Molecular tools in the evaluation of SIT programmes success against *Ceratitis capitata* in Spain: a review

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Abstract

Background: The success of sterile insect technique (SIT) programs against many tephritid fruit flies, including *Ceratitis capitata* (Wiedemann), relies on the mating success of released sterile males in the field. Since its´ development in an area-wide concept, this control program is evaluated by the recapture ratio of sterile versus wild flies. This measure neither takes into account the real target of the SIT that is the wild female, nor does it give any clue about the success of released sterile males. Thus, the contribution of released sterile males to reduce the wild population still remains as a key issue. In this work we review recent findings on how sterile males contribute to reduce wild populations by means of analyzing the sperm content of wild females, as the real target of the SIT program.

Methods: A mating competition test was initially performed in laboratory and then under semi-natural conditions, with different *Ceratitis capitata* Vienna-8 *tsl* release ratios. The efficacy of the SIT and its contribution to reduce wild population was assessed by determining the percentage of females mated with sterile male, with a sperm ID molecular test, and by linking to offspring production on sentinel hosts.

Results: Statistical methods have been developed with the obtained data of sperm ID in the spermathecae of captured females and with data of viable offspring produced in sentinel fruits, revealing that both can be predicted using release ratio and mean temperature. Moreover, humidity arose also as a factor influencing the female capture in lured traps. A strong negative relationship was established between the proportion of Vienna-8 mated females and *Ceratitis capitata* offspring production, being a key point for a model to predict the SIT program success.

Conclusions: The statistical models developed should contribute to enhance the efficacy of SIT programs against *Ceratitis capitata* by means of modulation of release ratios by season temperature and by checking wild female's sterile sperm content.

Keywords: Ceratitis capitata, offspring reduction, statistical models, SIT, sperm ID.

Ceratitis capitata control in Spain: a historical perspective and current trends

The Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann), is a key pest on citrus and other deciduous fruits produced in Spain (Beitia et al., 2003). *Ceratitis capitata* is believed to be of sub-Sahara African origin, which invaded the Mediterranean basin in a first step in 1842 finding the citrus as hosts, and receiving its common name from this first invaded area (Malacrida et al., 2007). This species has a great ability to disperse, to use alternative hosts and has a great developmental plasticity, which allows its survival in tropical and temperate regions (Vera et al., 2002; FAO/IAEA, 2013; Navarro-Llopis et al., 2014). The lack of natural enemies in the Mediterranean region altogether with the behavioural plasticity to avoid them that this species exhibits, may be the responsible for the outbreaks that give to this species its key pest status (Liquido et al., 1991; Beitia et al., 2003; Malacrida et al., 2007; Argov & Gazit, 2008, Sabater-Muñoz et al., 2009).

In Spain, and more precisely in the Valencia region, during the past decades the control of this fruit fly species has relied primarily on chemical control. However, the serious threat to the international trade market caused in 2001, linked to the appearance of malathion resistant populations (Magaña et al., 2009) and the establishment of the European normative 2009/128/CE and Horizon 2014-2020, led to the implementation of environmental safe techniques within the Integrated Pest Management (IPM) program established to control this pest (Beitia et al., 2003; Sabater-Muñoz et al., 2009; Urbaneja et al., 2010-15). In 2003 an area-wide IPM program was set-up by the Valencian Community government involving environmental safe procedures such as mass trapping, chemosterilization traps, surveillance nets, and the creation of a mass-releasing facility for the implementation of the Sterile Insect Technique (SIT) against this species. The SIT is based on the birth control system exerted by released sterile males. This birth control has been successful in several countries against multiple species (Dyck et al., 2005 and references herein). The characterization of parasitoids and potential predators was also a key point in this area-wide IPM program that promoted the use of these disvalued cultural practices, during the late century (Beitia et al., 2003; Monzó et al., 2007a,b, 2010, 2011; Urbaneja et al., 2010-15). In 2006, two facilities were set-up in the region, one for mass-rearing and another for mass-releasing which cover the nearly 182,000 Ha of citrus production. This citrus production area harbors more than 35 citrus varieties that are mainly for fresh fruit export market; a market that requires zero residues in fruit and no pest presence. The actual core of this IPM in the Valencian Community is the application of the SIT throughout the citrus production area. The program is funded by the community government whereas the mass-trapping and chemical control rely on the farmers with the aid of the GIP citricos web tool (Urbaneja et al., 2010-15).

The Sterile Insect Technique: principles, application and evaluation

The SIT relies mainly on the birth control exerted by released sterile males on the target population (Dyck et al., 2005). The released sterile males should mate with the wild females

and block the reproduction of the species, as the sterile sperm carry lethal dominant mutations that stop the development of viable next generation, imposing a birth control on the population and henceforth reducing its numbers. Its application requires of three steps (FAO/IAEA, 2003; Dyck et al., 2005): 1) ability to rear, sterilize and release enough insects to overflood the area with a high sterile:wild ratio; 2) the released sterile males should compete and mate with their wild counterparts; and 3) a monitoring system to follow up points 1 and 2, and a system to identify the sterile males. Nowadays the SIT is applied under the area-wide SIT umbrella, as the best option to control a certain pest species in a determined production area. Many species are the target of SIT (mosquitoes, lepidopterans, flies, screwworms, etc) but tephritid flies species remain as the key species both as good examples of success and as the best target species for SIT.

Despite the great success of SIT in the control of tephritid flies, the evaluation of the success of SIT against medfly relies only in the recapture ratio data. By using tridmelure baited traps the ratio sterile: wild of males can be determined after identification of sterile insects (Weldon, 2005), and when this ratio is 100:1 or higher, it is said that the SIT program has succeeded. But this ratio does not take into account the real target of SIT that is the wild females (Hendrich et al., 2002). In the past McInnis and coworkers (1993, 1994) also noticed this problem, solving it by making measurement of sperm heads accumulated in the female spermathecae or by determining the induced sterility index in the population by measuring the number of viable and unviable eggs in egg clutches in fruits in comparison with untreated areas. Even though both methods are a direct measure, they are long and tedious to put in practice on a daily manner. In 2007, our group (San Andrés et al., 2007) established a new methodology to determine the presence of sterile sperm in the female spermathecae, based on PCR-RFLP patterns (Fig. 1), shedding a new light on how to overcome this problem. Recently, we have improved this methodology (Juan-Blasco et al., 2013a,b,c) to reduce the handling time and improve the sperm ID accuracy.

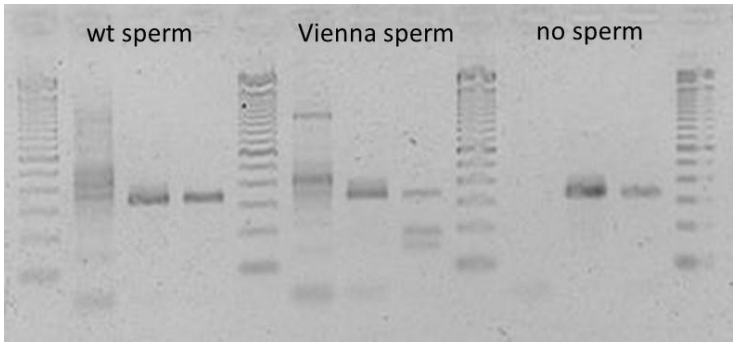

Fig. 1. SpermID method: PCR-RFLP pattern observed for *Ceratitis capitata* females mated with wild type males, sterile (Vienna) males, or unmated. Differences in the pattern allow to identify the sperm in the female spermathecae. For further explanation, see San Andrés et al., 2007.

Linking mating success of released sterile males with population reduction and fruit damage avoidance

The improvement of sterile males' success by boosting their mating achievement (Juan-Blasco et al., 2013a and references herein), and of the release procedures have rendered the aw-SIT a powerful environmentally friendly tool to control the medfly. However, despite the first studies (see Dyck et al., 2005 and references herein) no relationships between released sterile males, population and fruit damage reduction have been clearly established. Here we present a summary of the association observed among sterile males mating success (measured as sterile sperm identified in wild females' spermathecae), and the reduction of the offspring reduction and fruit damage (Juan-Blasco et al., 2013c, 2014). By means of semi-field trials, we have established a relationship between mating achievement, offspring production and fruit damage (Fig. 2). These studies included several fruit species, and different climatic conditions. Mating achievement has been determined by means of application of the SpermID method, as a direct measure of released males' success. The positive identification of sterile sperm in the female spermathecae was achieved even at 1:1 ratio (sterile: wild males) and at all natural conditions tested, opening a great window for the establishment of a direct measurement of SIT program success.

Fig. 2. Semi-field trial design. Single caged 20-years old clementine trees were used for testing sterile males' releases and spermID. In each tree, a Tephri-trap was hold, with 10 sentinel fruits (apples). Data from number of affected apples, and puparia obtained from each apple was recorded. Each captured female was subjected to spermID test by PCR and the presence of sterile sperm was recorded.

The proposed model has established for the first time a correlation between the percentage of sterile male-mated females and population reduction (Fig. 3). This model has enhanced the use of molecular techniques such as the PCR-RFLP in biological control of pest insects. Moreover, it has become a valuable tool for SIT programs mainly by indicating the success of

released males in achieving their role, to mate with wild females and by reducing the next generation.

The application of this sperm ID method to samples captured in the aw-SIT program has unveiled for the first time, that in some parts of the Valencian Community 50% of captured females were mated by the released males, indicating a correlated 50% of population reduction. Yet, these results require further analysis.

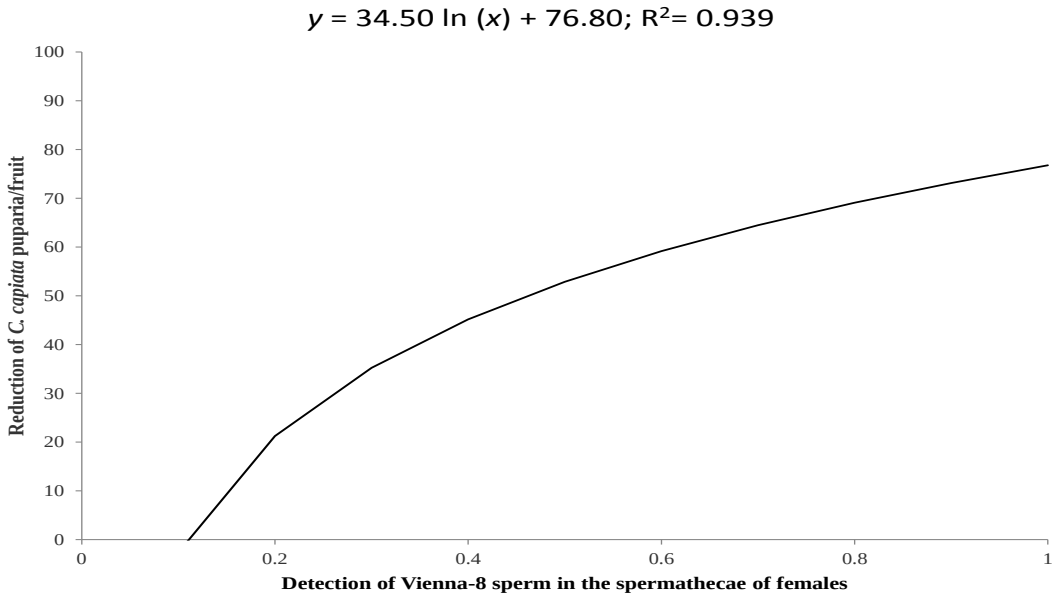

Fig. 3. Integrated correlation method describing the relationship between percentage of females mated by sterile males (indicated as percentage of females with sterile male sperm in their spermathecae) (x) and offspring reduction and fruit damage reduction (y).

Final Remarks

The SIT is one of the best environmental friendly tools for *Ceratitis capitata* control. Cost-benefit analyses have demonstrated the convenience of applying this control technique in an area-wide concept. The sperm ID method presented here is a valuable and direct measure of sterile males' success in open field conditions that contributes to a reduction of operational costs and therefore its adoption in different aw-IPM programs that relies in the SIT is highly recommended.

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