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Field Efficacy of a *Metarhizium anisopliae* Based-Attractant Contaminant Device to Control *Ceratitis capitata* (Diptera: Tephritidae)

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ABSTRACT

Biological control of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) using entomopathogenic fungi is being studied as a viable control strategy. The efficacy of a *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) based-attractant contaminant device (ACD) to control *C. capitata* was evaluated in a medium-scale (40 ha) 2-year field trial using a density of 24 ACD per ha.

Results showed that this density was adequate to efficiently reduce fruitfly populations and that the inoculation dishes (IDs) needed replacing mid-season to provide protection for the entire season. In this study, fungal treatment was even more effective than conventional chemical treatment. Population dynamics in fungus-treated fields along with the infectivity study of field-aged IDs in the laboratory found that the ACD remained effective for at least 3 months.

The results suggest *M. anisopliae* based-ACD can be used to control *C. capitata* in the field. The implications of its use, especially as a tool in an Integrated Pest Management program, are discussed.

Keywords *Metarhizium anisopliae*, biological control, entomopathogenic fungi, Autoinoculation device, Integrated control.

24 Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is one of
25 the most destructive pest of horticultural crops (Malacrida et al. 2007). It has a worldwide
26 distribution and has been recorded in more than 400 species of fruit and vegetables (Aluja
27 and Mangan 2008).

28 Nowadays, *C. capitata* control focuses on the development of effective, sustainable
29 and environment-friendly methodologies. Among the different alternatives being
30 developed or implemented, the Sterile Insect Technique (SIT) is effectively used, mainly
31 as part of area-wide integrated pest management (AW-IPM) programs (Dyck et al. 2005;
32 Vreysen et al. 2007). Other management methods include the use of: attractant-sterilant
33 devices containing the chemosterilant lufenuron (Navarro-Llopis et al. 2007, 2010);
34 oviposition deterrents (Arredondo and Diaz-Fleicher 2006); bait stations (Mangan and
35 Moreno 2007; Navarro-Llopis et al. 2013); and biological control with parasitoids
36 (Rendon et al. 2006). The AW-IPM aims at integrating control tactics against an entire
37 pest population within a delimited area. Accordingly, integration of several of the above
38 mentioned methods have been investigated including the use of parasitoids and SIT in
39 Brazil (Malavasi et al. 2007) and Mexico (Montoya et al. 2007), or the combined use of
40 field sanitation, protein bait sprays and/or traps, male annihilation and augmentative
41 parasitoids releases in Hawaii (Mau et al. 2007; Vargas et al. 2010)

42 Another important alternative being considered is the use of fungi as biocontrol
43 agents. Biological control with entomopathogenic fungi is experimentally long-standing
44 but inconsistent results in field trials, which are attributable to the biotic and abiotic
45 factors that influence fungus survival and activity, hindered their implementation as a
46 widely-used control methodology. However, over the last two decades, advances in
47 fermentation and formulations technologies (Prior et al. 1988; Bateman et al. 1993; Inglis

et al. 1997) have overcome some of the aforementioned problems and given rise to a renewed interest in application of fungal entomopathogens as biopesticides.

Recent approaches for introducing entomopathogenic fungi into fruit fly population range from cover sprays (Ortu et al. 2009; Daniel and Wyss 2010) to the integration of fungal pathogens into the SIT using sterile males as vectors (Toledo et al. 2007; Ekesi et al. 2007; Flores et al. 2013). Another strategic option in the use of entomopathogenic fungi is the soil inoculation to target prepupariating larvae and puparia (Ekesi et al. 2007; Garrido-Jurado et al. 2011). Nevertheless, the method that is perhaps being paid more attention of late is the attraction and contamination strategy.

The attraction and contamination strategy, also called "Lure & Infect", works by attracting an insect into an inoculation device where it becomes contaminated with the infective conidia before returning to the crop and, optimally, disseminates the pathogen to other insect of the population (Vega et al. 2007). Some important advantages can be highlighted from this strategy. The devices use specific lures, and therefore, they are pest target specific. Their use avoids spraying large quantities of fungus to reach the insects. In addition, they may protect the active agent from environmental factors increasing their persistence. Such devices have been evaluated for a number of insect and fungal species (see Baverstock et al. 2010 for a review), including those designed against *C. capitata* and other fruit flies (Primo-Yúfera et al. 2002; Moya 2003; Dimbi et al. 2003; Ekesi et al. 2007).

We report herein the results of a medium-scale field trial (40 ha) conducted over 2 consecutive years to evaluate the efficacy of an attractant-contaminant device based on *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) to control *C. capitata*.

Materials and Methods

Chemicals and Traps. Trimedlure (TML) plugs, 2,2-dichlorovinyl dimethyl phosphate (DDVP) tablets, Delta traps and plastic McPhail traps were provided by Econex (Murcia, Spain). Technical grade TML was supplied by Agrisense (Pontypridd, UK). The three component lures, ammonium acetate, trimethylamine hydrochloride and putrescine, (Biolure Medfly®) dispensers, were obtained from Suterra (OR, USA). Tephri-Trap traps were obtained from Utiplas S.L. (Madrid, Spain). Malafin (50% wt:vol malathion, Agrodan, Valencia, Spain) and Buminal (Bayer, Valencia, Spain) were also acquired and utilized for the experiments.

***Ceratitis capitata* Colony.** Mediterranean fruit flies were reared in our insectary in a 16:8 light:dark photoperiod, with 50-60% relative humidity and temperature of 27 ± 1ºC. Adult flies were fed a mixture of yeast autolysate and sucrose 1:4 (wt:wt). Larvae were reared on a mixture of wheat bran: sucrose: beer yeast: nipagin: nipasol: water: hydrochloric acid (20:5:1:0.5:0.5:10:0.1) by weight. This colony has been maintained in our laboratory since 1995. It is annually crossed with wild populations from infested Valencian orchard fruits, thus minimizing loss of biological similarity with the wild population usually associated with laboratory colonization (Joachim-Bravo et al. 2009).

Inoculation Dishes (ID) and Attractant-Contaminant Devices (ACDs)

Fungi. Treatments were carried out with a strain of *Metarhizium anisopliae* which was isolated from the soil of a citrus orchard (Moncada, Valencia, Spain) in 2002 and maintained in the Entomopathogenic Fungi Collection of the Centro Ecología Química Agrícola (UPV). The fungus was recently deposited in the Colección Española de Cultivos Tipo (CECT) under accession number CECT 20768.

95 The fungus was cultured in Petri dishes containing potato dextrose agar (PDA) at 96 26°C in the dark. The conidia from 7-day-old PDA cultures (3 plates) were suspended in 97 mineral oil and removed from each plate with a 10-ml pipette. Conidia concentration, 98 estimated using a haemocytometer (Improved Neubauer chamber), was adjusted to 1 x 99 10^6 conidia per milliliter and used as inoculum for the conidia mass production. A 0.1 100 milliliter aliquot of this suspension was spread onto PDA Petri dishes and incubated in 101 the same conditions as described above. After 7-8 days, yields of about 1 x 10^9 conidia 102 per plate were obtained.

103 **Inoculation Dishes (IDs).** These dishes make the contaminant part of the ACD and 104 were prepared according to Primo-Yúfera et al. (2002). Briefly, each ID consisted of the 105 bottom of a 9-cm-diameter Petri dish filled with a carboximethylcellulose-based semi-106 solid gel. This gel was used as the adherent material to support the infective/adsorbent 107 material and to maintain a suitable microenvironment for conidial persistence. The 108 adsorbent material was a porous material granular formulation containing 20% technical 109 TML (Corma et al. 2000) to ensure short-distance male attraction. It was also used as the 110 carrier for the *M. anisopliae* conidia suspended in mineral oil. This infective/adsorbent 111 material (1.45 g) was uniformly spread over the adherent material to achieve a dose of 1 112 x 10^9 conidia per dish and a total surface TML load of 200 mg per dish.

113 **Attractant-Contaminant Device (ACD).** The ID was placed inside a delta trap. To 114 ensure male attraction to the trap from long distances, a TML plug was placed in the 115 center of the ID. A Biolure Medfly® attractant was stuck on the inner surface of the delta 116 trap walls to also attract females. Trimedlure plugs were replaced every 3 months (usually 117 coinciding with the replacement of infective dishes). Biolure Medfly® attractants were 118 replaced every 45 days following the manufacturer's recommendations.

Infectivity Assays. During the first year of study, the infectivity of IDs aging in the field was periodically evaluated. Thus, every 20 days over a period of 100 days since the placement of the ACDs in the field, three IDs were taken to the laboratory. Each ID was put into a wire mesh cage (30 x 30 x 30 cm) containing 5-day-old, *C. capitata* males (50 flies per cage). Attraction was recorded every 10 min for 3 h by counting the number of males alighting on the dish. The percentage of attraction was obtained as the percentage of the average value during the attraction period. The ID was then removed and flies were provided with diet and water. Mortality was recorded daily and dead flies were removed. After surface sterilization using 0.3% sodium hypochlorite solution, cadavers were incubated in the darkness at 26°C to confirm mycosis, which was assumed when the sporulated mycelia of the fungus was observed on the cadaver surface.

Field Trials. They were conducted in a citrus orchard located in Casella Valley (GPS coordinates: 39°7'02" N, 0°21'30" W) (Alzira, Valencia, Spain) (Fig. 1A). Only the western side of the valley extended to other fruit orchards; the other sides were adjacent to mountains, with no fruit trees which could host medflies. Fungus-treated field (FTF) and the reference field, for which bait-malathion was applied, covered 40 and 11 ha respectively, the latter being located 1.8 km away from the FTF to avoid fruit fly intrusion between both areas (Navarro-Llopis et al. 2012). To further reduce the invasion from other untreated areas, a 50 m-wide barrier was set at least 30 m away from the FTF, according to Peck and McQuate (2000), who report 30 m as the minimum distance required to avoid influence of the barrier on the treated areas. One hundred and fifty traps (50 McPhail and 100 Tephri-trap) were placed on the barrier at a density of 50 traps per ha. Tephri-traps contained a Biolure Medfly® attractant and McPhail traps contained a TML plug, both with a DDVP tablet to kill fruit flies. TML plugs and DDVP were renewed every 3 months and Biolure every 45 days.

144 In the reference field, three plots of ≈4 ha each were established (Fig. 1A). Grooves
145 were: Plot C1, early mandarins *Citrus reticulata* (cultivar "Marisol"), Plot C2, sweet
146 oranges *Citrus sinensis* (cultivar "Navelina") and, Plot C3, late sweet oranges *Citrus*
147 *sinensis* (cultivar Valencia-late).

148 In the FTF (Fig. 1B), six plots of 5-7 ha each were set up. As in the reference field,
149 the cultivated varieties were: Plot 1 and 4, *C. reticulata* cult. Marisol; Plot 2 and 5, *C.*
150 *sinensis* cult. Navelina; Plot 3 and 6, *C. sinensis* cult. Valencia-late; Plots 1, 2 and 3 were
151 located in eastern part of the field and were named Fungus Treatment Field-East (FTF-
152 E). Plots 4, 5 and 6 were located in western part of the field, which were consequently
153 named Fungus Treatment Field-West (FTF-W). As varieties were the same in the western
154 and eastern part of the trials fruit fly evolution could be compared between them when
155 different treatments were applied in FTF-E and FTF-W.

156 **Biological and Chemical Treatments**. Main conditions of the fungal and chemical
157 treatments performed during the two-year trial in FTFs and reference field are
158 summarized in Table 1. ACDs were always placed at 1.5 m above the ground in the north-
159 east faces of trees to avoid maximum sunshine at midday. A density of 24 ACD per ha
160 was always used. All the devices contained the Biolure Medfly® attractant, but only one
161 of each three devices carried a TML plug inside. The distance between the TML
162 attractants was 3 times longer than that between the Biolure Medfly ® attractants due to
163 the greater efficacy of TML over long distances (Peck and McQuate 2000; Cohen and
164 Yuval 2000). Each year, two different fungal treatments were carried out. In 2004, ACDs
165 were placed in FTF-W (plots 4, 5 and 6) and IDs were replaced in July (two fungal
166 applications). In FTF-E (plots 1, 2 and 3), the IDs were not replaced in July (one fungal
167 application) in order to assess the efficacy of only one application of ACDs per year. In
168 2005, the treatments in areas FTF-W and FTF-E were reversed compared to 2004 in such

169 a way that FTF-E was subjected to two fungal applications while FTF-W was only treated
170 with the first fungal application (Table 1).

171 The reference plots were treated by aerial spraying of malathion using 20 liter/ha
172 which contained 10 milliliter/liter Malafin (50% wt:vol malathion) and 7.5 milliliter/liter
173 of the protein bait Buminal. Ground malathion treatments in the reference field were
174 exclusively carried out in the plot with Marisol cultivar (plot C1) by spraying 1 m^2 spots
175 on the south face of trees with a back-sprayer. The applied composition consisted of
176 malathion (2.5 g/liter) and Buminal (5.0 milliliter/liter) with a total expenditure of 200
177 liter/ha. The same ground treatments were performed on cultivar Marisol (plots 1 and 4)
178 in FTFs in order to exactly reproduce the conditions in the reference Marisol plot.

179 ***Ceratitis capitata*** **Population Monitoring.** Mediterranean fruit fly population
180 monitoring was performed using 40 McPhail plastic traps in the 40 ha treated with fungus
181 (20 in FTF-E and 20 in FTF-W) and 11 in the reference plots (one trap per ha trap grid).
182 Inside the traps, a TML plug and a DDVP strip were placed. The traps were monitored
183 weekly from February to December.

184 **Statistical Analysis**. To explore the effect of treatments on fruit fly catches, a
185 Generalized Linear Model (GLM) with repeated measures was conducted. Year, citrus
186 variety and treatment were considered as explicative variables and captures, over the 32
187 weeks of trial duration, as repeated measure. As designed plots have different size and
188 different number of traps (one trap per ha) we have used the average of fly captures in
189 each plot every week for analysis. Statistical analysis was carried out using SPSS 16.0
190 package (SPSS Inc. Chicago, USA).

191 Results

192 ***Ceratitis capitata* Population**. Neither citrus variety (F = 2.28; df = 2, 9; P = 0.158)
193 nor year of treatment (F = 1.12; df = 1, 12; P = 0.215) were significant predicting factors
194 for the population level. According to that, the three plots into each FTF were considered
195 replications of the same treatment and data from both years were considered in the same
196 analysis. Therefore 6 replications per treatment (untreated, treated with fungus once or
197 treated with fungus twice per year) were considered for statistical analysis. Treatment
198 with two fungal applications significantly reduced the fruit fly population (F = 13.39; df
199 = 2, 15: P < 0.001), showing reductions of 71 and 37% in the fruit fly population as
200 compared to those in the reference field and in the one fungal application, respectively.
201 In addition, significant differences were also observed between the one fungal application
202 treatment and the reference field, with the latter showing 2.19 times more population.
203 The Mediterranean fruit fly population dynamics in the two fungus treated areas
204 undergoing different fungal treatments (one or two applications of inoculation dishes) as
205 well as in reference field, during 2004, is shown in Fig. 2a. In the reference plots, the
206 population outbreak began in mid-June and reached its maximum peak in late June, just
207 as the population upward trend was interrupted by the first malathion aerial treatment
208 (27th June). A similar population upward trend was observed in FTF with one fungal
209 application, but was delayed by 1 month. The maximum population peak was reached in
210 late July (29th July). The increasing population in FTF with two fungal applications began
211 to be seen, as occurred with FTF with one fungal application, during the first half of July.
212 However, coinciding with IDs replacement during the second week of July (Table 1), this
213 increasing population was avoided and flies catches remained below 14 flies per trap per
214 day, while the population level reached values of over 50 flies per trap per day in the other
215 fields.

216 The population levels in plots with two fungal applications during the most
217 problematic period in terms of fruit damage (from September to November) were about
218 0.5 flies per trap per day, by mid-September, and were below 0.4 flies per trap per day,
219 from October to the end of the year (Fig. 2b). However, in plots treated with only one
220 fungal application the population level peaked over 1 fly per trap per day until the second
221 half of November. In the reference field, the level of 1 fly per trap per day was also
222 exceeded on occasions up to mid-October when the last of the five aerial treatments done
223 during the season (Table 1) was carried out.

224 Moreover, the fly population was always larger in the reference plots than in the
225 two fungal applications plots, although these differences were not always statistically
226 significant.

227 The fruit fly population dynamic observed in 2005 is shown in Fig. 3a. During
228 this season, the plots subjected to the different fungal applications were exchanged with
229 the aim of confirming the results of the previous year even whether the experimental
230 zones were reversed. The FTF now subjected to one fungal application showed a
231 population outbreak of over 40 flies per trap per day in July, while it did not reach 10 flies
232 per trap per day in FTF with two fungal applications (80% reduction in the maximum of
233 fruit fly population). The fruit fly population reduction noted in twice fungus applied plots
234 in relation to the population in reference plots was 86%.

235 During the most sensitive period for oranges, from September to the end of trial (Fig.
236 3b) *C. capitata* populations were 66% and 85% smaller in once or twice fungus applied
237 plots, respectively, than those recorded in the reference plots.

238 **Persistence of Conidia in the Inoculation Dish in the Field.** During the first year
239 of the field trial, the conidia viability in the IDs was evaluated in terms of infectivity in
240 the laboratory. As shown in Fig. 4, the IDs that remained in the field for 100 days were

241 still able to contaminate males in the laboratory (about 30%). Attractant release from
242 granular controlled-release emitter on the ID surface was too high, up to 60 days, to
243 induce adequate behavior in males under the laboratory conditions because of a saturation
244 effect. Saturation was evident when, after a brief response period, males became
245 motionless in the vicinity of, but not inside, the ID. Although a continuing decrease in the
246 saturation level over time could be seen, as shown by the continuing increase in mortality,
247 it was from day 60 of aging that saturation was not any longer detected. Thus, the 60-day
248 aging time was the inflection point from which mortality and attraction dropped in parallel
249 until day 80 of aging. From that moment, mortality lowered to a greater extent than
250 attraction showing that infectivity began to decay in the dish. In any case, loss of
251 infectivity was not complete because the mortality induced by the 100 aging-day IDs was
252 approximately half the greatest activity observed throughout the experiment (60%
253 mortality by 60 aging days) and when optimal performance of the ACDs in the field was
254 been recorded.
255
256
257 ## Discussion
258 The results obtained in this two year trial demonstrated that a density of 24 ACD per
259 ha suffices to efficiently control the fly population and that, in order to protect the entire
260 season, a mid-season replacement of IDs was necessary. Under these conditions, fungal
261 treatment obtained fruit fly population levels under 0.5 flies per trap per day from mid-
262 September to December, the most problematic period in citrus damage terms in Spain
263 because orange and mandarins are ripening at this time. Spain and the United States have
264 agreed that a regulated area with citrus orchards with <0.5 flies per trap per day of *C.*
265 *capitata* may be considered a low pest prevalence area for fruit flies FF-ALPP (USDA

266 2002). Therefore, the efficacy level obtained with the fungal treatment under these
267 conditions might be classified as highly efficient and, moreover, it was higher than that
268 obtained by conventional malathion treatment.

269 Two important conclusions can be drawn from this work. The initial placement of
270 inoculation dishes in the field was able to control pest populations but could not establish
271 a long-term epizootic in the field, as shown by the fly population recovery when not
272 replacing IDs. Long-term epizootics require a secondary infection of the fly population
273 through *C. capitata*-sporulated cadavers. Nonetheless, environmental requirements
274 (Zimmermann, 2007) do not enable this, and it is only, and then not always, achieved in
275 areas where high humidity conditions prevail, which are not usual in the Mediterranean
276 region.

277 The second important finding is related to the ACD lifetime. The population
278 dynamics in FTF plots, combined with the infectivity study in the laboratory allowed us
279 to estimate the useful life of the ACD. The field results showed device efficacy at about
280 3 months if we consider that an increase in the populations in both FTFs was evidenced
281 early in July, and in both year 1 and year 2. However, the laboratory study into the conidia
282 viability in the IDs demonstrated that those remaining in the field for 100 days still
283 maintained relatively high infectivity. Thus, loss of activity of the ACD in the field might
284 be due to the reduced proportion of viable conidia in IDs, but also to deficient attraction
285 to traps. Hence, what remains to be determined is the infectivity threshold in the device
286 that can offer an effective control in the field.

287 To our knowledge, this is the first report that describes an effective ACD in the field
288 to control *C. capitata* with a useful life regarding fungal activity of at least 3 months.
289 Previous similar studies include that of Dimbi et al. (2003), who designed an
290 autoinoculative device that proved efficient at the laboratory and field cages levels for

291 contaminating fruit flies. Later, Ekesi et al. (2007) reported the autodissemination of *M.*
292 *anisopliae* to suppress *Ceratitis cosyra* (Walter) (Diptera: Tephritidae) in mango
293 orchards. According to these authors, the *M. anisopliae* based-autoinoculative device was
294 able to control the population level of *C. cosyra* in a small-scale field trial (approximately
295 5 ha) and the fungal agent was able to persist in the device for 5 weeks (68% germination)
296 before lowering to 27% after 6 weeks.

297 The device evaluated herein is not strictly an auto-dissemination device, as is usually
298 defined, in that horizontal transmission is not achieved. The device contains oil-
299 formulated fungal conidia because we have previously proved that oil improves conidia
300 virulence and notably increases conidia persistence in the ACD in the field (Ibrahim 2002;
301 Moya 2003). Conversely, the assays carried out in our laboratory have demonstrated that
302 horizontal transmission through mating is inhibited (data not shown), which is likely
303 because oil strongly adheres the conidia to the lipophilic cuticle (Wraight et al. 2001),
304 hence avoiding its transfer by contact.

305 Our results suggest, however, that this auto-inoculation device could be used as an
306 efficient mycoinsecticide. The ACD was able to effectively attract the bulk of the
307 population (males and females) to the IDs as shown by the reduction of the population.
308 In these conditions, all the attracted insects receiving a high inoculum dose and a faster
309 and more homogeneous mortality response than that mediated by horizontal transmission
310 could be expected. Dimbi (2003) showed that *C. capitata* males and females exposed to
311 *M. anisopliae* became infected and exhibited 100% mortality at 5-6 days post-exposure.
312 However, when female inoculation was mediated by horizontal transmission (*M.*
313 *anisopliae*-treated males maintained for 24 h with untreated females under laboratory
314 conditions), female mortality was notably delayed ranging from 71% to 83% at 15 days

315 post-inoculation. According to that, an efficiently applied virulent mycoinsecticide might
316 counteract the lack of horizontal transmission.

317 On the other hand, the strong adherence of conidia to flies derived from the conidia-
318 oil-formulation reduces the dispersion of the fungal agent, thus providing a safer control
319 methodology from an environmental point of view.

320 The methodology described in this work could prove especially useful and viable if
321 it was considered in an IPM program, in line with the Directive 2009/128/EC of the
322 European Union which enacts the compulsory implementation of integrated pest
323 management practices (EC 2009) enhancing the uptake of “low risk” products for pest
324 control. Currently, there are in the market more powerful and long-lasting *C. capitata*
325 attractants emitters capable of effectively covering the entire fruit season in the case of
326 males attractants (Dominguez-Ruiz et al. 2008), or most of it (5 months) when
327 considering females attractants (Navarro-Llopis et al. 2008). By using these new
328 products, no replacement of attractants would be necessary. Moreover, an insecticidal
329 treatment, for example, performed at the beginning of the pest control period would
330 maintain a low fly population level in such a way that ACDs placement could be delayed
331 until the female attractants were able to cover the remaining period. Under these
332 conditions, only one replacement of IDs would be required throughout the period.

333 Other interesting and more ecological approach could be the integration of this
334 methodology in a Sterile Insect Technique (SIT)-based IPM program. The reduction of
335 *C. capitata* wild population achieved by using the ACDs might lead to a substantial
336 improvement in the efficacy of SIT, providing that sterile males are not impaired by the
337 fungus in terms of longevity and sexual performance. In this regard, studies recently
338 carried out to evaluate the effect of the ACD fungal agent against *C. capitata* Vienna 8

339 sterile males suggest that the combined strategy could prove viable (San Andrés et al.
340 2014).

341 In summary, ACDs can prove a useful tool for integrated *C. capitata* control because
342 it is 1) highly effective to reduce populations in the field, 2) highly selective in delivering
343 the fungus, which notably increases its environmental safety and 3) it is a highly persistent
344 product, which favors its economic feasibility. In addition, it would be exempt of
345 maximum residue limits and could be also used in rotation with more selective synthetic
346 insecticides to delay pest resistance.

347

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Table 1. Conditions of the fungal and chemical treatments performed during the two-year field trial

Year	Fungus Fields	Treatments in Fungus Treated Field		Chemical treatments in Reference Field	
		ACD placement (Date)	ID replacement (Date)	Ground treatments (Marisol cult.)	Aerial treatments
2004	FTF-E	March (4th wk)	No	5th, 12th, 19th, 26th September and 3rd, 10th October	27th June, 22nd July, 8th August, 10th September, 14th October
	FTF-W		Yes (July, 2nd wk)		
2005	FTF-E	April (1st wk)	Yes (July, 2nd wk)	5th, 19th September and 3rd, 10th October	27th June, 14th October
	FTF-W		No		

Fungus Treated Field (FTF) were treated with Attractant-Contaminant Devices (ACDs) at a density of 24 ACDs per ha.

Ground malathion treatments were exclusively applied in those plots containing the early variety Marisol, either in Marisol Reference plot or in Marisol fungus-treated plots (1 and 4).

FIGURE CAPTIONS

Fig. 1 Map of the experimental fields showing A) the reference (malathion-treated) plots (C1, C2 and C3), the barrier zone and both Fungus Treatment Fields (FTF-East and FTF-West) and B) their corresponding citrus varieties.

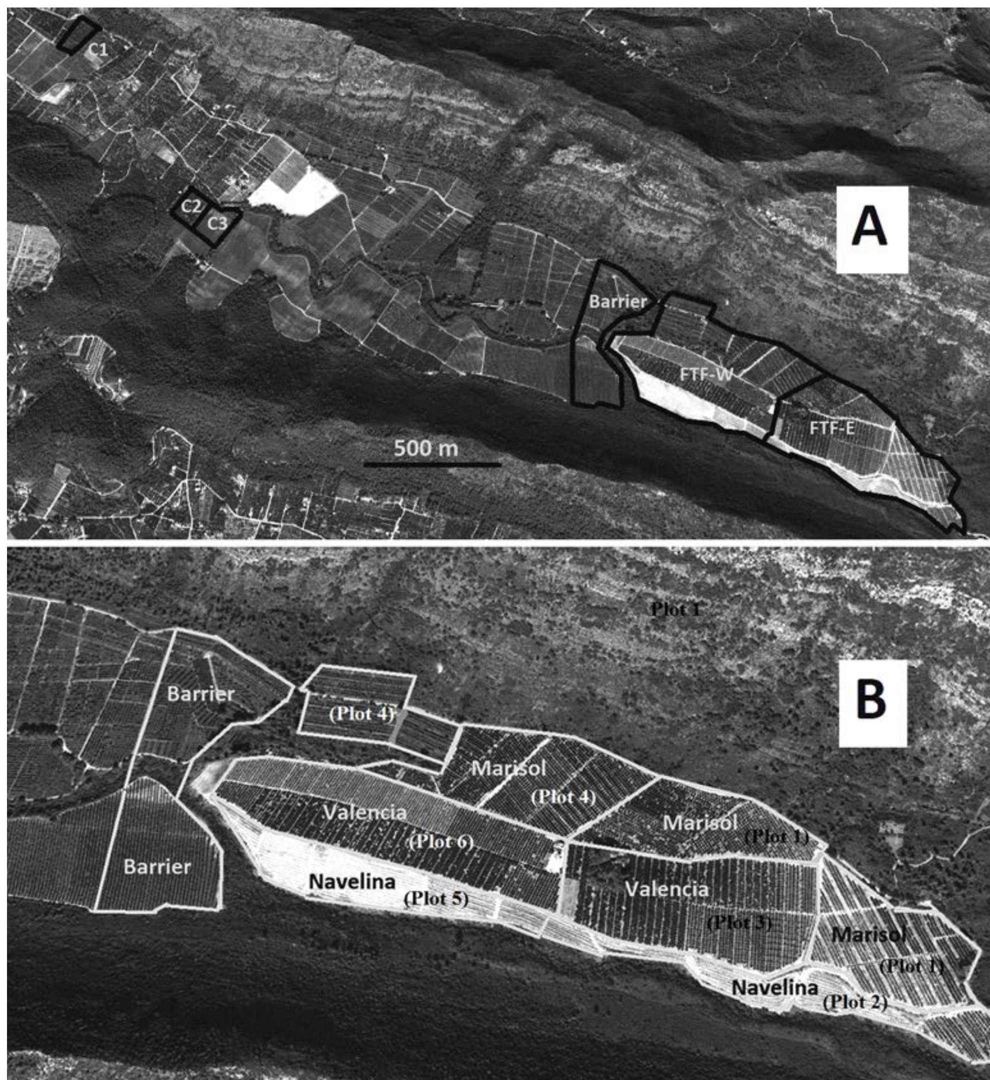

Fig. 2 Mediterranean fruit fly population dynamics in 2004 in fungus-treated fields (FTFs) and reference field over the period covering (a) all the season and (b) September to December.

Each point represents the average value ± standard error of flies captured per trap and day (FTD). FTF-E was treated with 24 ACDs per ha in March (4th wk) and inoculation dishes (IDs) were not replaced (Fungus 1 appl.). FTF-W was also treated with 24 ACDs per ha in March (4th wk) and IDs replaced in July (2nd wk) (Fungus 2 appl.) (Table 1)

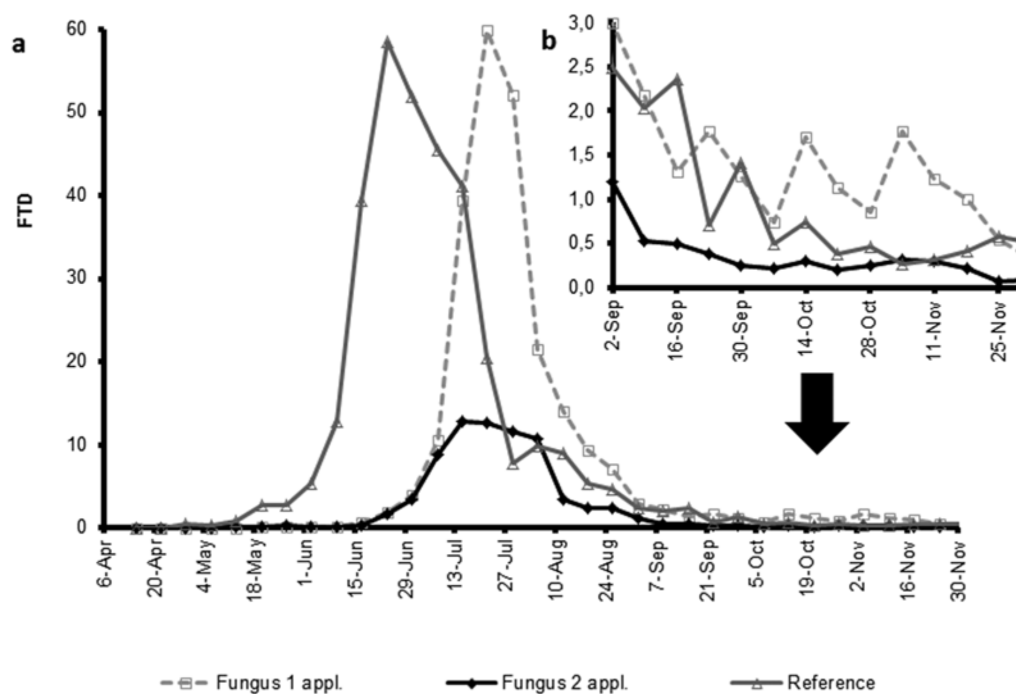

Fig. 3 Mediterranean fruit fly population dynamics in 2005 in fungus treated fields (FTFs) and reference field over the period covering (a) all the season and (b) September to December.

Each point represents the average value ± standard error of flies captured by trap and day (FTD). FTF-E was treated with 24 ACDs per ha in April (1st wk) and ID replaced in July (2nd wk) (Fungus 2 appl.). FTF-W was treated with 24 ACDs per ha in April (1st wk) with IDs being not replaced (Fungus 1 appl.) (Table 1)

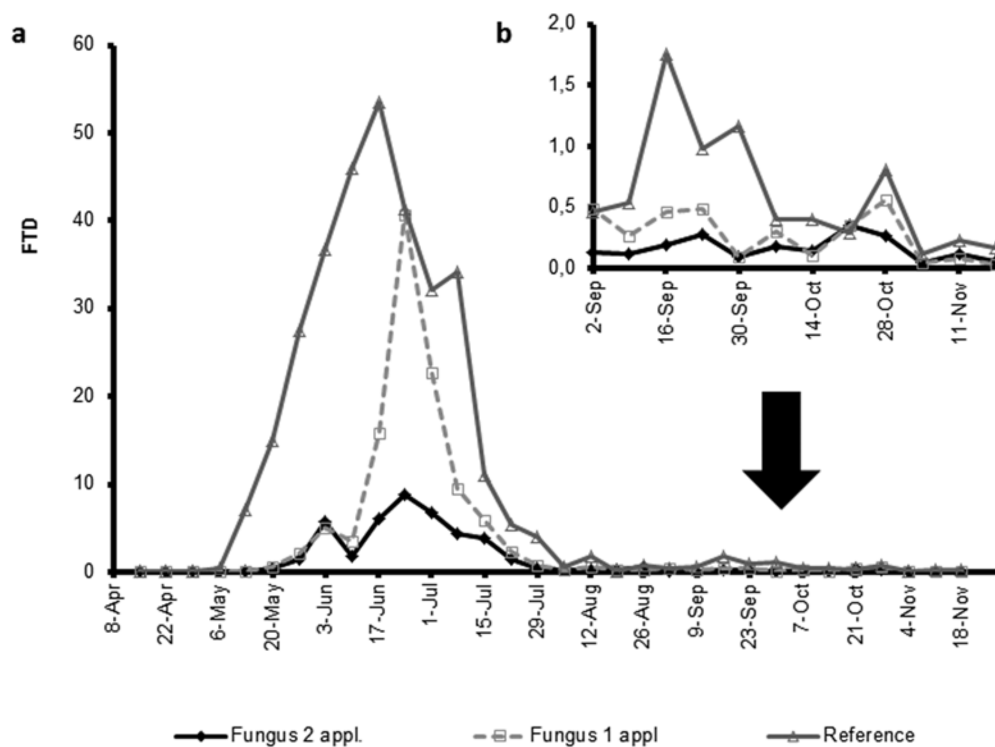

Fig. 4 Laboratory evaluation of the infectivity of the inoculation dish (ID) aging in the field. Results are shown as average values ± standard error.