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Efficacy of lufenuron as chemosterilant against *Ceratitis capitata* in field trials.

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Abstract: Two field trials in citrus orchards in Turis (Valencia, Spain) and Denia (Alicante, Spain) were performed in order to test the sterilant effect of the IGR lufenuron against wild medfly *Ceratitis capitata* (Wiedemann) populations. Two application methods for lufenuron were tested: spraying, in spots, an emulsion of lufenuron in a protein bait, and hanging delta traps that contained a proteinaceous gel with lufenuron (solid bait). The sterilant effect was measured as medfly population reduction, reduction of fruit damage in treated fields, and the number of eggs hatching in punctured fruits.

In order to assess the efficacy of lufenuron treatments, we recorded results obtained from two different zones in both trial fields: an outer zone, close to untreated fields, and an inner zone, in the center of lufenuron treated fields. We observed a minimum sterilant effect in the outer zone and a maximum sterilant effect in the inner one.

The maximum sterilant effect was observed in the inner zone, where a reduction of medfly population of 80.4% in the sprayed field and a reduction of 77.6% in the solid bait field was observed. In addition, the greater the distance from the untreated zones of the treated orchard (inwards), the lower the fruit damage and medfly population level. In this inner zone, fruit punctured by medfly developed significantly fewer larvae (38.8%) than punctured fruits from the outer zone (68.6%).

In addition, we recorded the decline in the activity of the lufenuron treatments with time. Lufenuron activity persisted in field for at least 2 weeks with spray applications, and for 3 months with bait gels.

1 INTRODUCTION

The Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is one of the pests that causes substantial economic losses in Mediterranean agriculture.[1] There are more than 300 plant species around the world, belonging to 67 different families, that are medfly hosts.[2] The zones most affected by the medfly are in tropical and subtropical latitudes, where daily temperatures in winter rarely go below 5ºC.

Currently, to maintain medfly populations at low levels in Spain, organophosphate insecticides (e.g. malathion) mixed with protein baits, are applied in aerial and terrestrial treatments. Spinosad is also being used in the USA and tested in Spain as a possible replacement for malathion.[3-5] Other current control methods used in Spain include the release of sterilised males using the Sterile Insect Technique (SIT),[6-8] mass trapping with new powerful attractants for females,[9-11] biological control with parasitoids[12] and the combination of several of these methods. In this paper, we report the results of two field trials conducted to investigate whether sterilization by means of an IGR could be considered as a new medfly control method.

The chosen IGR was Lufenuron, a phenyl-benzoylurea that inhibits chitin synthesis. Lufenuron is used for the control of fleas on pets and cockroaches in houses[13]. It is also used for control of lepidopteran and coleopteran larvae on cotton and vegetables, and against citrus whitefly and rust mites on citrus fruit.[14]

When female medflies ingest a bait containing 0.1% (w:w) lufenuron, the hatching of the subsequently laid eggs is prevented. Moreover, in laboratory experiments, females that mate with lufenuron treated males (0,5% (w:w) a.i. in diet) lay non-viable eggs.[15] In addition, our group has tested lufenuron in field caged grapefruits infested with medflies, observing an effective reduction of population density (unpublished results).

In the present work, field tests have been made to check the ability of lufenuron to reduce wild medfly populations, and to decrease fruit damage in oranges and mandarins.

2 MATERIALS AND METHODS

2.1 Chemicals employed were: Smellfol® from Dadelos (Valencia, Spain), Meliose 710B from Roquette Laisa (Benifaio, Valencia, Spain), sorbitol 70% from Guinama (Valencia, Spain), lufenuron tech. (99,1%) from Syngenta (Basel, Switzerland), Match® from Syngenta (Barcelona, Spain), Emulsogen EL from Clariant (Barcelona, Spain), trimethylamine hydrochloride from Aldrich (Madrid, Spain), putrescine from Acros (Geel, Belgium), ammonium acetate from Panreac (Barcelona, Spain) and Aralure® (emitters of trimedlure (TML)) from Aragonesas Agro S.A. (Madrid, Spain).

The bait had the following composition: Smellfol® 60%; meliose 30%; sorbitol 10% (vol:vol:vol). Smellfol® is a natural corn protein hydrolyzate with a protein content of 148 g kg^{-1}. Meliose 710B is a syrup, mixture of polysaccharides. The main components are glucose-fructose (39%:21% (w:w)) and the dry weigh of this meliose is 760 g kg –1. We have observed that meliose increases the ingestion of the bait by the flies (unpublished results).

2.2 Medflies were reared in our insectary in a 16:8 light:dark photoperiod with 50-60% relative humidity and a temperature of 27±1ºC. Adult flies were fed with standard artificial diet (a mixture of yeast autolisate 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 medfly colony has been maintained since 1995, with yearly additions of wild medfly pupae (30% of total pupal colony population) in order to maintain the genetic similarity of the colony to the wild population.

2.3 Treatments

Two different treatments were performed in order to test their efficacy: a spray treatment and a bait-gel treatment.

2.3.1 Spray treatment in *trial field 1*.

The treatments were carried out by spraying an area of 1 m^2 on the south side of all trees. The spraying pressure was 1500-2000 mmHg, and an 18 mm diameter diffuser was employed. The sprayed bait was a water based mixture of Smellfol (33 ml $litre^{-1}$) with 10 g. lufenuron per litre as formulated in Match®, and each tree was sprayed with 120 ml of this mixture. The treatment was carried out biweekly from May 6th to October 10th 2002, in order to ensure sterilant activity throughout the whole period.

2.3.2 Bait-gel treatment in *trial field 2*.

The treatments were performed by placing protein bait gels containing the sterilant substance (lufenuron) in the trial field. In the field, the protein bait attracts medflies and induces ingestion. In order to improve attraction we added to the bait gel 10 g $litre^{-1}$ of trimethylamine, 10 g $litre^{-1}$ ammonium acetate and one g $litre^{-1}$ of putrescine.[15] Gel was made with agar-agar at 1 g $litre^{-1}$. Lufenuron Technical (98% purity) was emulsified in the protein bait at 10 g $litre^{-1}$ with Emulsogen EL at 1 g $litre^{-1}$. The gels were placed in delta traps, which were suspended on the south-east face of trees, 1.5 metres above ground. We hung one trap per nine trees. The treatment began on January 25th and traps remained in place until May 25th 2002. The gels were replaced on April 25th in order to avoid any reduction in activity as the baits aged.

2.4 Trial fields: field trials were carried out in two fields: *Trial Field 1* and *Trial Field 2*.

Trial Field 1 was a 4.5 Ha field of mandarins (*Citrus reticulata* Blanco cv. Marisol), in Turís (Valencia, Spain). This field was surrounded by hills that prevent, to a great extent, the migration of medflies. Only one side of the field was open to other cultivated fields. The other three sides of the test field were adjacent to mountainous areas with no fruit trees or other possible medfly hosts (Fig. 1). In the lufenuron treated field, three zones were monitored: an “outer zone”, an “inner zone” and an “untreated field”. The "outer zone" was located less than 120 meters away from the neighbouring untreated fields with fruit trees. The "inner zone" was located more than 120 meters away from fruit trees not treated with lufenuron. In order to prevent mixed effects from populations of the inner and the outer zone, an area of 20 meters wide was established between them, and it was named “middle zone”. This middle zone was considered as a buffer zone and no monitoring traps were placed in this zone. The “untreated field” was located at 1.3 km from the “outer zone”. With this distribution, we expected to observe the maximum sterilant effect in the inner zone and a reduced effect in the outer zone. The “untreated zone” was expected to give the highest population of medfly.

Trial field 2 was located in Denia (Alicante, Spain) in a 5 Ha orange field (*Citrus sienensis* Osbeck cv. Valencia). This field was adjacent to other cultivated citrus fields on all perimeters. As in trial field 1, three zones were monitored: an "outer zone", located at less than 100 meters from the neighbouring untreated fields, an "inner zone", at more than 120 meters from any fruit tree that had not been treated with lufenuron, and an “untreated zone”. As in trial field 1, no monitoring traps were located between 100 and 120 metres from untreated fields.

2.5 Effectiveness of the method

2.5.1 Monitoring of the fly population

The medfly population was checked by means of captures in sticky traps. These traps were yellow, 20x20 cm^2, PVC sticky boards. In order to obtain the best index of the male population, an emitter of trimedlure (Aralure®) was placed at the top of the trap. The sticky boards were replaced biweekly, and emitters of TML were replaced every 45 days. In *Trial Field 1* (4.5 Ha) 12 monitoring traps were placed (6 in the inner zone and 6 in the outer zone). Captures were recorded biweekly from April to July and weekly from August to October. In *Trial Field 2* (5 Ha) the medfly population was checked with 27 traps, 9 for each monitored zone from February 1st to Jun 15th 2002.

2.5.2 Duration of the sterilizing activity of the spraying treatment

In order to test the duration of the sterilizing activity of the spray treatment, three trees were sprayed in *Trial field 1*, and the persistence of sterilizing activity was measured using laboratory tests as follows. Leaf samples from each sprayed tree were collected on the same day of spraying, and also 7 and 14 days later. The sprayed leaves were taken to the laboratory and placed in three Plexiglas cubic cages, 2 leaves of each tree per cage. Previously, we had introduced 5 mated medfly females (5-6 days old) into each cage. The cages measured 10x10x10 cm, with one side closed with fabric. Medflies laid eggs through the fabric and these were collected in a water-filled container placed under the fabric side of the cages. The leaves remained inside the cages freely available to the 5 females for 3 hours. During that time, the medflies could eat the lufenuron-bait of sprayed leaves. After this period treated leaves were removed, and normal dietary food was introduced in the cage. Then, the cages were taken to the insectary where flies could lay eggs through the fabric side of the Plexiglas cages. Eggs laid between 24 to 48 hours after the lufenuron-bait ingestion were collected with

a Pasteur pipette and seeded on a gel (3 g litre^{-1}agar in water). From each container we collected 150 eggs that were seeded on 3 petri dishes with agar, 50 eggs per petri dish. Three days after seeding, egg hatching was evaluated employing a stereoscopic microscope (Leica MZ75 - 40x). This test was replicated three times for each of the three periods of spray aging.

2.5.3 Duration of the sterilizing activity of the bait-gels

We tested the sterilant activity of bait gels aged for 0, 15, 30, 45, 60 and 90 days. Three gel dishes were collected from "Trial Field 2" at each aging date and were brought to the laboratory. Each gel was introduced in a 30x30x30 cm cage with 60 medflies (30 males and 30 females). The flies were 5 days old and they were starved for 24 hours before introducing the gels. The gels remained inside the cages, available to the flies, for 3 hours. During that time, medflies could eat the lufenuron-bait gel. After this time, sterilant gels were replaced with standard diet. Fifteen flies were caught from each cage and introduced into 3 Plexiglass cages as in the previous experiment. The eggs laid between 24 to 48 hours after the lufenuron-bait ingestion were collected with a Pasteur pipette and seeded on a gel (3 g agar l^{-1}). From each container we collected 150 eggs that were seeded on 3 petri dishes with agar, 50 eggs per petri dish. Three days after seeding, egg hatching was evaluated employing a stereoscopic microscope (Leica MZ75 - 40x). This test was replicated three times for each of the three periods of gel aging.

2.5.4 Fruit damage assessment

In order to evaluate fruit damage, we sampled 1% of treated trees, and all fruits from the selected trees were visually inspected.

In “Trial Field 1” 10,852 mandarins from 27 trees were inspected: 10 trees from the inner zone and 17 from the outer zone. Mandarins were checked on 5th October, 3 days before harvest.

In “Trial Field 2” 4,235 oranges from 12 trees were inspected : 6 trees from the inner zone, and 6 from the last row of trees in the outer zone (just at the beginning of untreated zone). Fruit of the selected 12 trees was left in the field until Jun 16th, one month after harvesting the rest of the oranges from the orchard. Therefore, the only receptive fruit in the whole orchard was the fruit of trees selected for this study of damage, and this could explain the elevated percentage of fruit damage in “trial field 2”.

The percentage of medfly-punctured fruits was recorded. Subsequently, a sample of 40 punctured fruits per zone was taken to the insectary, where they were maintained at 27±1ºC and 50-60% HR for 5 days. At the end of this period, fruits were checked to see how many of them contained developing larvae.

2.6 Statistical methods

In order to appreciate differences of population between different treated areas, we carried out a paired data analysis. The paired data t-test was employed in order 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 3 average values of the population: one for the treated outer zone, one for the treated inner zone and one for the untreated zone. Then we calculate, for each day, the differences between pairs of these values (outer versus inner, inner versus untreated and outer versus untreated). We obtain three series of data comparing outer versus inner zone, inner versus untreated zone and outer versus untreated zone. If the mean of each series divided by its standard error is a likely value of a t-student distribution of n-1 degrees of freedom (n is

the number of paired data of each series) then there are not significant differences between the efficacy of the treatments.

An ANOVA analysis was also carried out in order to compare population within periods, when variability of populations within these periods results lower.

In addition, one way ANOVA was performed in order to compare sterilising activity bait with aging.

Statistical analysis was performed using the package Statgraphics plus 5.1.

3 RESULTS AND DISCUSSION

3.1 Monitoring of medfly population

3.1.1 Trial Field 1

Figure 3 shows the changes in the medfly population in the field sprayed with lufenuron compared to the population in the untreated field. As can be noticed in Figure 3, the trend of pest population is very similar in both fields.

In order to analyse the development of the population, we distinguished three periods. The initial period (in which no reduction of medfly population was expected) extends from the beginning of spray treatments (May 4th) to June 14th. It should be possible to observe a reduction of medfly population from middle of June, because by this time, a whole generation of treated medfly would have been completed. Indeed, in the middle period, from June 15th to the end of August, we observed a significant reduction of medfly population (52.42 %) in the treated zone with respect to the untreated one. This observation was supported by the paired data t-test (P= 0.037, t=2.75, n=11). By the end of August, a spontaneous reduction of the population had taken place in all fields, and in September there was no significant difference between both zones in ANOVA test (F=0,3044; df=2,6; P=0.060). This reduction of medfly population usually takes place in August every year in Mediterranean regions. We must also

consider that, in September, ripening fruit is a great source of attraction for the flies from neighbouring fields. Thus, the medfly population in the lufenuron treated field could have been increased due to invasion from other fields, causing levelling of populations.

The results of the captures per period are shown in Table 1. The medfly population level was similar between untreated and treated fields at the beginning of field trial because the sterilising effect was not yet visible. In the second period, when it should have been possible to observe the sterilizing effect, the population level was lowest in the inner zone, medium in the outer zone, and highest in the untreated zone. Finally, from September to the end of the trial, no significant differences in medfly populations were observed between the different zones.

In figure 3 we can observe that medfly populations in treated and untreated field were very similar at the beginning of the trial, but 6 weeks after the first treatment, there were substantial differences between the populations. This is as expected, because medfly population should only be reduced by the sterilizing effect of lufenuron in the generation following the first treatment. The generation time of medflies under these conditions of humidity and temperature requires about 4 or 5 weeks.

3.1.2 Trial Field 2

Figure 4 shows the changes in the medfly populations in the lufenuron-gel treated zones (outer and inner ones) and in the untreated zones. According to Figure 4, the population trend of the pest was similar in inner and outer zones at the beginning of the trial.

In order to analyse differences in medfly population between zones we established 2 periods: a first period between March and mid April, when medfly population is very low and any sterilising effect is not noticeable and a second period, from mid April to the end of trial,

when medfly population increases quickly due to higher temperatures, and any sterilising effect is much more noticeable.

During the whole of the first period, the population was lower than 0.1 medflies/trap/day, and no significant differences between outer and inner zones could be observed (P=0.10 in paired data t-test) The medfly population in the untreated zone was significantly higher than in the treated zones during this period. In the second period we observed that in the outer and untreated zones, the medfly populations increased to more than 1 medfly/trap/day in a month, while in the inner zone the rate of population increase was lower, and the medfly population did not reach 0.4 medflies/trap/day. In Table 2 we can see that, while in the first period there were no significant differences between medfly populations in the inner and outer zones, in the second period the medfly population was significant lower in the inner zone (P=0.02 in paired data t-test). The maximum reduction in medfly population in the inner treated zone was 75.3% with respect to the untreated zone and 72.0% with respect to the outer treated zone at the end of the trial.

3.2 Duration of the activity of the sterilising baits

Table 3 show the percentage of eggs hatching relative to the aging time of the sprayed baits. Egg hatching was 6.65% when baits were ingested shortly after spraying. However, when the baits were aged for 14 days, egg hatching increased to 67.33%. This percentage is close to the control value (85.5%) although significant differences were still apparent (F=0.307, df=8, P<0.05). Nevertheless, with lufenuron-bait gels the medfly sterility rate remained above 58% after 1 month, and the baits were still able to sterilize after 3 months (Table 4).

In order to reduce medfly population with this sterilising technique, we must ensure that the sterilizing effect persists in the field, in order that, we are able to reduce the medfly population by interfering with successive generations. From these trials we first conclude that

it is necessary to perform biweekly spray treatments or, alternatively, to change the gel bait every 2 months to maintain the sterilising effect in field. Second, when sterilising gels are used, we only need to deploy the traps three times a year in order to affect medfly population in the Mediterranean region of Spain. Third, by hanging the traps with sterilising gels at the beginning of the medfly population outbreak, and replacing them every 2 and 4 months, nearly 5 out of 6 existing generations of medfly will be affected.

3.3 Assessment of fruit damage

Table 5 shows the results of medfly damage to fruits in the 2 described zones. Sampling of fruit damage in "Trial Field 1" was made when fruit was being harvested, so fruit damage was low because it was still ripening. In "Trial Field 2"a sample was taken of fruit that remain in the field one month after harvesting. Therefore, the only susceptible fruit in this field was in the control trees, and this fruit was overripe. This explains why the total percentage of damaged fruit was greater in "Trial Field 2" than in "Trial Field 1".

The percentage of fruit affected by medfly punctures was significantly lower in the inner zones than in the outer zones (near by lower 3 times in the inner zones), therefore we deduce that medfly population was lower in the inner zone. We also observed that the percentage of fruit that developed larvae was significantly higher in the outer zones (61% and 76%) than in the inner zones (38% and 39%).

4 CONCLUSSIONS

With this field trial we have proved that a method based on sterilising by chemicals is able to reduce the population of Mediterranean fruit fly. This method bases its effectiveness on the horizontal transmission of sterility so, by using the medflies capacity to find other medflies we can sterilise a significant part of the population even though it has not ingested the bait.[16]

First, we obtained a significant reduction of the population in the treated orchards with respect to the control orchard. Second, we observed that effectiveness of this sterilising method is greater when there is no medfly immigration from the untreated neighbouring fields. We observed that fields closer to untreated fields have significantly higher medfly populations than the most isolated treated zone.

Lufenuron-bait spray is probably the best method of application because a high percentage of the medfly population can be reached. However, biweekly applications are needed in order to obtain a sustained reduction in the fly population. As an alternative to this application method, the use of solid baits with much greater persistence in field was tested. This latter treatment showed similar efficacy to spray baits, but has two advantages. First, it is cheaper because less manual labour is necessary for the treatments. Second, the bait-gels attract mainly medfly, so other (beneficial) species are not affected by the treatments.

In addition, the use of chemosterilants is a method of controlling medfly compatible with other chemical treatments like malathion or spinosad bait spray. It could also work in conjunction with SIT as this method also sterilises wild flies and, therefore, the number of released sterile males required to compete with unsterilised wild medflies can be reduced.

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REFERENCES

1. Enkerlin W. and Mumford J. Economic evaluation of three alternative methods for control of the Mediterranean fruit fly (Diptera: Tephritidae) in Israel, Palestinian Territories, and Jordan. *J. Econ. Entomol*. 90 (5): 1066-1072 (1997).

2. Liquido, NJ, Barr, PG and Cunningham RT. Medhost: an encyclopaedic bibliography of the host plants of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). *Electronic database/program*. ARS-144. USDA-ARS, Washington DC. (1997).

3. Adan A; Estal PD, Budia F, Gonzalez M and Vinuela E. Laboratory evaluation of the novel naturally derived compound spinosad against *Ceratitis capitata*. *Pestic. Sci*. 48 (3): 261-268 (1996).

4. Peck SL, McQuate, GT. Field tests of environmentally friendly malathion replacements to suppress wild Mediterranean fruit fly (Diptera: Tephritidae) populations. *J. Econ. Entomol* 93 (2): 280-289 (2000).

5. Burns RE, Harris DL, Moreno DS, and Eger JE. Efficacy of spinosad bait sprays to control Mediterranean and Caribbean fruit flies (Diptera: Tephritidae) in commercial citrus in Florida. *Florida Entomol*. 84(4): 672-678 (2001).

6. Knipling EF. Possibilities of insect control or eradication through the use of sexually sterile males. *J. Econ. Entomol*. 48 (4): 459-462 (1955).

7. De Murtas ID, Cirio U, Guerrieri G and Enkerlin D. An experiment to control the Mediterranean fruit fly on the island of Procida by the sterile insect technique. In *IAEA [ed.], Sterile Male Technique for Control of Fruit Flies*. IAEA, Vienna. STI/PUB/276. Pp.:59-70 (1970).

8. Mellado L, Nadel DJ, Arroyo M, and Jiménez A. Mediterranean fruit fly suppression experiment on the Spanish mainland in 1969. In IAEA [ed.], *Sterile Male Technique for Control of Fruit Flies*. IAEA, Vienna. STI/PUB/276. Pp.:91-92 (1970).

9. Heath RR, Epsky ND, Dueben BD, Rizzo J, Jeronimo F. Adding methyl substituted ammonia derivatives to food based synthetic attractant on capture of the Mediterranean and Mexican fruit flies (Diptera: Tephritidae). *J. Econ. Entomol*. 90 (6): 1584-1589 (1997).

10. Epsky ND, Hendrichs J, Katsoyannos BI, Vasquez LA, Ros JP, Zumreoglu A, Pereira R, Bakri A, Seewooruthun SI and Heath RR. Field evaluation of female targeted trapping systems for *Ceratitis capitata* (Diptera: Tephritidae) in seven countries. *J. Econ. Entomol* 92 (1): 156-164 (1999).

11. Katsoyannos BI, Heath RR, Papadopoulos NT, Epsky ND, Hendrichs J. Field evaluation of Mediterranean fruit fly (Diptera: Tephritidae) female selective attractants for use in monitoring programs. *J. Econ. Entomol*. 92 (3): 583-589 (1999).

12. Wharton, RA. Biological control of fruit infesting Tephritidae. *Fruit flies of economic importance 87 proceedings of the CEC/IOBC* International Symposium, Rome 7-10, April 1987 Edited by R Cavalloro. p: 323 332 (1989).

13. Mosson, HJ, Short, JE, Schenker, R, Edwards, JP. The effects of the insect growth regulator lufenuron on Oriental cockroach, Blatta orientalis, and German cockroach, Blattella germanica, populations in simulated domestic environments. *Pestic. Sci*. 45 (3) p: 237-246. (1995)

14. Sechser, B. The fitness of some insecticides for integrated fruit production. *Acta Hortic*. v. (422) p. 295-299. (1996)

15. Katsoyannos BI, Papadopoulos NT, Heath RR, Hendrichs J, Kouloussis NA.. Evaluation of synthetic food-based attractants for female Mediterranean fruit flies (Dipt.: Tephritidae) in McPhail type traps. *Journal of Applied Entomology*. 123 (10): 607-612. (1999)

16. Casana-Giner V, Gangia-Balaguer A, Mengod-Puerta C, Primo-Millo, J and Primo-Yufera, E. Insect growth regulators as chemosterilants for *Ceratitis capitata* (Diptera: Tephritidae). *J. Econ. Entomol*. 92 (2): 303-308 (1999).

Fig 1. Turis trial field map

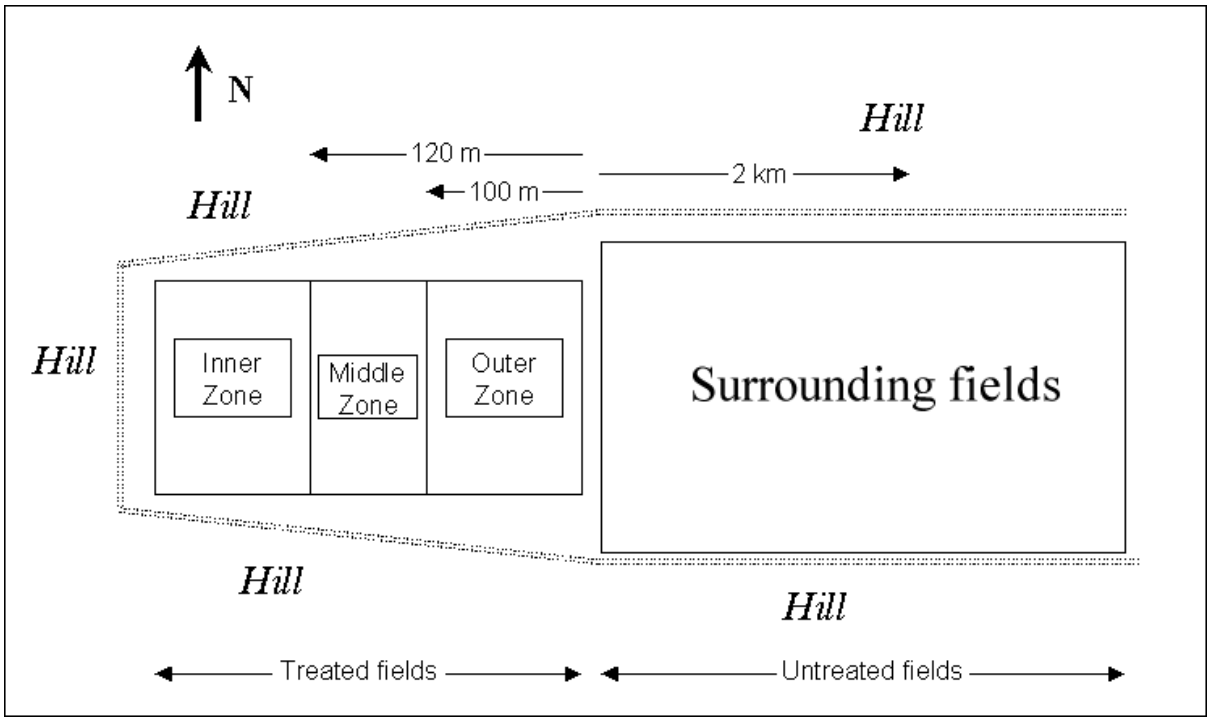

Fig 2. Denia trial field map

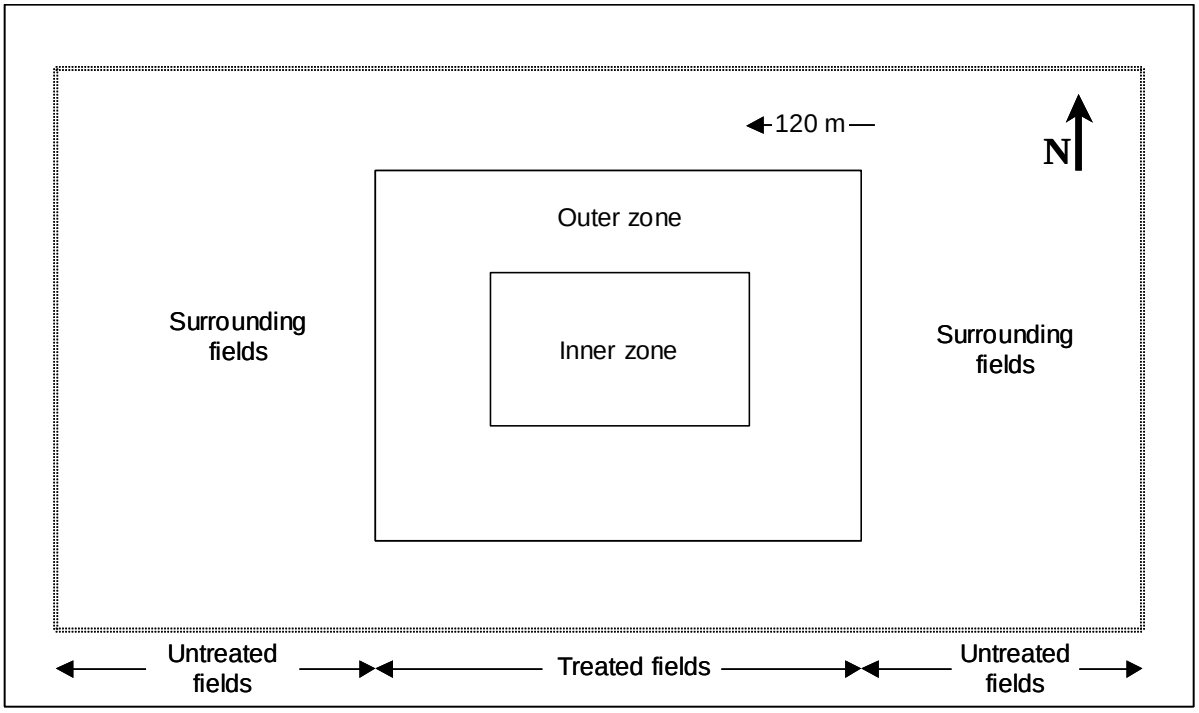

Fig. 3. Medfly captures in treated and untreated fields in "Trial field 1".

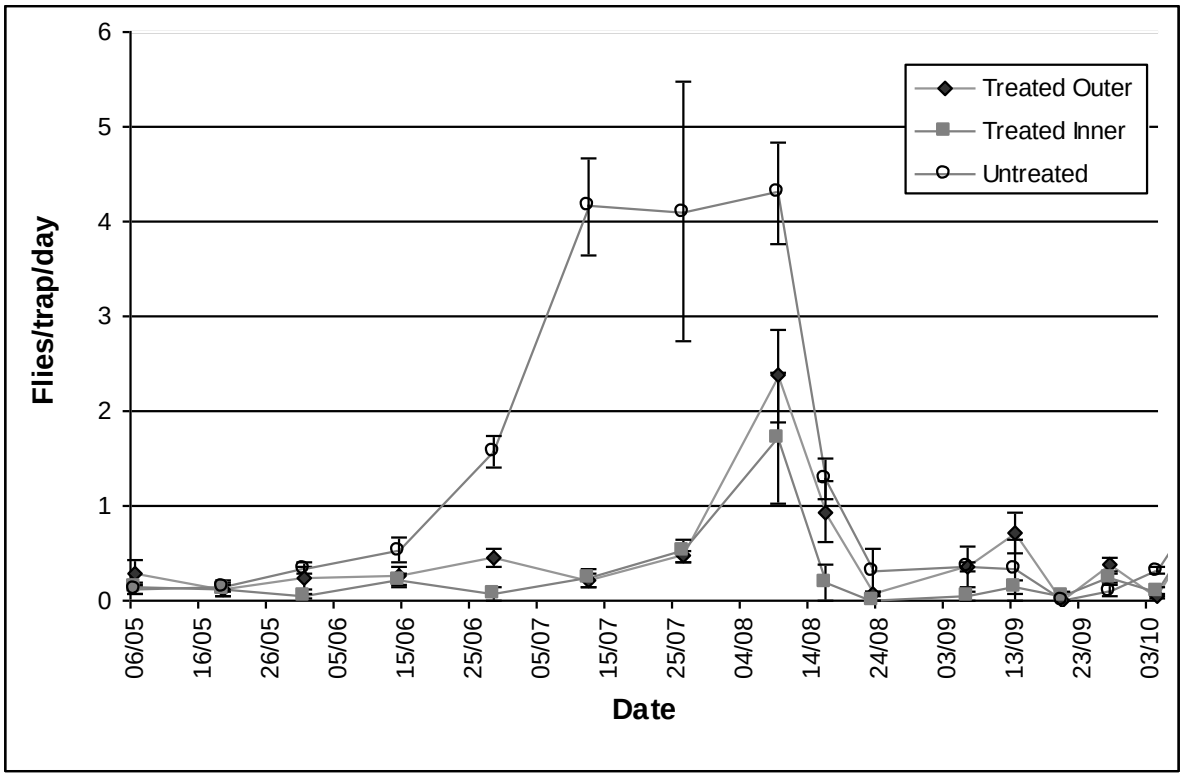

Fig. 4. Medfly captures in treated and untreated fields in "Trial field 2".

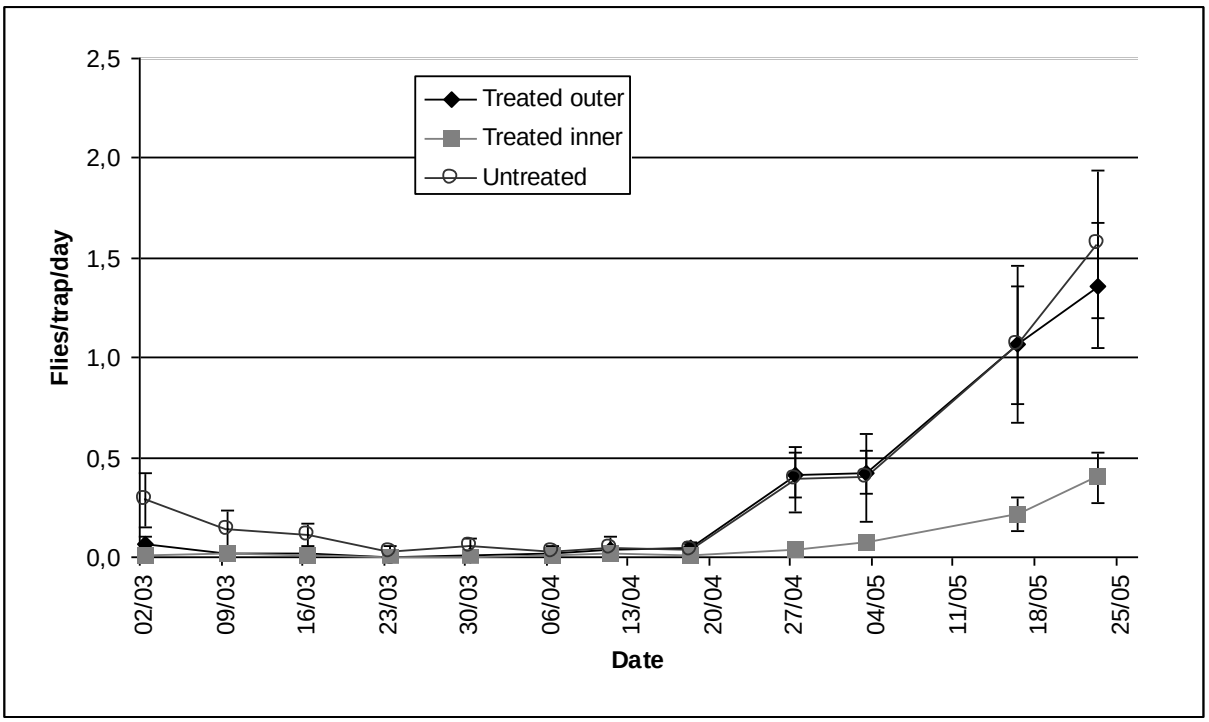

Table 1

Average daily captures (mean±SE) per zone and period in “Trial Field 1”.

Zone	Mean (flies/trap/day) (± SE)		
	From 4-May to 14-Jun	From 15-Jun to 31-August	From 1-Sept to 15-October
Treated outer zone	0.23 (±0.04)a	0.690 (±0.29)a	0.40 (±0.15)a
Treated inner zone	0.13 (±0.03)a	0.40 (±0.23)b	0.32 (±0.15)a
Untreated	0.28 (±0.09)a	2.3 (±0.69)c	0.37 (±0.16)a

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Table 2

Average daily captures (mean±SE) per zone and period in "Trial Field 2".

Zone	Mean (flies/trap/day) (± SE)	
	From 3-March to 14-April	From 15-April to 4-Jun
Treated outer zone	0.02 (±0.00)a	0.56 (±0.22)a
Treated inner zone	0.01 (±0.00)a	0.13 (±0.06)b
Untreated	0.11 (±0.02)b	0.58 (±0.25)a

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Table 3

Eggs hatching percentage from lufenuron-bait spray fed females versus normal bait fed females depending on bait age. (Percentage±SE).

	Bait spray age (Days)		
	0	7	14
Untreated	94.21 (± 3.91)a	98.36 (± 1.12)a	85.56 (± 3.39)a
Treated	6.65 (± 0.88)b	54.48 (± 2.73)b	67.34 (± 6.12)b

Table 4

Eggs hatching percentage from lufenuron-gel fed females versus gel without lufenuron fed females (± SE)

	Bait gel age (Days)					
	0	7	15	30	60	90
Lufenuron bait	5.1(±2.4)a	17.1(±5.5)a	23.8(±8.4)a	41.4(±9.2)a	76.6(±10.5)a	69.5(±9.4) a
Bait	98.7(±1.3)b	98.1(±1.4)b	96.7(±1.7)b	97.6(±1.1)b	88.7(±6.9)b	87.3±(10.3)b

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Table 5

Comparison of percentage of sting fruit and percentage of fruit with larvae development in two lufenuron sprayed zones.

Treatment	Zone	N	Percentage (%) (± SE)	
			% stung fruit	% (larvae develop./stung fruit)
Spray	Outer	17	0.99 (±0.22)a	61.11 (±7.20)a
	Inner	10	0.33 (±0.08)b	38.46 (±6.74)b
Gel	Outer	6	99.67 (± 0.33)a	76.14 (±9.32)a
	Inner	6	35.25 (±1.75)b	39.16 (±7.63)b

Figure legends

Figure 3

Treated outer: Area treated with lufenuron located less than 120 m away from untreated zones.

Treated outer: Area treated with lufenuron located more than 120 m away from untreated zones.

Figure 4

Treated outer: Area treated with lufenuron located less than 120 m away from untreated zones.

Treated outer: Area treated with lufenuron located more than 120 m away from untreated zones.

Table 1

Values of a period with the same letter within a column are not significantly different in paired data t-student test ($P \leq 0.05$).

Table 2

Values of a period with the same letter within a column are not significantly different in paired data t-student test ($P \leq 0.05$).

Table 3

Values of a period with the same letter within a column are not significantly different in t-student test ($P \leq 0.05$). Data were subjected arsine x transformation for analysis; untransformed data are presented.

Values are calculated as the mean of nine percentages, corresponding to three replications of 3 samples each. 50 eggs are tested per sample

Table 4

Values of a period with the same letter within a column are not significantly different in t-student test (P≤0.05). Data were subjected arsine x transformation for analysis; untransformed data are presented.

Values are calculated as the mean of nine percentages, corresponding to three replications of 3 samples each. 50 eggs are tested per sample

Table 5

Values with the same letter within a column are not significantly different in ANOVA test (P≤0.05)

Data were subjected arsine x transformation for analysis; untransformed data are presented.

Outer zone: trees in the edge between outer zone and untreated fields

Inner zone: located more than 120 m. away from untreated fields

N: number of inspected trees. % of stung fruit is calculated as the mean of percentages of N trees