Attract-and-kill systems efficiency against *Ceratitis capitata* (Diptera: Tephritidae) and effects on non-target insects in peach orchards

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**Abstract**
For control of the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae), the attract-and-kill or attracticide technique is an alternative to the spraying of traditional organophosphate pesticides. In this study, the effectiveness of Ceranock and AAL&K attract-and-kill bait stations was assessed for control of *C. capitata* in Tunisian peach (*Prunus persica*) orchards. Our results showed that, in orchards with early-ripening varieties, the numbers of *C. capitata* males and fruit damage were significantly lower in plots treated with Ceranock and AAL&K bait stations than in plots treated with conventional organophosphate and pyrethroid insecticides. In addition, the abundances of non-target insects in the Chrysopidae, Coccinellidae and Miridae were significantly greater in plots treated with the bait stations than in plots treated with the conventional pesticides; that is, the use of attract-and-kill bait stations had fewer negative effects than the application of conventional pesticides on the biological diversity in Tunisian peach orchards. Overall, the results indicate that Ceranock and AAL&K attract-and-kill bait stations are useful alternatives for the control of *C. capitata* in Tunisian peach orchards planted with early-ripening varieties.

**Introduction**
The Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is a highly polyphagous and multivoltine pest worldwide (Liquido et al. 1990). In Tunisia, *C. capitata* is one of the most economically important pests of citrus, peach, fig and apricot; the direct damage to fruits caused by *C. capitata* results in yield losses equivalent to 4.5 million U.S.$ (Driouchi 1990). Control of *C. capitata* has mainly relied on the application of organophosphate insecticides and especially malathion mixed with protein baits (Boulahia-Kheder et al. 2012). There are several problems with the use of these insecticides. For example, resistance to malathion was found in *C. capitata* populations in Spain (Magan et al. 2007). Also, malathion-based insecticides have negative effects on non-target insects (Michaud 2003; Michaud and Grant 2003) and can result in secondary outbreaks of other pests (Gerson and Cohen 1989). Moreover, the use of insecticides against *C. capitata* does not always prevent fruit damage. Alternatives to these organophosphate insecticides are clearly needed.

As an alternative to these insecticides, the attract-and-kill technique of mass trapping is used in many countries including Spain and Greece (Navarro-Llopis et al. 2008). Although mass trapping reduced *C. capitata* adult numbers and fruit damage in Tunisian orchards (Hafsi et al. 2015), the high costs of traps and attractants are considered limiting factors (Navarro-Llopis et al. 2013).
Other forms of attract-and-kill techniques need to be tested for the control of *C. capitata*, and one such technique is referred to as the bait station system. Bait station systems differ from mass trapping systems in that bait stations do not retain target insects in traps (El-Sayed et al. 2009; Navarro-Llopis et al. 2014). Various bait station systems have been developed and appear to be less expensive than current mass trapping systems (Navarro-Llopis et al. 2013) and effective at reducing fruit loss by maintaining adult *C. capitata* numbers under the economic threshold. Several studies proved the efficiency of this technique under low and high pest densities against pests in the Lepidoptera (Mansour 2009; Kroschel and Zegarra 2013) and Tephritidae (Martinez-Ferrer et al. 2010). Control with bait stations relies on a comprehensive understanding of the orientation behaviour and movement of adult flies with respect to oviposition and food resources (Miranda et al. 2001) and also relies on an understanding of insecticide efficacy (Manrakhan et al. 2013). The current study evaluates the use of two commercial bait station systems: the Ceranock and the AAL&K bait stations. Although the Ceranock attract-and-kill bait station system has been found to control *C. capitata* in Tunisian peach orchards (Bouagga et al. 2014), there are concerns regarding the effects of food-based attractants on the environment and on the non-target organisms (Burns et al. 2001; Barry et al. 2003). AAL&K bait stations have not been studied in Tunisia.

The first objective of our study was to compare the effects of Ceranock and AAL&K attract-and-kill bait stations with the spray application of deltamethrin and dimethoate insecticides on *C. capitata* numbers and fruit damage in early and mid-season variety. The second objective was to evaluate the effects of these treatments on the abundance of non-target insects.

**Materials and Methods**

**Attract-and-kill systems**

Two types of attract-and-kill bait stations were tested. The Ceranock® bait station, which was supplied by Russell IPM (United Kingdom), has three parts: a plastic hook, which is used to hang the device from a tree branch; a plastic case; and a sponge impregnated with the attractive bait (hydrolysed proteins and citrus plant extract at 5 g/bait station) and insecticide (alpha-cypermethrin at 0.01 g/bait station). The average ‘lifespan’ of this bait station is 4 months under normal field conditions. As recommended by the manufacturer, 400 Ceranock bait stations/ha were deployed in the peach orchards in this study.

The AAL&K® bait station, which was supplied by Atlas Agro (Switzerland), consists of a tube containing 150 g of attracticide paste, which is a mixture of attractive bait (enriched ginger oil as male *C. capitata* parapheromone at 10%), insecticide (permethrin at 6%) and stabilizer (at 84%). This product is applied as droplets of paste on the trees, and its average lifespan is 4 weeks under normal field conditions. As recommended by the manufacturer, it was applied in peach orchards with an average of 3000 droplets (225 g)/ha (6–7 droplets per tree), and droplets were placed on the undersides of leaves.

**Field experiments**

Experiments were conducted in three peach orchards in Tunisia. In two orchards, which were planted with early varieties (one with San-Pedro and the other with Florodastar), experiments were conducted from March to June 2013; these orchards were located in the Alelcha and Ourdanine areas in the coastal region of Tunisia (table 1). In one orchard, which was planted with a mid-season variety (Royal Glory), an experiment was conducted from May to July 2014; this orchard was located in north-eastern Tunisia (table 1). In each orchard, the efficacy of Ceranock and AAL&K bait stations was compared with a conventional approach that used an organophosphate insecticide (dimethoate at 100 ml/hl) and a pyrethroid insecticide (deltamethrin at 83 ml/hl) as cover sprays. Each of the three orchards had three plots of 1 ha each corresponding to the three treatments (the conventional insecticides and the Ceranock and AAL&K bait stations). The treatments are summarized in table 1. In plots treated with conventional insecticides, the insecticides were applied according to regular agricultural practices and label guidelines. The insecticides were applied with a high pressure sprayer equipped with turbulence chamber nozzles. Insecticides were sprayed at a pressure of 6–7 bars to run-off resulting in complete and even coverage of the tree canopies. The bait stations were deployed at the rates indicated in the previous section and on the dates indicated in table 1.

**Monitoring of *C. capitata* numbers**

To monitor the numbers of male *C. capitata* in all plots, we used ‘McPhail’ traps baited with trimedlure mixed with dichlorvos (DDVP) as a killing agent. Six of these traps were placed in each plot and were checked
weekly. The males of *C. capitata* captured in each trap were counted and removed weekly.

**Evaluation of fruit damage**

In each plot, 10 trees were randomly selected and marked. From each one, 20 fruits from each orientation (east, west, north and south) were marked and examined. The number of oviposition punctures per marked fruit was determined weekly. For each tree, the proportion of fruit damaged was calculated as the number of fruit with at least one oviposition puncture over the total number of examined fruits.

**Effect of Ceranock and AAL&K bait stations on non-target insects**

The effects of the two kinds of attract-and-kill bait stations on the abundance of non-target insects were assessed in the orchards at Alelcha and Ouardanine using beat sheet samples. Samples were collected 7 days before and 5, 12 and 19 days after the bait stations were deployed or after deltamethrin and dimethoate were applied. Each sampling was carried out on 15 trees per plot according to the method described by Fauvel et al. (1981). On each sampling date, five branches per tree were beaten, and insects were collected on a white rectangular beat sheet, giving a total of 75 sampled branches per treatment per sampling date per plot. The samples were kept in 90% alcohol and were identified to family and counted in the laboratory. The families of non-target insects selected for this study were Chrysopidae (Neuroptera), Coccinellidae (Coleoptera) and Miridae (Hemiptera). These families were selected because they contain species that are common and important in peach orchards in Tunisia.

**Statistical analysis**

For each orchard, a generalized linear model (GLM) with Poisson error (Log link) was used to determine how numbers of *C. capitata* males caught in the McPhail traps were affected by treatment (Ceranock bait station, AAL&K bait station or conventional approach), date and the interaction between treatment and date. We then compared the curves describing changes in male numbers over time for the three treatments by calculating area under disease progress curve (AUDPC) values, which were then analysed using analysis of variance (ANOVA).

A GLM with binomial error (logit link) was used to analyse the proportions of fruit damaged as a function of time by treatment.
of treatment, date and interaction between these two factors.

A GLM with Poisson error (Log link) was used to analyse the abundances of non-target insects as a function of treatment, date and their interaction. All statistical analyses were performed using R project for statistical computing (R Development Core Team, 2008).

Results

**C. capitata numbers**

As indicated by AUDPC values, the abundance of *C. capitata* males significantly differed among treatments in the Alelcha orchard (F$_2$, 15 = 129.81, P < 0.001), in the Ouardanine orchard (F$_2$, 15 = 43.18, P < 0.001) and in the Morneg orchard (F$_2$, 12 = 357.6, P < 0.001).

In the Alelcha orchard, the abundance of *C. capitata* males was affected by treatment (Dev$_2$, 243 = 818.6, P < 0.001), date (Dev$_14$, 229 = 6868.3, P < 0.001) and their interaction (Dev$_{24}$, 205 = 50.2, P = 0.0013). *C. capitata* males were significantly more abundant in plots treated with conventional insecticides than in plots treated with AAL&K and Ceranock bait stations (fig. 1a).

In the Ouardanine orchard, the abundance of *C. capitata* males was affected by treatment (Dev$_2$, 243 = 8477.1, P < 0.001), date (Dev$_{14}$, 229 = 7763.2, P < 0.001) and their interaction (Dev$_{24}$, 205 = 55.8, P < 0.001). The number of males was initially low and did not exceed 1.9 individuals/trap/day until 27 April 2013 (fig. 1b). After this date, *C. capitata* numbers increased steadily to a peak on 8 June 2013.

In the Morneg orchard, the abundance of *C. capitata* males was affected by treatment (Dev$_2$, 133 = 2571.1, P < 0.001) and date (Dev$_8$, 299 = 7077.00, P < 0.001) but was not affected by their interaction (Dev$_{16}$, 109 = 55.80, P = 0.40). Numbers of *C. capitata* males did not differ among treatments until 13 May 2014 (fig. 1c). After this date, *C. capitata* numbers increased more in plots treated with conventional insecticides than in plots treated with AAL&K bait stations (fig. 1a).

The numbers of *C. capitata* males differed among the three orchards (P < 0.001). The numbers were lower in orchards with the early-ripening varieties (in Alelcha and Ouardanine) than in the orchard with the mid-season ripening peach variety (in Morneg).

**Fruit damage**

The proportion of fruit damaged was affected by treatment and date in the Alelcha orchard (Dev$_2$, 537 = 439.44, P < 0.001; Dev$_{14}$, 529 = 2601.49, P < 0.001, respectively), in the Ouardanine orchard (Dev$_2$, 537 = 779.00, P < 0.001; Dev$_{14}$, 529 = 2601.49, P < 0.001, respectively) and in the Morneg orchard (Dev$_2$, 207 = 1275.50, P < 0.001; Dev$_8$, 201 = 3777.40, P < 0.001, respectively). The interaction between treatment and date was significant in the Morneg orchard (Dev$_{16}$, 189 = 15.60, P < 0.001) but not in the other two orchards.

![Fig. 1](image-url) Mean numbers of *C. capitata* males captured per McPhail tarp and per day in plots treated with AAL&K and Ceranock attract-and-kill bait stations and with conventional insecticides in three peach orchards in Tunisia. (a) the Alelcha orchard in 2013; (b) the Ouardanine orchard in 2013; and (c) the Morneg orchard in 2014. Arrows indicate when bait stations were deployed (light grey for AAL&K, dark grey for Ceranock) and when insecticides were applied (black).
In the Alelcha and Ourdanine orchards, the proportion of fruit damaged at harvest was about two times lower in plots treated with Ceranock and AAL&K bait stations than in plots treated with conventional insecticides (fig. 2a,b).

In the Morneg orchard, the proportion of fruit damaged at harvest was substantially greater in plots treated with Ceranock and AAL&K bait stations than in plots treated with conventional insecticides (fig. 2c).

Non-target insects
The abundance of the different families of non-target insects did not significantly differ among treatments on 04 November 2013, which was before insecticides were sprayed and bait stations were deployed ($P = 0.954$). The abundance of non-target insects did significantly differ among treatments after 04 November 2013 ($P < 0.001$).

Chrysopidae abundance significantly differed among treatments in the Alelecha orchard ($P < 0.001$, table 2A) and in the Ourdanine orchard ($P < 0.001$, table 2B) and among sampling dates within the treatments (table 2A,B). Chrysopidae were more abundant in plots treated with Ceranock bait stations than in plots treated with AAL&K bait stations or with conventional insecticides at the Alelecha and Ourdanine orchards (fig. 3a,b).

Coccinellidae abundance significantly differed among treatments in the Alelecha orchard ($P = 0.0021$, table 2A) and in the Ouardanine orchard ($P < 0.001$, table 2B) and among sampling dates within the treatments (table 2A,B). Coccinellidae were significantly more abundant in plots treated with Ceranock bait stations than with AAL&K bait stations or conventional insecticides (fig. 3a,b).

Miridae abundance did not significantly differ in plots treated with Ceranock and AAL&K bait stations, but Miridae abundance was significantly greater ($P = 0.012$, table 2B) in plots treated with Ceranock and AAL&K bait station than in plots treated with conventional insecticides (fig. 3).

Discussion
In this study, *C. capitata* numbers were lower in two orchards with early-ripening varieties (in Alelecha and Ouardanine areas) than in an orchard with a mid-season variety (in the Morneg area). In the orchards with the early-ripening varieties, fruit damage and numbers of *C. capitata* males were two-fold lower in plots treated with Ceranock and AAL&K attract-and-kill bait stations than in plots treated with conventional insecticides. The bait stations, however, were less effective than conventional pesticides in the orchard with a mid-season ripening variety and with high *C. capitata* numbers. We also found that Chrysopidae, Coccinellidae and Miridae families were more abundant in plots treated with Ceranock and AAL&K bait stations than in plots treated with conventional insecticides.

In Alelecha and Ouardanine orchards, *C. capitata* numbers were initially low (0.075 individuals/trap/day), increased (10.93 individuals/trap/day) with increasing temperature and fruit receptivity and then decreased to 0.91 individuals/trap/day at the end of...
the fruiting period because of harvest and the absence of other host fruits in the orchards.

In the Morneg orchard, *C. capitata* numbers remained low until the beginning of June. *C. capitata* numbers increased gradually as temperatures increased to about 29°C (Figure S1) and as host fruits, like those of figs and prickly pears, became available in nearby fields. *C. capitata* populations are greatly influenced by temperature and the presence of alternate host plants (Israely et al. 1997; Alemany et al. 2004; Campos et al. 2007). Thus, the low numbers of *C. capitata* observed in March, April and May under Tunisian conditions can explain why the attract-and-kill technique was effective in orchards with early-ripening varieties. Similar results were found by Bouagga et al. (2014), who reported that the use of Ceranock bait stations in Tunisian peach orchards reduced fruit damage by sevenfold compared to the untreated control. Khalaf et al. (2013) showed that the deployment of Ceranock bait stations decreased *C. capitata* numbers and fruit infestation in early ripening apricot. Reduced efficacy of attract-and-kill systems at high pest densities has been previously reported (Downham et al. 1995; Losel et al. 2000; Ebbinghaus et al. 2001). Other attract-and-kill systems have shown to be effective against *C. capitata* and other fruit fly species in other countries include the Magnet MED and the prototype L&K tube systems in Spain (Navarro-Llopis et al. 2013, 2014) and the GF-120 system in the USA (Prokopy et al. 2000). It would be useful to test these systems in citrus, peach and apricot orchards in Tunisia.

For early-ripening varieties, we showed that fruit damage caused by *C. capitata* was twofold lower in plots treated with either kind of bait station than in plots treated with conventional organophosphate insecticides (fig. 2). Other studies have also reported that the attract-and-kill technique is more effective than conventional insecticide sprays against *Bactrocera zonata* (Diptera: Tephritidae) in Mango orchards (Temerak et al. 2012) and against *C. capitata* in citrus orchards (Navarro-Llopis et al. 2010). The poor performance of AAL&K and Ceranock bait stations in terms of reducing *C. capitata* numbers and fruit damage in orchards planted with a mid-season variety suggests that AAL&K and Ceranock bait stations should be deployed at 400 and 500 bait stations per ha, respectively, when *C. capitata* numbers are very high. The performance of AAL&K and Ceranock bait stations might also be improved by deploying the stations earlier in the season to prevent *C. capitata* numbers from increasing to high levels later in the season. Early treatment was effective for the conventional insecticides in the orchard with the mid-season variety; at that orchard, insecticide treatments began on 19 March 2013 whereas bait stations were not deployed until about 2 months later. If the baits stations are deployed early, the lifespan of each kind of station should be considered.

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**Table 2** Statistics derived from generalized linear models with Poisson error for the effects of treatment, date and their interaction on the abundances of non-target insects (Chrysopidae, Coccinellidae and Miridae) in the Alelcha orchard (A) and the Ouardanine orchard (B).

<table>
<thead>
<tr>
<th>Taxa of non-target insects</th>
<th>Effect</th>
<th>d.f.</th>
<th>Deviance</th>
<th>Resid. d.f.</th>
<th>Resid. Dev</th>
<th>Pr(&gt;Chi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Chrysopidae</td>
<td>Date</td>
<td>5</td>
<td>24.92</td>
<td>48</td>
<td>41.06</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>2</td>
<td>14.29</td>
<td>46</td>
<td>26.76</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>Date x Treatment</td>
<td>10</td>
<td>23.40</td>
<td>36</td>
<td>3.36</td>
<td>0.002</td>
</tr>
<tr>
<td>Coccinellidae</td>
<td>Date</td>
<td>5</td>
<td>1.21</td>
<td>48</td>
<td>37.15</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>2</td>
<td>8.51</td>
<td>46</td>
<td>24.85</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Date x Treatment</td>
<td>10</td>
<td>9.46</td>
<td>36</td>
<td>7.84</td>
<td>0.074</td>
</tr>
<tr>
<td>Miridae</td>
<td>Date</td>
<td>5</td>
<td>2.34</td>
<td>48</td>
<td>24.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>2</td>
<td>5.32</td>
<td>46</td>
<td>18.99</td>
<td>0.070</td>
</tr>
<tr>
<td></td>
<td>Date x Treatment</td>
<td>10</td>
<td>9.14</td>
<td>36</td>
<td>4.59</td>
<td>0.155</td>
</tr>
<tr>
<td>B Chrysopidae</td>
<td>Date</td>
<td>5</td>
<td>37.72</td>
<td>48</td>
<td>32.90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>2</td>
<td>6.89</td>
<td>46</td>
<td>25.99</td>
<td>0.032</td>
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<tr>
<td></td>
<td>Date x Treatment</td>
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<td>14.22</td>
<td>36</td>
<td>11.78</td>
<td>0.163</td>
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<tr>
<td>Coccinellidae</td>
<td>Date</td>
<td>5</td>
<td>23.08</td>
<td>48</td>
<td>61.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>Treatment</td>
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<td>21.16</td>
<td>46</td>
<td>40.57</td>
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</tr>
<tr>
<td></td>
<td>Date x Treatment</td>
<td>10</td>
<td>35.66</td>
<td>36</td>
<td>4.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Miridae</td>
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<td>43.50</td>
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<td>34.55</td>
<td>0.011</td>
</tr>
<tr>
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<td>Date x Treatment</td>
<td>10</td>
<td>31.46</td>
<td>36</td>
<td>3.09</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
In addition to effectively controlling *C. capitata* numbers and fruit damage in orchards planted with early-ripening varieties, the attract-and-kill bait stations caused less harm to non-target insects than conventional insecticides. Chrysopidae, Coccinellidae and Miridae were more abundant in plots treated with Ceranock and AAL&K bait stations than in plots treated with conventional insecticides (fig. 3). This can be explained by the absence of organophosphate cover sprays in the attract-and-kill technique. Organophosphate insecticides are known to negatively affect non-target organisms (Chang et al. 2013). In conventional control, which depends mainly on the application of broad-spectrum insecticides, non-target insects are exposed to acute and long-term toxic effects of insecticides directly via contact and/or indirectly via ingestion of contaminated prey (Bostanian et al. 2009). In attract-and-kill systems, the use of insecticides (the killing agent) is limited to the treated devices. As a consequence, attract-and-kill bait stations have minimal lethal and sublethal effects on non-target insects. The use of a pheromone should increase bait station selectivity (Katsoyannos et al. 1999) and should maximize the number of flies attracted and the lifespan of the dispensers (Navarro-Llopis et al. 2008).

Our study demonstrates that attract-and-kill bait stations are more effective than conventional insecticides for the control of *C. capitata* in peach orchards planted with early-ripening varieties. The bait stations also caused less harm to non-target insects than conventional insecticide applications. These results indicate that attract-and-kill bait stations represent a good alternative to conventional insecticide sprays for the control of *C. capitata* in Tunisian peach orchards planted with early-ripening varieties.

**Fig. 3** Numbers of non-target insects (Chrysopidae, Coccinellidae and Miridae) in plots treated with AAL&K and Ceranock attract-and-kill bait stations and with conventional insecticides in Ailecha (a) and Ourdane (b) peach orchards. Non-target insects were sampled with beat sheets, and values are means ± SE per tree.
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**Supporting Information**

Additional Supporting Information may be found in the online version of this article:  
**Fig. S1.** Mean temperatures between March and July in Alelecha and Ouardanine orchards in 2013 and in the Morneg orchard in 2014.