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Abir Hafsi, Ridha Rahmouni, Soukaina Ben Othman, Khaled Abbes, Mohammed Elimem, Brahim Chermiti

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Mass trapping and bait station techniques as alternative methods for IPM of *Ceratitis capitata* Wiedmann (Diptera: Tephritidae) in citrus orchards

Abir Hafsi (b^{a,b}, Ridha Rahmouni^c, Soukaina Ben Othman^a, Khaled Abbes (b^a, Mohammed Elimem^d and Brahim Chermiti^a)

^aDepartment of Entomology and Biological Control, High Agronomic Institute of Chott-Mariem, Sousse, Tunisia; ^bDépartement d'Entomologie, CIRAD, UMR PVMBT, Saint-Pierre, La Reunion-France; ^cCitrus Technical Center (CTA) of Beni Khalled, Zaouiet Jédidi, Tunisia; ^dDepartment of Agricultural Production, High Agronomic Institute of Mograne, Zaghouan, Tunisia

ABSTRACT

Malathion bait sprays to control medfly, Ceratitis capitata (Diptera: Tephritidae), in Tunisian citrus orchards is expected to be phased out in the near future due to low residue tolerance in the fruits by consumers. Attract and kill strategy that encompasses mass trapping and bait station techniques has become a widespread method for controlling medfly. Effectiveness of two bait station methods (AAL&K® and Ceranock®) and two attractants for mass trapping (Ceratrap® and Starce®) was, respectively, compared with malathion and spinosad in Grombalia and Chott-Mariem experimental sites. Ceranock® and AAL&K® provide effective protection of citrus fruits from medfly attack as confirmed by low rate of damaged fruits of respectively 3.88 and 2.26% compared with malathion (21.63%). Medfly population and rate of fruit damage were significantly lower in Ceratrap® than in Starce® and spinosad treated plots. Rate of fruit damage on Starce® treated plots increased at least three times more than in Ceratrap®, and one-and-half times more than in spinosad treated plots. Ceranock® and AAL&K® bait station devices are a costeffective alternative to the use of insecticides in citrus orchards and can be used in IPM schemes against medfly in association with other environmentally friendly approaches.

ARTICLE HISTORY

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KEYWORDS

Attract and kill; organophosphate insecticide; medfly; pest management; mass trapping

Introduction

The Mediterranean fruit fly (medfly) *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) is the most serious pest species of citrus and stone fruits grown in tropical, subtropical and temperate regions (White and Elson-Harris 1992). *Ceratitis capitata* is a highly polyphagous species as larvae can develop in a wide range of fruits (Liquido et al. 1989) causing high economic yield losses and quarantine constraints (Oliveira et al. 2013) especially with fruits that lose their quality and even their marketability (Badii et al. 2015).

Citrus fruits are highly ranked among export commodities in Tunisia and represent a significant economic resource. However, the presence of *C. capitata* can restrict or close export markets and force farmers to carry out expensive disinfestation procedures (White and Elson-Harris 1992). For decades, the primary management strategy of *C. capitata* in various areas in Tunisia and in the world relied upon heavy applications of protein baits mixed with pyrethroid or organophosphate insecticides, notably malathion via aerial cover sprays (Burns et al. 2001; Boulahia-Kheder et al. 2012; Gazit and Akiva 2017).

Resistance to malathion was encountered in C. capitata populations in Spain (Magana et al. 2007). Application of organophosphate insecticides has become not only less effective in controlling damage caused by C. capitata (Hafsi et al. 2015a), but also a serious environmental and human health issue. In fact, it leaves high toxic residue levels in fruits, which ultimately threaten human health (Costa and Klein 2006; Magana et al. 2007). The maximum residue limit (MRL) of malathion in Tunisian citrus fruits destined for export was lowered from 0.05 mg per kg in 2001 to 0.01 mg per kg in 2013 (GIF 2013). Thus, there is a need for alternatives to the bait sprays currently used. In this context, attract and kill strategy that includes mass trapping and bait station techniques has become a widespread method for controlling Tephritidae species and particularly C. capitata. Different types of traps, bait station designs and food attractants have been developed and field-tested for the control of this pest (Leza et al. 2008; Navarro-Llopis et al. 2013, 2015). Various degrees of crop protection were achieved under low and high C. capitata population densities in most fruit-growing countries of the Mediterranean region (Navarro-Llopis et al. 2008; Jemâa et al. 2010; Martinez-Ferrer et al. 2012; Hafsi et al. 2015b). The efficacy of the attract and kill strategy depends on a number of factors which may occur in the female choice especially on the quality of food attractant (Epsky and Heath 1998; Broumas et al. 2002). A synthetic food-based female attractant consisting of putrescine, ammonium acetate, and trimethylamine (Epsky and Heath 1998; Heath et al. 2004, 2009) was developed as replacement to aqueous protein hydrolysate in order to increase the number of captured C. capitata females and decrease the number of captured beneficial insects (Katsoyannos 1994; Heath et al. 1997).

In Tunisia and in the countries where *C. capitata* causes serious damage, synthetic female attractants used in mass trapping and bait station techniques have proved to be more efficient than chemical treatments in reducing population level and fruit damage at harvest in orchards with minimum adverse effects on non-target arthropods (Bouagga et al. 2014; Jemâa et al. 2010; Hafsi et al. 2015b, 2016). In previous studies, mass trapping using Ceratrap[®] and Tripack[®] food attractant was found to be

more effective than insecticide bait sprays against *C. capitata* in citrus orchards in Tunisia (Jemâa et al. 2010; Hafsi et al. 2015b, 2016). Ceranock[®] bait station also was considered as effective as conventional applications of insecticide, if not better, against *C. capitata* (Bouagga et al. 2014). It was especially noted that Ceranock[®] and AAL&K[®] bait station devices were more effective than chemical treatments using organophosphate insecticides in peach orchards characterised by low density of *C. capitata* populations (Hafsi et al. 2016). It would be important to test the efficiency of Ceranock[®] and AAL&K[®] devices against *C. capitata* in citrus orchards in Tunisia.

Owing to the variability of information regarding bait station devices and food attractants used in mass trapping in the market (Jemâa et al. 2010; Navarro-Llopis et al. 2015; Hafsi et al. 2016), the main objective of the current study is to select the most effective form mass trapping and bait station methods to control *C. capitata* in order to reduce the use of controversial insecticides in citrus orchards. In this study, we tested for the first time the efficacy of novel attractant (Starce[®]) used in mass trapping, then we compared the efficacy of two attractants from different origins (Starce[®] from vegetal origin and Ceratrap[®] from animal origin) used in mass trapping to spinosad that is applied as ground cover spray in Chott-Mariem experimental site. The efficacy of bait station devices (Ceranock[®] and AAL&K[®]) was compared with the conventional approach using malathion as aerial cover sprays in Grombalia experimental site.

Materials and methods

Treatments description

Bait station method was performed in this study using two devices. Ceranock[®] and AAL&K[®] bait stations are both devices that attract target insects to a toxin. The Ceranock[®] (Russell IPM, London, United Kingdom) is a sponge soaked in attracting bait (95%) (hydrolysed proteins and citrus plant extract: 5 g per bait station) and low dose of toxicant (0.2%) (Alpha-Cypermethrin: 0.01 g per bait station) protected with a plastic case having a plastic hook. Ceranock[®] devices were installed on 16 September 2014. Devices were positioned on the south eastern part of canopies of citrus trees, at 1.5 m high above ground level, and were uniformly distributed in each orchard. The average lifetime of these devices is 4 months under normal filed conditions. According to the label guidelines, it is recommended to set an average of 400 devices/ha. The AAL&K[®] (Atlas Agro, Altendorf, Switzerland) bait station is a tube containing 150 g of a mixture of attractive bait (hydrolysed proteins and enriched ginger oil as *C. capitata* male parapheromone at 10%), insecticide (Permethrin at 6%)

and inert ingredients (stabiliser at 84%). It was applied for the first time on 16 September 2013. Three additional applications were carried out on 14 October, 11 November, and 9 December 2014. The average lifetime is 4 weeks under normal conditions. As recommended by the manufacturing company, it was randomly applied in citrus orchards with an average of 3000 droplets (\approx 225 g)/ha (6 to 7 droplets/tree placed on the undersides of leaves). Both bait station devices were previously described in Hafsi et al. (2016).

Mass trapping was performed in the current study using two synthetic food attractants (Ceratrap[®] and Starce[®]). Ceratrap[®] (Bioiberica, Barcelona, Spain) composed of enzymatic hydrolyzed protein of animal origin that releases a package of volatile compounds, mostly amines and organic acids, that are highly attractive to fruit flies (Orpella and Marín 2008). Starce® (Biagro, Valencia, Spain), which is a new tool against C. capitata, is a nutritive bait-solution of high concentration developed on the basis of an enzymatic protein hydrolysate of vegetal origin, as an attractant, enriched with nitrogen and phosphorus. Ceratrap® commercial traps were used in this study to applied Ceratrap® and Starce® food attractants in citrus orchards. This trap is commercially available and it consists of a transparent plastic bottle of 1 L volume containing 5 holes of 5 mm diameter each baited with 300 mL of each attractant. Traps were hung in the canopy of fruit trees at 1.5 m above ground level and applied at a density of 50 traps per ha. The average lifespan of these two attractants is four months under field conditions.

Malathion bait spray is an aerial cover spray using a solution composed of 1/3 of Fyfanon 50% EC (concentration 500 g per L) (Cheminova Agro A/S, Harboor, Denmark) and 2/3 of Lysatex SC protein bait (concentration 350 g per L) (Sepcam, Tunis, Tunisia) applied at the dose of 500 mL per ha. (Jemâa et al. 2010). In Tunisia, malathion aerial bait spray is the main treatment applied against *C. capitata* in citrus-growing region. This treatment is supervised by the government and is applied when the threshold record reaches three flies per McPhail trap per day or after heavy rainfall (Boulahia-Kheder et al. 2010). Treatment involves three full coverage ultralow volume aerial applications of the bait pray. The first treatment was applied on 16 September 2014, at that date the mean number of males captured per trap per day was 6.14, and fruits were still developing with green colour. Two additional applications were carried out on October 14 and 11 November 2014.

Spinosad bait spray 'Success APPÂT CB' (concentration 0.24 g per L) (Dow AgroSciences, Madrid, Spain) is a foliar cover spray applied at the dose of 1 L diluted in 20 L per ha. It was applied when the *C. capitata* population level reached three flies per McPhail trap per day or after heavy rainfall. This chemical treatment was applied with a high-pressure sprayer

equipped with turbulence chamber nozzles and was sprayed at a pressure of 6–7 bars until run-off resulting in complete coverage of the south eastern part of the canopies of citrus trees (Hafsi et al. 2016). Spinosad was applied three times during the season: on 6 September, 21 October, and 18 November 2014.

Field experiments

Experiments were conducted from August 27 to 30 December 2014 in two citrus-growing regions in the semi-arid coastal area of Tunisia. The first experimental site is in Grombalia (36.5461°N, 10.5002°W). The second one is located in the citrus farm of the Technical Center for Organic Agriculture (CTAB) in Chott-Mariem (35.8746°N, 10.5551°W).

In Grombalia, the efficacy of Ceranock[®] and AAL&K[®] and malathion used in aerial bait spray were compared in two neighbouring commercial citrus orchards. Each of the two orchards had three plots separated by 10 rows of 20-years-old citrus trees, corresponding to the three treatments (Ceranock[®] and AAL&K[®] food attractants and malathion). Each plot is planted only with Washington navel citrus variety that is the most susceptible variety to *C. capitata* with a lack of other host plants in or around these plots. The area of each plot was approximately 0.5 ha in size.

In Chott-Mariem, the efficacy of Ceratrap[®] and Starce[®] food attractants and spinosad used in ground bait spray were compared in two neighbouring citrus orchards. Each of the two orchards had three plots separated by 5 rows of 25-years-old citrus trees corresponding to the three treatments (Ceratrap[®] and Starce[®] bait stations and Spinosad). Plots planted with the 'Washington navel variety were considered for the study. The area of each plot was approximately 0.5 ha in size. Plots treated with conventional approach using malathion or spinosad were considered as a positive control because untreated commercial plots are not available as *C. capitata* is a major pest in citrus crops and can causes more than 90% of fruits loss in the absence of control (Braham et al. 2007).

Monitoring of C. capitata population

To monitor male populations of *C. capitata* in different treated plots, we placed McPhail traps (Biobest,Westerlo, Belgium) baited with mixture of the synthetic parapheromone trimedlure (Russel IPM, Flintshire, United Kingdom) and dichlorvos insecticide (10%) (Hercon Vaportape II, Emigsville, PA) used at 2 g per trap. McPhail traps were installed in citrus treated plots on 20 August 2014. The bait was replaced every five weeks. Five traps in each plot were maintained and checked weekly. Males of *C. capitata* captured in each trap were counted and removed after each sampling.

Damage assessment

Fruit damage rate was assessed every two weeks from the beginning of the ripening period (beginning of November), five times during the trial period. In each plot, 10 trees were randomly selected and from each one, 20 fruits by each cardinal orientation (East, West, North and South) were numbered and checked for oviposition punctures. For each tree, the fruit damage rate was calculated as the number of fruits with at least one oviposition puncture over the total number of checked fruit (800 citrus fruit).

Statistical analysis

All statistical analyses were performed using R project for statistical computing (R Development Core Team 2008). Population level of *C. capitata* on plots were compared among the two experimental sites before application of all treatments in order to check if the population differed or not between them and to confirm that the two trial were conducted in the same conditions. Then the population levels were compared between plots in each experimental site after application of all treatments. In each experimental site, the numbers of *C. capitata* males caught in traps were analyzed using a generalised linear model (GLM) with Poisson error (Log link function) as function of sampling dates, treatments (bait station, mass trapping, and insecticides) and interaction between these two factors.

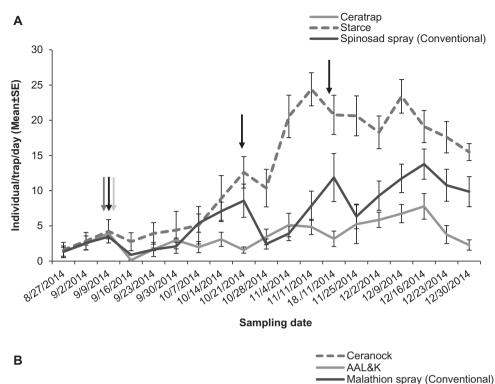
Fruit damage rate was analysed using a GLM with binomial error (logit link function) as function of sampling dates, treatments (bait station, mass trapping, and insecticides), and interaction between these two factors. We used the residual deviance " ΔDev " to measure model's predictions compared to the observed outcomes (i.e. a goodness of fit test of the model) (example: see Hafsi et al. 2016).

Results

Ceratitis capitata monitoring

Prior to treatments application (from 27 August to 9 September), the number of male *C. capitata* caught in McPhail traps did not differ significantly among plots neither in Grombalia ($\Delta Dev = 13.68$; df = 5, 12; *P* = 0.42) and nor in Chott-Mariem ($\Delta Dev = 19.17$; df = 5, 12; *P* = 0.31) (Fig. 1). In addition, there was no difference between plots within each of these two experimental sites.

After treatments application (from 4 September to 29 December), the number of *C. capitata* males differed significantly among treated plots in both Chott-Mariem ($\Delta Dev = 2174.50$; df = 2, 187; *P* < 0.001) and Grombalia ($\Delta Dev = 949.24$; df = 2, 206; *P* < 0.001) experimental sites (Fig. 1).



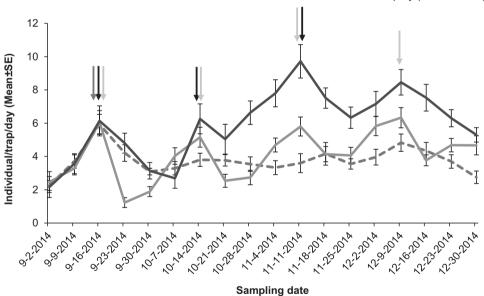


Figure 1. Mean numbers of *Ceratitis capitata* males captured in McPhail tap per day during 2014 in (A) plots treated with Ceratrap[®] and Starce[®] mass trapping and with conventional insecticide (spinosad) in six citrus plots in Chott-Mariem experimental site; (B) plots treated with Ceranock[®] and AAL&K[®] bait stations and with conventional insecticide (malathion) in six citrus plots in Grombalia experimental site. Arrows indicate when bait stations and traps were deployed (light grey for AAL&K[®] and Ceratrap[®], dark grey for Ceranock[®] and Starce[®]) and when insecticides (spinosad and malathion) were applied (black).

In Chott-Mariem, the number of *C. capitata* males was significantly lower in Ceratrap[®] mass trapping treated plots than in spinosad bait spray treated plots. The highest number of *C. capitata* males was recorded in Strace[®] treated plots (Fig. 1A).

In Grombalia, the number of *C. capitata* males was significantly lower in bait station (Ceranock^{*} and AAL&K^{*}) treated plots than in malathion bait spray treated plots, but did not differ significantly between Ceranock^{*} and AAL&K^{*} treated plots (Fig. 1B).

Damage assessment

In all plots, at the beginning of the ripening period, fruit damage rate started low and increased gradually as the fruit ripened until harvesting (Fig. 2). On the first assessment date (2 November 2014), the fruit damage rate did not differ significantly among treated plots in Chott-Mareim ($\Delta Dev = 100.85$; df = 2, 87; P = 0.071) and in Grombalia ($\Delta Dev = 82.98$; df = 2, 87; P = 0.22) experimental sites.

At the beginning of harvesting (1 December 2014), in Chott-Mariem, Starce^{*} treated plots had significantly higher fruit damage rate than Ceratrap^{*} and spinosad treated plots ($\Delta Dev = 147.73$; df = 2, 87; P < 0.001). At the end of harvesting (30 December 2014), fruit damage rate in Starce^{*} treated plots (21.25%) was three and one-and-half times higher than in Ceratrap^{*} (6.87%) and spinosad (11.25%) treated plots, respectively (Fig. 2A). In Grombalia, Ceranock^{*} and AAL&K^{*} treated plots had significantly ($\Delta Dev = 244.29$; df = 2, 87; P < 0.001) lower fruit damage rate than malathion treated plots (Fig. 2B). Fruit damage rate was maintained lower (less than 4%) in Ceranock^{*} (3.88%) and AAL&K^{*} (2.26%) treated plots and it was five times lower than in malathion (21.36%) treated plots.

Discussion

In this study, the efficacy of mass trapping techniques and attracticide devices was compared with bait sprays in order to provide information on the most effective tool against *C. capitata* and to reduce the amount of insecticide applied in the environment. Results confirm efficacy of Ceratrap[®] compared to Strace[®] mass trapping and spinosad bait spray, and showed that Ceranock[®] and AAL&K[®] attracticides are effective enough to keep the populations at low level and to protect citrus fruits from *C. capitata* attacks compared to conventional approach using malathion bait sprays.

Mass trapping was shown to be a very effective pest management tool under high and low population levels of *C. capitata* (Leza et al. 2008; Navarro-Llopis et al. 2008). The use of this technique as effective pest

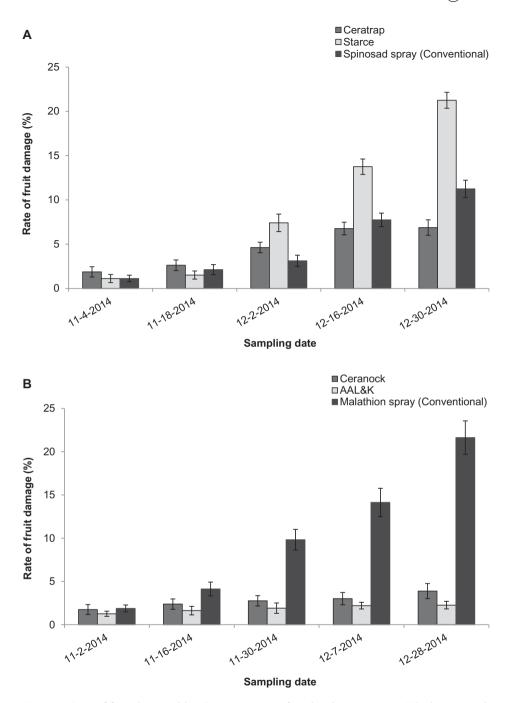


Figure 2. Rate of fruit damaged by *Ceratitis capitata* females during 2014 in (A) plots treated with Ceratrap[®] and Starce[®] mass trapping and with conventional insecticide (spinosad) in six citrus plots in Chott-Mariem experimental site; (B) plots treated with Ceranock[®] and AAL&K[®] bait stations and with conventional insecticide (malathion) in six citrus plots in Grombalia experimental sites.

management tool against C. capitata is increased in Tunisia and in the Mediterranean agro-systems (Navarro-Llopis et al. 2008, 2015; Jemâa et al. 2010; Hafsi et al. 2015b). Ceratrap® mass trapping used in pest management strategy against C. capitata population in biological and IPM citrus farmers in Tunisia has been proven to be efficient (Hafsi et al. 2015b). Results of this study confirm Ceratrap® mass trapping efficacy, showing that the population level and rate of fruit damage at harvesting can be considered low compared with spinosad-treated plots. The available data reported effectiveness of mass trapping technique is contradictory and significant differences in efficacy were found among the tested food attractants. The control failure of C. capitata in Starce[®] treated plots despite the low initial pest population may be attributed to the low efficacy of this treatment. Lasa et al. (2015) showed that the attractant from animal origin (Ceratrap[®]) is more effective than that from vegetal origin (Captor[®]), and that the type or/and the stability of the attractant can play a major role in the efficacy of mass trapping technique. In this study, the simple transparent colourless bottle trap baited with Ceratrap® was more effective than that baited with Starce[®] attractant. The efficacy of the transparent bottle and Ceratrap[®] combination was confirmed against several tephritid species (Lasa et al. 2015). Given that this type of trap behaved similarly to other more sophisticated commercial traps baited with Ceratrap[®] (Lasa et al. 2015), it would be important to test efficacy of Starce® using other commercial trap models under field conditions.

The population level in Starce[®] treated plots was maintained low during the first four weeks, until treatment application, but it increased drastically during the last period of assessment. The highest population level of C. capitata observed in Starce[®] treated plots indicates that this later was not working properly and lost efficacy four weeks after its application. During the assessment period, we noticed that the quantity of Starce[®] liquid attractant was decreased to half the original volume in traps six weeks after its application due to evaporation, a factor that was not observed in Ceratrap[®] traps. The high stability and particularly the low evaporation of the food liquid attractant is a key factor affecting the efficacy of liquid traps, given that the liquid serves as the attractant as well as the killing agent (Shelly et al. 2014). These same authors showed that after the evaporation of the contained liquid, the trap may still attract the flies, but the lack of liquid allows the flies to escape. Thus, to suppress C. capitata population in citrus plots, Starce[®] should be applied every four weeks, i.e. three times, and/or should be supported by insecticide sprays which increases the final treatment cost and harmful effects of insecticides on non-target insects. In addition to this low efficacy, effects of Starce® on non-target organisms are unknown. The cost for Starce[®] mass trapping control system used at a density of 50 traps per ha is estimated to be about 150 € ha⁻¹ (3 € for one baited trap). The use of Ceratrap[®] for mass trapping required 50 traps per ha to ensure less than 6.85% of damage, resulting in a total cost of 140 € ha⁻¹ (2.8 € for one baited trap). The cost of 3 chemical treatments using spinosad in ground application needed to keep fruit damage at 11.25% is 100 € ha⁻¹, 28.57% less than the cost associated with mass trapping using Ceratrap[®]. The 28.57% increase in mass trapping cost justifies the 40% decrease in fruit infestation, while avoiding chemical treatments and their adverse side effects on non-target organisms, human health and environment.

In recent years, efficacy of Ceranock[®] and AAL&K[®] attracticides was proved to be as effective as conventional organophosphate and pyrethroid insecticides to control low population density of *C. capitata* in early-ripening varieties of peach (Bouagga et al. 2014; Hafsi et al. 2016). This is the case also for citrus, where our study showed that these two attracticides were a valid standalone control methods against *C. capitata*. The population of *C. capitata* was kept at low level in Ceranock[®] treated plots during the 16 weeks following the application of treatments, indicating that the efficacy of this food attractant can persist for a long period. Whereas, we noticed that in AAL&K[®] treated plots, *C. capitata* population decreased after each application and increased again three weeks after the application of AAL&K[®] droplets indicating that the effectiveness of this attracticide device was a week less than the lifespan data as provided by the company. Thus, to keep fruit damage under 2%, AAL&K[®] should be applied every 3 weeks, i.e. four times per season in our case.

Ceratitis capitata has been traditionally controlled with organophosphate insecticides mixed with food attractant (Lysatex in Tunisia) applied as terrestrial or airlift ways (Boulahia-Kheder et al. 2010). In addition to the negative effects of this active ingredient on environment and human health (Michaud 2003; Michaud and Grant 2003), malathion was demonstrated low efficacy in C. capitata population reduction (Hafsi et al. 2015). The highest rate of fruit damage in malathion treated plots may be attributed to the nonefficiency of this insecticide and specifically to the resistance of C. capitata to this active ingredient as suggested by Magana et al. (2007). Efficacy of this technique against C. capitata and its effect on non-target insects have been evaluated recently in Tunisia (Hafsi et al. 2015b), but the question of resistance of C. capitata to malathion has never been discussed. Taking into account that four AAL&K® treatments were necessary to effectively protect citrus fruits, the final cost will increase to reach 114.28 € ha⁻¹. The projected cost of Ceranock® treatment necessary to ensure 3.88% citrus fruit damage is about 106.85 \in ha⁻¹. In our case, the three chemical treatments using malathion in aerial application resulted in 21.36% fruit damage with a final cost of $67 \in ha^{-1}$. Although that the final cost of mass trapping still higher than that of conventional approach using insecticides, it is clearly more effective in minimising fruit damage with 80-90% in citrus orchards.

Despite the relatively high cost of attract and kill techniques tested in this study, substantially less fruit damage was observed in mass trapping and attracticides treated plots than in insecticides treated ones, which resulted in cost-savings as less risks towards the environment. According to the analysis of cost-benefits of each control methods, we can deduce that the total cost of mass trapping and the rate of fruit damage were higher than those of attracticide devices. This result confirms previous studies reporting that the attracticide devices are alternative to mass trapping (Navarro-Llopis et al. 2015). In addition, the use of insecticides in attracticides and mass trapping techniques is limited to the devices or traps, which results in minimum adverse side-effects on non-target arthropods (Hafsi et al. 2016) and reduction of insecticide residues in citrus fruits and environment. The two experiments performed in this study show that the use of the most efficient attractant in attracticide devices (Ceranock® and AAL&K®) and mass trapping (Ceratrap^{*}) can be cost-effective and a safer alternative to conventional approach using insecticides in cover or aerial applications.

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Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Abir Hafsi () http://orcid.org/0000-0003-1859-5391 Khaled Abbes () http://orcid.org/0000-0001-5592-1892

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