Mediterranean Fruit Fly Supression Using Chemosterilants for Area-Wide Integrated Pest Management .

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Running title: Ceratitis capitata control using chemosterilants

Abstract

BACKGROUND: Chemosterilization technique has been demonstrated to reduce the population and fruit damage of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) in citrus orchards. Field trials showed efficacy by reducing the fruit fly population, that was progressively achieved by continuous application of lufenuron to several generations. Different authors have suggested that field trials should be carried out in isolated or wide areas in order to reduce fruit fly intrusion and obtain best results. In this way, a wide area trial over 3,600 hectares is under investigation in Valencia (Spain) since 2002 to validate the chemosterilization technique against the fruit fly. The whole area was treated with 24 traps per ha, using more than 86,000 traps in the field trial.

RESULTS: A continuous decrease of fruit fly population was observed along the four years under trial. Moreover, results show a significant reduction of persimmon damage in the chemosterilant treatment area compared with malathion aerial treated area. In the case of citrus damage, no significant differences were obtained between malathion and chemosterilant treatments.

CONCLUSION: The chemosterilant method reduces Mediterranean fruit fly populations, therefore it is a candidate treatment to replace aerial treatments with insecticides in order to suppress Mediterranean fruit fly populations. In addition, the efficacy of chemosterilant treatment increases year after year. The possibility of using this technique combined with other control methods is discussed.

Keywords: *Ceratitis capitata*, chemosterilization, lufenuron, fruit fly, sterile, trap

1 **INTRODUCTION**

New control techniques for *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) are currently being studied and developed in order to substitute organophosphate pesticide applications. Insecticides like malathion, fenthion or trichlorphon are not included in the EU Directive 91/414 and their use in the European community has been prohibited. Besides, a fruit fly resistance to malathion has been reported in Spain [1]. For this reason, this organophosphate is being replaced by other more environmentally friendly products like spinosad. However, spinosad formulations with bait (Spintor®) have only demonstrated an efficacy equal to that of malathion [2]. Moreover some foliage damage [3] and citrus fruit scars (Alfaro F., pers. comm.) have been described when Spintor® was applied in spots. Actually, scars appeared in the point where the bait spot touched the fruit. For this reason Spintor® cebo is currently applied to the top of the trees, and in this way, a reduction of foliage damage and green spot in fruit is achieved. Nowadays, there are more than 50,000 ha of citrus in Spain that are being treated with baited traps or a "lure and kill" method as a result of no alternative environmentally friendly available control methods. These techniques are applied in order to control isolated hosts like figs or in commercial orchards to reduce fruit fly population since 2 months before harvesting. The most common "lure and kill" trap is the M3 from Biagro SL (Valencia, Spain), but new developments are currently being tested in Spain like Magnet Med® from Agrisense Ltd (Pontypridd, UK) or EPALure&kill® from EPA SL (Carlet, Spain). All these devices are being tested in citrus, stone fruit, persimmon and apples in Mediterranean region of Spain like Valencia, Gerona, Huelva, Murcia and Balearic Islands. At the present time "lure and kill" devices are less used than traps for mass trapping. In fact, "lure and kill" treated surface only represents between 5 and 10% of mass trapping surface in Spain, but its use increases year after year.

There are some examples of insect growth regulators (IGRs) used for chemical sterilization. In Diptera, pyriproxifen [4] and triflumuron [5] have been effective to sterilize tse-tse flies *Glossina sp.*, while cyromazine, diflubenzuron and pyriproxyfen showed their activity against *Musca domestica* [6]. The effect of some IGR on *Ceratitis capitata* fecundity has been previously described: diflubenzuron added to the diet at 0.3% w/w reduced fecundity of adults [7] and also Budia and Viñuela also described that continuous feeding of cyromazine affected fecundity, fertility and larval development of the progeny [8]. In the case of pyriproxyfen, it has been proved to control whiteflies in greenhouses [9] or other Homoptera like aphids [10]. More recently, the non IGR substance pymetrozine (pyridine azomethine) demonstrated a detriment of egg hatching by 59.3% compared with controls, but this percentage could be increased with higher concentrations and exposing time [11].

In a reported study, the IGR lufenuron showed high activity in reducing egg hatch in *C. capitata*. When females ingested a bait containing 0.1% lufenuron, the hatching of eggs subsequently laid was prevented. Moreover, in laboratory experiments, females that mated with lufenuron treated males laid non-viable eggs [12]. Chemosterilization does not only affect those fruit flies that ingest the bait: these flies become vector sterilizing insects, reaching a higher percentage of population than other methods like mass trapping. In this method a caught male supposes a reduction of just one male in the whole population. By contrast, in chemosterilization a male that ingests lufenuron implies that several females can become sterilized.

Chemosterilization technique demonstrated the reduction of the Mediterranean fruit fly population in field trials, as well as the decrease of fruit damage in citrus orchards [13], including an 80 has trial during 4 years in an isolated valley [14]. These field trials showed efficacy by reducing the fruit fly population, whereas continuous application of lufenuron to several generations produced an improved control. This previous work showed that best results with

chemosterilant treatment are obtained when field trials are carried out in isolated or wide areas because it allows a reduction of fruit fly intrusion. The immigration of pests into a treated area prevents their effective suppression or eradication [15]. In the case of *Ceratitis capitata,* this issue is of particular relevance due to the high mobility of fruit flies. In order to reach an area-wide integrated pest management, trials should be carry out over large or very isolated areas and treat all the insect population of this area during a long-term planned program [16].

In this research, the lufenuron traps were tested in a wide area aimed at obtaining representative results to validate this technique as a *C. capitata* preventive control method for area-wide integrated pest management. For this purpose we have compared the efficacy of chemosterilant technique versus malathion aerial treatments, using fruit fly populations reduction and fruit damage assessment as efficacy indexes.

2 MATERIALS AND METHODS

2.1 Field trials

Trials were carried out in a 3,611 ha extension, located between Carlet and Alcudia in Valencia, Spain. This area is representative of Valencian agriculture, because it is characterised by a great number of small plots between 0.2 and 5 has extension with different species and varieties. Most orchards are sweet oranges of Navel group, *Citrus sinensis* (L.) Osbeck, mandarins *Citrus reticulata* Blanco (mainly cultivars "Satsuma", "Marisol" and "Clemennules"), persimmon *Diospyros kaki* L. as well as early varieties of stone fruits like peach *Prunus persicae* L., apricot *Prunus armeniaca* L. and plum *Prunus domestica* L. (referred hereafter as Prunus). Citrus fruits ripe between September and April depending on varieties, stone fruit between April and June and persimmon between September and November. This means that we are working in the worst scenario for Mediterranean fruit fly control because this insect can find ripen hosts during almost the whole year.

The trial field was divided in three areas (Figure 1). The lufenuron inner area was approximately a rectangle of 3.6 km × 4.5 km (1,650 ha) cultivated with citrus (1,082 ha), prunus (427 ha) and persimmon (150 ha). The lufenuron outer area was surrounding the inner area, 16 km long and 1.3 km wide, resulting a surface of 1,961 ha (1,173 citrus, 566 prunus and 214 persimmon). A dispersion model was applied to this trial, revealing that *C. capitata* intrusion was minimized by leaving an isolation area of 1300 meters wide (unpublished results). The chemosterilant treatment area of 3,611 ha was surrounded at the west side by mountains without hosts of the fruit fly and at north, east and south side by a buffer area treated with malathion in order to prevent pest intrusion. The check area is an extension of 400 ha (280 citrus, 65 prunus and 55 persimmon) located 2 km away from the lufenuron treated area. In the chemosterilant trial field, 62% of the area were citrus, 28% stone fruit and 10% persimmon.

2.2 Chemosterilant treatment

For the chemosterilant treatment a Chemosterilant Trap (CT) formed by: a SEVEP trap, a proteinaceous phagostimulant gel and three attractant dispensers was used. The SEVEP trap consisted in a yellow cylindrical trap, bottom opened with a 9 cm diameter dish with the bait gel at the bottom. The trap cover and the plate with the gel were joined by a cylindrical tube that contains the attractants. These attractants were released by small slots placed at the bottom of the cylinder, very close to the gel. The cover protects the gel and attractants from the rain. This design allows flies to get into the trap, ingest the gel with lufenuron and exit. The gel contained lufenuron a.i. at 30 g/l. For this trial lufenuron technical grade 99.4% purity from Syngenta Crop Protection AG (Basel, Switzerland) was used due to low purity (5%) of commercial formulations and the deterrent action of higher concentrations of solvents. The attractants are released by three types of mesoporous dispensers [14]. For males attraction we used a trimedlure (TML) dispenser (1.8 g of TML) with a life span of more than 6 months [15]. For female attraction we used two dispensers: a N-methylpyrrolidine dispenser (0.5 g of methylpyrrolidine) and an ammonium acetate dispenser (2 g), all supplied by Ecología y Protección Agrícola SL (Valencia, Spain). Both female dispensers remain active in field during more than 4 months which covers the main period of the *C. capitata* season from June to October [16]. The activity of female attractants decreased in October. Nevertheless, although the protein bait gel resulted less active, it remained in field as female attractant and phagostimulant during all the year.

Treatments were made by placing in the chemosterilant area 24 SEVEP traps per hectare which were hung on the south-east side of the trees, 1.5 metres above ground. Approximately one trap per 15-20 trees was hung in this way. The bait remained in the field inside the trap during the whole season.

The treatment began on May 2002 and the traps remained in field until next year. Every year, gel with lufenuron and dispensers were replaced before the first annual fruit fly population outbreak (between 15 April and 15 May). In the third year, May 2004, all the trap devices including the trap covers were replaced. Bait and lures were only replaced in May 2005.

2.3 Check area treatments

All the surface surrounding the chemosterilization area, including the check area, was treated with malathion by aerial spraying of 8 litre/ha with 7.5 g of malathion per litre, Malafin® 50% from Agrodan SA (Valencia, Spain) and 12 g of protein bait per litre, Buminal® from Bayer CropScience AG (Andernach, Germany). The airplane stripe spray was 20 meters wide, leaving 50 meters untreated between treated rows. The number of sprays was based on thresholds determined by captures of adults in traps and fruit ripen status. When fruit was receptive to egg laying and adult captures went over 0.5-1 fly per trap and day an aerial bait spray was made. For this reason, a different number of aerial treatments was done every year: eight in 2002 (17th and 28th of June, 18th and 30th of July, 21st of August, 5th of September, 5th of October and 5th of November), six treatments in 2003 (18th of June, 15th and 30th of September, 8th and 22nd of October and 5th of November), four treatments in 2004 (30th of August, 13th , 22nd and 30th of September) and six treatments in 2005 (1st and 21st of September, 6th, 19th and 28th of October and 8th of November).

The chemosterilant treatment is intended for preventive control in wide areas, therefore it should remain in field during the entire year. By contrast, chemical treatments are curative and they can be applied in small fields, hence they are applied as many times as weeks with fruit fly presence and receptive fruit. Ordinary treatments in Valencia against *C. capitata* include bait spraying every 5 days with malathion or lambda-cyhalothrin and every 7-10 days with spinosad since fruit starts ripening one month before harvest. This means within 4 and 8 treatments depending

on harvesting using 50-150 ml per tree with 0.3-1% of insecticide (malathion or lambda-cyhalothrin) and 0.5-1% of hydrolysed protein, or 1-1.5 litre of Spintor® per hectare. If harvesting is delayed, total cover sprays with malathion are necessary to protect the fruit, using 500 ml per tree with 0.5-1% of malathion. Malathion is the pesticide most commonly applied for Mediterranean fruit fly control all over the world including Spain. Although malathion was still authorized during this trial, currently malathion has been excluded from the annex I of directive 91/414 EEC and its use has been prohibited since December 2008. However spinosad and lambda-cyhalothrin are included in the Anex I and can be applied in the EU.

2.4 Fruit fly population monitoring

Mediterranean fruit fly population was monitored by means of 180 traps in the 3611 hectares treated with lufenuron and 20 traps in the check area (1 trap/20 hectares). All traps were Tephri-trap® from Utiplas SL (Madrid, Spain) which contained one Aralure® plug of TML from Agrisense BCS Ltd. (Pontypridd, UK) and a dichlorvos (DDVP) strip from Econex SL (Murcia, Spain). Each monitoring trap was placed 450 m apart from one another in a different plot. Thus, 180 plots were monitored in the lufenuron treated area (108 citrus, 27 persimmon and 45 -prunus plots) as well as 20 plots in the check area (14 citrus, 3 persimmon and 3 prunus plots), each one with a Tephri-trap®.

During all the trial, traps were inspected weekly from April to December. Since fruit fly population decreased from January to March, just the pest population during these winter months in the first two years was recorded. Aralure® dispensers and DDVP strips from the monitoring traps were replaced every two months. DDVP has been excluded from Annex I of the 91/414 EU Directive from December 2007 (2007/387 EC), although an essential use has been accepted in Spain during 2008 for mass trapping and monitoring traps as a period of grace granted by Member States in accordance with the provisions of Article 4(6) of Directive 91/414/EEC.

Therefore no more DDVP strips can be used for this purpose and other insecticides are in study to replace the DDVP.

2.5 Analysis of fruit fly population

Figure 2 shows the weekly evolution of fruit fly catches along the four years of the trial. Data in the first weeks were not considered for the statistical analysis because practically they are null, as reflected in Figure 2. Two peaks of population can be observed for each year, a first peak in July and another in October. Two different approaches can be applied for the data analysis: a generalized linear model or non-linear regression. Taking into account that the evolution of pest population versus time is clearly non-linear, to use a non-linear regression model was decided. Moreover the coefficients can be interpreted more easily than in the case of a generalized linear model.

Given that peaks are symmetrical, we tried a bimodal Gaussian model (equation 1) with six parameters. Three of them provide information about peak 1 (m_1, t_{p1}, w_1), while m_2, t_{p2} and w_2 account for peak 2.

$$\log_{10}(1+FTD) = m_1 \cdot \exp\left[-\left(\frac{t-t_{p1}}{w_1}\right)^2\right] + m_2 \cdot \exp\left[-\left(\frac{t-t_{p2}}{w_2}\right)^2\right] \qquad \text{(eq. 1)}$$

The dependent variable is a logarithm transformation of the number of fly catches per trap and day (FTD). This type of transformation was used because data are not normally distributed. The independent variable, t, indicates the week of the year at which the catch data was collected, and it ranges from 1 to 52.

The m_i is a parameter related to the height of peak i, while t_{pi} indicates the week at which the dependent variable reaches the relative maximum. The parameter w_i is proportional to the width of peak i. But this parameters interpretation is false if both peaks are very close to each other, which occurred in 2005. Actually, if $t=t_{p1}$, the dependent variable is equal to m_1 only if the

second addend of equation 1 is negligible. Thus, m_1 can be interpreted approximately as the maximum value reached by the dependent variable, and m_2 as the second relative maximum.

For each year, different models were obtained depending on the selected data: (i) all data, (ii) data from the chemosterilant inner area treated with lufenuron, (iii) data from the lufenuron outer area, and (iv) data from the control area treated with malathion. The optimisation tool Solver of Excel was used in order to determine the values of the 6 parameters that achieve the best fit of equation 1 to the observed data (Figure 2), according to the least-squares criterion. For verification purposes, it was checked that the same results were obtained using the nonlinear regression option of Statgraphics plus 5.1 (Statistical Graphics Corp., Herndon, VA, USA).

In order to study the differences of fruit fly population between citrus, persimmon and prunus orchards, four additional models were fitted for each type of cultivation: (i) selecting all data from the three areas, (ii) using data from the lufenuron inner area, (iii) lufenuron outer area and (iv) malathion treated area. Each model was fitted using the weekly average data collected during the years 2002 to 2004. Data from 2005 were disregarded in this case because the population followed a different pattern, as discussed below.

Efficacy of lufenuron versus malathion treatments was calculated for every week as the percentage of the fruit fly population reduced, according to the Abbott formula [20] as follows:

$$Efficacy = \left(\frac{FTDmal - FTDluf}{FTDmal} \right) \times 100 \qquad \text{(eq. 2)}$$

FTDmal and FTDluf are the number of fly catches per trap and day in one week in the malathion and lufenuron inner treated fields, respectively. Both parameters were predicted for each year by implementing the fitted regression models (eq. 1) into a spreadsheet of Excel. Then, equation 2 was applied.

2.6 Fruit damage

A second way of measuring the efficacy of the lufenuron treatment was assessing the damage in citrus and persimmon fruits. One sample of collected fruit was taken for each consignment. Fruit sampling was 2% of the incoming fruits when the consignment was more than 50,000 fruits (almost 1000 fruits per sampling) and 5% when the consignment was less than 10,000 fruits (almost 500 fruits per sampling). The samples were visually inspected at the entry of the warehouse and damaged fruit was put aside for detailed inspection. In this inspection, punctured fruit was recorded as fruit fly damage and percentage of fruit damage was calculated. The number of sampled consignments are detailed in Table 3.

In the year 2005, instead of persimmon inspection in the warehouse, a field inspection in persimmon plots was conducted. This inspection was conducted in 32 fields of the lufenuron treated area and in 15 fields of the malathion treated area. In each plot we visually inspected 40 fruits (10 fruits per orientation, North, South, West and East) per tree in 20 trees (i.e., 800 fruits sampled per plot). Inspection was carried out one week before harvesting at the end of October. One corner of the field was randomly selected and, next, we took randomly one tree among the 4 first lines and rows. Once the first tree was selected, the number of trees in the diagonal of the plot was divided by 20 and was subtracted by 1. The resulting value was the number of trees (entire value) we left among sample trees.

Percentage of fruit damage was transformed to arcsin(sqrt(x)) and effect of treatment was studied for each year and type of cultivation with a two-sample comparison t-test. The analysis was carried out using Statgraphics plus 5.1.

3 RESULTS

Figure 2 shows the dynamics of Mediterranean fruit fly population in lufenuron and malathion treated areas during 2002, 2003, 2004 and 2005. The efficacy of lufenuron treatment increased year after year. In the first year of treatment relevant differences of *C. capitata* population between the malathion and the lufenuron treated areas were not observed. However, Figure 2 shows that the population level always remained lower in lufenuron treated areas than in malathion treated ones during the second, third and fourth year. In addition, this population reduction is clearly observed during the months of October, November and December, when citrus and persimmon are ripening and fruit damage can occur. Although the fruit fly population of the malathion-treated areas decreased in the fourth year, lufenuron treated areas achieved a higher reduction. After 4 years of chemosterilant treatment, the fruit fly population in these areas was below 2.5 flies per trap and day at the maximum level.

The results of the nonlinear regression are shown in Table 1. The high values of R^2 indicate that equation 2 fits the weekly data properly in most cases. Values of the regression parameters reflect the differences among the fitted models. The values of t_{p1} and t_{p2} are very similar for the years 2002 and 2004, which indicates a remarkable synchronization of the pest population dynamics. In 2003 there was a lag of about one week. Values of w_1 and w_2 resulted higher in 2003 than in 2002, which indicate that the Gaussian model became wider in 2003. In contrast, peak 2 in 2004 was the narrowest. The parameter FTD_i, indicated in Table 1, is the estimated value of FTD reached at $t=t_{pi}$ according to the fitted equation. The values of FTD_i probably provide the most relevant information contained in Table 1, because they allow a gross comparison of the pest population among the fitted models.

A severe frost occurred in the last week of January 2005 that damaged the citrus crop. As a result, the two peaks of fruit fly population observed in the previous years were not clearly distinguished in 2005 (see Figure 2). Due to this different pattern of pest dynamics, the goodness-of-fit (R^2) was lower than in the rest of the cases (Table 1). Since the model was unable to determine the exact position of the peaks, we fixed t_{p2}=41, which is the average value of the previous years. The results suggest that the FTD evolution in 2005 could be interpreted as two peaks of a similar height (i.e. $FTD_1 \approx FTD_2$) merged together due to a delay of peak 1 in about 2 weeks. Actually, the value of $t_{p1\text{-}2}$ is clearly lower in 2005.

The nonlinear analysis carried out does not indicate if the differences between the fitted curves are statistically significant. For that purpose, we conducted an ANOVA with factors *period* and *area*. The latter has three levels: lufenuron inner area, lufenuron outer area and control. Data corresponding to t<22 and t>49 were disregarded for this analysis because the pest population was very small. One outlier was identified and eliminated. The log-transformation of FTD was used as dependent variable.

The factor *period* was obtained as follows: given that two peaks of fruit fly population were observed in most years, the value of t at which the dependent variable reaches a relative minimum that was called t_{min} was calculated. From 2002 to 2004, $t_{min}\approx 36$ (Table 1). Thus, weeks 22 to 35 were regarded as first period, while weeks 36 to 49 were assigned to second period. In 2005, a single period was considered as the two peaks were not clearly distinguished.

The resulting two-way ANOVA revealed that both factors as well as the interaction were statistically significant (P<0.0001). The interaction plot (Figure 3) indicates the average population in each period. The LSD (least significant differences) intervals, calculated at a confidence level of 95%, indicate that the fruit fly population was significantly lower in the chemosterilant area except in the first period of 2002, and this difference is particularly relevant

in the first peak. FTD_1 resulted higher than FTD_2 in all years, and a clear trend is observed. The chemosterilant treatment achieved a progressive reduction of the fruit fly population along the years. But this was not the case with the malathion treatment, because no reduction of FTD_1 was clearly observed. Thus, the chemosterilant treatment was more effective than the malathion aerial applications since the first year of trial. The slight increase of FTD_2 in 2005 compared to FTD_2 in 2004 is difficult to explain due to the frost that changed the experimental conditions.

A parallel evolution of pest population corresponding to the lufenuron inner and outer areas is observed, but it is not very clear from Figure 3 if the differences are statistically significant. In order to further investigate this issue, the ANOVA was repeated after discarding data from the malathion treatment. Factor area was statistically significant (P=0.002), which indicates that the pest population in the lufenuron inner area was significantly lower than in the outer area.

The weekly evolution of the efficacy is showed in Figure 4. Equation 2 becomes indeterminate when the population is near to zero. For this reason only the efficacy between week 20 and 45 was calculated. The efficacy was moderate during the first year of treatment. In 2003, it increased up to nearly 70% as well as 2004, and it became slightly higher than 80% in 2005. Results indicated that, after two years of chemosterilant treatment, the fruit fly population was reduced in average about 70-80% in the lufenuron inner area if compared with the population in the control area treated with malathion.

3.1 Population dynamics according to type of cultivation

Additional models were fitted to study the differences of fruit fly population among citrus, persimmon and prunus orchards. The regression parameters are displayed in Table 2. Attempting to better understand the differences among the models, those corresponding to the inner and malathion areas are depicted in Figure 5. The second peak is nearly absent in the case of prunus. The model explains this different pattern as an earlier second peak which is smaller and wider

than in the other types of cultivation (Table 2). Interestingly, despite the different shape of prunus curves, the coefficient of determination is slightly higher than most R^2 values in Table 1.

Results indicate that the fruit fly population was lower in the lufenuron inner area than in the outer area, and the highest number of catches resulted in the malathion area. For each cultivation, the most different parameter in Table 2 according to type of area is m_i. Conversely, w_i and t_{pi} are rather similar, which indicates that there is a common pattern for each type of cultivation. Regarding the first peak, there is an interesting negative correlation between m_1 and t_{p1}. The highest peak corresponds to prunus, and the maximum is reached about one week earlier than in the case of citrus. Persimmon models present the lowest peak, which is delayed about one week compared with citrus. Values of FTD_i indicate that the fruit fly population was higher in citrus orchards than in persimmon plots, but the second peak was earlier in the latter. The presence of this second peak in persimmon might be related to the availability of hosts, given that this fruit ripens from September to November.

3.2 Fruit damage

Results of fruit damage in citrus and persimmon are shown in Table 3. We can observe that there are no statistically significant differences in citrus damage along the last 3 years of the trial between the fruit from the malathion treated area and from the lufenuron treated area ($P>0.7$). However, this is not the case for persimmon. In the second year of treatments, the percentage of fruit damage in the lufenuron area is not clearly lower than in the malathion area ($F=2.73$; df=1: $P=0.094$). However, in the third ($F=4.77$ df=1 $P=0.031$) and fourth year ($F=5.55$ df=1 $P=0.023$) of lufenuron treatment, persimmon damage differences are statistically significant between malathion and lufenuron treated areas considering a significance level $\alpha=0.05$. This result is consistent with the decrease of fruit fly population along the years of chemosterilant treatment which can be observed in Figure 3. Differences in persimmon damage between field sampling in 2005 and storehouse sampling were not statistically significant ($F=0.77$, df=1 $P=0.3823$).

4. DISCUSSION

The Area-Wide Integrated Pest Management (AW-IPM) is defined as "IPM against an entire pest population within a delimited geographic area, with a minimum size, large enough or protected by a buffer zone so that natural dispersal of the population occurs only within this area" [12]. This definition includes the common thread in all AW-IPM programmes: to control all foci of infestation from which recruits emerge in order to avoid re-establishment of damaging densities of the pest population in areas of concern. It is essential to reach the total pest population in all the control strategies, particularly in wide areas when we look for a pest suppression or eradication [21]. Chemosterilant treatment fits with this requirement as it acts during all the year over the whole population of a wide area. Chemosterilant treatment focuses on the preventive management of pest population because it acts before pest population increases, though this method should be applied during several years for optimal results. The AW-IPM control methods are preventive and require multi-year planning [16]. The results obtained in this research indicate that the chemosterilization effect is cumulative year after year and, therefore, best results will be obtained after successive seasons. Current insecticide treatments with malathion or spinosad are punctual and their effect remains in field during no more than 10 days [22]. However, the effect of lufenuron treatment with CT remained in field all the season (from May to November) under our experimental conditions [14] and it produced a continuous reduction of fruit fly population year after year.

The mechanism of sterility induction of IGRs is to disrupt the development of any instar of the insect by interfering with the endocrine mechanisms [15]. But in order to achieve this effect as an insect control technique we need to develop a target that has to remain active in field during all the year and that has to affect fruit flies as long as possible. Pyriproxifen was demonstrated to sterilize tse-tse flies [4], although the developed target for field application did not remain active in

field for more than fourth months [23]. Triflumuron is another IGR used for chemical sterilization of tse-tse flies, and a 6 months active life span of the targets with triflumuron was achieved [5]. This compound causes the sterility after fly contact with the target and the transmission of sterility from males to females only remained 2 days. CT represents an improvement of this system as it remains active in field during all the season and the transmission of sterility from males to females lasts more than 15 days [12].

Chemosterilant treatment represents a new way to reduce fruit fly population in wide areas as an alternative to insecticide treatments. In addition this treatment has several advantages regarding aerial bait sprays. First of all, chemosterilant treatment is specific for the target pest as it uses certain attractants for fruit flies. By contrast it is well known that bait sprays attract other dipera, specially Drosophilidae[24,] or chalcidoid parasitoids[25]. Moreover, CT does not leave insecticide residues in fruit [26] and it is more safe for applicators. By using the same insect specie to fight against itself, the introduction of exotic agents or new genetic material which occurs with other biological control methods is avoided [27].

The fruit damage study reported here showed no significant differences in citrus along the trial. It is important to emphasize that we are comparing 4 to 6 malathion aerial treatments each year with CT and no significant differences has been obtained between them. It indicates that both treatments obtained the same efficacy in citrus and therefore that CT is an alternative to current aerial treatments with insecticide. Evaluation of fruit damage over stone fruit was not possible because only very early varieties are cultivated in this area (harvested between April and May) and no important fruit damage was detected in these months before fruit fly outbreak. However a very sensitive crop like persimmon showed less fruit damage in CT fields that in malathion treated fields from the second year of treatments.

Fruit damage minimization is the main objective of any control method in fruit flies. Regarding the viability of chemosterilant method, it is of interest to discuss if its price is competitive. In this

case, a 2% reduction of fruit damage was obtained in the 363 has of persimmon which results in a direct saving of more than 50,000 euros only in 2004. This save does not include indirect costs like rejected shipments or the transport of damaged fruit. Taking into account that the total cost of the chemosterilant treatment for those 363 has was about 43,560 euros in 2004, we conclude that the treatment is economically profitable.

The present work demonstrates that CT is effective to reduce fruit fly population measured as fly catches and to reduce fruit damage in persimmon. For this reason, a commercial development of these chemosterilant traps has been recently introduced into the market by Syngenta in some Mediterranean countries under the name of Adress®. Chemosterilization provides one advantage over mass trapping.; although attractants activity is maintained the main part of the season, the chemosterilization traps remain active much longer because of the attractant effect of protein baits [14]. Keeping a chemosterilization activity along the year, fruit fly population recovery is avoided year after year, resulting in an accumulative reduction of fruit fly population as it is shown in Figures 3 and 4.

It would be possible to reduce the wild population of Mediterranean fruit fly with the combination of SIT and Chemosterilization, that is, with chemosterilization for two or three years followed by application of SIT in a more efficient and economic way. This trial is being conducted in Valencia in the same area where chemosterilization has been applied during the last 7 years.

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Table 1. Results of the non-linear regression analysis to compare a chemosterilant with a malathion aerial treatment.

		Coefficients of the model (eq. 1)[a]												
		Peak 1			Peak 2									
Area[b]	**Year**	**m_1**	**w_1**	**t_{p1}**	**m_2**	**w_2**	**t_{p2}**	**R^2 [c]**	**FTD_1[d]**	**FTD_2**	**$date_1$[e]**	**$date_2$**	**t_{min}[f]**	**$t_{p1\text{-}2}$[g]**
all	2002	0.87	3.88	28.5	0.46	6.20	41.8	0.952	6.47	1.86	19/07	20/10	34.9	93.1
inn	2002	0.87	3.99	28.5	0.44	6.03	42.2	0.949	6.53	1.76	19/07	23/10	35.2	95.9
out	2002	0.83	3.73	28.6	0.46	6.31	41.6	0.922	5.91	1.89	20/07	19/10	34.7	91.0
mal	2002	0.95	3.86	28.2	0.57	5.90	40.8	0.909	7.95	2.75	17/07	13/10	34.3	88.2
all	2003	0.82	4.57	26.9	0.21	8.78	40.0	0.955	5.98	0.62	8/07	8/10	35.6	91.7
inn	2003	0.75	4.39	26.8	0.16	10.08	39.4	0.955	5.13	0.46	7/07	3/10	35.8	88.2
out	2003	0.84	4.27	27.4	0.23	8.01	40.5	0.950	6.26	0.71	11/07	11/10	35.2	91.7
mal	2003	1.06	5.22	26.5	0.40	7.22	39.8	0.909	10.98	1.50	5/07	6/10	35.0	93.1
all	2004	0.69	4.21	28.6	0.16	4.96	41.2	0.983	3.86	0.46	19/07	15/10	36.1	88.2
inn	2004	0.60	4.22	28.6	0.13	5.08	41.5	0.985	2.96	0.32	19/07	17/10	36.4	90.3
out	2004	0.64	4.01	28.8	0.18	5.09	40.8	0.960	3.33	0.52	20/07	12/10	35.8	84.0
mal	2004	1.14	4.42	28.4	0.32	4.49	41.0	0.967	12.74	1.11	17/07	14/10	36.0	88.2
all	2005	0.32	5.61	30.8	0.29	7.23	41.0	0.811	1.27	1.00	4/08	15/10	–	71.3
inn	2005	0.25	5.32	30.7	0.25	6.72	41.0	0.793	0.86	0.80	3/08	15/10	–	72.0
out	2005	0.27	5.54	31.1	0.24	8.16	41.0	0.712	1.12	0.77	6/08	15/10	–	69.5
mal	2005	0.69	6.41	30.9	0.55	7.62	41.0	0.825	5.08	3.05	5/08	15/10	–	70.7

FTD=fly catches per trap and day

[a] Equation 1 predicts the logarithm of (1+FTD), being FTD the number of fly catches per trap and day

[b]For each year, 4 models were fitted depending on the FTD data selected: all data collected in the year (all); data from the lufenuron inner area (inn), lufenuron outer area (out), and malathion area (mal).

[c]coefficient of determination.

[d]maximum estimated value of FTD reached at t_{p1}, which is obtained at t=t_{p1} according to the fitted model.

[e]date corresponding to t_{p1} of the year indicated in the second column.

[f]week of the year between t_{p1} and t_{p2} at which the model reaches a relative minimum.

[g]number of days between $date_1$ and $date_2$, which can also be calculated as $7\times(t_{p2}-t_{p1})$.

Table 2. Results of the non-linear regression analysis according to type of cultivation in a comparison test among a chemosterilant and a malathion aerial treatment.

		Coefficients of the model (eq. 1)[a]												
		Peak 1			Peak 2									
Cult[b]	Area[c]	m_1	w_1	t_{p1}	m_2	w_2	t_{p2}	**R^2 [d]**	**FTD_1[e]**	**FTD_2**	**$date_1$[f]**	**$date_2$**	**t_{min}[g]**	**$t_{p1\text{-}2}$[g]**
citr	All	0.76	4.39	28.1	0.346	6.37	42.1	0.980	4.78	1.22	16/07	22/10	35.3	98.0
citr	Inn	0.74	4.18	28.3	0.315	6.29	42.3	0.981	4.54	1.06	18/07	24/10	35.4	98.0
citr	Out	0.67	4.40	28.1	0.354	6.65	42.4	0.944	3.73	1.26	16/07	24/10	35.2	100.1
citr	Mal	0.99	4.95	27.6	0.481	5.96	41.0	0.966	8.96	2.03	13/07	15/10	35.1	93.8
pers	All	0.63	3.51	28.9	0.251	5.94	40.4	0.965	3.31	0.78	22/07	10/10	34.9	80.5
pers	Inn	0.53	3.42	28.7	0.199	5.60	39.0	0.939	2.44	0.58	20/07	1/10	34.5	72.1
pers	Out	0.68	3.58	29.2	0.284	5.59	41.0	0.941	3.83	0.92	24/07	15/10	35.3	82.6
pers	Mal	0.85	3.55	28.6	0.437	5.09	41.3	0.906	6.03	1.73	20/07	17/10	34.7	88.9
prun	All	0.90	4.52	27.2	0.142	8.20	37.1	0.987	7.51	0.41	10/07	17/09	–	69.3
prun	Inn	0.78	4.47	27.0	0.115	9.56	35.7	0.982	5.84	0.36	8/07	6/09	–	60.9
prun	Out	1.03	4.16	27.7	0.213	6.23	38.3	0.984	9.96	0.64	12/07	27/09	–	74.2
prun	Mal	1.42	5.31	27.4	0.171	6.00	38.6	0.975	25.68	0.54	10/07	25/09	–	78.4

FTD=fly catches per trap and day

[a] Equation 1 predicts the logarithm of (1+FTD), being FTD the number of fly catches per trap and day.

[b]Type of cultivation: citrus (citr), persimmon (pers) or prunus (prun).

[c]For each cutivation, 4 models were fitted: data collected in all three areas (all); data from the lufenuron inner area (inn), lufenuron outer area (out), and malathion area (mal). In each model, weekly data were averaged for the years 2002 to 2004.

[d]coefficient of determination.

[e]maximum estimated value of FTD (fly catches per trap and day) reached at t_{p1}, which is obtained at t=t_{p1} according to the fitted model.

[f]date corresponding to t_{p1} of the year indicated in the second column.

[g]week of the year between t_{p1} and t_{p2} at which the model reaches a relative minimum.

[h]number of days between $date_1$ and $date_2$, which can also be calculated as $7\times(t_{p2}-t_{p1})$.

Table 3. Effect of chemosterilant and malathion aerial treatment on fruit damage .

Treatment	% of fruit damage ± SE.											
	2003				2004				2005			
	N	Citrus #	N	Persimmon #	N	Citrus #	N	Persimmon #	N	Citrus #	N	Persimmon θ
Lufenuron	196	0.61±0.21a	166	1.31±0.19a	249	0.12±0.06a	52	2.43±0.55a	35	0.46±0.09a	32	2.01±0.47a
Malathion	122	0.52±0.19a	105	1.61±0.21b*	208	0.14±0.05a	54	4.24±1.10b	125	0.42±0.08a	15	4.14±1.23b

a,b Percentage of fruit damage with the same letter within the same cultivar and year are not significantly different in two-sample comparison t-test ($P \leq 0.05$).

* Show significant differences in two-sample comparison t-test ($P \leq 0.1$).

N: Number of samples

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

Fruit sampling carried out in the storehouse.

θ Fruit sampling carried out in field.

Figure 1. Trial fields diagram

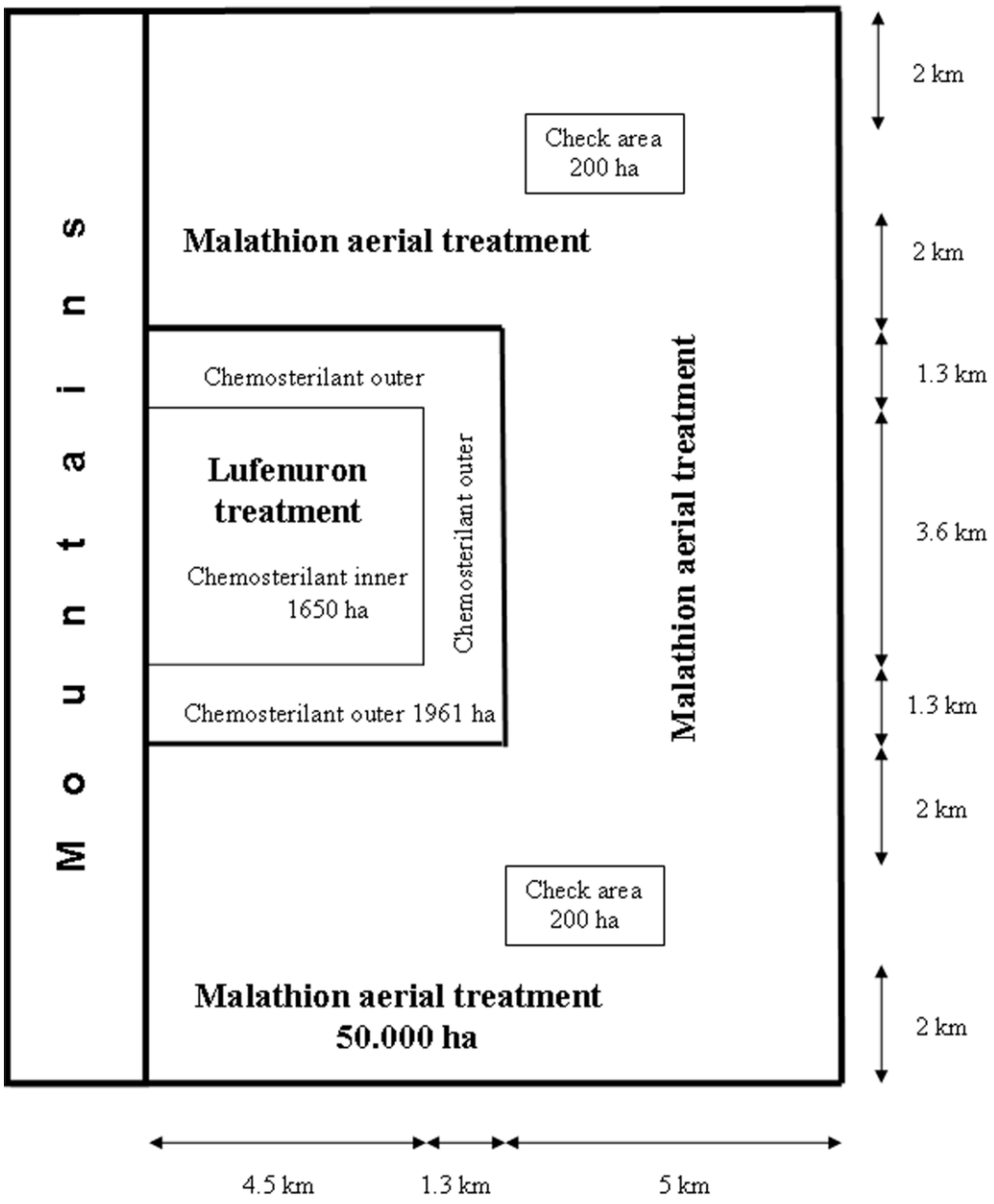
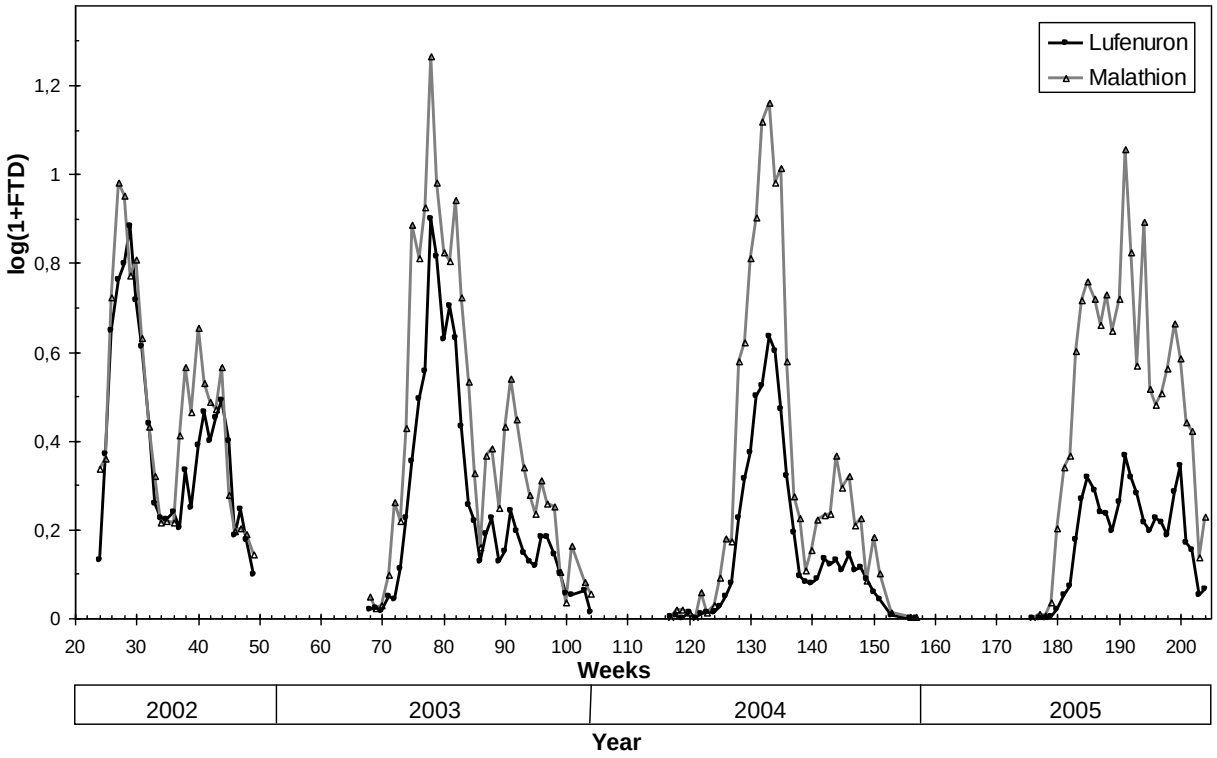
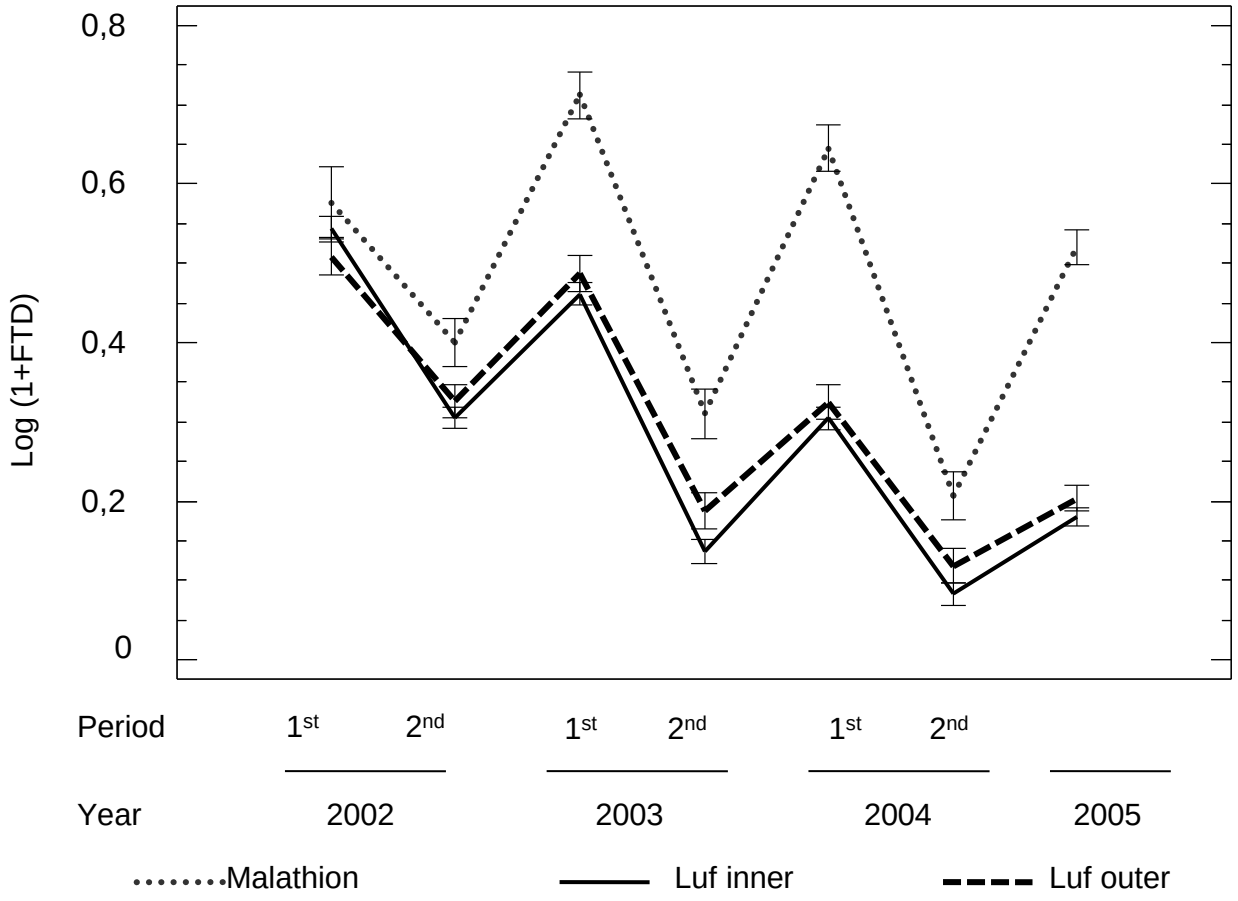


Figure 2. Time series of fruit fly catches per trap and day (FTD) in lufenuron and malathion treated fields from 2002 to 2005.



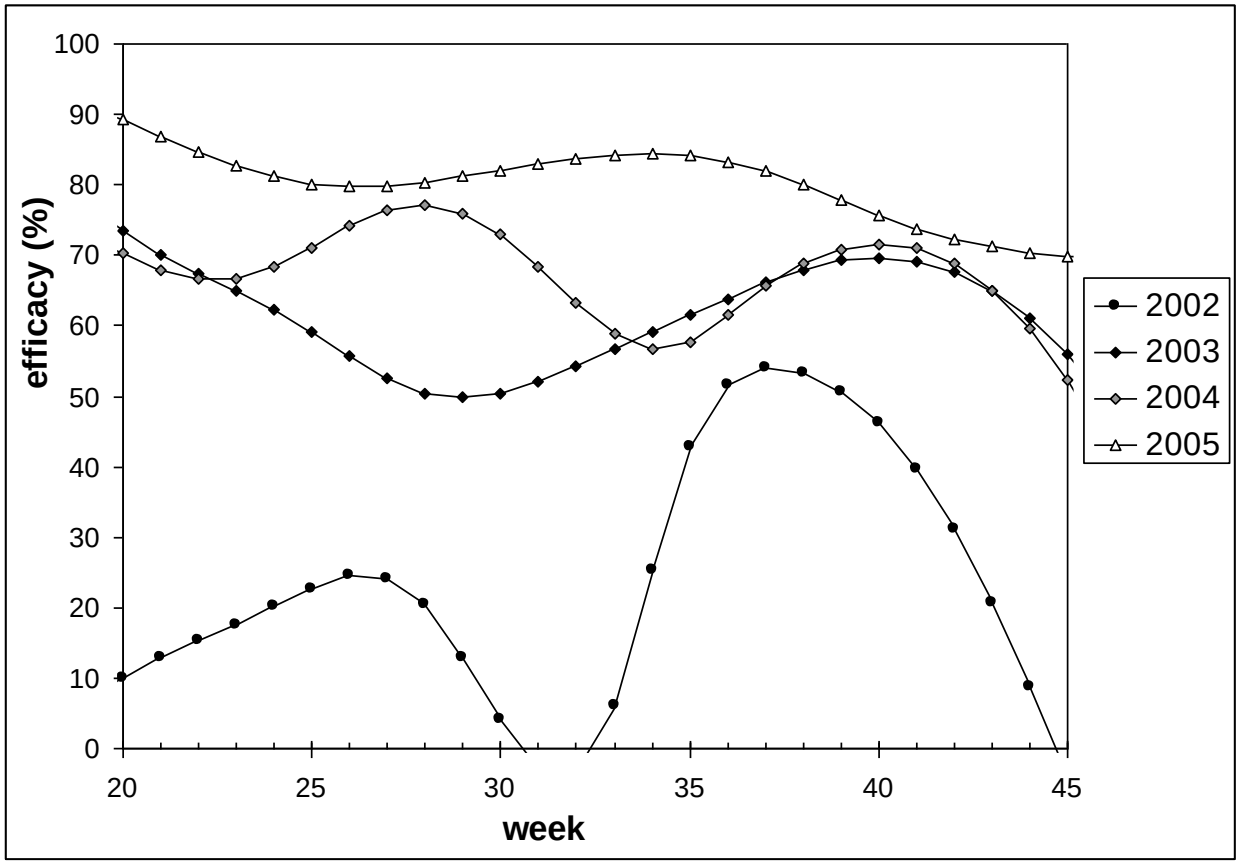
FTD=fly catches per trap and day

Figure 3. Interaction plot and 95% LSD intervals of the ANOVA carried out to study the effect of factors treatment area and period in the number of fly catches per trap and day (FTD).



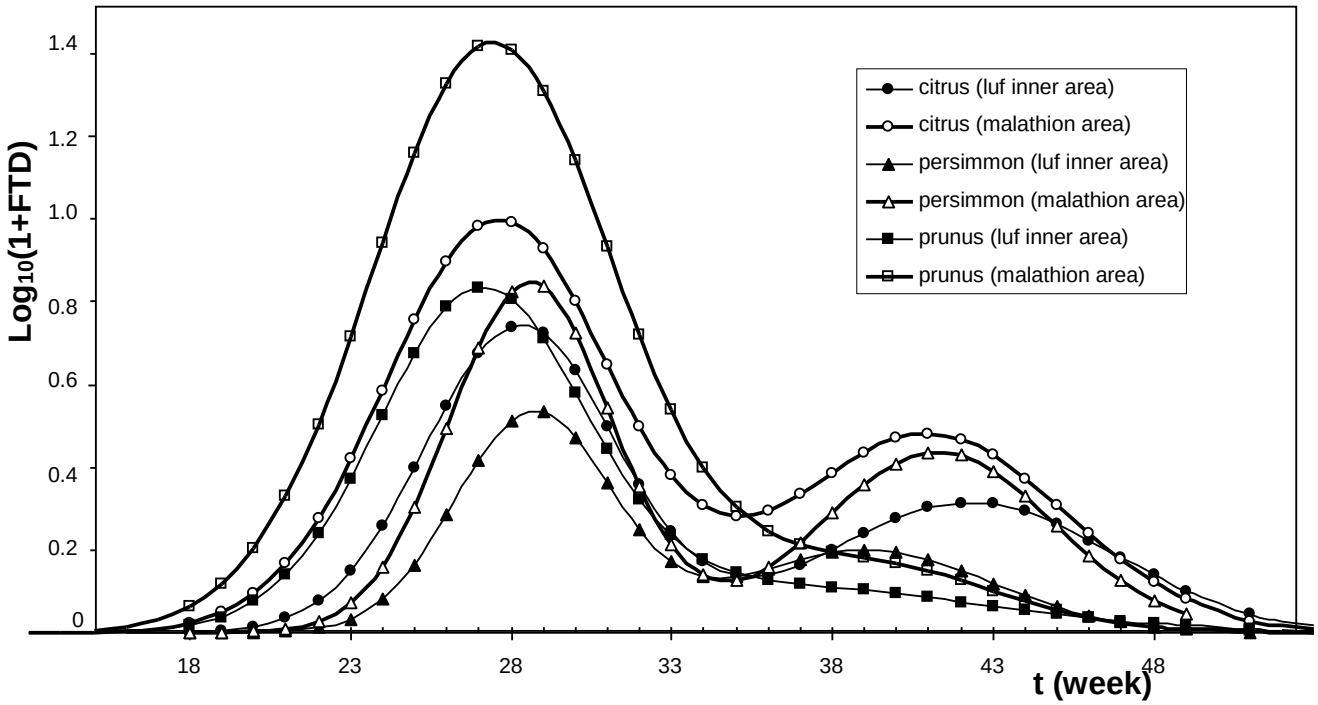
FTD=fly catches per trap and day
For years 2002 to 2004, two periods were considered. The first one accounts for weeks 22 to 35, and the second period, weeks 36 to 49.

Figure 4. Time course of efficacy (equation 1) for the different years



Efficacy was calculated according to equation 2 ((FTDmal-FTDluf)/FTDmal)x100,
FTDmal: number of fly catches per trap and day in the malathion treated area
FTDluf: fly catches per trap and day in lufenuron treated area.
FTD values were estimated for each week according to equation 1, using the coefficients in Table 1.

Figure 5. Predicted dynamics of fruit fly population in citrus, persimmon and prunus orchards. Each curve was obtained by means of equation 1 using the estimated values of the coefficients indicated in Table 2 (weekly data averaged for the years 2002 to 2004).



FTD=fly catches per trap and day