

## Laboratory Evaluation of Natural Pyrethrins, Pymetrozine and Triflumuron as Alternatives to Control *Ceratitis capitata* Adults

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Three insecticides, pyrethrins + piperonyl butoxide (PBO), pymetrozine and triflumuron, were tested as potential alternatives for controlling the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). The compounds were administered to adult flies in the laboratory by different uptake methods at the recommended rates currently used in fruit crops in Spain. Pyrethrins + PBO (80 + 320 ppm a.i.) exhibited a comparable knock-down effect to malathion (1,500 ppm a.i.) in the laboratory, irrespective of the method of application used. After these results were obtained, the number of concentrations tested was increased to carry out a dose-response analysis. Pymetrozine (300 ppm a.i.) and triflumuron (150 ppm a.i.) did not kill adults at the concentrations tested. However, pymetrozine diminished the fecundity, especially when adults were fed the insecticide; egg hatch was decreased by 59.3% compared with controls. Further experiments showed that increased period of ingestion and higher concentrations had a clear effect in reducing both fecundity and fertility. The possible use of pyrethrins + PBO and pymetrozine to reduce populations of *C. capitata* is discussed. **KEY WORDS:** *Ceratitis capitata*; Mediterranean fruit fly; medfly; pymetrozine; natural pyrethrins; piperonyl butoxide; triflumuron; malathion.

### INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), causes serious damage in temperate and subtropical regions worldwide, attacking over 200 varieties of cultivated fruit crops, of which more than 100 are of economic importance (10,12). The fight against this pest is generally carried out by aerial bait spraying of hydrolyzate proteins with organophosphates such as malathion (1,7), which is still considered a leading compound for controlling *C. capitata*. However, public concerns over spraying of these broad-spectrum insecticides is the cause of protests by local inhabitants and ecological groups (5). More environmentally safe methods such as the use of bait sprays with low-toxicity compounds (17) or autosterilization, which uses traps baited with sterilants and specific attractants are currently being studied (9), in addition to the sterile insect technique or pure biological control.

Among the compounds with a better environmental profile to use against *C. capitata* are natural pyrethrins, a mixture of esters obtained from flowers of *Tanacetum*, with insecticidal activity. The natural pyrethrin insecticides have the desirable environmental properties of possessing both low toxicity to mammals and low persistence (18). Pyrethrins can be formulated with piperonyl butoxide (PBO), employed primarily as an insecticide synergist,

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to inhibit certain mechanisms of enzymatic detoxification. Along with that, PBO, at high concentrations, exhibits acaricidal and insecticidal properties. It affects the penetration of insecticides through the insect cuticle, has synergized unidentified resistance mechanisms, and has been described also as affecting development of insects (11).

Triflumuron is a benzoylphenyl urea, registered in Spain to control psyllas and leafminers in peach orchards with a reported effect as a chemosterilant of *C. capitata* at much higher concentrations than those used currently in crops (9). The first benzoylphenyl urea commercialized, diflubenzuron, has already shown a remarkable effect of reducing fecundity when ingested by *C. capitata* (19).

Pymetrozine is a new insecticide, highly active and specific against sucking insect pests (13). It is the only commercial representative of the pyridine azomethines, a new class of insecticides, and it is currently being developed worldwide for control of aphids and whiteflies on, among other crops, citrus and deciduous fruit (14). This compound affects the nerves controlling the salivary pump of some sucking pests and causes irreversible cessation of feeding within a few hours of application, followed by starvation and death (20). Today, triflumuron and pymetrozine are being applied to control different pests in fruit trees, with their potential effect on medfly survival and reproduction not yet known.

Our objective was to evaluate the potential role of the three insecticides in the management of Mediterranean fruit flies. Experiments were conducted to identify the toxicity of the insecticides *via* different uptake routes on adult flies in the laboratory. We characterized the response of the new insecticides in comparison with a reference control: malathion.

## MATERIALS AND METHODS

**Chemicals** Alsystin®25 (25% triflumuron, WP) Bayer Spain, Pelitre Hort® (4% natural pyrethrins + 16% PBO, EC) Q. Massó Spain, and Plenum®25 (25% pymetrozine, WP) Syngenta Spain, were used in the experiments. Malafin®50 (50% malathion, EC) Agrodan Spain was the reference control.

The following maximum field concentrations recommended in Spain were tested: 150 ppm active ingredient (a.i.) triflumuron; 300 ppm a.i. pymetrozine; 80 a.i. natural pyrethrins + 320 a.i. PBO; and 1,500 a.i. malathion. Because of its high toxicity, a broad range of doses was used to determine the LC<sub>50</sub> of natural pyrethrins + PBO and malathion by topical and ingestion treatment at 72 h.

**Insects** *Ceratitis capitata* was routinely reared in our laboratory at 25±2°C, 75±5% r.h. and a photoperiod of 16:8 (L:D). There was no history of insecticidal exposure. Adult flies were fed on a diet consisting of a mixture of autolyzed yeast and sugar (1:4, wt:wt).

**Comparative effects of insecticides on fly survival and reproduction** Fresh dispersions of the compounds in distilled water for the residual and ingestion tests and acetone for topical application tests (8), were prepared prior to the assays. Control groups were supplied with distilled water or acetone. Groups of five newly emerged pairs of flies (♀/♂) were transferred to plastic cages (11 cm diam, 5 cm high) with a hole on the upper side (7.5 cm diam) covered with gauze as described in detail by Budia and Viñuela (6). Food was supplied in small plastic containers (5 ml). Water was offered in glass troughs covered with Parafilm® with a piece of Spontex® wiper. Both containers were fixed at the bottom of the cage with plasticine. Exposure to low temperatures (approx. 4°C) for 3 min was

used for adult handling in order to avoid CO<sub>2</sub> enhancing the effects of neurotoxicants (22). Each experiment consisted of four or five replicates per concentration tested. Mortality was recorded on a daily basis, but only representative data are given.

To study fecundity, eggs were collected and counted daily for a period of 5–7 days from the beginning of oviposition. The mean number of eggs per female and day during 5–7 days was recorded to compare fecundity among different compounds.

Fertility was assessed on the 5<sup>th</sup> day from the flies' emergence; four replicates of 50 eggs per dose and day, chosen at random among all the eggs laid in every dose, were placed on moistened black filter paper in petri dishes. The number of emerged larvae was counted 48 h later.

**Residual treatment** Glass surfaces (11.8×11.8 cm) were treated under a precision spray Potter Tower at the concentration tested. One ml of every test solution at a pressure of 55 kPa was sprayed over each glass surface to obtain a homogeneous deposit of 1.54±0.12 mg fluid per cm<sup>2</sup>. Treated glass surfaces were stored to obtain the different residues in the climate-controlled chambers where the rearing and experiments were carried out: 25±2°C; 75±5% r.h.; photoperiod of 16:8 (L:D) and under illumination by fluorescent tubes: Sylvania F-18W/Grolux; two tubes per cage ~20 cm above the cage (2,500 lux). Three residue ages were evaluated: 2-h, 3-days and 7-days-old. As soon as the residues were of the appropriate age, treated glasses were adjusted to a round glass frame with two bolts (modified from Jacas and Viñuela (15)) and five pairs of newly emerged flies were introduced per cage. These cages were connected to forced ventilation to avoid fume accumulation. Ten replicates per residue age were used. We obtained data to evaluate mortality after 24 h of exposure from five replicates. The rest of the replicates (five per compound) were exposed to the corresponding age residue for 3 consecutive days (stopping just before the beginning of oviposition). Afterwards, the survivors of these replicates were transferred to the non-treated plastic cages ordinarily used to evaluate fecundity and fertility.

**Topical treatment** Newly emerged adults (≤24 h old) were topically treated with 0.5 μl of an acetic solution of insecticides in the pronotum. An Arnold hand microapplicator (Burkard, UK) equipped with a 1 ml glass syringe fitted with a 30-gauge hypodermic needle was used to dispense the insecticide. Acetone alone was used as control. Mortality data at 24 h were recorded. Fecundity and fertility were assessed as described above.

**Ingestion treatment** Newly emerged adults (≤24 h, pre-oviposition) were exposed continuously to the test compound *via* the drinking water. Mortality data at 24 h were recorded. Fecundity and fertility were assessed as described above.

**Influence of exposure time and pymetrozine concentration on reproduction** Since the adverse effects of pymetrozine in the reproduction of *C. capitata* were basically observed in the ingestion treatment, further experiments were performed. First, a group of adults were fed the insecticide (300 ppm a.i.) only during the preoviposition period (3 days under the environmental conditions of climate chambers). Second, a different group was fed pymetrozine (300 ppm a.i.) continuously for 8 days, which implies a continuous exposure that includes the preoviposition and oviposition periods. Third, a concentration three times the maximum recommended (900 ppm a.i.) for 8 days was also tested. Our objective with this experiment was to determine the effect on the reproductive parameters of different exposure periods and higher concentrations of pymetrozine.

**Statistical analysis** Parameters recorded were analyzed with the statistical software package Statgraphics (21). One-way analysis of variance (ANOVA) and the LSD multiple range tests were performed on the data to determine significant ( $P < 0.05$ ) differences between concentrations. When premises of ANOVA were violated, proportional data were normalized through arcsine transformation and those expressed as percentages through log transformation. The Kruskal-Wallis test was applied only when ANOVA premises could not be fulfilled, even after transformations. Means and standard errors were calculated from the nontransformed data. Mortality, when possible, was subjected to PoloPlus 1.0 analysis to estimate probit regressions (16), lethal concentrations and 95% fiducial limits were calculated. The criterion of overlapping fiducial limits of relative potencies of lines was used to establish whether lines were significantly different or not ( $P = 0.05$ ).

## RESULTS AND DISCUSSION

**Mortality** Treatments to control *C. capitata* in the field must be performed on adults because eggs and larvae live in the fruit and pupae in the soil. The toxicity of three insecticides at the maximum field concentration to newly emerged adults of *C. capitata* was compared with that of malathion (Table 1) in the laboratory, using different uptake routes. Significant differences between toxicities were observed. Natural pyrethrins + PBO showed excellent activity compared with the positive control on residual and topical application and a slightly delayed effect after ingestion treatment. As early as 24 h after treatment, both chemicals were highly effective against adults (100% mortality) when insects were treated by contact. The efficacy was similar even when adults were exposed to 7-day-old residue. Over 90% mortality was achieved 3 days after ingestion of malathion or natural pyrethrins + PBO. This can be explained by taking into account that flies need time to contact the insecticide when it is offered at drinking sites. Pymetrozine and triflumuron were harmless, irrespective of the mode of insecticide application.

Concentrations of malathion and natural pyrethrins + PBO lethal to 50% and 90% of the population and relative potencies for topical and ingestion treatment after 72 h are given in Table 2. In both assays, the hypothesis of equal slope could be accepted ( $X^2 = 0.17$ ;  $df = 1$ : ingestion and  $X^2 = 1.43$ ;  $df = 1$ : topical).

Malathion was as effective as natural pyrethrins + PBO when topically treated (relative potencies = 1.09), with natural pyrethrins + PBO being less toxic when ingested (relative potencies = 0.22).

Pyrethrins, although very effective against many pest insects, are known to be rapidly deactivated under sunlight. On potato leaves, half-life ( $T_{1/2}$ ) values of pyrethrin-I and pyrethrin-II, the major insecticidal components of the pyrethrum daisy, were significantly shorter (several hours) than PBO residues (several days). Differences between residues detected on potato leaves and consequently their half-lives are due to the different physical properties of each active ingredient, indicating greater persistence of PBO (3). Angioni *et al.* (2) studied the behavior of pyrethrins and PBO on peaches after field treatment. At concentrations nearly 5.4 times lower than those applied herein with a similar commercial formulation, the initial deposition was below the maximum residue level, and the half-life time calculated for total pyrethrins was 2.3 days. It was shown that the trend of dissipation was within days, whereas Antonious *et al.* (3) found that it was within hours. This means that pyrethrins can be useful during some days in fruit crops. We have obtained similar degrees of comparison between the effectiveness of malathion and pyrethrins + PBO at all

TABLE 1. Mortality (%) of *Ceratitis capitata* adults exposed to different uptake routes (residual, topical, ingestion) with various insecticides at the maximum recommended field concentration in Spain (Data are expressed as means  $\pm$  S.E.)

Compound	Concn. (ppm a.i.)	Residual treatment			Topical treatment	Ingestion treatment
		2 h <sup>z</sup>	3 days <sup>z</sup>	7 days <sup>z</sup>		
Mortality at 24 h						
Control	0	0a <sup>y</sup>	0a	2.5 $\pm$ 2.5a	1.1 $\pm$ 1.1a	1.1 $\pm$ 1.1a
Malathion	1,500	100b	100b	100b	100b	42.6 $\pm$ 5.8b
Pyr. <sup>x</sup> + PBO	80	100b	100b	95.0 $\pm$ 2.8b	90.0 $\pm$ 5.5b	6.0 $\pm$ 6.0a
Pymetrozine	300	5.0 $\pm$ 2.8a	5.0 $\pm$ 2.5a	2.5 $\pm$ 2.5a	4.0 $\pm$ 2.4a	0a
Triflumuron	150	0.0 $\pm$ 0.0a	2.5 $\pm$ 2.5a	2.5 $\pm$ 2.5a	0a	0a
Mortality at 48 h						
Control	0	0a	0a	2.5 $\pm$ 2.5a	4.0 $\pm$ 1.6a	1.1 $\pm$ 1.1a
Malathion	1,500	100c	100b	100b	100b	100 c
Pyr. + PBO	80	100c	100b	95.0 $\pm$ 2.8b	90.0 $\pm$ 5.5b	72.0 $\pm$ 3.7b
Pymetrozine	300	15.0 $\pm$ 2.9b	7.5 $\pm$ 2.5a	2.5 $\pm$ 2.5a	6.0 $\pm$ 4.0a	0a
Triflumuron	150	7.5 $\pm$ 2.5a	2.5 $\pm$ 2.5a	2.5 $\pm$ 2.5a	0a	0a
Mortality at 72 h						
Control	0	0a	0a	2.5 $\pm$ 2.5a	4.0 $\pm$ 1.6a	1.1 $\pm$ 1.1a
Malathion	1,500	100c	100b	100b	100b	100b
Pyr. + PBO	80	100c	100b	95.0 $\pm$ 2.8b	100b	93.3 $\pm$ 6.0b
Pymetrozine	300	15.0 $\pm$ 2.9b	7.5 $\pm$ 2.5a	2.5 $\pm$ 2.5a	8.0 $\pm$ 5.8a	0a
Triflumuron	150	7.5 $\pm$ 2.5a	2.5 $\pm$ 2.5a	2.5 $\pm$ 2.5a	0a	0a

<sup>z</sup> Age of residue.

<sup>y</sup> Within columns, means followed by the same letter do not differ significantly; ANOVA, LSD (P>0.05).

<sup>x</sup> Pyr. = pyrethrins.

TABLE 2. Comparative toxicity at 72 h of malathion and natural pyrethrins to *Ceratitis capitata* adults after topical and ingestion treatments

Compound	n	Slope (means $\pm$ S.E.)	LC <sub>50</sub> (ppm; 95%FL)	LC <sub>90</sub> (ppm; 95%FL)	Relative potencies	$\chi^2$ (df)
<i>Ingestion Treatment</i>						
Malathion	350	3.0 $\pm$ 0.4	11.1 (9.2-13.2)	28.7 (23.0-39.2)		1.94 (3)
Pyrethrins+PBO	300	3.3 $\pm$ 0.8	50.6 (41.2-62.3)	130.5 (100.6-188.4)	0.22 (0.16-0.28)	1.01 (3)
<i>Topical Treatment</i>						
Malathion	400	7.0 $\pm$ 0.9	29.9 (27.8-32.3)	48.3 (43.2-56.1)		2.15 (4)
Pyrethrins+PBO	400	5.6 $\pm$ 0.7	27.2 (25.4-29.2)	43.9 (39.6-50.4)	1.09 (0.99-1.21)	3.33 (4)

Mortality curves were constructed with six or seven concentrations. All concentrations used fall in the range of 5% and 95% mortality. Each concentration was tested with at least five replicates of ten adults each.

n = number of individuals.

FL = fiducial limits.

residue levels. However, it is doubtful that the same values of mortality can be repeated in an open field when the residue is older than a few days. In spite of this, it should be considered a valuable tool to be used in the management of the medfly because of its rapid knock-down, especially when restrictions of environmental quality and consumer safety are the most important requirements. As such, pyrethrins are one of the few insecticides allowed for the control of tephritids on organic farms.

TABLE 3. Number of eggs per female and day laid by *Ceratitis capitata* exposed to different uptake routes of pymetrozine and triflumuron (Data are expressed as means±S.E.)

Compound	Residual treatment			Topical treatment	Ingestion treatment
	2 h <sup>z</sup>	3 days <sup>z</sup>	7 days <sup>z</sup>		
Control	30.4±2.0a <sup>y</sup>	35.0±1.2a	34.5±3.0a	31.3±1.9a	28.1±2.3a
Pymetrozine 300 ppm a.i.	23.0±2.4a (24.3%) <sup>x</sup>	29.2±1.1a (16.6%)	32.3±1.1a (6.4%)	13.4±2.5b (57.2%)	12.5±2.6b (55.5%)
Triflumuron 150 ppm a.i.	27.2±3.1a (10.5%)	32.9±2.3a (6.0%)	33.0±2.3a (4.3%)	31.5±2.4a (-0.6%)	31.4±2.5a (-11.7%)

<sup>z</sup> Age of residue.

<sup>y</sup> Within columns, means followed by the same letter do not differ significantly; ANOVA, LSD (P>0.05).

<sup>x</sup> Numbers in parentheses represent the percent reduction in comparison with the corresponding control.

TABLE 4. Eggs hatch (%) of *Ceratitis capitata* exposed to different uptake routes of pymetrozine and triflumuron (Data are expressed as means±S.E.)

Compound	Residual treatment			Topical treatment	Ingestion treatment
	2 h <sup>z</sup>	3 days <sup>z</sup>	7 days <sup>z</sup>		
Control	90.1±6.8a <sup>y</sup>	91.7±4.3a	89.5±3.6a	86.2±2.2a	60.0±5.9a
Pymetrozine 300 ppm a.i.	38.3±19.1b (57.4%) <sup>x</sup>	84.0±2.0a (8.4%)	89.8±2.0a (-0.3%)	65.6±11.4a (23.8%)	24.4±13.9b (59.3%)
Triflumuron 150 ppm a.i.	92.0±5.6a (2.1%)	91.7±4.4a (0.0%)	89.9±2.1a (-0.4%)	76.8±8.8a (10.9%)	61.2±11.9a (-2.0%)

<sup>z</sup> Age of residue.

<sup>y</sup> Within columns, means followed by the same letter do not differ significantly; ANOVA, LSD (P>0.05).

<sup>x</sup> Numbers in parentheses represent the percent reduction in comparison with the corresponding control.

**Effects on reproduction** The prevention of fecundity or eclosion is one of the most desired effects to control *C. capitata* because the absence of larvae prevents most of the fruit damage. As such, the autosterilization of flies in their natural habitat by using localized traps with specific lures for males and females and food mixed with a sterilant is an alternative control method (10), because theoretical studies have suggested that sterilization of a proportion of flies will be more effective in population reduction than simply killing the same number of flies (4).

Pymetrozine diminishes fecundity to a certain extent (57.2% reduction compared with the control in topical treatment and 55.5% in the ingestion treatment) (Table 3). A slight,

TABLE 5. Effects of ingestion of pymetrozine on reproduction of *Ceratitis capitata* (Data are expressed as means±S.E.)

Concn. (ppm a.i.)	Eggs/female/day	Egg hatch (%)		Total reduction (%) <sup>v</sup>
		4th day	6th day	
Control	45.8±1.9a <sup>x</sup>	94.8±1.5a	92.5±5.2a	
300 <sup>z</sup>	26.8±2.7b (41.5%) <sup>w</sup>	66.9±5.1ab (29.4%)	93.3±2.1a	12.2
300 <sup>y</sup>	17.5±3.5c (61.8%)	45.8±17.6bc (51.7%)	59.9±11.9b	31.9
900 <sup>y</sup>	19.5±2.6bc (57.4%)	25.6±17.5c (72.9%)	45.3±19.4b	41.9

<sup>z</sup> Exposure during the first 3 days (preoviposition).

<sup>y</sup> Continuous exposure.

<sup>x</sup> Within columns, means followed by the same letter do not differ significantly; ANOVA, LSD (P>0.05).

<sup>w</sup> Numbers in parentheses represent the percent reduction in comparison with the corresponding control.

<sup>v</sup> Total reduction is calculated as inhibition of oviposition (%) × inhibition of egg fertility (%).

but non-significant, reduction in egg-laying could be detected after exposure to a 2-h-old residue. After 3 days, females on pymetrozine residues of 300 ppm produced the same number of eggs as those laid by females exposed to distilled water. The lack of effect detected in residual treatments regardless of the residue age, in comparison with topical assay and ingestion, can be attributed to two factors: first, it is likely that adults of *C. capitata* would not have contacted the residue in a sufficient amount to cause an effect on reproduction, especially when they were exposed to a fresh residue; second, it is possible that an old residue had become degraded.

By preventing egg hatching, the fruit cannot be destroyed by feeding; hence, the best results are those that affect egg output and eclosion. None of the tested compounds prevented egg hatching completely. Pymetrozine reduced egg hatching by 57.4%, 23.8% and 59.3% compared with the control in residual (2-h-old), topical and ingestion treatments, respectively (Table 4). Triflumuron did not reduce hatching, which is in contrast to the results obtained by Casaña *et al.* (9), who found triflumuron to cause total suppression of egg hatch of females when administered mixed with their food at 10,000 ppm for 3 days. Obviously, we have tested a dose 66.6-fold lower. Several investigators (7,9) have tested compounds in the search for sterilizing effects without too much success. The reduction, unless close to 100%, is not sufficient to control the pest in the field. Although the reduction in fecundity and fertility attributed to pymetrozine found herein is too slight to consider it as a control method, it can contribute to a partial reduction in the number of medflies in fields that are treated with pymetrozine for sucking insects control. The joint reduction of fecundity and fertility is not negligible and, when combined with a more efficient method, can significantly control medfly populations.

As such, taking into account that ingestion seems to be the most effective mode of application, the use of localized traps with a specific lure and pymetrozine at higher concentrations as a sterilant can be subjected to a more detailed study. After 3 days of ingestion of pymetrozine at the maximum concentration rate during the preoviposition period (Table 5), the mean number of eggs laid per female and the egg hatch at day 4 was significantly reduced in comparison with the controls (by 41.5% and 29.4%, respectively, with a total reduction of 12.2%). Nevertheless, the effects on fertility observed at day 4 disappeared at day 6, with egg hatch similar to the control. When the period of exposure was increased to 8 days at 300 ppm a.i. pymetrozine, more significant differences between the controls and fecundity and fertility were detected (a total reduction of 31.9%), being more acute in the shorter ingestion period. In conclusion, a longer period of exposure (8 days) and higher concentrations (900 ppm a.i.) reduced the mean number of eggs per female and day and also the egg hatch more effectively (a total reduction of 41.9%), the effect attributed to the increase of concentration being higher. A three-fold rate of pymetrozine ingested continuously for 8 days yielded a reduction of 57.4% in fecundity, 72.9% in egg hatch at day 4 and of 57.2% at day 6.

The effects of pymetrozine on *C. capitata* offer a valuable prospect for incorporating this chemical into IPM programs. It might be applied to reduce populations to levels that are more manageable by natural enemies or to diminish the number of treatments currently being used. Nevertheless, more detailed studies have to be carried out to conclude that pymetrozine can be taken seriously into account as an effective control agent.

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