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Chemosterilants as control agents of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in field trials

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Abstract

Lufenuron is a chitin synthesis inhibitor, which can interrupt Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) reproduction. In laboratory assays, lufenuron prevented the hatching of eggs laid by females following ingestion of the compound. Moreover, in previous field trials lufenuron showed efficacy by reducing the *C. capitata* wild population, whereas continuous application of lufenuron to several generations of fruit fly gave improved control. A field trial in an isolated valley of 80 hectares during 4 continuous years was carried out. In order to maintain the sterilizing effect in the field during a whole year, a new lufenuron bait-gel was developed. The bait gel was introduced in delta traps suspended in the trees at a density of 24 traps per Ha. Traps were replaced once every year during the field trial. Monitoring of the adult *C. capitata* population was made in order to assess the effect of the chemosterilant treatment. In the first year of treatments with sterilizing traps a reduction of *C. capitata* population was

observed, indicating that the traps reduce population from first generation. In the second, third and fourth year a continuous and progressive reduction of the adult Mediterranean fruit fly population was observed when comparing chemosterilant with aerial malathion treatments. The possibility of using this method alone or combined with sterile insect technique is discussed.

Introduction

Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is one of the most destructive pests in fruit orchards (Liquido et al., 1997). Usually, the main control method used to control this pest is application of conventional insecticides like organophosphates. In Mediterranean countries, insecticide treatments are made by aerial spraying, affecting non target insects and vertebrates. Moreover, aerial applications over high-density residential areas, like the Mediterranean coast, provokes public concern.

Traditional biological control is one of the possible ways to fight against *C. capitata* and new parasitoids and predators are studied. A new African collection of *C. capitata* hymenopterous parasitoids (Copeland et al., 2002; Lopez et al., 2003), including *Fopius ceratitivorous* Wharton, and *Aganaspis daci* (Weld) seems to be efficient biological control agents of *C. capitata* (Papadopoulos & Katsoyannos, 2003). Moreover, the use of microbiological control agents in laboratory has been widely studied (Castillo et al., 2000; De La Rosa et al., 2002; Dimbi et al., 2003) although field trials with this microorganisms are not so extended. Nowadays our group is

carrying out some field trials using *Metarhizium anisopliae* as a new weapon to control this pest (unpublished results).

Possibility of insect control or eradication through the use of sexually sterile males was describe in 1955 (Knipling, 1955) and this is applied currently in the Sterile Insect Technique (SIT). Sterile Insect Technique has demonstrated to be able to reduce fruit fly population, and reduce fruit damage. Usually, for successful application of SIT, *C. capitata* populations should have been previously reduced by aerial chemical treatments (Batkin, 1995), mass trapping or lure and kill methods (Katsoyannos & Papadopoulos, 2004) or biological control (Wong et al., 1992), and in this way, a large number of released sterile males compete with small number of wild males. But in Mediterranean regions *C. capitata* populations are very high and it means that the preceding treatments are not enough to reduce the fruit fly population. For this reason our group searched for a method at the end of 1990's to reduce efficiently Mediterranean fruit fly populations. First, trials were conducted to look for an Insect Growth Regulator (IGR) that reduced fertility or fecundity in *C. capitata.* Lufenuron showed a high activity, reducing egg hatching. When females ingested a bait containing 0.1% (w:w) lufenuron, the hatching of the subsequently laid eggs was prevented. Moreover, in laboratory experiments, females that mated with lufenuron treated males (0.5% (w:w) a.i. in diet) laid non-viable eggs (Casana-Giner et al., 1999).

After this study, several field trials were made testing the minimum required surface in order to obtain representative results, the optimum bait composition and the isolation grade of the orchards for optimal field trials (Navarro-Llopis, 2002). Lufenuron application studies and first extended field trials were conducted, resulting in a bait trap application of lufenuron. There was a significant *C. capitata* population reduction and significantly less stung fruit in lufenuron-

treated orchards than in untreated orchards (Navarro-Llopis et al., 2004). Moreover this study showed that barriers for reducing Mediterranean fruit fly population intrusions into lufenuron-treated fields were needed. A new study over an extensive and isolated area during almost four years was designed to verify the efficacy of this method and the possible cumulative effect on *C. capitata* populations.

In this paper, the use of chemosterilization as a method of reducing *C. capitata* populations is discussed with reference to the results from a four years field trial comparing chemosterilant treatment versus malathion plus protein bait treatment in citrus orchards. The possibility of combining chemosterilization and SIT in high population areas is discussed.

Materials and Methods

Trial fields description

Trials were carried out in a citrus orchard located in the Casella Valley (Alzira, Valencia, Spain) with sweet oranges of Navel group, *Citrus sinensis* (L.) Osbeck, and mandarins *Citrus reticulata* Blanco (cultivar “Marisol” and “Clementina Fina”), as cultivated species (Figure 1 shows a site map of the trial fields). The east side of the trials field extended to untreated fruit orchards and the west side extended to another trial field where microbiological control of *C. capitata* was carried out. The trial area was surrounded by mountains without fruit trees which could host Mediterranean fruit flies on the North and South sides. In the selected malathion-treated field, early mandarins *C. reticulata* (cultivar “Marisol”), and sweet oranges *C. sinensis* were cultivated. In the lufenuron-treated fields, the main fruit trees cultivated were early mandarin *C. reticulata*

(cultivar “Marisol” and cultivar “Clementina Fina”) and sweet orange *C. sinensis* (cultivar New-Hall).

In the trial fields we have compared two types of treatment against Mediterranean fruit fly: a chemosterilant treatment using traps with a lufenuron bait (lufenuron treatment) and a serial of aerial applications of malathion with bait (malathion treatment). The lufenuron treated fields occupied 80 hectares and the malathion fields 11 hectares, 0.5 km away from lufenuron treated fields. In the lufenuron-treated area, 10 plots from 3 to 9 ha in size were established (Table 1) with similar–characteristics using criteria such as irrigation technique, variety of the trees and cultural management. Separation between neighbouring plots was between 10 and 100 meters, using roads or ravines as natural boundaries. In seven plots there were mandarin varieties and in three plots there were orange varieties. The plot located at the west side of the trial, neighbouring the barrier between malathion treated orchards and lufenuron treated area, was considered as a buffer area. Two check areas were delimited: one 6 ha plot of mandarins and one 5 ha plot of oranges. In 2004, the mandarin orchards *lufenuron 3* and *lufenuron 7* were removed from the trial because the trees were dug up.

Traps, attractants and baits

In the field trial we have used three types of traps: delta traps, Tephri traps and International Pheromone McPhail traps (IPMT). Delta trap was a yellow trap of rectangular base of 15x10 cm and two rectangular sides of the same dimensions that formed a triangular profile.Delta traps were provided by Econex (Murcia, Spain). Tephri traps from Utiplas S.L. (Madrid, Spain) (Katsoyannos, 1994) consisted of a yellow invaginated base 5 cm deep, fitted with an opaque lid (3.5 cm high). The total height of the trap was 14 cm and diameter at the junction of lid and base was 12 cm. Four fly entry holes, 2.1 cm in diameter were placed 90º to each other, 1 cm from the

top of the trap base. IPMT from Econex (Murcia, Spain) (Katsoyannos, 1994) is a container made of a yellow base (7 cm tall) and a clear top (11 cm tall) and is 17 cm in diameter at its widest point.

Attractants used were: 1,1-dimethylethyl 4(or 5)-chloro-2-methylcyclohexanecarboxylate plug, common name trimedlure (TML), a synthetic sexual attractant for males (Beroza et al., 1961), from Econex (Murcia, Spain) and Biolure, a synthetic food-based attractant, attractive to both males and female Mediterranean fruit flies, consisting of separate chemical release packets for ammonium acetate, trimethylamine and putrescine, from Suterra (OR, USA).

Phagostimulant bait was a proteinaceus gel from Ecologia y Protección Agrícola (EPA) (Valencia, Spain) that contained 30 g/l a.i. of lufenuron technical grade ((RS)-1-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]-3-(2,6-difluorobenzoyl)urea) from Syngenta (Basel, Switzerland).

Chemosterilant, monitoring and barrier traps

The proteinaceus phagostimulant bait with lufenuron and attractants (TML and/or Biolure) were placed in Delta traps. In the following description, the delta trap including the chemosterilant bait and attractants will be referred to as the *Chemosterilant trap*.

In order to monitor *C. capitata* population we used IPMT baited with a TML plug and a 1.5 g tablet with 20% dichlorvos (DDVP) from Econex (Murcia, Spain) to kill *C. capitata* The IPMT with a TML plug and a DDPV tablet were hung in the lufenuron treated area and check field at a density of one per hectare. These traps will be referred to as the *Monitoring traps.*

In order to avoid *C. capitata* intrusion, a mass trapping barrier of Tephri-trap and IPMT traps 100 m wide, was placed at the east and west side of the trial area (Cohen & Yuval, 2000).

Tephri-traps contained a Biolure attractant and a DDVP tablet to kill fruit flies as in the mass-trapping technique. IPMT traps contained a TML plug and a DDVP tablet. One hundred and fifty traps (50 IPMT traps and 100 Tephri-trap) were placed in each barrier (east and west sides of the trial field) at a density of 30 traps per hectare and they will be referred to as the *Barrier traps* (Figure 1).

Mediterranean fruit fly population monitoring.

C. capitata population monitoring was performed with 80 IPMT in the 80 hectares treated with lufenuron and 11 in the check field (one trap per hectare). Inside the traps, one plug of TML and a DDVP tablet were placed.

During 2001, traps were inspected weekly from April to 15 of August, twice per week from 16 of August to 7 of October, and weekly again from 8 of October to the end of the trial. The purpose of this monitoring was to follow *C. capitata* population dynamics all year round, paying special attention to the middle period when fruit is ripening. During 2002, 2003 and 2004, the traps were monitored weekly from February to December. Trimedlure emitters from the barrier and the monitoring traps were replaced every two months. Tri-pack attractants and DDVP strips were replaced every 45 days.

Lufenuron treatment with sterilizing traps

Treatments were made placing in field 24 delta traps per hectare. Each delta trap carried within it a 9 cm petri dish with the bait gel and an attractant. A Biolure attractant was placed inside each trap to increase the attraction for females and only one of each three traps carried a TML dispenser inside, just in the centre of the petri dish, to maintain the attraction of males. In this case, the distance between TML attractants was three times than the distance between Biolures

due to the superior efficacy of TML over large distances. In this way, males and females were attracted in order to obtain greatest possible effect on the *C. capitata* population. Direct visual observation in the field showed that males and females were attracted to the traps. Males were attracted mainly by TML because they landed directly on the TML plugs. Normally, after a short time, the males went down the plug, walked on and fed on bait. However, females went directly to the bait at the edge of the petri dish and fed on bait. Finally, both males and females leave the trap and fly away.

The gel with lufenuron was introduced into the 9 cm diameter petri dish at around 80 ml of gel per dish and placed in delta traps, which were hung on the south-east side of the trees, 1.5 meters above ground. Approximately one trap per 15 trees were hung in this way. The bait remained in the field inside the trap during the whole season

Chemosterilant traps were placed before the first annual *C. capitata* population outbreak (between 15 May and 15 June).The treatment began the 10 May 2001 and traps remained in field until 25 April 2002, when they were replaced with new traps. These traps were replaced in 20 April 2003 and remained in the field until May 2004, when they were replaced with new ones which lasted until the end of the trials in November 2004. Moreover, during 2001 and 2002, 50 chemosterilant traps were placed at the entry to the valley, about 2 km away from the lufenuron-treated area for aging trials.

Check field and insecticide treatments.

Check field was aerially sprayed at 20 liters per hectare with 7.5 g malathion per litre (Malafin 500 g/l from Agrodan, Valencia, Spain) and 12 g of protein bait (Buminal, 300 g of protein per litre, from Bayer Crop Science Andernach, Germany) per litre in order to reduce fruit fly population. Aerial bait spray of malathion was carried out once in 2001, on the 28 of August ,

five times in 2002, the 27 June, 22 July, 8 August, 10 September and 14 October, seven time in 2003, the 9 July, 20 August, 16 and 30 September, 11 and 22 October and 15 November and eleven times in 2004, the 3 and 16 of August, 1, 15 and 23 of September, 1, 9, 18 and 27 October and 4 and 16 November. Increasing aerial treatments are the result of the USA-Spain protocol for mandarin export to USA which defines the need for application with *C. capitata* populations over 0.5 flies per trap and day.

The normal local treatments were made in the check field and lufenuron treated areas, consisting of one treatment of chlorpyriphos in April-May against *Aonidiella auranti* (Maskell) (Homoptera: Diaspididae). Moreover, all *Marisol* mandarin areas (from the check field and the lufenuron-treated area) were treated terrestrially with malathion against *C. capitata* three times per year during September and October in order to avoid fruit damage. These treatments corresponded to treatments that the farmers performed in most Spanish citrus areas and were carried out by spraying one square meter spots on the south side of the trees with backpack sprayers. Applications were made with Buminal and Malafin and each treatment consumed 200 litres per hectare of the following composition: malathion 2.5 g /l and Buminal, 5 ml/l.

Laboratory sterilant trials.

C. capitata, was reared in our insectary, in a 16:8 light:dark photoperiod, 50-60% relative humidity and a temperature of 27±1ºC. Adult flies were fed with *standard diet*, a mixture of yeast autolysate from Sigma-Aldrich (Steinheim, Germany) and sucrose 1:4 (w:w). Larvae were reared on a mixture of wheat bran: sucrose: beer yeast: nipagin: nipasol: water and hydrochloric acid (20: 5: 1: 0.5: 0.5: 10: 0.1) by weight. Our *C. capitata* colony has been maintained since 1995, however, each year, wild pupae (50% of total pupae colony population) are added to maintain the biological similarity of the colony with the wild population.

In order to test the loss of activity of the baits with aging, laboratory tests were conducted as follows. For aging the baits, 50 delta traps, each one including a petri dish with bait, 25 with lufenuron and 25 without lufenuron, were hung in 50 trees. Traps were placed 1 km away from the malathion-treated area and about 2 km away from lufenuron-treated area at the beginning of May of 2001 and 2002. Petri dishes with aged bait gels were collected from the field every month (0, 30, 60, 90, 120, 150, 180, 210 and 240 days) and tested in laboratory assays. For each date, three bait dishes were collected from field and three bait dish without lufenuron were introduced into six 30x30x30 cm cages with 60 Mediterranean fruit flies (30 males and 30 females). The flies were five days old and were starved for 24 hours before introducing the gels. The gels remained inside the cages, available to the flies, for three hours. During that time, flies could eat the lufenuron-bait gel. After three hours, the three dishes with gels with lufenuron and the three gels without lufenuron were replaced with standard diet. Fifteen flies were caught from each cage and introduced into 3 plexiglass cages (five flies per cage) in order to obtain 3 measurements of fertility per aged bait. In total 18 cages were prepared, nine for the bait with lufenuron (three cages per bait) and nine for the control without lufenuron), five females per cage. Females lay eggs through the fabric of the plexiglass cages, and eggs fall to a plastic container with water. One hundred and fifty eggs per cage, laid between 24 to 48 hours after the bait ingestion were collected with a Pasteur pipette and placed onto three petri dishes with agar gel (3 g/l), 50 eggs per petri dish. Three days after eggs were positioned, egg hatching was evaluated employing a stereoscopic microscope (Leica MZ75 - 40x). This test was replicated during 2002.

Statistical Analysis

In order to analyze population differences we used “Statgraphics plus 5.1” to carry out paired t-test. Paired data analysis was performed in three pre-defined periods in order to avoid a great standard error due to normal population variation along the year. The first period was established from 15 April to 15 June, when the first *C. capitata* generation occurs. The second period is from 15 June to 15 of August when the maximum population level was achieved. The third and last period was from 15 August to 30 November, when fruit fly populations were low but fruit was ripening and fruit damage was occurring.

The paired data t-test was employed to avoid variability of data due to date, because if we had taken the population mean during a period of time, we would be introducing variability due to normal population variation. For each monitoring day we obtain 15 average values of the population, 10 for mandarin fields (7 lufenuron fields, one the average of lufenuron fields, the check field and one buffer area) and 5 for orange fields (3 lufenuron fields, one the average of lufenuron fields and one the check field) (Table 3).. Then we calculated, for each monitoring day, the differences between fruit fly population of each field versus all the other fields of the same cultivar, obtaining 45 pairs of values in mandarin orchards (combinations of 10 elements in pairs) and 10 pairs in orange orchards (combinations of 5 elements is pairs). We obtained 55 series of data (each series was as long as the number of monitoring days) comparing each field with all the other fields of the same cultivar. For each pair, if the mean of each series of data divided by its standard error was a comparable value to a t-student distribution of n-1 degrees of freedom (n is the number of paired data of each series) then there were no significant differences between plots.

Assuming that each year the *C. capitata* population distribution is different during the year, we needed to create an index to evaluate the annual efficacy of the chemosterilant. The index would

count the reduction of fruit fly population in lufenuron areas with respect to malathion-treated areas. The annual amount of fruit flies in each field would be calculated as the sum of weekly averages of catches from 15th April to 30th November. Therefore, the annual efficacy index of the lufenuron treatment would be calculated as follows:

$$Annual\ Efficacy = \left(1 - \frac{\sum_{n=1}^{33} LufenWA}{\sum_{n=1}^{33} MalatWA}\right) \times 100$$

n=number of weeks from 15 April to 30 November

LufenWA= Weekly average of Mediterranean fruit fly catches in flies/trap/day) in the lufenuron-treated area

MalatWA= Weekly average of Mediterranean fruit fly catches in flies/trap/day in the malathion-treated area.

Results and discussion

Figures 2A, 2B, 2C and 2D show the *C. capitata* population evolution in lufenuron and malathion treated areas in 2001, 2002, 2003 and 2004 respectively. In 2001, lufenuron traps were placed in the fields on 10 of May, therefore population reduction could not be expected until the following generation. With the normal temperature of this area in May-June, one generation will be completed in 45 days. Actually, since the first monitoring date until the end of June, there were no differences in Mediterranean fruit fly population. However, from the end of June until the end of 2001, the population was significantly lower in the lufenuron treated areas than in the malathion treated area.

During 2002 until 2004, a continuous population reduction was observed in the lufenuron-treated area. In 2002, the fruit fly population peak was delayed in lufenuron-treated areas by between 15 and 21 days with respect to malathion-treated areas, but it was observed that the maximum population in the lufenuron-treated area was half that of the malathion-treated area. During 2003 and 2004, the lufenuron area population remained always below that in malathion area.

Looking at the figures 2, it can be seen that from 2001 till 2004 a continuous population reduction is achieved in both the malathion and the lufenuron treated areas. Effectively, in the first year in malathion-treated fields, the maximum population level reached 73 flies per trap and day, meanwhile in 2002 it was 52, in 2003 it was 49 and in 2004 it was 26. This reduction could be explained by the increasing number of malathion aerial treatments (from only one treatment in 2001 to 11 treatments in 2004). However, *C. capitata* population in lufenuron treated areas is always, excepting the end of the year in 2002 and three weeks in 2003, under the fruit fly population in malathion-treated areas. The maximum population reduction was achieved after four years of continuous lufenuron application obtaining a Mediterranean fruit fly maximum level of 13 flies per trap day.

In order to evaluate the efficacy of lufenuron we have established an “Annual Efficacy Index” which takes into account the fruit fly population of two different treated areas during the year. In this case, we are comparing lufenuron treatment versus a malathion treatment. Table 2 shows the result of calculating this index, obtaining an increasing efficacy of this method year after year, reaching in the last year an efficacy of 60%. In this index we did not include the increasing number of aerial treatments in the malathion-treated area so, when we study the cumulative efficacy of lufenuron treatments from year to year, we are looking at the real efficacy of lufenuron treatments. Progressive increase in efficacy from year to year in the field trials has

resulted in statistical significance for the first specie risk of 5%. A linear correlation between efficacy and years of treatment is observed, obtaining a correlation ratio of r=0.97 with a p-value=0.0295, lower than 0.05. These results prove that the chemosterilization effect is cumulative and, therefore best results will be obtained after successive seasons and applications.

Table 3 shows Mediterranean fruit fly population in the seven mandarin lufenuron-treated areas and the buffer area compared to malathion, and the three orange lufenuron-treated areas compared to the malathion one. Only in one orange field in 2002, was the annual fruit fly population higher in the lufenuron field than in malathion field (Orange –Lufenuron1-2002). However no significant differences could be observed (F=4.22, df=12,117, P=0.00). In all 37 other cases annual *C. capitata* population of lufenuron-treated areas was lower than in malathion treated ones (28 cases with significant differences and 9 cases with no significant differences between malathion and lufenuron). When individual fields were analyzed it was observed that in the central period, between June and August, when fruit fly population were highest, there was less fruit fly population in lufenuron-treated areas. Buffer areas were located in the first 100 meters of lufenuron treatment, closer to malathion treatment areas. These buffer areas had intermediate results and only in 2002 did *C. capitata* populations differ significantly from malathion treated areas. In this way it is normal that in surrounding fields, fruit fly populations were higher than in the central treated field (Navarro-Llopis et al., 2004). In order to avoid this effect of fruit fly invasion, insecticide application (McQuate et al., 2005) or perimeter mass trapping (Cohen & Yuval, 2000) would be used. When we compare in the same year malathion-treated areas versus lufenuron-treated areas (fields 1 to 7) in mandarins we obtain in all the years significantly less Mediterranean fruit fly population in the lufenuron-treated areas than in malathion. Similarly, in oranges, the *C. capitata* population was significantly lower in lufenuron-

treated areas except in one year (2002). In this case, although fruit fly population was 40% lower in the lufenuron area, no significant difference is observed in the paired t-test (t=1.28, n=64, P=0.21) or ANOVA test (F=1.47, df=1,62, P=0.23).

In this field trial the lufenuron bait stations have been replaced only once per year. In order to ensure the activity of bait gels, they were tested in laboratory conditions after field aging. Results are shown in Table 4. During the seven months that *C. capitata* activity occurs in Mediterranean conditions the gels remain active, reducing fertility below 8%. The bait stations were replaced every year but attractants should be replaced every two or three months. Currently new dispensers based on zeolites and other micro and mesoporous inorganic materials are being developed in order to reach a constant emission for at least seven months (Munoz-Pallares et al., 2001). With this development it will be possible to continuously reduce *C. capitata* populations with the same dispenser all the year round.

All these results show a better efficacy of the chemosterilant technique than aerial malathion spraying. Moreover, the chemosterilization technique achieves better results year after year so, theoretically, continuous application over large areas should suppress fruit fly populations. The chemosterilization technique is very specific to *C. capitata* because specific attractants are used. Effectively, during the four years of field trials no pest resurgence has been detected in chemosterilization areas and it could mean that beneficial insects and non-target pests were not affected by this treatment, although more ecological studies are necessary to prove this.

The main advantage of this method over SIT is that chemosterilization affects wild males and females, reducing the Mediterranean fruit fly population, independently of the overall population. *C.capitata* population is very high in Mediterranean countries and a large quantity of irradiated males is required for SIT success. With combination of the two methods it should be

335 possible to reduce the wild Mediterranean fruit fly population with chemosterilization during two or three years and then apply SIT in a more efficient and economic way. This combination of chemosterilization with SIT is now being studied in a field trial and first results will be obtained in 2007.

References

Batkin, T.A. (1995) Impact of the Mediterranean Fruit Fly, Ceratitis capitata (Wiedemann), on California agriculture. *Light-Activated Pest Control* **616**, 70-81.

Beroza, M., Gertler, S.I., Miyashita, D.H., Green, N. & Steiner, L.F. (1961) Insect Attractants - New Attractants for Mediterranean Fruit Fly. *Journal of Agricultural and Food Chemistry* **9**, 361-&.

Casana-Giner, V., Gandia-Balaguer, A., Mengod-Puerta, C., Primo-Millo, J. & Primo-Yufera, E. (1999) Insect growth regulators as chemosterilants for Ceratitis capitata (Diptera : Tephritidae). *Journal of Economic Entomology* **92**, 303-308.

Castillo, M.A., Moya, P., Hernandez, E. & Primo-Yufera, E. (2000) Susceptibility of Ceratitis capitata Wiedemann (Diptera : Tephritidae) to entomopathogenic fungi and their extracts. *Biological Control* **19**, 274-282.

Cohen, H. & Yuval, B. (2000) Perimeter trapping strategy to reduce Mediterranean fruit fly (Diptera : Tephritidae) damage on different host species in Israel. *Journal of Economic Entomology* **93**, 721-725.

Copeland, R.S., Wharton, R.A., Luke, Q. & De Meyer, M. (2002) Indigenous hosts of Ceratitis capitata (Diptera : Tephritidae) in Kenya. *Annals of the Entomological Society of America* **95**, 672-694.

De La Rosa, W., Lopez, F.L. & Liedo, P. (2002) Beauveria bassiana as a pathogen of the Mexican fruit fly (Diptera : Tephritidae) under laboratory conditions. *Journal of Economic Entomology* **95**, 36-43.

Dimbi, S., Maniania, N.K., Lux, S., Ekesi, S. & Mueke, J.K. (2003) Pathogenicity of Metarhizium anisopliae (Metsch.) Sorokin and Beauveria bassiana (Balsamo) Vuillemin, to three adult fruit fly species: Ceratitis capitata (Weidemann), C-rosa var. fasciventris Karsch and C-cosyra (Walker) (Diptera : Tephritidae). *Mycopathologia* **156**, 375-382.

Katsoyannos, B.I. (1994) Evaluation of Mediterranean Fruit-Fly Traps for Use in Sterile-Insect-Technique Programs. *Journal of Applied Entomology-Zeitschrift fur Angewandte Entomologie* **118**, 442-452.

Katsoyannos, B.I. & Papadopoulos, N.T. (2004) Evaluation of synthetic female attractants against Ceratitis capitata (Diptera : Tephritidae) in sticky coated spheres and McPhail type traps. *Journal of Economic Entomology* **97**, 21-26.

Knipling, E.F. (1955) Possibilities of Insect Control Or Eradication Through the Use of Sexually Sterile Males. *Journal of Economic Entomology* **48**, 459-462.

Liquido, N.J., Barr, P.G. & Cunningham, R.T. (1997) Medhost: an encyclopaedic bibliography of the host plants of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). *Electronic Database Program.ARS-144.*

Lopez, M., Sivinski, J., Rendon, P., Holler, T., Bloem, K., Copeland, R., Trostle, M. & Aluja, M. (2003) Colonization of Fopius ceratitivorus, a newly discovered African egg-pupal parasitoid (Hymenoptera : Braconidae) of Ceratitis capitata (Diptera : Tephritidae). *Florida Entomologist* **86**, 53-60.

McQuate, G.T., Sylva, C.D. & Jang, E.B. (2005) Mediterranean fruit fly (Dipt., Tephritidae) suppression in persimmon through bait sprays in adjacent coffee plantings. *Journal of Applied Entomology* **129**, 110-117.

Munoz-Pallares, J., Corma, A., Primo, J. & Primo-Yufera, E. (2001) Zeolites as pheromone dispensers. *Journal of Agricultural and Food Chemistry* **49**, 4801-4807.

Navarro-Llopis, V. (2002) Nuevos métodos de lucha contra Ceratitis capitata (Wiedemann) basados en la aplicación de cebos atrayentes combinados con un IGR esterilizante. *Ph.D.dissertation.*(Universidad Politécnica de Valencia) Valencia. Spain.

Navarro-Llopis, V.N., Sanchis-Cabanes, J., Ayala, I., Casana-Giner, V. & Primo-Yufera, E. (2004) Efficacy of lufenuron as chemosterilant against Ceratitis capitata in field trials. *Pest Management Science* **60**, 914-920.

Papadopoulos, N.T. & Katsoyannos, B.I. (2003) Field parasitism of Ceratitis capitata larvae by Aganaspis daci in Chios, Greece. *Biocontrol* **48**, 191-195.

Wong, T.T.Y., Ramadan, M.M., Herr, J.C. & McInnis, D.O. (1992) Suppression of A Mediterranean Fruit-Fly (Diptera, Tephritidae) Population with Concurrent Parasitoid and Sterile Fly Releases in Kula, Maui, Hawaii. *Journal of Economic Entomology* **85**, 1671-1681.

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Table 1. Description of different plots in lufenuron and malathion treated area

Variety	Treatment	Field	Area (Ha)	Variety
Mandarin	Malathion	1	6	Marisol
	Lufenuron	1	6	Clementina fina
	Lufenuron	2	8	Clementina fina
	Lufenuron	3	9	Clementina fina
	Lufenuron	4	5	Clementina fina
	Lufenuron	5	7	Marisol
	Lufenuron	6	4	Marisol
	Lufenuron	7	5	Marisol
	Buffer area	1	6	Marisol
Oranges	Malathion	1	5	New-Hall
	Lufenuron	1	6	New-Hall
	Lufenuron	2	5	New-Hall
	Lufenuron	3	3	New-Hall

Table 2. Annual efficacy index during 4 years depending of treatment type.

	Aggregated fruit fly captures			
Treatment	**2001**	**2002**	**2003**	**2004**
Lufenuron annual	314	160	133	79
Malathion annual	537	290	309	200
Annual efficacy index	41%	45%	57%	60%

Lufenuron Aggregated= Sum of weekly averages of Mediterranean fruit fly catches in flies/trap/day in the lufenuron treated area

Malathion Aggregated= Sum of weekly averages of Mediterranean fruit fly catches in flies/trap/day in the malathion treated area.

Annual efficacy index: One minus the quotient of Lufenuron aggregated divided malathion aggregated in percentage.

Table 3. Mediterranean fruit fly population (mean ± SEM) per period in malathion and lufenuron treated fields from 2001 till 2004 in different cultivars.

Year	Cultivar	Treatment	Field	n	Date			Annual
					15April-15 June	15Jun-15Agos	15-August 30 November	
2001	Mandarin	Malathion	1	6	19.53 ± 7.91a	16.88 ± 4.16a	0.71 ± 0.24abc	4.17 ± 1.53a
		Lufenuron	1	6	27.68 ± 12.10a	3.40 ± 1.40b	0.23 ± 0.06bc	0.91 ± 0.38b
		Lufenuron	2	8	17.75 ± 7.08a	1.51 ± 0.67b	0.62 ± 0.14abc	0.81 ± 0.19b
		Lufenuron	3	9	9.50 ± 4.01a	2.83 ± 1.21b	0.33 ± 0.06bc	0.65 ± 0.46b
		Lufenuron	4	5	8.57 ± 4.99a	2.88 ± 2.01b	0.05 ± 0.01c	0.65 ± 0.46b
		Lufenuron	5	7	25.46 ± 7.55a	1.49 ± 0.39b	1.27 ± 0.23a	1.32 ± 0.20b
		Lufenuron	6	4	13.66 ± 2.16a	1.58 ± 0.69b	1.36 ± 0.34a	0.72 ± 0.23b
		Lufenuron	7	5	9.24 ± 5.67a	2.38 ± 0.75b	0.27 ± 0.06bc	1.41 ± 0.30b
		Lufenuron	1-7	44	15.98 ± 5.66a	2.30 ± 0.98b	0.70 ± 0.24c	0.96 ± 0.24b
		Buffer area	1	6	19.97 ± 4.69a	1.23 ± 0.41b	1.17 ± 0.32a	1.19 ± 0.26b
	Orange	Malathion	1	5	21.78 ± 7.78a	11.36 ± 3.24a	1.60 ± 0.54a	3.69 ± 1.09a
		Lufenuron	1	6	24.12 ± 8.23a	1.61 ± 0.34b	0.83 ± 0.22ab	0.99 ± 0.19b
		Lufenuron	2	5	4.12 ± 2.34a	5.54 ± 2.24b	0.19 ± 0.03c	1.33 ± 0.61b
		Lufenuron	3	3	14.16 ± 3.36a	3.12 ± 0.95b	0.39 ± 0.09bc	0.97 ± 0.30b
		Lufenuron	1-3	14	11.00 ± 3.67a	3.43 ± 1.11b	0.47 ± 0.09b	1.10 ± 0.32b
2002	Mandarin	Malathion	1	6	0.29 ± 0.14a	26.60 ± 6.90a	1.40 ±0.54bcd	9.00 ± 2.99a
		Lufenuron	1	6	0.10 ± 0.04a	13.22 ± 3.49bc	0.74 ± 0.19cd	4.48 ± 1.49abc
		Lufenuron	2	8	0.28 ±0.12a	16.92 ± 4.54ab	3.69 ± 1.25bc	6.97 ± 1.92ab
		Lufenuron	3	9	0.14 ± 0.08a	18.01 ± 4.49ab	1.22 ± 0.44bcd	1.04 ± 0.39c
		Lufenuron	4	5	0.02 ± 0.01a	3.11 ± 0.99c	0.15 ± 0.08d	6.20 ± 1.98ab
		Lufenuron	5	7	0.18 ± 0.07a	2.83 ± 0.56c	1.83 ± 0.33bcd	1.73 ± 0.29c
		Lufenuron	6	4	0.69 ± 0.30a	8.16 ± 1.81bc	5.21 ± 1.40a	5.07 ± 1.14abc
		Lufenuron	7	5	0.04 ± 0.02a	10.63 ± 2.51bc	3.97 ± 1.05ab	5.00 ± 0.96abc
		Lufenuron	1-7	44	0.20 ± 0.08a	10.41 ± 2.24b	2.40 ± 0.39c	4.35 ± 1.02b
		Buffer area	1	6	0.33 ± 0.13a	10.14 ± 3.44bc	8.98 ± 3.05e	7.18 ± 1.81ab
	Orange	Malathion	1	5	3.08 ± 1.29a	24.09 ± 6.49a	1.69 ± 0.38b	9.04 ± 2.70a
		Lufenuron	1	6	0.24 ± 0.11b	25.43 ± 6.49a	5.44 ± 1.22a	10.39 ± 2.75a
		Lufenuron	2	5	0.02 ± 0.01b	6.03 ± 2.08b	1.55 ± 0.22b	2.56 ± 0.77b
		Lufenuron	3	3	0.14 ± 0.09b	6.30 ± 1.53b	2.97 ± 0.94b	3.30 ± 0.74b
		Lufenuron	1-3	14	0.14 ± 0.06b	12.59 ± 2.98a	3.32 ± 0.57a	5.42 ± 1.29a
2003	Mandarin	Malathion	1	6	1.49 ± 1.06a	26.92 ± 6.33a	0.57 ± 0.09a	8.15 ± 2.72a
		Lufenuron	1	6	0.43 ± 0.23a	15.54 ± 4.72b	0.17 ± 0.04a	4.55 ± 1.77b
		Lufenuron	2	8	0.35 ± 0.19a	13.29 ± 4.86b	1.24 ± 0.91a	4.46 ± 1.71b
		Lufenuron	3	9	0.24 ± 0.09a	9.82 ± 1.98b	0.26 ± 0.07a	2.94 ± 0.93b
		Lufenuron	4	5	0.12 ± 0.08a	9.81 ± 3.39b	0.11 ± 0.02a	2.84 ± 1.20b
		Lufenuron	5	7	0.38 ± 0.20a	4.51 ±0.90b	0.32 ± 0.09a	1.51 ± 0.42b
		Lufenuron	6	4	0.62 ± 0.24a	19.81 ± 5.67ab	1.43 ± 0.44a	2.08 ± 0.57b
		Lufenuron	7	5	0.45 ± 0.25a	6.59 ± 0.91b	0.27 ± 0.12a	6.44 ± 2.15ab
		Lufenuron	1-7	44	0.37 ± 0.17a	11.34 ± 2.93b	0.54 ± 0.09a	3.55 ± 1.18b
		Buffer area	1	6	1.35 ± 0.72a	9.74 ± 1.95b	0.64 ± 0.15a	3.33 ± 0.90b
	Orange	Malathion	1	5	6.38 ± 4.08a	33.57 ± 5.19a	1.85 ± 0.53a	11.94 ± 3.03a
		Lufenuron	1	6	0.29 ± 0.14b	7.48 ± 1.86b	0.57 ± 0.12bc	2.46 ± 0.76b
		Lufenuron	2	5	0.27 ± 0.12b	4.00 ± 0.81b	0.16 ± 0.06c	1.26 ± 0.38b
		Lufenuron	3	3	0.36 ± 0.13b	5.87 ± 1.08b	1.15 ± 0.28ab	2.33 ± 0.52b
		Lufenuron	1-3	14	0.31 ± 0.09a	5.79 ± 1.18b	0.63 ± 0.11b	2.02 ± 0.53b
2004	Mandarin	Malathion	1	6	0.23 ± 0.12a	14.26 ± 3.87a	0.67 ± 0.12bc	4.64 ± 1.61a
		Lufenuron	1	6	0.02 ± 0.01a	7.61 ± 2.49bc	0.92 ± 0.48ab	2.71 ± 0.96ab
		Lufenuron	2	8	0.05 ± 0.02a	7.12 ± 1.13bc	3.86 ± 1.36a	3.95 ± 0.85ab
		Lufenuron	4	5	0.06 ± 0.02a	2.41 ± 0.47c	0.45 ± 0.30c	0.95 ± 0.26b
		Lufenuron	5	7	0.09 ± 0.07a	10.41 ± 3.21ab	1.32 ± 0.29bc	3.76 ± 1.24ab
		Lufenuron	6	4	0.12 ± 0.05a	4.21 ± 0.75bc	0.55 ± 0.10c	1.55 ± 0.39b
		Lufenuron	1-6	30	0.06 ± 0.02a	6.87 ± 1.09b	1.30 ± 0.31b	2.68 ± 0.62a
		Buffer area	1	6	0.33 ± 0.08a	10.13 ± 2.09ab	2.02 ± 0.38b	4.06 ± 0.98b
	Orange	Malathion	1	5	12.54 ± 7.76a	20.30 ± 4.81a	1.37 ± 0.46a	9.45 ± 2.55a
		Lufenuron	1	6	0.22 ± 0.09b	2.66 ± 0.48b	1.22 ± 0.29a	1.42 ± 0.25b
		Lufenuron	2	5	0.05 ± 0.03b	13.29 ± 3.71a	0.52 ± 0.15a	4.24 ± 1.53b
		Lufenuron	3	3	0.83 ± 0.35b	7.27 ± 1.64b	0.81 ± 0.17a	2.76 ± 0.73b
		Lufenuron	1-3	14	0.37 ± 0.12b	7.74 ± 1.57b	0.85 ± 0.12a	2.81 ± 0.75b

a, b Values of the same period with the same letter within the same cultivar and year are not significantly different in the paired data t-test (P≤0.05).

n: monitoring traps number

Table 4. Eggs hatching (% ± SEM) from lufenuron bait-gel fed females and non lufenuron bait gel fed females in laboratory.

Bait composition	Bait aging days							
	0	**30**	**60**	**90**	**120**	**150**	**180**	**210**
(+) Lufenuron	0.9 ± 0.5a	5.1 ± 1.9a	4.9 ± 0.9a	3.3 ± 1.0a	6.7 ± 1.8a	3.6 ± 1.9a	8.0 ± 3.2a	7.8 ± 1.1a
(-) Lufenuron	98.0 ± 1.2b	98.7 ± 1.3b	92.0 ± 4.2b	98.0 ± 1.1b	97.3 ± 1.7b	96.7 ± 1.7b	98.0 ± 1.1b	96.7 ± 1.8b

(+) Lufenuron. Protein bait gel containing 30 g/l of lufenuron

(-) Lufenuron. Protein bait gel containing without lufenuron.

a,b Values within the same aging with the same letter are not significantly different in Student t-test (P≤0.05)

Data were subjected to arcsin (sqrt(x)) transformation for analysis; untransformed data are presented.

Figure 1. Trial field map.

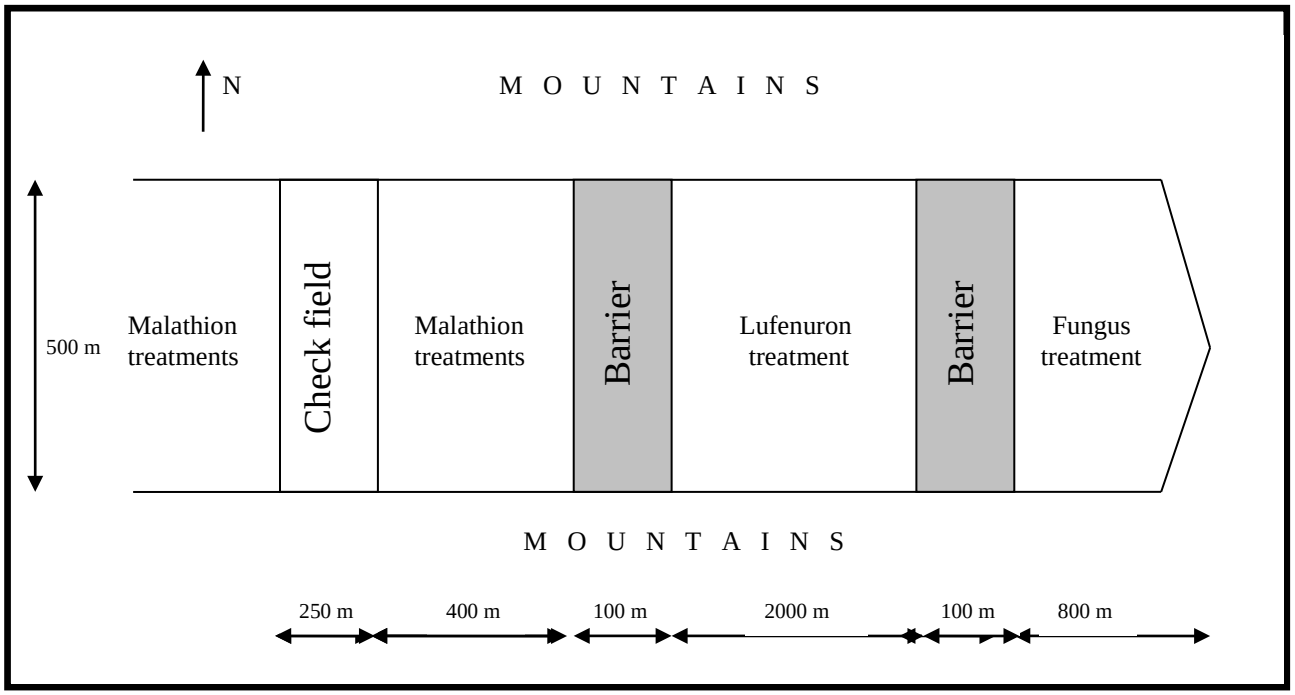

Figure 2. Evolution of Mediterranean fruit fly population in lufenuron and malathion treated fields from 2001 till 2004.

Figure 2A. 2001

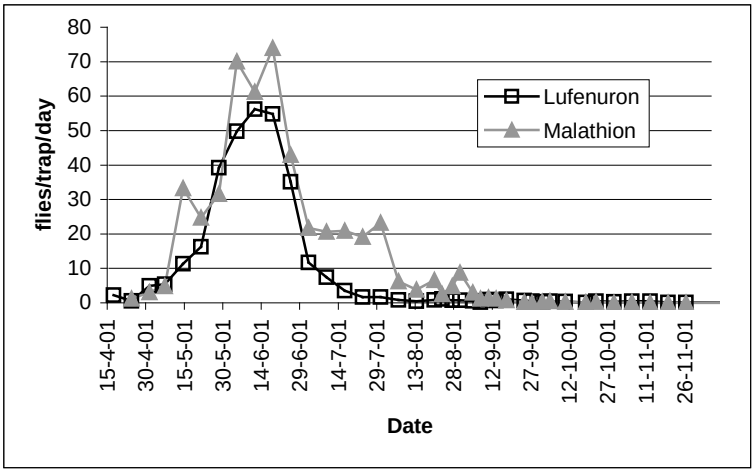

Figure 2B. 2002

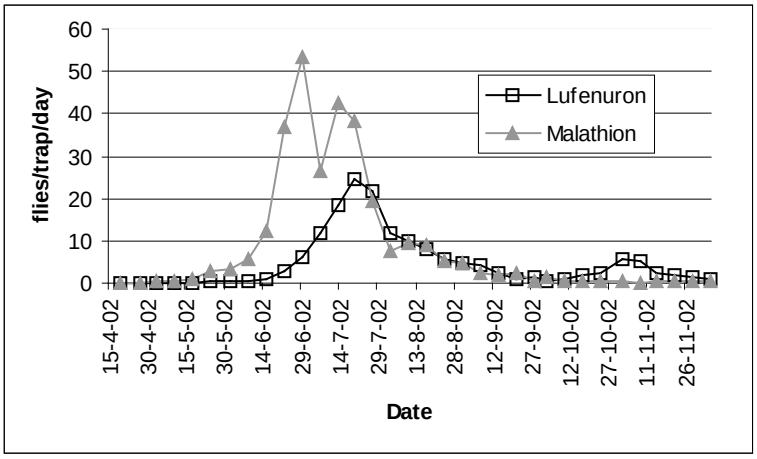

Figure 2C. 2003

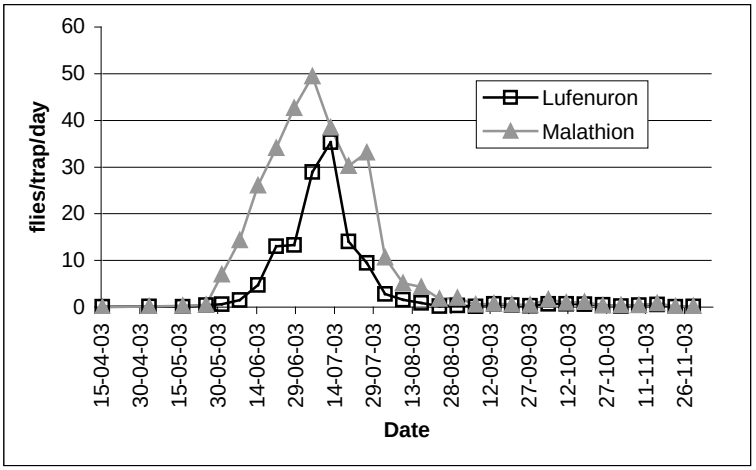

Figure 2D. 2004

455

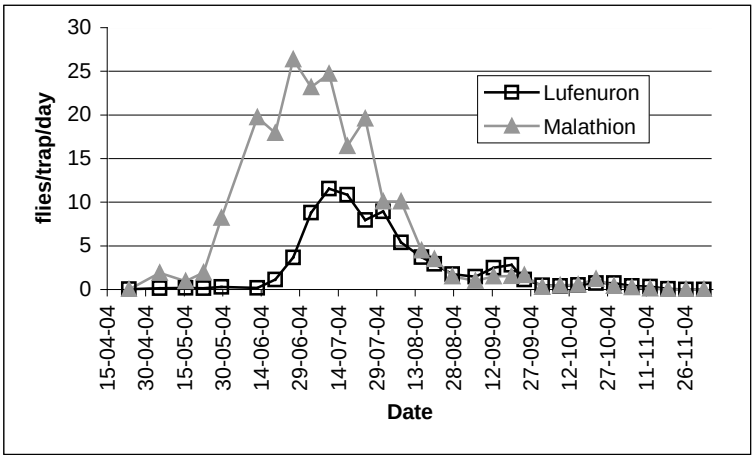

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Figure 1. Trial field map.
Check field was inside aerial malathion treated area and was treated as the malathion area