle est présente dans toutes les régions agrumicoles du Maroc, avec de sérieux problèmes pour l'exportation marocaine. Le contrôle de ce ravageur repose principalement sur la lutte chimique, ce qui entraîne plusieurs problèmes de résistance du ravageur, la pollution de l'environnement et nuit à la santé humaine. L'objectif de cette étude est de suivre l'évolution des mâles stériles de la mouche méditerranéenne au cours des années 2019 et 2020 dans des vergers d'agrumes (dans la région de Berkane), par un suivi hebdomadaire à l'aide de deux pièges à phéromones pour les mâles et de deux pièges à attractif alimentaire pour les femelles. Les pupes des mâles stériles de cette mouche proviennent d'un laboratoire à Valence (Espagne), puis ont été conservées dans une salle d'élevage à l'INRA (Qualipole de Berkane) jusqu'aux dates des lâchers au champ. Les lâchers d'insectes ont été effectués en 2017, 2018 et 2019. Le sexage des adultes et l'identification des mâles stériles ont été effectués au laboratoire à l'aide d'une loupe binoculaire et la lumière ultraviolette. Nos résultats ont indiqué que les mâles ont enregistré les taux de captures les plus élevés en comparaison avec les femelles de mouche, avec la capture de 2607 mâles et 55 femelles en 2019 et 707 mâles contre 29 femelles en 2020. De plus, les mâles sauvages ont été plus capturés que les mâles stériles en 2019, avec une somme totale de 2396 mâles sauvages contre 211 mâles stériles, Cependant, aucun mâle stérile n'a été identifié en 2020, puisqu'aucun lâcher n'a été effectué au cours de cette année.

Mots clés : *Ceratitis capitata*, Mouche méditerranéenne des fruits, agrumes, mâle stérile, pièges à phéromones.

مراقبة أعداد ذباب الفاكهة المتوسطية المعقم (*Ceratitis capitata* Wiedemann, 1824) في بساتين الحمضيات في منطقة ملوية

خالد الخفيف، فؤاد مقريني ومحمد السباغي

ملخص

تعتبر ذبابة الفاكهة المتوسطية من أخطر الحشرات على ثمار الحمضيات في العالم وتوجد في جميع مناطق زراعة الحمضيات في المغرب، مع تشكيل مخاطر على التصدير المغربي. تعتمد مكافحة هذه الآفة بشكل أساسي على المكافحة الكيميائية مما يؤدي إلى العديد من المشاكل المتعلقة بمقاومة الآفات وصحة الإنسان وتلوث البيئة. الهدف من دراستنا هو رصد تطور الذبابة المتوسطية وكذلك ذكورها العقيمين خلال عامي 2019 و 2020 في بساتين الحمضيات (منطقة بركان)، من خلال المراقبة الأسبوعية باستخدام اثنين من الفيرمونات للذكور واثنين من مصائد الغذاء للإناث. جاءت شرنقة ذكور الذبابة العقيمة من المختبر في فالنسيا (إسبانيا)، ثم تم الاحتفاظ بها في غرفة تربية في مختبر بركان (INRA) حتى موعد إطلاقها في الحقول. تم إطلاق الحشرات خلال الأعوام 2017 و 2018 و 2019. وتم تحديد جنس البالغين وتحديد الذكور العقيمة في المختبر باستخدام عدسة مكبرة ثنائية العين وضوء فوق بنفسجي. أشارت نتائجنا إلى أن الذكور كانوا الأكثر تعرضاً للقبض مقارنة بالإناث خلال العامين الماضيين، حيث بلغ المجموع 2607 للذكور و 55 للإناث خلال عامي 2019 و 707 مقابل 29 للذكور والإناث على التوالي خلال عام 2020. علاوة على ذلك، كانت الذكور البرية الأكثر اصطياداً من تلك العقيمة خلال عام 2019 بإجمالي 2396 ذكراً برياً مقابل 211 عقيماً خلال عام 2019، في حين لم يتم التعرف على أي ذكر عقيم لعام 2020، في غياب إطلاق البالغين المعقمين في هذا العام.

الكلمات المفتاحية :

ذبابة الفاكهة المتوسطية، حمضيات، ذكر عقيم، مصيدة فرمون.

Introduction

According to FAOSTAT database (2021), world citrus production amounted to more than 158.4 Mt (million tonnes) during 2020 with production of over 44.6 Mt for China as 1st world producer. In Morocco, a remarkable increase in citrus production was noticed between 2006 and 2018 (Figure 1). This sector is considered as a strategic sector that contributes to the trade balance, with production reaching more than 1.7 Mt in 2020 (MAPMDREF, 2019; FAOSTAT, 2021). According to MAPMDREF (2019), in 2018-2019, the national citrus production has reached the record of 2.62 Mt and showed an increase of 15% over the previous marketing year.

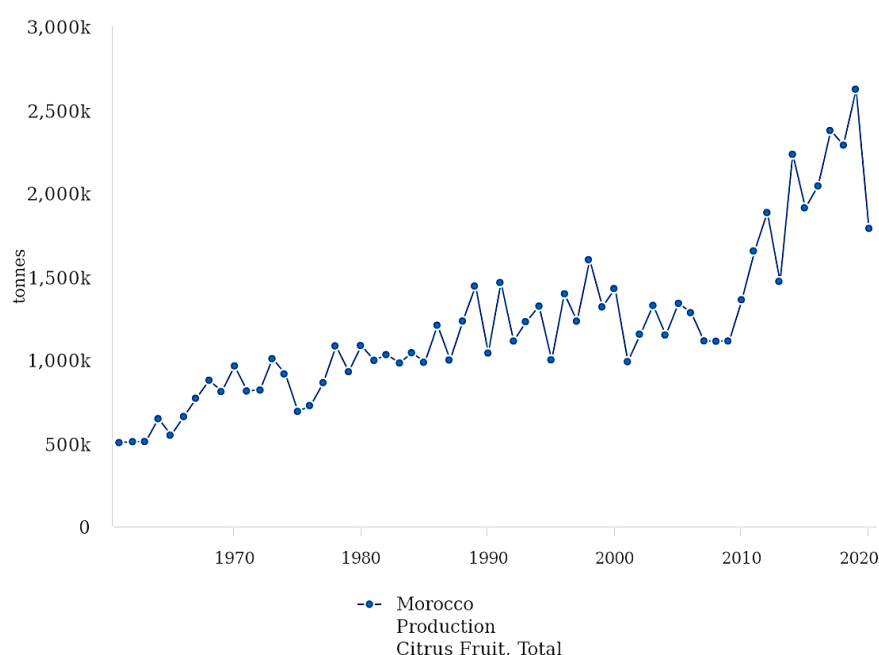


Figure 1. Citrus production in Morocco over time (FAOSTAT, 2021)

The main production regions are: Souss with 31% of the national citrus area, followed by Gharb (20%), Oriental (17%), Haouz (13%), Tadla (13%), Loukkos (2%) and other regions (4%) (Maroc Citrus, 2020). The Moulouya irrigated perimeter, characterized by its semi-arid Mediterranean climate, is cultivated mainly by citrus (ORMVAM, 2017). During 2019, the citrus area in this region was 21,518 ha (Maroc Citrus, 2020).

According to MAPMDREF (2019), citrus export during 2018-2019 reached a record of 715,450 tonnes (Maroc citrus, 2020). The main exports are destined, mainly, to the Russian, European, Canadian and American markets (Maroc citrus, 2020). The two Russian and American markets require citrus fruit undamaged from phytosanitary problems, including the medfly, considered as a quarantine insect in the United States and an endemic pest in Morocco. The detection of larvae on Moroccan clementines can result into a temporary suspension of all Moroccan citrus exports (Lyoussi, 2016).

Ceratitis capitata or the Mediterranean fruit fly (medfly) is considered as the most dangerous insect on citrus fruits that exists in all citrus-growing areas of Morocco. The damage caused by this pest on fruits is the egg-laying bites caused by the females and the galleries generated by the larvae (Macedo et al., 2008). These galleries constitute a pathway for the penetration of fungi and bacteria, responsible of the decomposition

and premature drop of the fruits. Chemical control is the most widely used method to manage and control this pest. However, the intensive use of chemicals (8 to 12 treatments against the medfly) leads to residues accumulation on fruits, leading to real problems for human health and environmental pollution (Macedo et al., 2008; Benelli et al., 2012).

Hence, the major concern nowadays is to find alternative control methods, respecting the environment and allowing the reduction of pest population. Sterile Insect Technique (SIT) is an excellent example of a biological control: this promising technique is based on the biological characteristic of the medfly who mates only once during its entire life. Once males are exposed to ionizing radiation, they become sexually sterile due to the introduction of dominant mutations in the sperm (Bounfour, 2010). When sterile males are released, the female mates with a sterile male and therefore she lays sterile eggs allowing a significant reduction in the population level of the next generation (Hendrichs et al., 2002). An optimal ratio between sterile males and wild males is necessary to reach a TIS effectiveness (Shelly and McInnis, 2016; Zavala-Lopez and Enkerlin, 2016).

The biological material of medfly sterile males used the last years in Morocco was supplied by the mass production factory from Valencia, Spain. Recently in 2020, shipment of sterile insects has been imported from Argentina, meanwhile the start of the production unit of medfly sterile males in the region of Souss-Massa (Morocco), expanded the treated area over 45,000 ha through the production of over than 165 million sterile males weekly (ONSSA, 2014; MAPMDREF, 2019).

The objective of the present study is to monitor the evolution of sterile males during two campaigns in 2019 and 2020 as a control technique to be incorporated into an integrated management program against *C. capitata* in Moroccan conditions.

Materials and methods

Study site

The study was conducted during 2019 and 2020, in the Zniber Domain (Riad de la Clementine), province of Berkane (Morocco) about ten kilometers from Madagh. The site is 767 ha with 360 ha dedicated to export. Two citrus plots, planted 40 years ago, of about 1 ha each, were chosen for our monitoring the "Berkane" clementine. They have been drip irrigated and have received standard fertilization and pesticide treatments. The insecticides used during both study periods were: *Tau-Fluvalinate*, *Triclopyr*, *Acetamiprid*, *Flonicamid*, *Lambda-Cyhalothrin* and *Abamectin*.

Monitoring of medfly population

The weekly monitoring of the medfly population concerns, males (wild and sterile) and also the females. In each plot, the monitoring was completed using one pheromone trap for the males and one food attractant trap (protein hydrolyzate), Flycap type (Figure 1) for the females, and have been changed every three months. The two types of traps used contained an insecticide and were suspended to trees at a height of 1.5 to 2 meters, facing South-East. The medfly monitoring started from mid-March to the

beginning of September during 2019, while in 2020 it was performed from the beginning of July to the end of December.



Figure 1. Flycap trap

Identification of medfly released

The pupae of medfly's sterile males came from a laboratory in Valencia, Spain, then were kept in a rearing room at INRA (Qualipole of Berkane) at a temperature of $23 \pm 2^{\circ}\text{C}$ and $60 \pm 10\%$ of humidity, until the release moment according to the calendar of Maroc Citrus. The sterile male releases were carried out during each week, from mid-October to the end of December during 2017, and from the beginning of March to the end of December during 2018 and from mid-February to the end of November during 2019. For 2020, no release has been conducted.

The adults sexing was done with a binocular magnifier and the identification of sterile males becoming orange under ultraviolet light had been all done at the Plant Protection laboratory of INRA (Qualipole of Berkane).

Data analysis

Data analysis were performed by R (version 4.1.2) and means differences were tested at $P < 0.05$ by Student's t-test.

Results and discussions

During 2017, 2018 and 2019, Maroc Citrus has conducted the operation of medfly's SIT in Zniber Domain (Riad de la Clementine) and released about 114.678.000 sterile males in this Domain. The average number of sterile males released per week was 1.978.200 in 2017, versus 2.174.571 in 2018 and 1.907.129 during 2019. With an average of sterile males per hectare of 2579 during 2017 and 2835 and 2486 during 2018 and 2019, respectively (Figure 2).

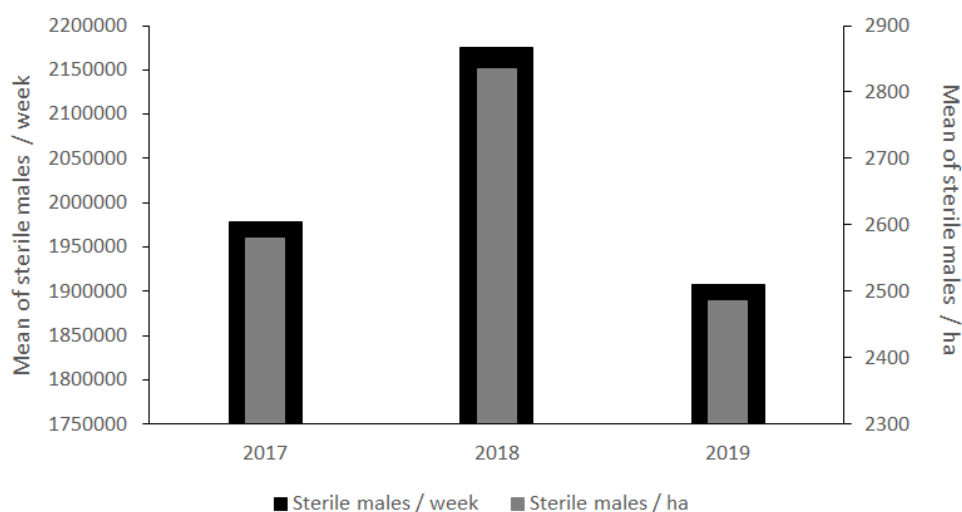


Figure 2. Average number of sterile males per week and per hectare during 2017, 2018 and 2019.

Figure 3 showed the fluctuation of medfly population during 2019 for both males (sterile and wild), and females. Our result indicated that the males were the most caught compared to the females, with a total sum of 2607 males and 55 females during 2019. The reduction of female population had been explained in the study of Bounfour (2010) as a consequence of 2 years of medfly sterile male releases in Souss region (Morocco). The encouraging results of SIT had reduced the number of treatments from 30 to 50% in this trial area. Eight generation peaks have been observed, with a maximum peak of 185 males per trap weekly at the beginning of study during the middle of March 2019. The maximum peak for females was observed at the middle of May by 12 adults per trap weekly (Figure 3).

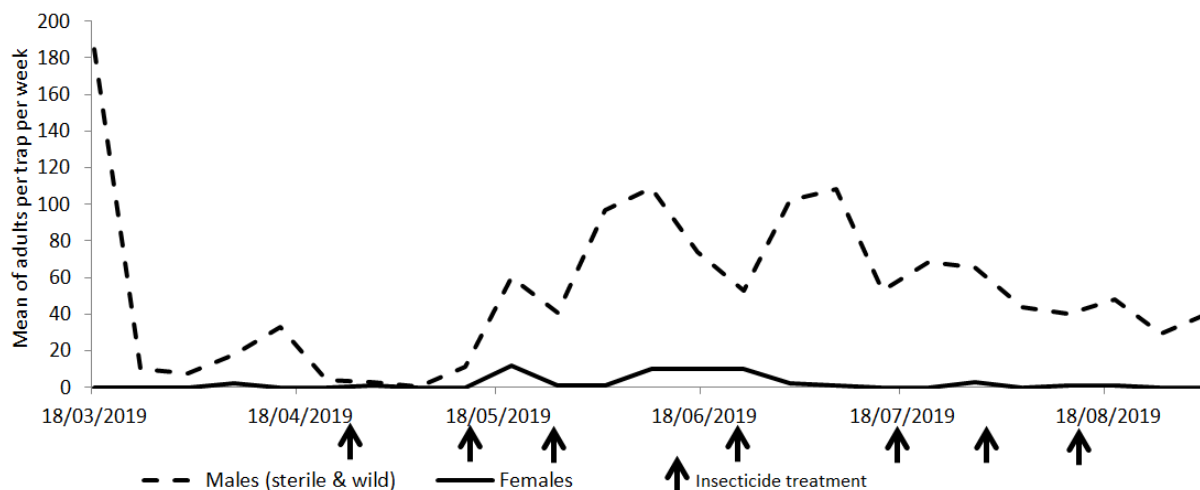


Figure 3. Mean of adults per trap and per week during 2019.

The wild males were, significantly, more caught than sterile ones, with a total sum of 2396 wild males compared to 211 sterile ones during 2019 ($t(24) = -6.913$, $p\text{-value}=0.000$), (Figures 4 and 5). The density of the sterile male population in the field, which fluctuates in relation to the release frequency and the sterile male mortality rate, should not decrease below that needed to maintain the critical overflooding ratio (Barclay, 2005; Kean et al., 2005).

During the study conducted in 2018 at Moulouya Perimeter of Morocco, Ben Yazid et al. (2020) had confirmed the effectiveness of the sterile males release in reducing the fly population in the TIS area. The authors calculated the fly/trap/day of sterile and wild males, in five citrus orchards using Maghreb-med trap, with a pre-determined release of 0, 500, 1000 and 3000 sterile males per hectare. Their results showed that the fly/trap/day for sterile males were related to the release density, and to the ratio of the sterile to the wild insects.

Several parameters can affect the number of recaptured medfly males and may lead to different population estimates comparing to our study, such as the trap and attractants type, climatic conditions and the management techniques in citrus orchards (Shelly, 2010).

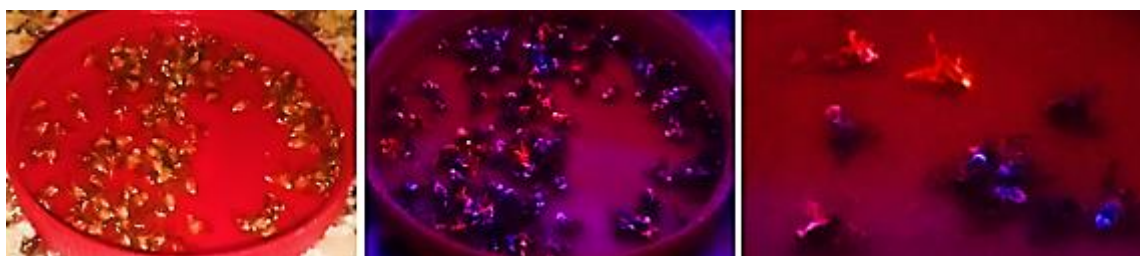


Figure 4. Male adults of medfly caught (left), with orange colored ones under ultraviolet (middle) and zoom-in on sterile male (right).

The eight peaks were mainly observed for the wild adults with a maximum peak at the middle of March recording 236 males per trap/week for wild adults, and 134 sterile adults/trap/week (Figure 5).

The quality and ability of sterilized males to mate can be a key factor in the effectiveness of SIT (Rull et al., 2005; Orozco-Davila et al., 2007). Furthermore, the mating success can correlate with the quality of diets as reported by De Aquino et al. (2016). Their results indicated that males maintained on diets enriched with guarana powder showed higher copulation success than males fed only with the basic diet, probably because of its stimulant properties, which may represent a new and viable option for increasing SIT effectiveness. The exposure of *C. capitata* males to the odor of either orange oil or ginger root oil increases their mating performance (Shelly and McInnis, 2001; Papadopoulos et al., 2006). These studies clearly showed that the prerelease exposure of medfly males to the aroma of ginger root oil (GRO) (*Zingiber officinale* Roscoe), containing the known male attractant α -copaene significantly increases the mating competitiveness of sterile males (Shelly et al., 2005; Shelly et al., 2007; Paranhos et al., 2008; McInnis, 2011; Shelly, 2016). The study of Paranhos et al. (2010) showed that ginger root oil can be used to treat sterile medfly males without interfering with their dispersal or survival in the field.

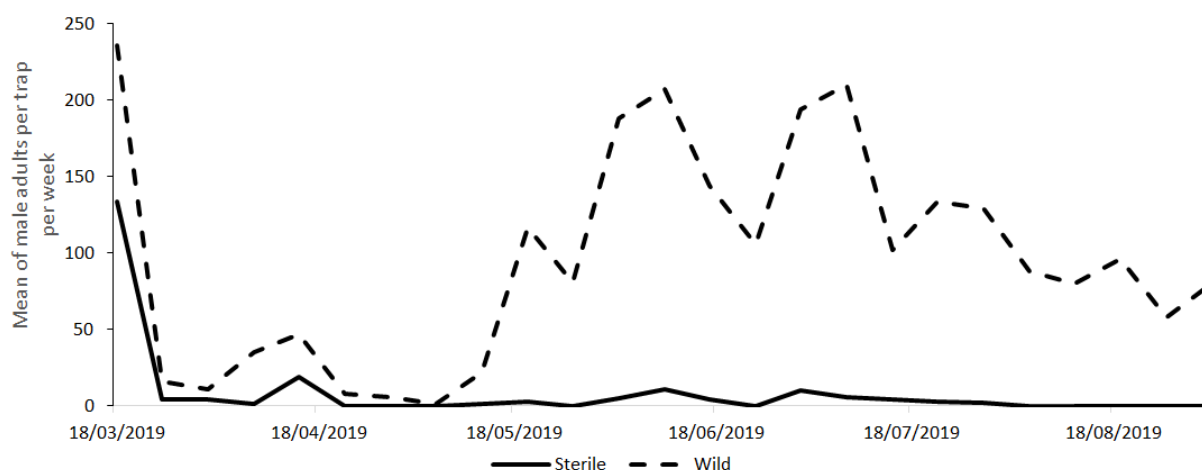


Figure 5. Mean of male adults per trap per week during 2019

During 2020, the study has been conducted until the end of December, and showed other peaks during this period, with a drastically decrease of male population from the beginning of August to November. During this year, males were the most caught with a total sum of 707 compared to the females with 29 catches. No sterile male has been identified, as there was no release done for this year. Five peaks were observed recording a maximum of catches at the middle of July: 197 males/trap/week and 12 females/trap/week (Figure 6).

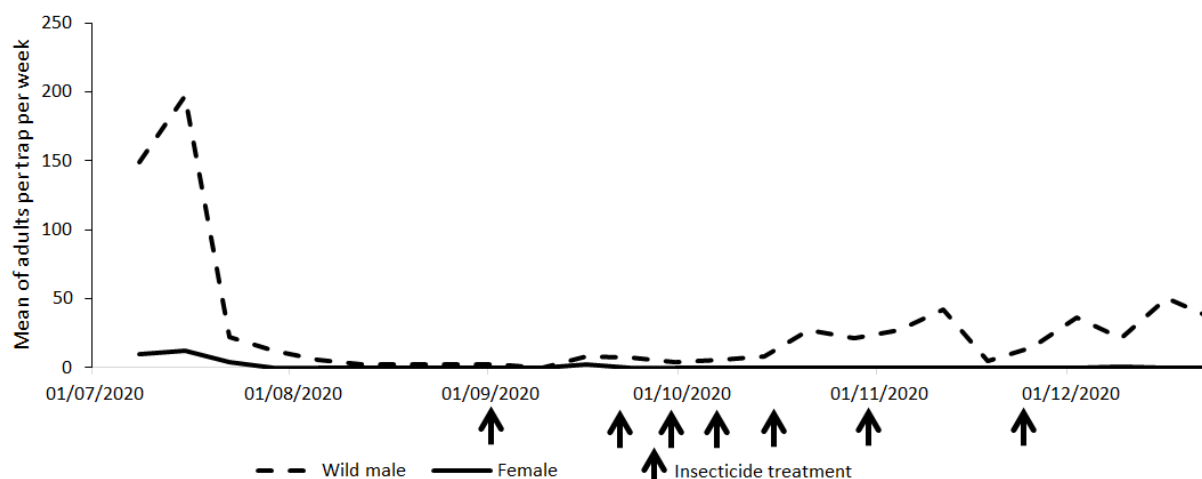


Figure 6. Mean of adults per trap per week during 2020

A project has been conducted to suppress medflies using SIT in approximately 5,000 ha in the Souss Valley, Morocco. Each week, 8 million sterile male flies were released; they had been initially imported from Madeira in Portugal (2008-2011) and later starting from 2013 they had been imported from the mass-rearing facility in Valencia, Spain (Mazih, 2015). Between 2010 and 2011, the level of female medflies in this area decreased in comparison with the levels during 2009 (Albra, 2015). It confirms our data of the low number of females caught in both years 2019 and 2020. According to Albra (2015), the reduction rate of chemical sprays in this area was also observed during the 2008/2009 and 2009/2010 campaigns and ranged from 25 to 75%.

Conclusion

SIT is considered as one of the promising genetic and biological control methods of the fruit medfly *C. capitata*. Our monitoring has showed a low number of females caught compared to the males. The number of sterile males recaptured was lower compared to the wild ones. Environmental conditions, the release, the storage and irradiated larvae rearing should be taken into consideration to improve and increase efficacy and effectiveness of this technique.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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