Chemosterilant Bait Stations Coupled with SIT: An Integrated Strategy to Control the Mediterranean Fruit Fly (Diptera: Tephritidae)

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ABSTRACT During 2008 and 2009, the efficacy of the combination of two Mediterranean fruit fly control techniques, the Sterile Insect Technique (SIT) and a chemosterilant bait station system (Adress®), was tested in three crops: citrus, stone fruit and persimmon. Two thousand sterile males were released per ha and week in the whole trial area (50,000 ha, SIT area). In 3,600 ha, inside of the whole trial area, 24 Adress® traps per ha were hung (SIT+Adress® area). Ten SIT+Adress® plots and 10 SIT plots in each of three different fruit crops were arranged to assess Mediterranean fruit fly population densities and fruit damage throughout the trial period. In order to evaluate the efficacy of each treatment, the male and female populations were each monitored from August 2008 to November 2009 and injured fruit was assessed before harvest. Results showed a significant reduction in the *Ceratitis capitata* Wiedemann population in plots treated with both techniques vs plots treated only with the SIT. Likewise, a corresponding reduction in the percentage of injured fruit was observed. These data indicate the compatibility of these techniques and suggest the possibility to use Adress® coupled with SIT to reduce *C. capitata* populations in locations with high population densities, where SIT alone is not sufficiently effective to strongly suppress them.

RESUMEN. En este trabajo se ha evaluado la eficiacia resultante de la combinación de dos métodos de control de la mosca del Mediterráneo, la técnica del insecto esteril (TIE) y el sistema quimioesterilizante Adress® basado en estaciones cebo. Estos ensayos se realizaron durante 2008 y 2009 sobre tres cultivos: cítricos, frutales de hueso y caqui. Semanalmente, en las 50.000 ha de ensayo se liberaron 2.000 machos por ha (Area TIE). En una zona de 3.600 ha, situada en el interior del Area TIE, además de los machos estériles se colgaron 24 unidades Adress® (Area TIE+Adress®). Dentro de cada una de las areas descritas se seleccionaron 10 parcelas de cada uno de los cultivos citados. En cada una de las 30 parcelas de cada área se evaluó la población de mosca y porcentaje de fruta atacada por la mosca durante el período de ensayo. Se realizó un seguimiento de la población de machos y hembras de mosca desde agosto de 2008 hasta noviembre de 2009 y se evaluó el porcentaje de fruta dañada en cada parcela justo antes de la recolección de la fruta. Los resultados mostraron que hay una reducción de la población de moscas en las areas tratadas con ambas técnicas cuando se compara con las poblaciones de mosca del area tratada sólo con TIE. En concordancia a este resultado también se observó una reducción del daño en fruta en el area tratada con ambas técnicas. Estos resultados muestran la compatibilidad del sistema Adress® y el sistema TIE en la reducción de poblaciones de mosca en zonas con elevada población, donde el sistema TIE por sí solo no es suficiente para suprimir las poblaciones de *Ceratitis capitata*.

KEY WORDS Sterile insect technique, Adress®, chemosterilant, lufenuron, *Ceratitis capitata*, low prevalence area

Due to increasing demand for organically produced food and progressively more stringent restrictions on use of insecticides in the European Union, new environmental friendly techniques for the control of *Ceratitis capitata* are currently being studied and developed. The possibility of controlling certain pest species by using sexually sterile males was described by Knipling (1955). In recent years, the sterile insect technique (SIT) has become a suitable control technique as part of area-wide integrated pest management program (AW-IPM) for some agricultural pests (Hendrichs et al. 2005). Nowadays, SIT is included in several *C. capitata* AW-IPM programs according to major control strategies conditioned by the degree of ecological isolation of the target area, and whether the invasive has become established , i.e., as population suppression programs being conducted in Israel and Jordan, Madeira, South Africa, Spain and Tunisia; as the containment program of Argentina, Chile and Mexico; and as the prevention programs of the pre-emptive sterile male releases underway in California and Florida (USA) (Hendrichs et al. 2005).

SIT is effective control only against sparse populations (Klassen 2005). Several authors indicate that the key factor to achieve high efficacy is the ratio between the numbers of released sterile males and wild males (FAO 2007). Therefore, in high density population areas, it is advisable the combination of SIT with another treatment to reduce the density of the target population, i.e., chemical treatments (Enkerlin and Mumford 1997), mass trapping, lure and kill methods (Katsoyannos and Papadopoulos 2004) or biological control (Wong et al. 1992).

Rearing of sterile fruit flies in Spain began at Caudete de las Fuentes (Valencia) in 2006. Since April 2007, sterile males have been continuously released in a wide area of about 50,000 ha in Valencia, where,during the first years, the SIT was combined with chemical insecticide treatments and mass trapping (Argiles and Tejedo 2007).

The SIT generally used against fruit flies involves rearing large number of males, exposing them to gamma or beta rays to induce the sexual sterility, and realising them into the field. In order to avoid the need to mass-rear *C. capitata,* a chemosterilant bait station has been devised, which uses male and female attractants to bring both sexes of the pest species in the wild to feed on a bait laced with a non-mutagenic chemosterilant (Navarro-Llopis et al. 2004, 2007). Through collaboration of public and private sector scientists, Syngenta International AG (Basel, Switzerland) developed the Adress® chemosterilant bait station (Mas and Gonzalez 2009). The chemosterilant used in this device, the chitin synthesis inhibitor, lufenuron (N-{2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenylaminocarbonyl}-2, 6-difluorobenzamide) is formulated in a phagostimulant gel; the male attractant is trimedlure, and the 2-component female attractant consists of N-methyl pyrrolidine and ammonium acetate (Navarro-Llopis et al, 2010).

Field studies demonstrated that the chemosterilisation with the Adress® device caused a reduction in the Mediterranean fruit fly population densities, as well as a decrease in fruit damage in citrus orchards (Navarro-Llopis et al. 2004). The same result was obtained in 80-ha trial over four years in an isolated citrus valley (Navarro-Llopis et al. 2007). This work also showed that the best results with chemosterilant treatments were obtained either in isolated or in wide areas, because in this situations the chances of intrusions by fruit flies were reduced. In general, migration of pest – specially gravid females- into the treated area reduces the effectiveness of the sterile male treatments (Klassen 2005). The efficacy of the chemosterilant bait station method to control the *C. capitata* population in an area-wide trial was demonstrated recently (Navarro-Llopis et al. 2010).

Since 2005, this chemosterilant bait station method has been commercially available as the Adress® system (Syngenta Agro S.A., Madrid, Spain). The commercial availability of this invention prompted us to test the combination of both induced sterility techniques: the

SIT and the Adress® system. Thus, the aim of this work was to determine whether the combination of these two sterile insect treatments was more efficacious that the SIT alonein an area-wide *C. capitata* management program.

Materials and methods

Field experiment. The field experiment was carried out in a wide area (50,000 ha) in Spain, located between Carlet and Alcudia in Valencia. This area was characterized by a numerous small orchards between 0.2 and 5 ha in size with different tree fruit species and varieties. Most tree fruits were mandarins *Citrus reticulate* Blanco (mainly "Satsuma", "Marisol" and "Clemenules") and persimmon *Diospyros kaki* L., as well as early varieties of stone fruit, such as peach *Prunus persicae* L., apricot *Prunus armeniaca* L. and plum *Prunus domestica* L. (referred to hereafter as prunus). Citrus fruits ripen between September and November, depending on the variety, stone fruits between April and June, and persimmon ripens between mid-September and mid-November. Thus, this was the worst scenario for Mediterranean fruit fly control, because this pest had access to ripening hosts during most of the year.

During 2008-2009, a 50,000 ha area was treated with SIT, and 3,612 ha within this area were also treated with the Adress® chemosterilant bait station system (SIT+Adress® area). The SIT+Adress® area was approximately a rectangle of 6.2 x 4.5 km, which included 2,255 ha of citrus, 993 ha of stone fruit and 364 ha of persimmon. The check fields treated only with SIT were located within the 50,000 ha surrounding area and almost 2 km away from the SIT+Adress® area to avoid migration of flies from SIT plots to SIT+Adress® area (Figure 1).

Adress® chemosterilant bait station treatment. Adress® system was supplied by Syngenta Agro S.A. (Madrid, Spain). The bait station consisted of a yellow vertical cylinder containing the slow release formulations of the above-mentioned *C. capitata* attractants with slots near the bottom to emit the attractant odors. A 9 cm diameter plate containing the gel formulation of a phagostimulant and 3% lufenuron was attached to the bottom of the cylinder, so that the flies could readily feed on the gel.. The system was covered with a wide yellow bottomless cone to protect the gel and attractants from rain and other elements. The attractants were released by three types of mesoporous dispensers (Muñoz-Pallarés et al. 2001). For male attraction, a 1.8 g trimedlure (TML) dispenser was used, and for female attraction, a 0.5 g N-methyl pyrrolidine dispenser and 2 g ammonium acetate dispenser were used. The Adress® system remained effective for more than one year. Twenty four Adress® devices per ha were applied for the treatment. Each of these bait stations was hung on the south-east side of the trees, 1.5 m above the ground. The treatments began in June 2008 and the Adress® devices were replaced on June 2009.

SIT treatment. Sterile male flies of the Vienna 8 strain, also named GS1/D53 or T(Y;5D30C) (Franz 2002) were obtained from the mass rearing facility in Valencia, Spain. Vienna 8 is a "male-only" strain containing a *tsl* (temperature sensitive lethal) mutation which allows the elimination of females at the egg stage. Males used in this trial were dyed and irradiated as pupae, 2 days before emergence under hypoxia at 95 Gy beta irradiation. Environmental conditions were 25 ± 4ºC, 75 ± 5% relative humidity and L16:D8 photoperiod in a climate chamber. For the SIT treatment, 2,000 sterile males per ha per week were released. Adults were introduced in a chilling box inside an Aircraft Cessna 206. Then, insects were carried out of the box by a worm gear and the airplane speed was controlled by GPS in order to release males at the calculated flow.

Fruit fly population monitoring. Mediterranean fruit fly populations were monitored within 30 orchards inside SIT+Adress® area and within another 30 inside SIT area. Among these 30 orchards, 10 were persimmon, 10 were stone fruit species and 10 were citrus. Two monitoring traps, supplied by Probodelt S.L. (Tarragona, Spain) were placed in each orchard, one baited with trimedlure (Zentinel® TML, EPA S.L., Valencia, Spain) as male attractant and one baited with BIOLURE® (Suterra Biocontrol, S.L., Barcelona, Spain) as female attractant. The insecticide used in both cases was a 500 mg dichlorvos (DDVP) strip (Agrisense BCS Ltd, Pontyprid, UK). Monitoring traps were checked weekly from May to November and once per month for the rest of the year because populations were very low from December to April. The trial period was from 1 September 2008 to 28 November 2009 in citrus and persimmon orchards, and from 15 October 2008 to 28 November 2009 in stone fruit orchards.

Assessment of fruit damage. The ultimate proof of superior efficacy of SIT+Adress® treatment vs SIT alone was the assessment of fruit damage in citrus, persimmon and stone fruit. Each sampling period involved checking 25 fruits per tree from 20 trees per plot, for a total of 500 fruits per plot. Each fruit with oviposition punctures was taken to the laboratory and the number of larvae that emerged was recorded. Ten plots per each tree fruit crop were evaluated in each treated area, which involved a total of 10,000 fruit per crop, 5,000 fruit per crop in each area of the two treatments (SIT alone vs SIT+Adress®). Fruit damage was assessed on May 2009 in stone fruit orchards, when the fruit was susceptible to being punctured by *C. capitata.* In citrus and persimmon, the damage was evaluated in the most susceptible period, which was during the harvest (September to November 2009).

Statistical analysis. One-way ANOVA followed by LSD (95%) test was used to study the effect of SIT+Adress® treatment vs SIT treatment in suppressing the Mediterranean fruit fly population. In order to normalize the data distribution, the population data were transformed by applying the quadratic root transformation (i.e., $x^{0.5}$). In order to test the significant differences in fruit damage between the SIT and SIT+Adress® treatments, a one-way ANOVA model was employed. The Statgraphics 5.1 package was used for all the statistical analyses (Statpoint INC. 2000).

Results

Population monitoring. Fig. 2 shows the trends of the male *C. capitata* population monitored in both treatments in each crop: citrus (1A), stone fruit (1B) and persimmon (1C), during the period from 1 September 2008 to 28 November 2009. The male population in the SIT+Adress® treatment was lower than in SIT only treatment for all the crops during the entire period. Considering from 1 July to 1 September is the main flight period, the mean percentage reduction in number of males in the SIT+Adress® treatment compared to the SIT was around 82% in the three crops (Table 1). Even in persimmon (Fig. 2C), where the male population reached 70 males per trap per day in the SIT only treatment, control was still maintained by the SIT+Adress® treatment. Analysis of males per trap per day captured among the two treatments during 1 July to 1 September), showed that the male population was significantly lower in SIT+Adress® treatment than in SIT only treatment for the three crops (F=26.24; df=1; P<0.001, F=55.78; df=1; P<0.001, F=9.94; df=1; P=0.002, statistical values for citrus, stone fruits and persimmon respectively).

Fig. 3 shows the trends of the *C. capitata* female populations in both treatments for each crop: citrus (2A), stone fruit (2B) and persimmon (2C). In contrast with males, the female population reduction varied among the three crops during the main flight period, as shown in Table 1. The most significant reduction in female catches in the SIT+Adress® treatment compared to the SIT only treatment was observed in stone fruit, (i.e.80%) (F=19.37; df=1; P <0.01). In citrus, SIT+Adress® treatment achieved a female catch reduction of 60% (F=4.90; df=1; P =0.04). However in persimmons, the reduction in female captures in the SIT+Adress® treatment compared to the SIT only treatment was only 28%, and these differences between the two treatments were not significant (F=1.05; df=1; P =0.32). During the period from September to October, the female population densities were similar in both treatments for citrus orchards, probably because the farmers had employed mass trapping with protein-baited traps one month before harvest.

In stone fruit orchards, the relative percent reduction of females decreased to 22%, and female populations in both treatments were very low during this period shortly before harvest. However, in persimmon, the relative percent reduction of number of females increased to 70% during the summer period. Because the sensitive period of persimmon is from mid-September to mid-November, strong reduction of the female population is essential to achieve a high degree of fruit protection with either of the two techniques. Focusing our attention in Fig. 3, the female population never surpassed one female per trap per day in the SIT+Adress® treatment, and this is a very important outcome.

Fruit damage. Fig. 4 shows the mean percentage of *C. capitata* damaged-fruit of the three crops tested in orchards treated either with SIT alone or with SIT+Adress®. Almost no punctured stone fruits were found, probably because the female population density was very low (less than 1 female per trap per day) in both treatments during the harvest period, when the fruit was very susceptible. A reduction in percent damaged citrus fruit was observed in SIT+Adress® treatment relative to that in the SIT only treatment. However, these differences were not significant due to the high variability in the data sets (*F*=1.53; df=1; *P*=0.22). The best fruit protection was observed for persimmon, where the most significant fruit damage reduction was found: 1.4% of fruits evaluated in orchards treated with SIT only treatment was damaged compared to only 0.4% in SIT+Adress® treatment (*F*=17.22; df=1; *P*<0.001).

Discussion

Under the International Plant Protection Convention, the Food and Agricultural Organization of the United Nations (FAO) has developed International Standards for Phytosanitary Measures (ISPMs), some of which serve as guidance on establishing areas of low pest prevalence for fruit flies (FF-ALPP). In this regard, the most relevant ISPMs are Nos. 22, 26, 29 and 30. ISPM No. 29, "Recognition of pest free areas and low prevalence areas" (FAO 2007) asserts that in order to establish a FF-ALPP for the purpose of exporting fruit to another country, the National Plant Protection Organization (NPPO) of the exporting country must negotiate mutually acceptable criteria of low prevalence and the protocol for properly managing the FF-ALPP with the NPPO of the prospective importing country. ISPM No. 30, "Establishment of Area of Low Prevalence for Fruit Flies (Tephritidae)", (FAO 2008) provides guidance for establishing and maintaining FF-ALPPs by the NPPO with the aim to facilitate trade by minimizing the risk of introduction or spread of regulated fruit flies. An important criterion of low prevalence is the no. of flies caught per trap per day (FTD); and the protocol for calculating this statistic is provided in Enkerlin (2007).

The USA, a major importer of fruit, has recognized FF-ALPPs not only in Spain, but also in Costa Rica, El Salvador, Guatemala and Panama. These, FF-ALPPs have facilitated the export of a variety of fruits as well as bell pepper (Capsicum annuum L.) and vine ripe tomato (Lycopersicon esculentum Mill.) (Reyes et al. 2007). Spain and the USA have agreed that a regulated area with citrus orchards having no more than 0.5 FTD of C. capitata may be considered as a FF-ALPP (USDA 2002).

However the above FTD value of 0.5 is not a sufficient condition to secure the entry of ‘Clementines” into the USA. In order for ‘Clementines’ to be exported to the USA, the level of infestation of fruits by C. capitata larvae must not exceed 1.5%, because at higher levels of infestation the cold treatment applied during trans-ocean shipment of ‘Clementines’ sometimes fails to achieve probit 9 mortality (USDA 2002). Thus the purpose of the FF-ALPP in Spain is to assure that freshly harvested ‘Clementines’ will reliably meet the requirement of having less than 1.5% larval infestation.

The Food and Agricultural Organization of the United Nations (FAO) establishes the areas of low pest prevalence for fruit flies (FF-ALPP) defined according to the number of flies caught per trap and day (FTD). In this way, areas with less than 0.5 FTD are considered ALPP for *C. capitata* in citrus orchards, whereas in mango orchards this limit is established in less than 1 FTD ((FAO) Food and Agricultural Organization of the United Nations 2007). These levels are also required by the USA export of fruits from Spain (USDA 2002) and employed for other authors to establish the level of low pest prevalence (Reyes et al. 2007).

The results of this work show a significant reduction in male and female populations in SIT+Adress® treatment versus the SIT only treatment. The SIT+Adress® treatment generally contained the female population density near or below one female per trap per day. In citrus, this treatment maintained the female population below 0.5 females per trap per day, except during two weeks in 2009, i.e., 16 July and 31 October). Moreover, in persimmon (Fig. 3C), one month before harvest (14 August to 14 September) the female population reached 1.0 FTD, but during the harvest period (15 September to15 November), the female population was below 0.5 females per trap per day. These values are even more restrictive than those proposed by the FAO because are only referred to females.

In the trial area citrus fruits ripen between September and November, depending on the variety, stone fruit between April and June and persimmon between 15 September and 15 November. Under the weather conditions of the Valencian Comunity (Spain), the *C. capitata* season takes place from April to December (Martinez-Ferrer et al. 2010), with the main period being June to October (Navarro-Llopis et al. 2008). Therefore, the trial area presents the worst scenario for Mediterranean fruit fly control, because this pest is able to find ripening hosts during its entire season.

The percentage of fruit damage was significantly lower in persimmon treated with both techniques than in persimmon treated only with SIT. This result was expected, because the female population reduction was higher during the period when the fruit was most susceptible to infestation. Neverthless, during September and October, in persimmon orchards treated with SIT+Adress®, the female population was maintained below 0.5 females per trap per day, whereas in persimmons treated only with SIT, the population was higher. In citrus and in stone fruits both treatments strongly suppressed densities of female population, so that the densities of females in the two treatments were not significantly different in these two crop groups. In stone fruit orchards, the female population was very low in the period when the fruits were ripening (May-June).

Some control methods are more effective against dense and moderately dense populations, while others, such as the SIT and sex pheromones, are effective only against sparse populations (Klassen 2005). Nowadays, SIT is included in several fruit fly AW-IPM programmes (Hendrichs et al. 2005). In most of the countries, where AW-IPM programmes are employed to control fruit flies, SIT is always combined with other control methods (Reyes et al. 2007, Hendrichs 2005), such as male annihilation technique (MAT) (Jessup et al. 2007, Mau et al. 2007), insecticidal protein bait sprays (Mau et al. 2007, Montoya et al. 2007, Gonzalez et al. 2007, Reyes et al. 2007), augmentative parasitoids releases (Mau et al.

2007, Montoya et al. 2007) and other cultural and post-harvest treatments. SIT is never employed as a standalone control method against known infestations (Klassen 2005), but it is used to prevent the establishment of *C. capitata* in southern California and Florida that arrive in smuggled fruits (Reyes et al. 2007, Hendrichs 2005).

The Mediterranean fruit fly has long been an important pest in Spain, achieving population levels higher than 50 flies per trap per day in Valencia (Navarro-Llopis et al. 2007); where in the recent years, mass trapping, malathion aerial treatments, protein bait sprays and cultural practices have been integrated into effective an AW-IPM program (Primo et al. 2003).

During 2002 to 2006, the efficacy of chemosterilant bait station (Adress® system) was tested in Valencia (Navarro-Llopis et al. 2010), and in 2007, SIT was incorporated in the AW-IPM program (Argilés y Tejedo 2007). Given that the Adress® technology and the SIT are a form of birth control through sterilization of flies, and taking into account the importance for reducing population in order to improve the efficacy of SIT, the evaluation of the joint use of the SIT and the Adress® system was of interest. This is especially interesting in countries with high populations of Mediterranean fruit fly because in these countries Wide-Area programs including air bait spray and other methods should be implemented. However, the new EU regulation 1107/2009 banned air spray of insecticides from June 2011. Therefore, other methods that could help the implementation of SIT should be considered. In addition, Adress® sterilize a proportion of wild males and females and therefore, in theory, it will work independently of *C. capitata* population level. However in the SIT, sterile males are released to compete with wild males and therefore, it will work better with low populations. The combination of both techniques during the first years may help the implementation of SIT in areas with high population levels.

Nonetheless, the cost of these treatments should be taken into account. SIT production cost in the Valencian Community, releasing 2,000 males per ha during 52 weeks over 150,000 ha, is

6.5 Million € per year, which results in 43-44 € per ha (GVA 2010). Facilities depreciation cost, maintenance and R&D will add around 23 € per ha; thus, the final cost of this treatment will be around 67 € per ha. The cost of Adress® treatment is around 155 € per ha. Obviously, the sum of the costs cannot be assumed by farmers and the combined strategy of these methods only can be assumed at the beginning of a Wide-Area program.

This work concluded that the SIT and the Adress® system were compatible and the combination of both techniques improved the control of the Mediterranean fruit fly. In addition, Adress® is compatible with biological control because it is residue free, environmental friendly, non toxic for growers and so long lasting that the bait stations need be replaced only once per year. This last feature reduces labour in comparison with other methods which require the replacement of the attractants. Moreover, the two sterilization techniques have a cumulative effect in achieving year-round suppression of *C. capitata*.

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Table 1. Mean percentage reduction of male and female populations in the SIT+Adress® treatment compared to the SIT only treatment in citrus, persimmon and stone fruits during 1 July and 1 September 2009.

Crop	Population reduction (%)	
	Males	Females
Citrus	86.3	59.5
Persimmon	78.6	27.7
Stone fruit	82.3	79.7

Figure 1: Sketch showing the experimental site: SIT, SIT+Adress® and buffer areas. In the boxes labelled with the corresponding type of crop, n indicates the number of plot (from 1 to 10).

Figure 2A: Male C. capitata population density trend in citrus orchards treated either with SIT alone, or with SIT+Adress®. Ripening period delimited by arrows.

Figure 2B: Male *C. capitata* population density trend in stone fruits orchards treated either with SIT alone, or with SIT+Adress®. Ripening period delimited by arrows.

Figure 2C: Male *C. capitata* population density trend in persimmon orchards treated either with SIT alone, or with SIT+Adress®. Ripening period delimited by arrows.

Figure 3 A: Female *C. capitata* population density trend in citrus orchards treated either with SIT alone, or with SIT+Adress®. Ripening period delimited by arrows.

Figure 3B: Female *C. capitata* population density trend in stone fruits orchards treated either with SIT alone, or with SIT+Adress®. Ripening period delimited by arrows.

Figure 3C: Female *C. capitata* population density trend in persimmon orchards treated either with SIT alone, or with SIT+Adress®. Ripening period delimited by arrows.

Figure 4: Mean percentage of *C. capitata*-damaged fruit of stone fruits, persimmon, or citrus in orchards treated either with SIT alone, or with SIT+Adress®.