

1 **Efficacy of attract-and-kill devices for *Ceratitis capitata* control**

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12 Running title: Attract-and-kill devices for *Ceratitis capitata*

13

14 **Abstract**

15

16 **BACKGROUND:** The control of *Ceratitits capitata* Wiedemann used to rely on chemical
17 control with organophosphate insecticides. New European Directives have banned the use
18 of many substances, so the development of new control methods is essential to manage this
19 pest. Bait sprays with spinosad, mass trapping and lure-and-kill techniques have been the
20 basis for new integrated pest management programmes. This study planned two one-year
21 field trials in two citrus areas to test the efficacy of attract-and-kill devices against mass
22 trapping and spinosad plus bait treatments.

23 **RESULTS:** The Magnet® MED attract-and-kill device, Spintor® treatments and mass
24 trapping achieved good control of *C. capitata* populations, confirmed by low percentages of
25 damaged fruit in the harvest assessments. Conversely, the fly population levels on plots
26 treated with another attract-and-kill prototype device increased three times more than the
27 populations recorded in other treated plots. The same was observed for fruit damage, with
28 from six to eight times less damage with Magnet® MED and spinosad treatments,
29 respectively, vs. the attract-and-kill prototype devices.

30 **CONCLUSION:** With an effective attractant, conventional trapping systems can be
31 replaced with cheaper, more user-friendly attract-and-kill devices. The efficacy of these
32 devices and their advantages in relation to conventional mass trapping systems are
33 discussed.

34

35 **Keywords:** *Ceratitits capitata*; attract and kill; bait station; mass trapping; fruit fly

36

37 **1 INTRODUCTION**

38 Currently, new *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) control techniques are
39 being studied and developed to replace traditional organophosphate pesticide applications.

40 In Spain, fruit fly resistance to malathion has been reported¹ and insecticides such as
41 malathion, fenthion or trichlorphon are currently banned in the EU. Replacement of
42 organophosphates with other more environmentally friendly products, such as spinosad, is
43 taking place. Spinosad formulations with bait (Spintor®) have proved to be as effective as
44 malathion,² but some fruit damage problems were described in 2004 and 2005 in citrus and
45 other crops when Spintor® was applied in spots (Alfaro, personal communication). In fact,
46 scars appear at the point where the bait spot touches the fruit or the leaves.³

47 The use of attract-and-kill techniques has increased in recent years as these devices attract
48 insects to a killing agent and avoid having to spray large quantities of insecticides to affect
49 insects. The attract-and-kill tactic is called lure-and-kill when insects are not retained inside
50 a device, whereas mass trapping refers to the use of a trap that retains pests.⁴ Bait stations
51 are defined as discrete containers of attractants and toxins whose insecticide attracts pests⁵
52 but, in this case, the toxin can kill, sterilise⁶ or infect the target insect. The application of
53 bait sprays with insecticide should be considered as a lure-and-kill method, but with larger
54 amounts of insecticide.⁷

55 In Spain, there are currently more than 50 000 ha of citrus being treated with bait, mass
56 trapping or a lure-and-kill method because there are no other available environmentally
57 friendly control methods. Bait stations are a cost-effective way of mass trapping which
58 could replace current mass trapping methods. We should take into account that mass
59 trapping costs around 3-4 euros per trap, which includes the trap, the attractant and the

60 insecticide. This means a total amount of 200 euros per ha. This cost is not redeemable for
61 most crops and only public subsidies enable the use of mass trapping. However, this cost
62 can be cut to 2 euros per trap using bait stations (around 100 euros per ha), which means
63 that this method can be applied to European crops cost-effectively.

64 Bait stations have been developed for other tephritids, such as *Bactrocera cucurbitae*
65 (Coquillett) or *B. dorsalis* (Hendel);⁸⁻¹⁰ *B. carambolae* Drew & Hancock,¹¹ *Rhagoletis*
66 *pomonella* (Walsh),¹² *R. mendax* Curran,¹³ *Anastrepha suspensa* Loew.⁵ In most cases
67 however, the efficacy of these devices has been tested in laboratory or field cages, but not
68 under real conditions. Devices are designed to remain active in the field for as long as
69 possible with no maintenance, and to attract flies effectively.

70 Both mass trapping and bait stations should be applied in isolated or wide areas in order to
71 reduce fruit fly intrusion. It is intuitively obvious that immigration of pests into a treated
72 area prevents their effective suppression or eradication.¹⁴ For *C. capitata*, this intuitive
73 affirmation is an even better example of such a case due to fruit flies' high mobility. In
74 order to achieve "area-wide integrated pest management", trials should be carried out over
75 large or very isolated areas, and should affect the whole population of this treated area
76 during a long-term planned campaign.^{14,15} For this reason, we planned field trials in plots of
77 around 1 ha to monitor fruit fly populations and to assess the fruit damage in the centre of
78 each plot.

79 This work reports the efficacy of two attract-and-kill devices with two parameters: fruit fly
80 population reduction and fruit damage obtained with each treatment. The efficacy of these
81 devices is compared with a standard treatment with insecticides and/or with the mass

82 trapping technique. This study will allow the recommendation of alternatives to reduce the
83 use of insecticides and to cut the cost of mass trapping systems.

84

85 **2 MATERIAL AND METHODS**

86 **2.1 Field trials**

87 Field trials were carried out in the years 2010 and 2011 in early-clementine orchards
88 (*Citrus reticulata* Blanco, variety Marisol). All the traps or lure-and-kill devices (LK
89 hereafter) were placed at a density of 50 traps ha⁻¹ two months before the harvest started.

90 *2.1.1 2010 Trial*

91 Four treatments were assessed from mid-July to October 2010: two types of LK, Magnet®
92 MED (Suterra LLC, Bend, OR, USA) and a new lure-and-kill device design developed in
93 our laboratory (L&K Tube hereafter), mass trapping and spinosad spray. A field trial was
94 carried out in a 20-year-old 7.6-ha orchard located in Sagunto (N 39° 39' 51''; W 0° 17'
95 31'', 20 km north of the city of Valencia, Spain). The orchard was divided into 12 plots,
96 with three plots per treatment, as shown in Figure 1 and in Table 1. The untreated plots
97 inside the orchard were non-productive plots or non-early varieties which ripen several
98 weeks after Marisol clementines.

99 *2.1.2 2011 Trial*

100 Two treatments were assessed from mid-July to November 2011: Magnet® MED (Suterra
101 LLC, Bend, OR, USA) and mass trapping. A field trial was carried out in a 18-year-old 7.3-
102 ha orchard, located in Gandía (N 38° 58' 7''; W 0° 15' 59'', 60 km south of the city of
103 Valencia, Spain). The orchard was divided into 8 plots, including three plots per treatment
104 and two untreated plots, as shown in Figure 2 and in Table 1.

105

106 **2.2 Description of treatments**

107 Magnet® MED, supplied by Suterra Europe (Barcelona, Spain), is a paper envelope attract-
108 and-kill device impregnated with deltamethrin which contains two membrane dispensers,
109 with trimethylamine and ammonium acetate as attractants (Fig. 3A).

110 The L&K Tube is a prototype lure-and-kill device consisting of a yellow-coloured cylinder.
111 There is a protein bait that contains cypermethrin at the bottom of the cylinder, and there
112 are several small holes around the bait container which allow attractants to be released.
113 There are two mesoporous dispensers inside the tube containing ammonium acetate,
114 trimethylamine and methyl-pyrrolidine (Fig. 3B).

115 Mass trapping was carried out with Tephri-Traps®, baited with a three-component lure
116 called Biolure® Unipack™ (Suterra LLC, Bend, OR, USA) and with a 500 mg dichlorvos
117 strip (Suterra Biocontrol España, Barcelona, Spain).

118 “Spintor® cebo” is a commercial formulation from Dow Agrosiences LLC (Madrid,
119 Spain) with 0.024 % w/v of spinosad in the protein bait. The product was diluted in three
120 parts water and was sprayed in spots on the south face of trees with a backpack sprayer,
121 using 1 L of “Spintor® cebo” per ha. In the 2010 field trials, plots 8, 10 and 12 (Fig. 1)
122 were treated weekly from 5 weeks before harvesting until the end of the trials (from 2
123 September to 30 September).

124

125 **2.3 Fruit fly population monitoring and fruit damage assessment**

126 Two parameters were used in both the 2010 and 2011 trials to assess the efficacy of each
127 treatment: fruit fly population and fruit damage assessment per plot.

128 In order to follow the fruit fly population, one monitoring trap was placed in each plot in
129 the second week of July. Mosquisan® fly-traps (SANSAN Prodesing SL, Valencia, Spain)
130 baited with Biolure® (Suterra LLC, Bend, OR, USA) and a 500 mg dichlorvos strip were
131 employed for monitoring purposes. Traps were hung in the centre of each plot and were
132 checked weekly in 2010 and biweekly in 2011, and the number of males and females
133 caught were recorded.

134 Fruit damage was assessed for 4 weeks before harvesting when fruit began to ripen until the
135 harvesting date. Twenty trees per plot and 20 fruits per tree were sampled weekly and
136 scrutinised with a Linen Tester. The first and last three rows of each plot were discarded to
137 avoid the influence of the outer population. The location of the trees selected for sampling
138 was randomised before starting each assessment.

139

140 **2.4 Statistical analysis**

141 In order to analyse the fruit fly population data, two periods were established in the 2010
142 trial; the first from 21 July 2010 to the 7th week of the trial (7 September), when attract-
143 and-kill devices were placed in the field and no Spintor® treatments had yet been applied.

144 The second period started from the 8th week until the end of the 2010 trial, when fruits were
145 ripening. Spintor® was applied weekly on the corresponding plots. In the 2011 trial, two
146 periods were considered: an initial period before treatment application to ensure that all the
147 plots had the same population; a second period started after traps were placed to assess the
148 treatments' efficacy.

149 A one-way ANOVA was employed to compare the fruit fly populations in each period
150 (LSD test at $P < 0.05$). The square-root transformation of the number of catches was used

151 to normalise the data.

152 For fruit damage, a one-way ANOVA with the log-transformed data [$\log(x+1)$] from the
153 last two assessments was employed to compare the effect of the different treatments.
154 Statistical analyses were done using the Statgraphics plus 5.1 package (Statpoint
155 Technologies, Warrenton, VA, USA).

156

157 **3 RESULTS**

158 **3.1 Fruit fly population**

159 *3.1.1 Trial 2010*

160 According to the treatment employed, the fruit fly population dynamics is shown in Figure
161 4. During the first seven weeks, the medfly population increased in the L&K Tube plots,
162 with significant differences found between the L&K Tube plots and the Magnet® MED
163 and mass trapping-treated plots ($F = 15.21$; $df = 3,83$; $P < 0.001$) (Table 2). However, L&K
164 Tube plots did not significantly differ from the Spintor® plots which, during this period,
165 had not yet been treated. This means that mass trapping and Magnet® MED significantly
166 reduced fruit fly populations in comparison to untreated plots. However, the L&K Tubes
167 did not significantly reduce the fruit fly population if compared with an untreated field.

168 From the 7th week to the 10th week, corresponding with the ripening period, the Spintor®,
169 Magnet® MED and mass trapping-treated plots showed no significant differences in
170 population levels, whereas the population in the L&K Tube plots increased until the 10th
171 week and became significantly higher than in the other plots ($F = 10.32$; $df = 3,32$; $P <$
172 0.001) (Table 2).

173 *3.1.2 Trial 2011*

174 At the beginning of the trial, before trap placement, no significant differences in fruit fly
175 populations were noted between treatments ($F = 0.1$; $df = 2,16$; $P = 0.90$), according to the
176 population dynamics depicted in Figure 5. In contrast, after placing traps and lure-and-kill
177 devices, the fruit fly population increased in the untreated plots, with significant differences
178 found with the Magnet® MED and mass trapping-treated plots ($F = 8.28$; $df = 2,88$; $P <$
179 0.001) (Table 3). This means that mass trapping and Magnet® MED significantly reduces
180 fruit fly populations in comparison to untreated plots.

181

182 **3.2 Fruit damage**

183 *3.2.1 Trial 2010*

184 Fruit damage was assessed weekly from 8 September 2010, 4 weeks before harvesting, but
185 no fruit damage was recorded until 29 September, 1 week before harvesting. Fruit damage
186 evolution is shown in Figure 6. In the L&K Tube-treated plots, the number of damaged
187 fruits increased as fruits ripened, whereas the fruit damage for other treatments remained at
188 acceptable levels.

189 The fruit damage assessment during the harvest period is shown in Table 4. The L&K Tube
190 plots displayed significantly more damage than the other treatments ($F = 4.66$; $df = 3,284$;
191 $P = 0.0034$). Magnet® MED and mass trapping treatments proved to be as effective as
192 weekly Spintor® sprays from the 5th week before harvesting, with no significant
193 differences among them.

194 *3.2.2 Trial 2011*

195 Although in this case, the fruit remained unharvested in fields for 1.5 months more than in
196 the 2010 trials, fruit damage was below 0.05% in all the plots. This fruit damage was

197 negligible and did not allow a statistical analysis.

198

199 **4 DISCUSSION**

200 This study has compared the efficacy of the mass trapping technique, attract-and-kill
201 devices and bait sprays. In recent years, the mass trapping technique has proved to be as
202 effective as insecticide sprays to control *C. capitata* in citrus orchards.^{16,17} The results of
203 this work confirm mass trapping efficacy and also show that attract-and-kill devices are
204 effective enough to reduce *C. capitata* populations while remaining under the economic
205 threshold. In fact, these devices had a similar efficacy as mass trapping or the Spinosad®
206 weekly treatments. However, significant differences were found in the efficacy achieved by
207 the different tested devices.

208 Two types of attract-and-kill devices were compared in the 2010 trial versus standard
209 treatments, but there was no true control plot. The existence of this true control plot without
210 treatments would provide information about pest pressure in the trial fields; unfortunately
211 the cost of 3 ha of fruit losses would make the trial economically unfeasible. Nonetheless,
212 the efficacy of new control methods can be compared with well-known treatments.
213 Moreover in this trial, one of the treatments (L&K Tube) was significantly less effective
214 than the others, with high percentages of fruit damage in the three plots where this
215 treatment had been applied. These plots cannot be considered true control plots, but their
216 high percentage of fruit damage and their significantly larger fruit fly population allow us
217 to detect the strong pest pressure in this field and to validate the efficacy of the other
218 control methods. With the 2011 trial, we could arrange three untreated plots because fruit
219 damage was very low and no important economic losses were expected. On this occasion,

220 we observed that *C. capitata* populations were 6 times higher in untreated plots than in the
221 attract-and-kill plots.

222 A direct relationship between the percentage of fruit damage and the population level
223 detected in monitoring traps was found for the 2010 data. In these trials, the female
224 population reached 9.2 females per trap and day (FeTD) and fruit damage was over 1.5%.
225 In accordance to this correlation, plots with smaller fruit fly populations (below 3 FeTD)
226 had significantly lower percentages of fruit damage (under 0.3%). However in the 2011
227 trial, those plots with female populations over 6 FeTD did not present significant fruit
228 damage, even though the fruit remained in the field and over-ripened for two months more
229 than in the 2010 trial. The number of flies per trap and day (FTD) is an index that is widely
230 used to establish Areas of Low Prevalence (ALPP).¹⁸ With *C. capitata*, USDA and the
231 Spanish Government agreed on a limit of 0.5 FTD to consider ALPP. However, there is no
232 published FTD index to ensure that fruit damage remains below a defined limit. The results
233 of the present work show that this index cannot be easily obtained.

234 Previous experiments carried out in 2009 demonstrated the good efficacy of the L&K Tube
235 prototype in reducing fruit fly populations (unpublished results), with similar fruit damage
236 reductions to those of insecticidal treatments. However, the efficacy of this device in the
237 2010 trials was significantly lower than in the chemical and mass trapping treatments. This
238 difference between 2009 and 2010 could be attributed to the changes made in the devices
239 tested, as the holes through which attractants are released had a different position. This fact
240 means that the role of attractants is essential as to how the device performs and, therefore,
241 any change made in how the device is manufactured must be tested under field conditions.

242 The mass trapping technique was as effective as the Magnet® MED treatment using a

243 similar attractant. This means that, by using an effective attractant, a conventional trapping
244 system can be replaced with a device that is cheaper and easier to handle. Moreover, the
245 lifespan of Magnet® MED was 15 weeks, implying a lifespan of over 100 days; however,
246 this observation should be confirmed in future trials.

247 The mass trapping technique has been employed for *C. capitata* control in Spain since the
248 beginning of the 20th century.¹⁹ The efficacy of this technique has been assessed in the last
249 80 years and its economic viability has been discussed in several articles.^{17,20,21} Yet the
250 most important improvements in this technique have come about through the development
251 of synthetic female attractants,²² and via the use of cheaper, more efficient traps.²³
252 Nevertheless, new improvements are required to prolong the lifespan of attractant
253 dispensers. The dispensers for female attraction currently in use offer a lifespan of 4
254 months (Suterra LLC, Bend, OR, USA), whereas those for males can last 8 months in the
255 field.²⁴

256 The main advantages of the attract-and-kill systems over mass trapping techniques are: (1)
257 less manpower required for their field application, (2) absence of an expensive device that
258 retains flies, and (3) non-saturation of traps. In these trials, the assembly and hanging of
259 traps, filled with attractants and insecticide, in mass trapping plots required 1.5 hour per ha,
260 whereas the attract-and-kill systems needed about 40 min. In this case, the financial savings
261 are more important for growers than the absence of insecticides.⁴ The cost of the attract-
262 and-kill treatment for 1 ha (50 devices ha⁻¹) has been estimated to be between 100 and 150
263 euros with 40 min manpower, whereas one Spintor® treatment takes 90 min and costs 18
264 euros per ha. By bearing in mind that the number of Spintor® treatments varies between
265 three and six per year, the final cost would be between 48 and 96 euros and between 270

266 and 540 min manpower per ha. Currently, the cost of manpower in Spain is around 8 euros
267 per hour; therefore, the mean cost of treatment in Spain would be around 130 euros per ha
268 with attract-and-kill and 126 euros per ha with Spintor®. Therefore, the cost of the attract-
269 and-kill technique would not prove to be an obstacle for the implementation and success of
270 this control method.

271

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356 **Figures**

357 **Fig. 1.** Sketch of plot distribution in 2010 field trial. Plots: 1,3,6 Magnet Med; 7,9,11 Mass
358 trapping; 8,10,12 Spinosad; 2,4,5 L&K tube.

359 **Fig. 2.** Sketch of plot distribution in 2011 field trial. Plots: 1,4,8 Magnet MED®; 2,5,7
360 Mass trapping; 3,6 Untreated

361 **Fig. 3.** Magnet MED® (A) and L&K Tube (B) devices

362 **Fig. 4.** Dynamics of fruit fly population according to treatment, as number of females
363 caught per trap and day in 2010 field trial. Arrows point out treatments with spinosad
364 applied only in Spintor® plots.

365 **Fig. 5.** Dynamics of fruit fly population according to treatment, as number of females
366 caught per trap and day in 2011 field trial. Arrow points out mass trapping and Magnet
367 MED® device placement.

368 **Fig. 6.** Dynamics of fruit damage in the different plots, as percentage of damaged fruit.
369 Fruit damage assessments carried out on 23 September (1), 30 September (2), 7 October (3)
370 and 14 October (4).

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372

373

374 **Tables**375 **Table 1**

376 Plot treatments and characteristics

2010				2011			
Plot #	Treatment	Size (ha)	# devices	Plot #	Treatment	Size (ha)	# devices
1	Magnet® MED	1.1	57	1	Magnet® MED	0.8	46
2	L&K tube	0.9	49	2	Mass trapping	0.9	48
3	Magnet® MED	1.3	73	3	Untreated	1.0	0
4	L&K tube	1.1	63	4	Magnet® MED	1.1	56
5	L&K tube	0.8	40	5	Mass trapping	0.8	45
6	Magnet® MED	0.9	46	6	Untreated	0.8	0
7	Mass trapping	0.3	14	7	Mass trapping	0.9	49
8	Spintor®	0.2	0	8	Magnet® Med	1.0	51
9	Mass trapping	0.4	24				
10	Spintor®	0.2	0				
11	Mass trapping	0.3	12				
12	Spintor®	0.3	0				

377

378

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380

381 **Table 2**

382 Mean and standard error (\pm SE) of female *C. capitata* population registered in trial 2010.

Treatment	Females per trap and day (\pm SE)	
	21 July to 1 Sept. (weeks 1 to 7)	2 to 30 Sept. (weeks 8 to 10)
Magnet® MED	0.42 (\pm 0.09) ^a	1.89 (\pm 0.61) ^a
L&K Tube	2.76 (\pm 0.39) ^c	7.94 (\pm 1.29) ^b
Mass trapping	1.35 (\pm 0.33) ^{ab}	2.38 (\pm 0.42) ^a
Spintor®	2.73 (\pm 0.74) ^{bc}	1.81 (\pm 0.34) ^a

383 Means within a column followed by different letters are significantly different (LSD

384 test at $P < 0.05$).

385 The square root transformation of the number of catches was used to perform the

386 ANOVA. Untransformed data are presented.

387

388

389 **Table 3**
 390 Mean and standard error (\pm SE) of female *C. capitata* population registered in trial 2011.

Treatment	Females per trap and day (\pm SE)	
	22 June to 19 July	20 July to 9 Nov.
Magnet® MED	0.53 (\pm 0.11) ^a	0.43 (\pm 0.07) ^a
Mass trapping	0.58 (\pm 0.18) ^a	0.40 (\pm 0.05) ^a
Untreated	0.30 (\pm 0.11) ^a	2.55 (\pm 0.66) ^b

391 Means within a column followed by different letters are significantly different (LSD
 392 test at $P < 0.05$).

393 The logarithmic transformation of the number of catches was used to perform the
 394 ANOVA. Untransformed data are presented.

395

396

397 **Table 4**

398 Percentage of fruit damage and standard error (\pm SE) according to treatment, during the
399 harvest period, obtained in trial 2010.

Treatment	% Fruit damage (\pm SE)
Magnet® MED	0.21 (\pm 0.12) ^a
L&K tube	1.18 (\pm 0.47) ^b
Mass trapping	0.21 (\pm 0.16) ^a
Spintor®	0.14 (\pm 0.10) ^a

400 Percentages followed by different letters are significantly different (LSD test at $P < 0.05$,
401 with untransformed data).

402 The logarithmic transformation of the number of punctured fruits was used to perform
403 the ANOVA. Untransformed data are presented

404

405