Double-attraction Method to Control *Frankliniella occidentalis* (Pergande) in Pepper Crops in Tunisia

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Abstract

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Blue sticky traps with and without pheromone capsules were used to monitor and control *Frankliniella occidentalis* Pergande (1895) (Thysanoptera; Thripidae) in pepper crop greenhouses in Tunisia. Traps containing pheromone capsules could catch significantly more thrips than those without capsules, proving their efficiency in attracting and controlling this pest. In fact, greenhouses with traps with pheromone capsules caught 585.1, 526.7, and 668.8 adults/trap in a greenhouse with 10 traps with pheromone capsules on April 12, 19, and 26, 2012, respectively. The capture in a greenhouse with five traps with pheromone capsules was about 456.2, 412.8, and 431 adults/trap while in a greenhouse with five traps without pheromone capsules, the capture of *F. occidentalis* adults numbered 198, 257, and 302 adults/trap for the same three dates. Furthermore, traps with pheromone capsules captured males and females in equal numbers while traps without capsules caught much fewer males than females: 218.2 males/trap on April 26, 2012 in a greenhouse with five traps with pheromone capsules and 212.8 females/trap, 322.4 males/trap, and 342.3 females/trap in a greenhouse with 10 traps with pheromone capsules. In neither case were the differences significant. In contrast, in a greenhouse with five traps without pheromone capsules, the number of adults was about 88.6 males/trap, a significantly lower amount than the number of females/trap (213.4). This indicates that this pheromone plays a double role as a sex pheromone and as an aggregation pheromone by attracting both sexes. In fact, this double attraction approach improved the efficiency of these capsules by preventing the mating of untrapped females with males. This induced a high percentage of males and led to a decline in the thrips population on pepper flowers. Ten blue sticky traps with pheromone capsules reduced the thrips population more effectively in one week only than the use of five sticky traps, which took two weeks to achieve the control.

Keywords: aggregation pheromone; attraction; control; traps; sex pheromone

Frankliniella occidentalis Pergande (1895) (Thysanoptera; Thripidae), also known as western flower thrips (WFT), originates from California in the USA, and is a very harmful thrips species. Since the 1960's, it had a limited dispersion in the northwestern USA, Canada and Mexico. However, it has spread to many countries around the world since the 1970's (Anonymous 2002; Kirk & Terry 2003). In Tunisia, WFT is still considered to be a quarantine pest following its introduction in the early 1990's due to indirect damage caused by the transmission of viruses such as *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot wilt virus* (INSWV). WFT is a polyphagous thrips species that may attack a large range of plant species belonging to several botanical families (Belharrath *et al.* 1994; Kirk 2001; Kirk & Terry 2003; Cloyd 2009).

The control of WFT is possible by various means even though this pest is usually controlled by insecticides. However, this pest is able to acquire some resistance to several insecticide families, making chemical treatments ineffective. Thus, the alternation of different insecticides every 2–3 weeks is strongly recommended, although this depends on the biology of the pest, the season, environmental factors and the number of WFT generations (Grasselly 1996; Shelton *et al.* 2003, 2006). WFT may also be controlled by biological management using beneficial insects. Among them are parasitoids such as species belonging

to the genus *Ceranisus*, including *C. menes* Walker, *C. americensis* Girault and *C. lepidotus* Graham, which can efficiently eliminate WFT (Loomans & van Lenteren 1995; Lacasa *et al*. 1996; Loomans 2006; Bosco *et al.* 2008). Predators are a promising means of controlling WFT, particularly members of the family Anthocorridae. Most species of this family that are used to control WFT belong to the genus *Orius*, including *O. laevigatus* Fieber, *O. insidiosus* Say, *O. majuscules* Reuter, and *O. tristicolor* White, particularly in greenhouse-grown crops such as cucumber, pepper, sweet pepper and rose (Loomans & van Lenteren 1995; Parker *et al.* 1995). Some predatory mites have also been applied as a biological agent to control WFT populations in greenhouses, mostly belonging to the family Phytoseiidae, including *Amblyseius cucumeris* Oudemans, *A. swirskii* Athias-Henriot, *A. andersoni* Chant, and *Euseius ovalis* Evans (Jones *et al.* 2005; Pijakker & Ramakers 2008). The use of plant extracts is another effective means of controlling WFT. For example, a plant extract-based insecticide including *Platycladus orientalis* L., *Stemona japonica* (Kunth), *Chenopodium ambrodioides* L., and *Sophora flavescens* (Ait) used in rose crop greenhouses could control WFT with 80% efficiency (Elimem & Chermiti 2011). The majority of the thrips species attacking flowers in particular, including *F. occidentalis*, prefer white traps that have a better reflection of light than other trap colours such as blue or yellow (Hoddle *et al.* 2002). However, Roditakis *et al*. (2001) noted that trap colours that are the most attractive to WFT are blue and fuchsia rather than yellow or other trap colours. Sticky traps, however, seem to be an effective way to control and monitor WFT populations. For instance, the use of yellow sticky traps in cucumber greenhouses attracted a large number of WFT adults and could be used to directly control or monitor WFT populations (Zepa-Coradini *et al.* 2010). In that study, the number of captured thrips varied as a function of time, most thrips being captured at the first reading and the least at the third reading, indicating that the WFT population decreased. Sampson *et al.* (2012) indicated that thrips in general (and WFT in specific) use scent and colour to find host flowers. For this reason, the choice of trap colour is important to catch WFT. In fact, among many trap colours that were used by Sampson *et al.* (2012), blue sticky traps caught the highest number of WFT with highly significant differences between yellow, clear and black traps. Those traps may contain semiochemical substances such as kairomones or pheromones which can improve their effectiveness (Gómez *et al.* 2006; van Tol & de Kogel 2007). Kairomones, which are chemical substances released by an organism that affect other organisms in a food chain, may attract males and females of many thrips species such as *F. occidentalis* and *Thrips tabaci* Lindeman. However, a pheromone is a chemical substance that triggers a variety of behavioural responses in another member of the same species (van Tol & de Kogel 2007; Broquier & Lacordaire 2008; Uchida *et al*. 2008; Harbi *et al.* 2013). WFT is a thrips species that responds to odours such as pheromones produced by individuals of the same species or that may be of different types, particularly alarm pheromones, which are produced by second instar larvae (Teerling *et al*. 1993; MacDonald *et al.* 2003), and sex and aggregation pheromones, which are produced by adult males (Hamilton *et al.* 2005; Kirk 2009). According to Hamilton *et al*. (2005), Mound (2009), and Kirk (2009), adult males of many thrips species have structures known as pore plates or *areae porosae* on the underside of the abdomen. These pore plates are associated with large internal glandular structures indicating that their function is to release pheromones. On the other hand, sex pheromone produced by WFT males also plays a role as an aggregation pheromone, thus attracting both sexes (Hamilton *et al*. 2005; Kirk personal communication). Furthermore, Hamilton *et al.* (2005) reported that WFT males produce a pheromone consisting of two major components, (*R*)-lavandulyl acetate and neryl (*S*)-2-methylbutanoate, substances that may play a major role in thrips control. In fact, a WFT aggregation pheromone has been identified and commercialised (Hamilton *et al*. 2005; Gómez *et al*. 2006). The use of this pheromone showed an increase in WFT captures on sticky traps up to three times more than on traps without it (Gómez *et al.* 2006). Moreover, Natwick *et al*. (2007) concluded that the use of blue sticky traps containing the WFT sex pheromone enhanced the potential of mass trapping. Sampson *et al.* (2012) noted that regardless of the sticky trap colour, WFT was significantly more attracted to traps with pheromones than to traps without pheromones.

The aim of this study was to evaluate the effectiveness of pheromone capsules (Lures Atlas-Agro®; Atlas-Agro AG, Altendorf, Switzerland) on both WFT sexes in pepper crop greenhouses and to assess their effect on the WFT population development and increase using two blue sticky trap densities (five and ten per greenhouse) with and without pheromone capsules. The efficiency of pheromone capsules was tested as an integrated method in agricultural practice to protect pepper crops. At present, no such method to control WFT populations exists.

MATERIAL AND METHODS

Experimental site. The present study was carried out in four pepper crop greenhouses (G1, G2, G3 and G4) situated in the Teboulba region (35°36'02.24''N, 11°00'20.38''E, elevation 10 m a.s.l.), which belongs to the Monastir Governorate located on the Eastern Central Coast of Tunisia, from March 1 to May 31, 2012. Each greenhouse had an area of about 500 m^2. In all greenhouses there were four rows, each of which consisted of two lines of the Tunisian pepper cultivar Chargui. Inter-row distance was about 1 m. On May 7, all four greenhouses were closed to make them insect-proof to prevent the entry of thrips from the outside.

Sampling of flowers. The WFT population on pepper flowers was monitored weekly by sampling from March 1 to May 30, 2012. For this study, four greenhouses in total were used. Greenhouse G1 served as the control while the other greenhouses (G2, G3, and G4) were used for different treatments (traps with or without pheromone). Each greenhouse was divided into four blocks and each block into five experimental units making the total number of repetitions 20 in each greenhouse. From each sampling unit, a pepper plant was randomly selected from which three fully opened flowers were collected. Every single pepper plant that was used for sampling was marked to avoid its sampling in the next week. Each sampled flower was placed into a plastic bag on which the number of sampling units and strata had been marked.

Trapping of adults. The trapping of adults started on April 4, 2012 in the four greenhouses using blue sticky traps (Koppert, Berkel en Rodenrijs, the Netherlands) that are 25 cm long and 10 cm wide. Only greenhouses G2, G3, and G4 served for this part of the study. Greenhouse G2 was used as the control in which five blue sticky traps were installed evenly throughout the greenhouse (5 ST). In greenhouse G3, five blue sticky traps containing sex pheromone capsules (5 ST + SP) (Lures Atlas-Agro®, Atlas-Agro AG, Altendorf, Switzerland) were placed evenly throughout the greenhouse. Finally, 10 blue sticky traps containing sex pheromone capsules (10 ST + SP) were positioned evenly in greenhouse G4. Traps were suspended above pepper plants at a height of about 30 cm. During this study, blue sticky traps were renewed weekly while the sex pheromone capsules were renewed monthly. Specifically, sex pheromone capsules were replaced on April 4 and May 3, 2012.

Development rate of WFT. The development rate of *F. occidentalis* was calculated according to the Ramade (2003) formula:

$$D_r = d^t - d^{t-1}/d^{t-1} \times 100$$

where: D_r – development rate; d – population density; t – sampling date; t–1 – previous sampling date

Statistical analysis. Statistical analyses were performed by SPSS (Statistical Package for the Social Sciences) version 17. This program was used for analysis of variance (ANOVA) and Duncan's multiple range test to determine significant differences ($P \leq 0.05$) between the numbers of thrips encountered on traps with and without sex pheromone capsules.

RESULTS

This study reports on the first-ever method to reduce a WFT population in a pepper greenhouse using pheromone-treated sticky traps.

Development of thrips population in sampled flowers. When WFT on pepper flowers was monitored in the four greenhouses, large variations and differences were observed (Figure 1). The greenhouse control was characterised by an increase in the number of thrips/flower between March 1 and March 31, 2012, whereas the pheromone trap treatments all resulted in a decrease in the number of WFT in this order of effectiveness: 10 ST + SP > 5 ST + SP > 5 ST. In fact, in the greenhouse with five blue sticky traps without pheromone capsules (5 ST), the number of thrips increased from 5.47 to 8.51 thrips per flower on March 1 and March 29, 2012, respectively. However, since the traps were installed on April 5, 2012, the thrips population showed a more or less constant development during the first two dates on April 19 and 26 with 8.73 and 7.21 thrips per flower, respectively. Beyond May 3, 2012 until the end of the study period, the thrips population decreased slightly reaching average values of about 6.43 and 5 thrips per flower on May 24 and May 31, 2012, respectively. In the greenhouse containing five blue sticky traps treated with pheromone capsules (5 ST + SP), there was a progressive increase before the traps were installed. However, a decrease in the thrips population was observed two weeks after the installation of five blue sticky traps with pheromone capsules. This decrease continued progressively until the end of the study period, reaching low average values. In the greenhouse with ten blue sticky traps (10 ST + SP) with pheromone capsules, the average number of thrips before the traps were installed varied between 7.38 and 10 thrips per flower on March 1 and March 15, 2012, respectively. A decrease in the *F. occidentalis* population was observed one week

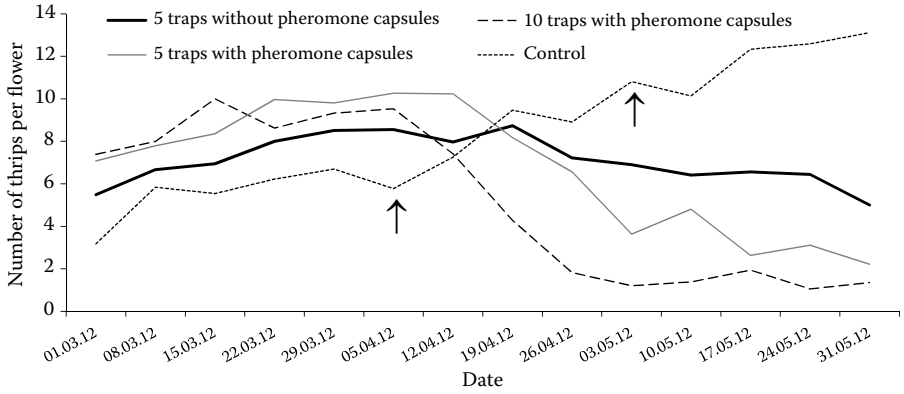

Figure 1. Mean number of thrips population in sampled flowers in pepper crop greenhouses (Arrows indicate dates on which sexual pheromone capsules were renewed)

after the traps were installed. The number of thrips in this greenhouse continued to decrease until relatively low values were reached. This trend clearly indicated the superior nature of pheromone-treated sticky traps in reducing the WFT population. Both densities used for traps with pheromone (i.e. 10 ST + SP and 5 ST + SP) were able to significantly reduce the thrips population, the only difference being the time taken: one week with 10 ST + SP and two weeks with 5 ST + SP.

Development of thrips population on blue sticky traps. The development of adults on blue sticky traps showed differences between the three greenhouses (Figure 2). The traps containing the pheromone capsules attracted more thrips than traps without pheromone, allowing for the effective control of WFT. In fact, during the first three weeks of observation, traps that captured the highest number of thrips were those treated with pheromone capsules. In this case, the respective average values on April 12, 19, and 26, 2012 were 585.1, 526.7, and 668.8 adults per trap in the greenhouse where ten traps with pheromone capsules were installed. In the greenhouse with five blue sticky traps with pheromone capsules, 455.2, 412.8, and 431 adults per trap were counted while in the greenhouse with five blue sticky traps not treated with pheromone capsules the number of captured adults was 198, 257, and 302 adults per trap for the same three dates. These values between the greenhouses were significantly different. However, after the fourth week after the traps were installed (April 5, 2012), the number of adults on traps not treated with pheromone capsules increased progressively, being highly significant compared to the other greenhouses where the number of thrips on traps with pheromone capsules was characterised by a continuous decrease until the end of the study period. Regarding the development of both sexes on blue sticky traps, the traps without pheromone capsules had significantly more females (Figure 3). However, in greenhouses where the traps with pheromone capsules were installed, the number of males and females was frequently insignificantly different (Figures 4 and 5). The capture of both sexes on traps with pheromone capsules was higher than on traps without pheromone capsules during the first week of observation and before the thrips population decreased in greenhouses where the traps with pheromone capsules were installed (Figures 3–5).

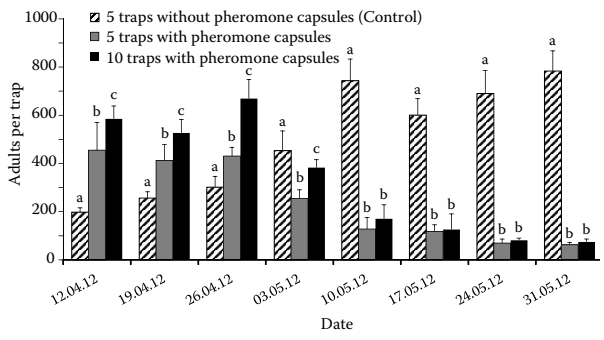

Figure 2. Average values of thrips adults number in traps in different greenhouses (G2, G3, and G4)

Means followed by the same letters are not significantly different at $P \leq 0.05$, $F = 34.99$ and degrees of freedom (df) = 2

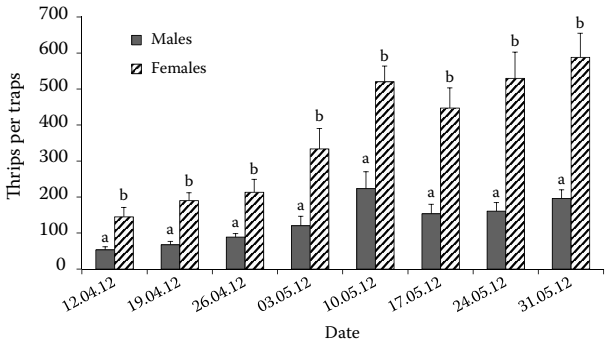

Figure 3. Mean number of *F. occidentalis* of both sexes on blue sticky traps not associated with pheromone capsules (greenhouse G2)

Means followed by the same letters are not significantly different at $P \leq 0.05$, $F = 56.01$ and degree of freedom (df) = 1

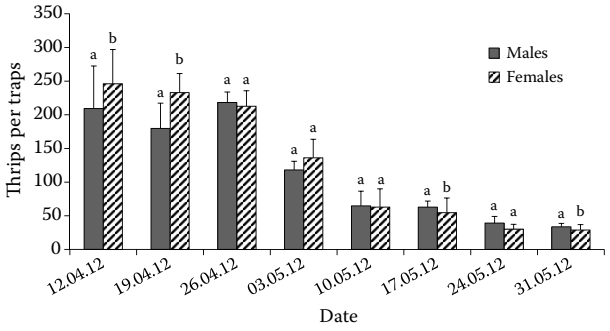

Figure 4. Mean number of *F. occidentalis* of both sexes on blue sticky traps associated with pheromone capsules in the greenhouse in which five traps were installed (greenhouse G3)

Means followed by the same letters are not significantly different at $P \leq 0.05$, $F = 1.02$ and degree of freedom (df) = 1.

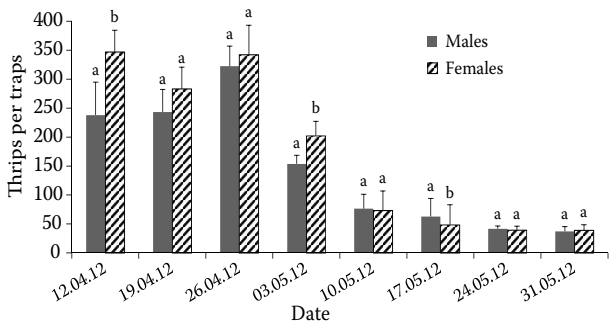

Figure 5. Mean number of *F. occidentalis* of both sexes on blue sticky traps associated with pheromone capsules in the greenhouse in which 10 traps were installed (greenhouse G4)

Means followed by the same letters are not significantly different at $P \leq 0.05$, $F = 21.69$ and degree of freedom (df) = 1.

DISCUSSION

The results obtained in the control greenhouse are consistent with those reported by Elimem and Chermiti (2009) and Elimem *et al.* (2011), who showed that WFT in rose and pepper crop greenhouses in Tunisian conditions and without pesticide treatments tended to increase as the hot season approached, reaching high levels. The use of pheromones to reduce WFT populations in pepper greenhouses in this study was as effective as the use of some thrips predators such as *Orius laevigatus* (Fieber), which decreased the WFT population in a short period of time (1–2 weeks), maintaining it at very low numbers (Sánchez & Lacasa 2002, 2006). Harbi *et al.* (2013) also indicated that the use of kairomone capsules to control WFT improved thrips capture and maintained the predator population at a very low level. Elimem and Chermiti (2011) could also use an organic insecticide based on plant extracts to reduce the WFT population as effectively as pheromones until very low values of infection (0.95–1.8 thrips/flower) were reached. The thrips population on pepper flowers in greenhouses in which traps with pheromone were installed (especially in the greenhouse with ten traps) decreased until it approached the harmful economic threshold of potential damage to a pepper crop which was estimated by Bán *et al.* (2012) to be one thrips per pepper flower.

The results obtained in this study show that traps containing pheromone capsules attract thrips more effectively than traps without such capsules. Gómez *et al.* (2006) showed that sexual aggregation pheromone has the ability to increase WFT captures on blue sticky traps by up to three times more than traps without pheromone. Furthermore, Natwick *et al.* (2007) observed that blue sticky traps combined with sex pheromone could further enhance the potential of WFT mass trapping. Moreover, Sampson *et al.* (2012) reported that WFT localises flowers by using scent and colour although blue sticky traps attracted more thrips than traps of other colours, and even more so when combined with pheromone. Cloyd (2009) stated that the threshold of WFT may vary from 10 to 40 WFT/sticky trap/week. For *Dianthus caryophyllus* L., for instance, the threshold was about 20 WFT adults/blue sticky trap/week, the value that could be used to determine insecticide applications in an integrated pest control program. In this study, the average number of thrips on blue sticky traps without pheromone capsules exceeded the economic threshold during the last two weeks. However, the average number of thrips on blue sticky traps with pheromone capsules decreased progressively until the values close to the harmful economic threshold were reached.

Elimem and Chermiti (2009) reported that the percentage of WFT females on blue sticky traps was much higher than the percentage of males throughout the whole year in a Tunisian rose crop greenhouse.

Hamilton *et al.* (2005) noted that aggregation pheromone increased the ability of sticky traps to capture WFT females and males by 1.9- and 1.5-fold, respectively, relative to the control (which had no traps).

The pheromone produced by the male sternal gland plays a double role, firstly as a sex pheromone that attracts females for mating and also as an aggregation pheromone that attracts males (Hamilton *et al.* 2005; Kirk personal commununication). Kirk (2009, person. commun.) described the unusual and complex mating behaviour of WFT. Kirk claimed that

males are generally unlikely to find a mate or a female unless they are in an aggregated condition, that it is important for a male to have other males mating with females, and that this is likely to be mediated by the aggregation pheromone. This substance draws both males and females to swarms and appears to induce mating behaviour rather than feeding or egg laying, which would explain their attraction to traps treated with pheromone capsules (Figures 4 and 5).

The attraction of both WFT sexes by traps with pheromone capsules led to a higher decrease in the thrips population than on traps without pheromone capsules. Indeed, the lower number of males in pepper flowers consequently prevented further mating between both sexes, which led to a thrips population in which males become important due to arrhenotokous parthenogenesis in which unfertilized eggs develop into males and fertilized eggs develop into females (Fraval 2006), consequently leading to a decrease in thrips populations in greenhouses.

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