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**Monitoring resistance of *Cydia pomonella* (L.) Spanish field  
populations to new chemical insecticides and the mechanisms involved**

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**Abstract**

**BACKGROUND:** A widespread resistance of *Cydia pomonella* to organophosphates was demonstrated in populations from the Spanish Ebro Valley area that showed high levels of enzymatic detoxification. To determine the efficacy of new insecticides, neonate larvae bioassays were carried out on twenty field codling moth populations collected from three different Spanish apple production areas. Synergist bioassays were performed to detect the enzymatic mechanisms involved.

**RESULTS:** The least active ingredients were methoxyfenozide, with 100% of the populations showing significantly lower mortality than the susceptible strain, and lambda-cyhalothrin, with very high resistant ratios (872.0 for the most resistant field population). Approximately 50% of the populations were resistant or tolerant to thiacloprid. By contrast, tebufenozide was very effective in all the field populations, as was chlorpyrifos-ethyl despite its widespread use during the last few years. Indoxacarb, spinosad and chlorantraniliprole also provided high efficacy, as did emamectin and spinetoram, which are not yet registered in Spain.

**CONCLUSION:** The resistant Spanish codling moth populations can be controlled using new reduced-risk insecticides. The use of synergists showed the importance of the concentration applied and the difficulty of interpreting the results in field populations that show multiple resistance to different active ingredients.

**Keywords:** *Cydia pomonella*, insecticide, resistance, synergist

## 1 INTRODUCTION

Since the 1990s the integrated pest management (IPM) program in apple orchards in Spain has focused on biological control of *Panonychus ulmi* (Koch) (Acari: Tetranychidae), using naturally occurring phytoseiid populations and achieving great success in most orchards.<sup>1</sup> Since the late 1990s, the main pest to control has been the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). Organophosphates (OPs), especially azinphos-methyl, have been the most important chemical insecticide group used to control codling moth in apple, pear and walnut orchards. They have been used intensively for the last thirty years in Spain and in other apple production regions of the world.<sup>2-6</sup> The present control strategy for codling moth relies mainly on mating disruption, especially in apple and walnut orchards, where the pest is more difficult to control, but chemical insecticides are widely used to reinforce the system. In Spain, the characteristics of some parts of the production area, with small orchards and mixed crops, make the application of this strategy more complex and the use of insecticides more necessary.

Codling moth resistance to pesticides is well documented and began a long time ago with arsenate and DDT.<sup>7,8</sup> It now affects almost every class of synthetic insecticides and is spread throughout the world's apple production regions.<sup>3,5,9-18</sup> The number of available insecticides against codling moth has fallen drastically in the EU since the re-registration of active substances covered by Directive 91/414/EEC, and some of the products most used to control codling moth are no longer available. When azinphos-methyl was prohibited in Spain in 2008, widespread resistance to the product and to OPs in general was demonstrated in problematic orchards of the Ebro Valley area (Catalonia and Aragon, NE Spain).<sup>19-20</sup> Chlorpyrifos-ethyl then became the most widely used insecticide and was very active against neonate larvae, the main target instar of

codling moth, despite also belonging to the chemical class of OPs.<sup>20</sup> Negative cross-resistance between chlorpyrifos-ethyl and two other OPs, azinphos-methyl and methylparathion, was observed in field populations of codling moth adults in California by Dunley and Welter,<sup>3</sup> and also in neonate larvae of Spanish populations by Rodríguez *et al.*<sup>20</sup> During the last few years, the use of pyrethroids has also increased due to the low prices of fruits and the attempt of growers to reduce production costs. Knight *et al.*<sup>21</sup> report a gradual increase in the use of lambda-cyhalothrin since 2005. OPs and other broad spectrum insecticides, such as pyrethroids and carbamates, act by contact and/or ingestion and are effective against multiple pests and different target instars, but they have low selectivity to natural enemies and high mammalian toxicity, and cause environmental contamination.

A number of codling moth insecticides classified as reduced-risk or OP alternatives have been registered in Spain since the 1980s and are recommended by IPM programs: the insect growth regulator fenoxycarb, the neonicotinoid thiacloprid, the ecdysone receptor agonists methoxyfenozide and tebufenozide, the voltage-dependent sodium channel blocker indoxacarb, the nicotinic acetylcholine receptor allosteric modulator spinosad and, recently, the ryanodine receptor modulator chlorantraniliprole. Codling moth cross-resistance among some of these reduced-risk insecticides groups and OPs and pyrethroids have been detected in some European countries,<sup>12,14,22</sup> the USA<sup>23,24</sup> and Canada,<sup>25</sup> even with pesticides that have been registered recently<sup>12,26</sup> or have not yet been registered.<sup>27</sup> No codling moth susceptibility study has been reported with these new chemical insecticides, except thiacloprid, which has been tested in Spanish field populations, showing general high levels of enzymatic detoxification, mainly due to cytochrome P450 polysubstrate monooxygenases (PSMO) (in neonate larvae, adults and

post-diapausing larvae), but also due to glutathione S-transferases (GST) (in adults and post-diapausing larvae) and esterases (EST) (in post-diapausing larvae).<sup>19,20</sup>

The objectives of this work were to evaluate the insecticide resistance of *C. pomonella* in three areas of apple production in Spain with two completely different management systems and to determine the efficacy of some new insecticides in controlling codling moth neonate larvae, paying special attention to populations that showed insecticide resistance with the most commonly used products. French field populations from the neighboring area were also tested. Tests with synergists were performed on some field populations to explain the enzymatic mechanisms that may be involved in resistance.

## 2 MATERIALS AND METHODS

### 2.1. Insects

Twenty field populations of codling moth were collected as diapausing larvae in 2010, 2011 and 2012 (Table 1). The populations came from three different Spanish apple production areas: eleven from Catalonia, three from Aragon (both located in the Ebro Valley, northeast Spain, with a maximum distance of about 190 km between orchards) and three from Asturias (northern Spain). Three field populations from the southeast of France (Provence-Alpes-Côte d'Azur region) were also studied. The populations were mostly from IPM orchards, but the population Coll (from Catalonia) was an organic orchard with codling moth control problems. The three Asturian orchards produced cider-apples and the rest produced table apples, so we had two, well-distinguished pest management systems. The susceptible strain S\_Spain was collected from an abandoned apple orchard in Lleida in 1992 and has been reared since then using a semi-artificial dehydrated apple diet at the joint IRTA (Institute for Food and Agricultural Research and Technology) and UdL (University of Lleida) laboratory (Lleida, Spain).

### 2.2. Insecticides and synergists

Ten insecticides (expressed below with mode of action followed by chemical class) were tested using commercial formulations (Table 2). Two of them are or were commonly used in the IPM orchards to control codling moth: the acetylcholinesterase inhibitor organophosphate chlorpyrifos-ethyl (prohibited at present), and the sodium channel modulator pyrethroid lambda-cyhalothrin. Four of them are hardly used: the nicotinic acetylcholine receptor competitive modulator neonicotinoid thiacloprid, the ecdysone receptor agonist diacylhydrazines methoxyfenozide and tebufenozide, and the voltage-dependent sodium channel blocker oxadiazine indoxacarb. Other products tested were recently or not yet registered in Spain at the moment of field population collection: the nicotinic acetylcholine receptor allosteric modulator spinosyns spinosad (registered in 2013) and spinetoram (not yet registered), the ryanodin receptor modulator diamide chlorantraniliprole, or Rynaxypyr, (registered in October 2011), and the glutamate-gated chloride channel allosteric modulator avermectin emamectin benzoate (not yet registered).<sup>28</sup> The synergists used were piperonyl butoxide (PBO, 90% purity, distributed by Fluka) as a microsomal monooxygenase inhibitor (PSMO), diethyl maleate (DEM, 97% purity, distributed by Sigma Aldrich) as a GST inhibitor, and S,S,S-tributyl phosphotriothioate (DEF, 98% purity, distributed by Sigma Aldrich) as an EST inhibitor. The insecticides were diluted in distilled water and the three synergists in 96% acetone.

### **2.3. Insecticide efficacy bioassays**

The bioassay to test the insecticide efficacy of each product was performed using the diagnostic concentrations that produced approximately 90% mortality in the susceptible population, S-Spain, hereinafter LC<sub>90</sub>. These concentrations (Table 2) were previously determined from a concentration-mortality curve and were corroborated every year in the susceptible population during the field population treatments (from 2012 to 2014).

Microplate wells were filled with 150  $\mu$ L of artificial diet (Stonefly Industries Ltd) and 6  $\mu$ L of each insecticide's LC<sub>90</sub> was applied to the surface of the diet. Distilled water replaced the insecticide in the controls. Thirty minutes after the treatment, newly hatched larvae (0-24 h old) were individually placed in each well and transferred to controlled conditions ( $25 \pm 1^\circ\text{C}$  and 16:8 [L:D] h photoperiod). Mortality was recorded after 4 days. Larvae were considered dead when they did not respond to a probe with dissecting forceps. Missing larvae were subtracted from the initial number. Fourteen Spanish (eight from Catalonia, three from Aragon and three from Asturias) and two French field codling moth populations were treated. Depending on the neonate larvae obtained in the progenies, 3–10 insecticides per population were tested.

#### **2.4. Synergist bioassays**

The synergists were dissolved in acetone and the concentrations used were 2.5 mg a.i./L for PBO, 10 mg a.i./L for DEM and 5 mg a.i./L for DEF. The concentration of each synergist to be applied was previously calculated with the laboratory population, S-Spain, and was the one that produced approximately 10% mortality. The neonate larvae were exposed by contact to the synergist for 1 h before feeding on the treated diet following the same methodology as in the insecticide efficacy bioassays. The insecticide concentrations used are shown in Table 2 and were those that produced approximately 50% mortality in S-Spain, hereinafter LC<sub>50</sub>. Six Spanish (five from Catalonia and one from Aragon) and one French field codling moth populations were treated. Only three field populations had a sufficient number of progeny for insecticide and synergist bioassays to be performed. The insecticides chlorpyrifos-ethyl and lambda-cyhalothrin and the synergist PBO were prioritized in the assays.

#### **2.5. Data analysis**

The mortalities were corrected using Abbot's formula.<sup>29</sup> To calculate the insecticide efficacy, the correction factor was the mortality of the solvent-treated control (water), and for the synergistic effect the correction factor was the mortality produced by the synergist. In the insecticide efficacy studies, the difference between the efficacies of each insecticide in the field populations was compared with that in S-Spain, tested in the same year of the field population bioassay using a Pearson  $\chi^2$  test. Resistance ratios (RR) were determined by dividing the mortality of S-Spain, obtained with the LC<sub>90</sub>, by that of the field population. Populations were classified as resistant ( $RR \geq 10$ ), tolerant ( $1 < RR < 10$ ) and susceptible ( $RR \leq 1$ ).<sup>25</sup> To assess the degree of synergism, synergistic ratios (SR) were calculated by dividing the mortality obtained by the LC<sub>50</sub> of the insecticide plus synergist application by that of the insecticide alone. The differences between the corrected mortality obtained by each insecticide was compared with that obtained by the synergist plus the insecticide using a Pearson  $\chi^2$  test.

### 3. RESULTS

#### 3.1. Insecticide efficacy bioassays

During each year of the study the S-Spain population showed similar mortality levels when tested with the LC<sub>90</sub> concentration of each individual insecticide (Table 3). The only exception was during the 2013 bioassays, when methoxyfenozide produced 84.2% mortality, which was significantly lower than that obtained in the 2012 and 2014 bioassays, 92.4% and 97.1%, respectively.

The level of susceptibility of the codling moth field populations to the tested insecticides differed greatly depending on the apple production area. The field populations from Asturias were in general as susceptible as, or even significantly more susceptible than, the S-Spain population in three out of 19 bioassays (AstAb to lambda-cyhalotrin and chlorantraniliprole:  $dF = 1$ ,  $\chi^2 = 4.43$ ,  $p = 0.0354$  and  $dF = 1$ ,  $\chi^2 = 4.43$ ,  $p$

= 0.0012, respectively). The products that showed significantly lower mortality than S-Spain in some field populations were chlorpyrifos-ethyl (AstN:  $dF = 1$ ,  $\chi^2 = 4.39$ ,  $p = 0.0362$ ) and methoxyfenozide (AstN and AstC:  $dF = 1$ ,  $\chi^2 = 8.11$ ,  $p = 0.0044$  and  $dF = 1$ ,  $\chi^2 = 4.33$ ,  $p = 0.0373$ , respectively). The Asturian population with the lowest susceptibility was AstN, which was significantly different from S-Spain in two of the seven bioassays.

In the efficacy bioassays, the three field populations from Aragon showed significantly lower mortality than S-Spain in five of the 18 bioassays tested. The Tamarite and La Almunia populations were as susceptible as S-Spain in all the bioassays, but the Albalate de Cinca (ADC) population was significantly less susceptible than S-Spain in five of the ten bioassays: lambda-cyhalothrin ( $dF = 1$ ,  $\chi^2 = 57.47$ ,  $p = 3.43 \times 10^{-14}$ ), methoxyfenozide ( $dF = 1$ ,  $\chi^2 = 34.68$ ,  $p = 3.90 \times 10^{-9}$ ) thiacloprid, ( $dF = 1$ ,  $\chi^2 = 34.68$ ,  $p = 27.85 \times 10^{-7}$ ), indoxacarb ( $dF = 1$ ,  $\chi^2 = 11.36$ ,  $p = 0.0008$ ) and chlorantraniliprole ( $dF = 1$ ,  $\chi^2 = 10.45$ ,  $p = 0.0012$ ). However, the ADC population was significantly more susceptible than S-Spain to spinosad ( $dF = 1$ ,  $\chi^2 = 7.71$ ,  $p = 0.0301$ ).

In the efficacy bioassays the eight field populations of Catalonia showed significantly lower mortality than S-Spain in 24 of the 61 bioassays tested. The populations SAS, Mir7/84 and Tossal showed lower susceptibility than S-Spain in two out of four of the products tested and PuigverdC in seven out of ten. Linyola was the only field population as susceptible as S-Spain to all the five products tested. Methoxyfenozide showed significantly lower mortality than S-Spain in all the six Catalan field populations: SAS ( $dF = 1$ ,  $\chi^2 = 40.09$ ,  $p = 8.71 \times 10^{-11}$ ), PuigverdB ( $dF = 1$ ,  $\chi^2 = 13.93$ ,  $p = 0.0002$ ), Poalbud ( $dF = 1$ ,  $\chi^2 = 11.78$ ,  $p = 0.0006$ ), PuigverdC ( $dF = 1$ ,  $\chi^2 = 59.55$ ,  $p = 1.19 \times 10^{-14}$ ), Mir7/84 ( $dF = 1$ ,  $\chi^2 = 60.55$ ,  $p = 7.19 \times 10^{-15}$ ) and Tossal ( $dF = 1$ ,  $\chi^2 = 50.92$ ,  $p = 9.60 \times 10^{-13}$ ). Lambda-cyhalothrin showed significantly lower mortality than S-Spain in

six out of eight populations: SAS ( $dF = 1, \chi^2 = 30.30, p = 3.70 \times 10^{-8}$ ), PuigverdB ( $dF = 1, \chi^2 = 67.61, p = 1.99 \times 10^{-16}$ ), PuigverdC ( $dF = 1, \chi^2 = 73.63, p = 9.42 \times 10^{-18}$ ), Mir7/84 ( $dF = 1, \chi^2 = 73.63, p = 9.42 \times 10^{-18}$ ), Tossal ( $dF = 1, \chi^2 = 52.17, p = 5.10 \times 10^{-13}$ ) and Paradet ( $dF = 1, \chi^2 = 5.39, p = 0.020$ ). Thiacloprid showed significantly lower mortality than S-Spain in four out of six populations: SAS ( $dF = 1, \chi^2 = 9.87, p = 0.0017$ ), PuigverdB ( $dF = 1, \chi^2 = 6.99, p = 0.0082$ ), PuigverdC ( $dF = 1, \chi^2 = 46.10, p = 1.12 \times 10^{-11}$ ) and Mir7/84 ( $dF = 1, \chi^2 = 23.40, p = 1.32 \times 10^{-6}$ ). Two out of four populations tested with indoxacarb obtained significantly lower susceptibility than S-Spain (PuigverdC:  $dF = 1, \chi^2 = 1.17, p = 0.0074$ , and Mir7/84:  $dF = 1, \chi^2 = 5.95, p = 0.0147$ ). All the populations tested with spinosad were as susceptible as S-Spain, but two populations of the five tested with spinetoram were significantly less susceptible than S-Spain (PuigverdB:  $dF = 1, \chi^2 = 4.38, p = 0.0363$  and PuigverdC:  $dF = 1, \chi^2 = 17.02, p = 3.70 \times 10^{-5}$ ). Despite being hardly used, chlorpyrifos-ethyl showed significantly lower mortality than S-Spain in just two of the seven populations tested: PuigverdC ( $dF = 1, \chi^2 = 21.17, p = 4.20 \times 10^{-6}$ ) and Mir7/84 ( $dF = 1, \chi^2 = 6.21, p = 0.0127$ ). This was the same proportion as for the new active ingredient chlorantraniliprole in SAS ( $dF = 1, \chi^2 = 26.39, p = 2.79 \times 10^{-7}$ ) and PuigverdC ( $dF = 1, \chi^2 = 11.72, p = 0.0006$ ). Emamectin obtained significantly lower mortality than S-Spain in only one of the eight field populations treated, SAS ( $dF = 1, \chi^2 = 12.91, p = 0.0003$ ). In contrast with the methoxyfenozide results, all five field populations tested with tebufenozide were as susceptible as S-Spain.

The two field populations from France were significantly less susceptible than S-Spain in seven of the 14 bioassays. Neither of them was treated with chlorpyrifos-ethyl due to the number of available neonate larvae. Both the Pompiers and Le Thor populations were significantly less susceptible than S-Spain to lambda-cyhalothrin ( $dF = 1, \chi^2 =$

12.79 and  $p = 0.0003$  and  $dF = 1$ ,  $\chi^2 = 19.52$  and  $p = 9.93 \times 10^{-6}$ , respectively), methoxyfenozide ( $dF = 1$ ,  $\chi^2 = 38.87$ ,  $p = 4.54 \times 10^{-10}$  and  $dF = 1$ ,  $\chi^2 = 13.64$  and  $p = 0.0002$ , respectively) and thiacloprid ( $dF = 1$ ,  $\chi^2 = 19.82$ ,  $p = 8.53 \times 10^{-6}$  and  $dF = 1$ ,  $\chi^2 = 9.27$  and  $p = 0.0023$ , respectively), and both were as susceptible as S-Spain to indoxacarb, spinetoram and emamectin. Only Le Thor was treated with tebufenozide and spinosad, and tebufenozide produced a significantly lower mortality in the field population than in S-Spain ( $dF = 1$ ,  $\chi^2 = 13.63$ ,  $p = 0.0002$ ).

According to these results, methoxyfenozide was the least effective insecticide, obtaining significantly lower mortality than in S-Spain in 100% of the field populations tested, regardless of their origin, followed by lambda-cyhalothrin and thiacloprid, which obtained significantly lower mortality in 60% and 54% of the populations, respectively. The most effective insecticides were spinosad and emamectin, which were as effective as in S-Spain in 100% and 94% of the field populations, respectively, while tebufenozide, spinetoram and chlorpyrifos-ethyl were significantly less effective in 13%, 20% and 23% of the treated populations, respectively. Indoxacarb and chlorantraniliprole obtained significantly lower mortality than in S-Spain in 33% and 25% of the populations, respectively.

Considering RRs, lambda-cyhalothrin was the insecticide with most resistant field populations (PuigverdC, Mir7/84 and PuigverdB, with RRs of 872, 148 and 15.4, respectively). PuigverdC and Mir7/84 were also resistant to methoxyfenozide (RRs of 14.6 and 15.9, respectively) and PuigverdC was also resistant to thiacloprid (RR of 11.2). All the populations treated with methoxyfenozide were resistant or tolerant to the product. Of the field populations 81% and 87% were susceptible to emamectin and spinosad, respectively ( $RR \leq 1$ ). For the rest of the products, the percentage of susceptible field populations ranged from 42% to 60%.

### 3.2. Synergist bioassays

Only three field populations in which insecticide efficacy bioassays were performed had enough larvae to also allow bioassays to be carried out with synergists (Tamarite, PuigverdC and PuigverdB). The field populations named Tossal were collected in two different years and were considered distinct populations. Table 4 shows the corrected mortality produced by the LC<sub>50</sub> used in the synergist bioassays. The mortality obtained in the control treatment of the eight field populations ranged between 0.00% and 11.11%. Lambda-cyhalothrin was the product with a highest number of populations with significant lower mortality than S-Spain in PuigverdB (dF = 1,  $\chi^2 = 43.75$ ,  $p = 3.73 \times 10^{-11}$ ), PuigverdC (dF = 1,  $\chi^2 = 20.04$ ,  $p = 7.59 \times 10^{-6}$ ), Torregrossa (dF = 1,  $\chi^2 = 5.03$ ,  $p = 0.0249$ ) and Tossal (dF = 1,  $\chi^2 = 41.86$ ,  $p = 9.80 \times 10^{-11}$ ), and chlorpyrifos-ethyl showed significantly lower mortality than in S-Spain only in PuigverdC (dF = 1,  $\chi^2 = 5.63$ ,  $p = 0.0177$ ). By contrast, some emamectin treatments showed significant higher mortality than in S-Spain: PuigverdC (dF = 1,  $\chi^2 = 9.47$ ,  $p = 0.0021$ ), Torregrossa (dF = 1,  $\chi^2 = 14.29$ ,  $p = 0.0002$ ) and Tossal (dF = 1,  $\chi^2 = 18.01$ ,  $p = 2.20 \times 10^{-5}$ ).

The corrected mortality obtained with the application of the different synergists before the treatment with chlorpyrifos-ethyl, lambda-cyhalothrin, emamectin and chlorantraniliprole to some field populations is shown in Table 5. In no cases did the application of a synergist modify the mortality obtained in the susceptible population, S\_Spain. A significant synergistic effect was observed with PBO only in a few field populations when it was applied before any tested insecticides. The corrected mortality significantly increased with the synergist in one of the eight field populations treated with chlorpyrifos-ethyl, Torregrossa (dF = 1,  $\chi^2 = 13.79$ ,  $p = 0.0002$ ), and in one of the eight field populations treated with lambda-cyhalothrin, Tossal (dF = 1,  $\chi^2 = 21.86$ ,  $p = 2.90 \times 10^{-6}$ ). A significant increase in mortality was also observed when PBO was

applied before emamectin in the field population PuigverdB ( $dF = 1$ ,  $\chi^2 = 3.94$ ,  $p = 0.0470$ ). No significant synergistic effect was observed with DEM in lambda-cyhalothrin treatments. In two field populations treated with chlorpyrifos-ethyl, treatment with DEM resulted in a significant increase of mortality: Noves ( $dF = 1$ ,  $\chi^2 = 4.12$ ,  $p = 0.0424$ ) and Torregrossa ( $dF = 1$ ,  $\chi^2 = 8.42$ ,  $p = 0.0037$ ). The same occurred in two other treatments of emamectin: Tamarite ( $dF = 1$ ,  $\chi^2 = 15.86$ ,  $p = 6.80 \times 10^{-5}$ ) and PuigverdB ( $dF = 1$ ,  $\chi^2 = 10.77$ ,  $p = 0.0010$ ). The synergist DEF significantly increased mortality to chlorpyrifos-ethyl and emamectin in the same field populations as DEM did. It increased mortality to chlorpyrifos-ethyl in Noves ( $dF = 1$ ;  $\chi^2 = 4.01$ ,  $p = 0.0453$ ) and Torregrossa ( $dF = 1$ ;  $\chi^2 = 10.01$  and  $p = 0.0016$ ), and it increased mortality to emamectin in Tamarite ( $dF = 1$ ,  $\chi^2 = 15.86$ ,  $p = 6.80 \times 10^{-5}$ ) and PuigverdB ( $dF = 1$ ,  $\chi^2 = 6.31$ ,  $p = 0.0120$ ). The mortality also increased significantly when DEF was applied to the Torregrossa field population before the treatment with lambda-cyhalothrin ( $dF = 1$ ,  $\chi^2 = 4.68$ ,  $p = 0.0306$ ), which showed significantly less susceptibility than S-Spain. The highest SR obtained was 17.1, when PBO was applied before lambda-cyhalothrin in the field population Tossal. For the rest of the field populations and treatments in which the application of synergists significantly increased the mortality, the SR ranged between 2.0 (PBO + chlorpyrifos-ethyl in Torregrossa) and 1.1 (DEM or DEF + chlorpyrifos-ethyl in Noves). The SRs obtained when chlorantraniliprole was applied after a synergist ranged between 0.6 and 1.0. Significantly lower mortality was obtained in all the field populations previously treated with PBO and in two of the three field populations treated with DEM.

#### **4. Discussion**

##### **4.1. Insecticide efficacy bioassays**

As was expected, the field populations from Asturias were susceptible to all the insecticides tested, unlike the field populations from the other three areas of production. The Asturian apple orchards are for cider production and the most important pests to control are the rosy apple aphid, *Dysaphis plantaginea* Pass. (Homoptera: Aphididae), and the codling moth.<sup>30</sup> In the production area of Asturias, the codling moth has 1.5 generations per year, while in the other areas it has 2.5 generations. Since the early 1990s, in the new semi-intensive Asturian orchards, using mating disruption and selective insecticides, such as granulovirus and insect growth regulators (IGR), the level of codling moth damage has been maintained below 2% and biological control of the European Red Mite (*Panonychus ulmi* (Koch) (Acari: Tetranychidae)) has been achieved with phytoseids.<sup>31</sup> To control rosy apple aphid, neem derivatives are currently used, though some less selective treatments are applied occasionally. The efficacy bioassays showed a significantly lower susceptibility of the Asturian field populations to methoxyfenozide, although the RR in these populations ranged between 1.3 and 1.2. These values are very low, and it should be noted that with S-Spain it was also possible to calculate a ratio of 1.2 when the mortalities obtained with methoxyfenozide were compared during the years studied (Table 3). In addition, an RR of 1.1 or 1.2 was found in many of the efficacy bioassays where no significant differences from the susceptible strain were found. These results imply that RR values ranging between 1.1 and 1.2 cannot be attributed to the population's tolerance to the insecticide but may be due to assay variability coupled with population response variability.

The field populations from Catalonia and Aragon showed a similar low susceptibility to the tested insecticides. In both areas it was possible to find field populations as susceptible as S-Spain to all or almost all the products (Tamarite and La Almunia in Aragon, and Linyola and Poalbud in Catalonia) and populations that had low

susceptibility to half or more than half the insecticides used in the bioassay (ADC in Aragon and PuigverdC, Mir7/84 and some others in Catalonia). No French field population was susceptible to all the products but few orchards were tested, and a similar situation to that of Catalonia and Aragon can be expected.<sup>12</sup>

In the apple production area of Lleida (Catalonia), since azinphos-methyl use was prohibited, 24% and 16% of the chemical applications against codling moth have been with chlorpyrifos-ethyl and lambda-cyhalothrin, respectively (data obtained from the record of treatments of 2875 ha of apples and pears during the year 2008 in Lleida). Chlorpyrifos-ethyl was surprisingly effective and the susceptibility of the field populations had not diminished in the last five years in spite of its high frequency of use. This high efficacy was also found by Rodríguez *et al.*<sup>20</sup> in field populations collected in the same area in 2006 and 2007, in which a great loss of susceptibility to azinphos-methyl was detected. Reyes *et al.*<sup>32</sup>, in laboratory selected populations, found that the azinphos-methyl-resistant laboratory strain was significantly more susceptible to chlorpyrifos-ethyl than the sensitive strain. Dunley and Welter<sup>3</sup> found a negatively correlated cross-resistance between chlorpyrifos-ethyl and azinphos-methyl in codling moth and suggested the possibility of developing a resistance management strategy based on it, which was in fact done by advisors and growers some years ago in Spain when azinphos-methyl was banned. On the other hand, a high frequency of populations tolerant or resistant to lambda-cyhalothrin, the insecticide with the highest RRs, was found particularly in the production area of Catalonia, where 148- and 872-fold resistance was found in two orchards. The use of pyrethroids, specifically lambda-cyhalothrin, has gradually increased in the last few years in Spanish apple production areas. It is applied not only to control codling moth, particularly near harvest, but also to control *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) and other pests. This practice is

currently threatening the mite control strategy with indigenous phytoseids, widely established in the area since the 1990s, and is being favored by the stringent market requirements in terms of number of active substances detected and their level in fruit at harvest, obviating the need for active ingredient rotation for resistance management. According to the results, methoxyfenozide was the least effective insecticide, obtaining significantly lower mortality than in S-Spain in all the field populations tested, regardless of their origin. The product is not registered in France against codling moth,<sup>33</sup> but both the French populations tested were tolerant to the insecticide. The highest methoxyfenozide RRs were found in field populations from Catalonia but this active ingredient was hardly used in the area. This finding may suggest a cross-resistance with organophosphates, particularly azinphos-methyl (heavily used before 2008) and phosmet, which was suitably demonstrated in previous works.<sup>12,19</sup> Cross-resistance of methoxyfenozide with organophosphates has been proven by several authors in codling moth (in North Carolina (USA)<sup>23</sup>, in Michigan (USA)<sup>24</sup> and in Canada<sup>25</sup>) and other tortricid pests (obliquebanded leafroller, *Choristoneura rosaceana* (Harris) in New York (USA)<sup>34</sup> and Michigan (USA)<sup>35</sup>, and *Planotortrix octo* Dugdale in New Zealand).<sup>36</sup> The Spanish field populations resistant to methoxyfenozide were susceptible to tebufenozide but the French population tolerant to methoxyfenozide was also tolerant to tebufenozide. Reyes *et al.*<sup>12</sup> suggested cross-resistance between azinphos-methyl and tebufenozide in codling moth field populations from Switzerland, and this cross-resistance has been proven in other tortricids<sup>34,35</sup>, but it was not the case for Spanish field populations. A high selection pressure with IGRs (mainly diflubenzuron) has occurred in southern France since the 1980s and has produced a cross-resistance with tebufenozide, even when it was a new mode of action.<sup>27</sup> Therefore, the tolerance in the tested populations may be due to an intensive use of the product or to a cross-resistance

previously described in the area. Tolerance or resistance to thiacloprid was found in 50% of the field populations. The product is rarely used in apple orchards of the Ebro Valley but it is often applied to control aphids or psylla in pears. The residual activity of the insecticides and the flux of populations between neighboring orchards may also contribute to these results. Brunner *et al.*<sup>37</sup> obtained high levels of mortality of *C. pomonella* neonates even 28 days after the treatment in the field with different neonicotinoids. The possibility of a negative cross-resistance between thiacloprid and chlorpyrifos was also pointed out by Isci and Ay<sup>18</sup> in some field populations from Turkey. The field population PuigverdC showed an 11.2-fold resistance to thiacloprid and an 872.0-fold resistance to lambda-cyhalothrin, and the rest of the populations tolerant to thiacloprid coincided with those tolerant or resistant to lambda-cyhalothrin. Reyes and Sauphanor<sup>38</sup> found a significant decrease in susceptibility to thiacloprid in neonate larvae of laboratory strains resistant to azinphos-methyl, diflubenzuron and the pyrethroid deltamethrin. In Canada and Greece, Grigg-McGuffin *et al.*<sup>25</sup> and Vodouris *et al.*<sup>22</sup>, respectively, obtained low susceptibility to thiacloprid in neonate larvae and fifth-instar diapausing and non-diapausing larvae when the product was recently registered. These field populations, as in the present study, showed tolerance to multiple active ingredients. The Spanish populations of this study were not exposed to indoxacarb (not used to control codling moth) and chlorantraniliprole (registered in Spain at the end of 2011). However, 33% and 36% of the tested populations were tolerant to these products, respectively, although with RR of only 1.2 to 2.2. Some codling moth field populations tolerant to indoxacarb were also found in Michigan (USA)<sup>24</sup> when it was a new compound. No resistance to chlorantraniliprole has been reported in codling moth<sup>21,25</sup>, but a slight reduction in susceptibility was found in some of our field populations when the LC<sub>90</sub> was applied. The three field populations tested

with the LC<sub>50</sub> of chlorantraniliprole (Table 4) were as susceptible as the laboratory population, but when they were treated with the LC<sub>90</sub> (Table 3), one of them (PuigvertC) showed an RR of 1.5. Previous studies found an increase in RR at higher concentrations for some populations of *C. pomonella* and in other Lepidoptera, which can lead a population to be susceptible or tolerant, or tolerant or resistant, according to the discriminant concentration applied.<sup>16,24,39</sup> Sial *et al.*<sup>40</sup> also found some chlorantraniliprole-tolerant field populations of *C. rosaceana* in Washington (USA), and high levels of resistance were reported in some field populations of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in China after only 2 years of intensive use or misuse of the product<sup>41</sup>, showing the importance of a management resistance strategy in pests with a strong ability to develop resistance.

Neither of the insecticides from the spinosyn group, spinetoram (not yet registered in Spain) or spinosad (registered in Spain in 2013), had ever been used against the tested field populations. Two of them were significantly less susceptible to spinetoram than S-Spain, with RR of 1.3 and 2.2, but spinosad was very effective with all the field populations and, in some cases, even more than with S-Spain. Mota-Sanchez *et al.*<sup>24</sup> also found no resistance to spinosad in some field populations from Michigan (USA) that were resistant to the organophosphates azinphos-methyl and phosmet, the pyrethroid lambda-cyhalothrin and the diacylhydrazine methoxyfenozide. In Washington (USA), codling moth field populations that had high tolerance to azinphos-methyl and acetamiprid showed low susceptibility to spinosad and methoxyfenozide when they were not widely use.<sup>21</sup> Sial *et al.*<sup>40</sup> found a high correlation between spinetoram and spinosad resistance at the level of LC<sub>50</sub> in *C. rosaceana* and also suggested the possibility of cross-resistance. In our case, the most tolerant field population to spinetoram (PuigverdC), in which the efficacy of the product was 40%

mortality, was resistant to lambda-cyhalothrin (872.0-fold), methoxyfenozide (14.6-fold) and thiacloprid (11.2-fold), but a second population (Mir7/84), also with high resistance levels to lambda-cyhalothrin (148.1-fold) and methoxyfenozide (15.9-fold), was susceptible to spinetoram, which rules out cross-resistance with these products. In addition, all the populations were susceptible to spinosad, even more than S-Spain. Emamectin (not yet registered in Spain), together with spinosad, was the most effective product, being as effective as in S-Spain in 94% of the field populations treated. In the only tolerant population, SAS, the mortality produced by emamectin was over 70.0%. These results were similar to the ones obtained by Reyes *et al.*<sup>12</sup> with different field populations from European countries, mainly France.

#### 4.2. Synergist bioassays

To evaluate the involvement of the enzymatic systems in insecticide resistance, the maximum concentration of the synergists that produces a minimum larval mortality should be used. We used the concentration of the synergist that produced a maximum mortality of 10% in S-Spain. These values were not perhaps the optimum ones for the field populations suspected, in general, of being less susceptible than S-Spain. Reyes *et al.*<sup>32</sup> found high differences in the concentrations of synergists to apply in laboratory resistant populations compared with a susceptible population (2.7-fold with PBO and DEF, and 80-fold with DEM in the field population) when they were seeking the maximum concentration to apply that did not produce larval mortality. However, obtaining enough progeny to do all the tests is usually a limiting factor for the field populations.

The sensitivity of the S-Spain strain to all the tested insecticides was not significantly modified by the synergists, as happened in other susceptible populations previously tested.<sup>32</sup> The same response was obtained with the ecological field population Coll

when it was treated with any of the synergists tested plus chlorpyrifos-ethyl and lambda-cyhalothrin.

Generalized enhanced levels of the metabolic detoxifying system PSMO were found in several studies of codling moth resistance in field populations from the same Spanish area in adults, post-diapausing larvae and neonate larvae.<sup>19,20</sup> Therefore, a general increase in the mortality produced by the LC<sub>50</sub> of some insecticides was to be expected when PBO was applied, particularly in the pyrethroid and organophosphate treatments, which are proven to be detoxified with PSMO.<sup>20,22,27</sup> Lambda-cyhalothrin obtained the lowest mortalities in both bioassays (LC<sub>90</sub> and LC<sub>50</sub> treatments). However, with the application of the synergist, the mortality produced by lambda-cyhalothrin increased numerically in Noves and Torregrossa but significantly only in Tossal (SR = 17.1), where the resistance to the product was heavily overcome with the previous application of PBO, reaching 51.4% mortality with the LC<sub>50</sub>, so PSMO seems to be the main mechanism involved in this case of resistance. PuigvertC and PuigvertB, which were very resistant to lambda-cyhalothrin, with RRs of 872.0 and 15.4, respectively, did not increase their mortality with the previous application of PBO. This may be because the insects did not receive a sufficient amount of synergist to block their PSMO enzymatic system activity, and they were able to detoxify the insecticide. Increasing concentrations of PBO applied before the application of a diagnostic dose of temephos in the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae) produced a significant mortality increase in a naturally resistant strain<sup>42</sup>, but the lower concentration did not increase the mortality, the same happened with *Rhizopertha dominica* (F.) (Coleoptera: Bostrychidae) and deltamethrin<sup>43</sup>. Two major enzymatic systems are involved in the metabolism of pyrethroid insecticides: PSMO and EST.<sup>44</sup> Only one lambda-cyhalothrin-tolerant field population was tested with the product after being previously treated with DEF,

Torregrossa, and a significant mortality increase was obtained. Previous results also reported a possible involvement of EST in the pyrethroid resistance of codling moth larvae.<sup>22,45</sup>

The only chlorpyrifos-ethyl-tolerant field population, PuigverdC, showed no significant increase with the application of the synergists. DEM and DEF significantly increased the mortality obtained in the field populations Torregrossa (SR = 1.7 and 1.8, respectively) and Noves (SR = 1.1 for both synergists), although in Noves the increases were very low, and DEF also produced mortality increases with SRs of between 1.4 and 1.5 in PuigverdC and PuigverdB. PBO produced a significant increase only in Torregrossa, but the results in the rest of the field populations were very variable, as happened with the application of DEM. Reyes and Sauphanor<sup>38</sup> found a positive correlation between PSMO activity and chlorpyrifos-ethyl tolerance in neonate larvae from resistant laboratory populations, but no correlation with EST and GST in these life stages. These two mechanisms were not involved as a generalized insecticide-resistant mechanism in neonate larvae from some field populations collected in 2006 and 2007 in the production area of Lleida,<sup>20</sup> though both were detected, but were generalized in the post-diapausing larvae.<sup>19,46</sup> By contrast, a significant correlative association between lower EST activity in adults and fifth-instar larvae and resistance to organophosphates was found in European codling moth field populations.<sup>12,19,22,47</sup> These variable results were attributed in some cases to the different affinity for the substrates used in the studies but confirm the results of Reyes *et al.*,<sup>38</sup> who found no correlation for EST activity between the developmental stages neonate larvae, diapausing larvae and adults, and no correlation for GST with the larval stages.

None of the Spanish field populations tested with the synergists were resistant to the new active ingredient emamectin, which was the expected result. By contrast, three of

the five field populations treated with the LC<sub>50</sub> were significantly more susceptible than S-Spain (Table 4). The use of DEM and DEF caused a significantly increased mortality in PuigvertB and Tamarite, which were as susceptible to the product as S-Lleida, so, GST and EST seem to influence the efficacy of the product in these field populations. If the susceptible field populations are exposed to a mixture of synergist+insecticide, the specific detoxification pathway will be blocked and the small proportion of resistant insects to the insecticide will die as if they were susceptible. For this reason, when treating our laboratory susceptible population, we obtained SR lower than 1 in all cases. Nevertheless, DEM had no significant influence in PuigverdC, which was very susceptible to the product (85.2%), even more than the reference susceptible population (55.6%). Reyes *et al.*<sup>12</sup> linked the efficacy reduction of emamectin in some European field populations to EST, despite the fact that it was the most effective product, with a mortality over 83% in all cases. Unfortunately, we had insufficient larvae to test these synergists with the other field populations. PSMO had no effect on the response to the insecticide in four of the five field populations tested, except in PuigvertB, which had a low SR (1.3). Civolani *et al.*<sup>48</sup> suggested that monooxygenases were not responsible for emamectin benzoate detoxification in *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), but opposite results were found with other Lepidoptera.<sup>49,50</sup> The LC<sub>50</sub> concentration of chlorantraniliprole, the other new insecticide for the Spanish field populations, was very effective against the three field populations tested, even in PuigverdC, which was tolerant to the LC<sub>90</sub> concentration. However, the application of PBO and DEM led to a surprising significant decrease in mortality in three and two field populations treated, respectively. In a selected chlorantraniliprole-resistant laboratory population of *C. rosaceana*, EST was responsible for detoxifying the

product<sup>51,52</sup> and the application of DEF increased the mortality even in the susceptible laboratory population, although not significantly.

## **CONCLUSIONS AND FURTHER DIRECTIONS**

Control of *C. pomonella* in Spain is possible with the use of mating disruption combined with reduced-risk insecticides already registered such as tebufenozide, spinosad, indoxacarb and chlorantraniliprole, which have been shown to be effective in controlling resistant populations in the area. As the not yet registered insecticides emamectin and spinetoram are also effective, their registration would be valuable. If a rational strategy of resistance management is to be achieved in IPM production, growers need to have several insecticides at their disposal, and the excessively strict residue requirements of commercial market, far higher than the legal ones, need to be reduced.

The methodology to be applied when trying to determine the enzymatic systems involved in insecticide resistance with synergists need further refinement. One difficulty is the need to adjust the concentrations to be applied for each population and synergist in order to obtain credible information, and another is the interpretation of the results, particularly in field populations, in which co-occurrence of different resistance mechanisms may be present and interactions between them may occur.

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Table 1. *C. pomonella* field population name and origin, year of collection, orchard management system and assay performed with them. The neonate larva generation treated is also indicated.

Name	Country / Region	Collection year	Generation treated	Assay	Management
Puigverd B	<i>Spain / Catalunya</i>	2011	F1	Synergists / bioassays	IPM
Puigverd C	<i>Spain / Catalunya</i>	2011	F1	Synergists / bioassays	IPM
Tossal	<i>Spain / Catalunya</i>	2010	F2	Synergists	IPM
Tossal	<i>Spain / Catalunya</i>	2012	F2	Bioassays	IPM
Torregrossa	<i>Spain / Catalunya</i>	2010	F1	Synergists	IPM
Coll	<i>Spain / Catalunya</i>	2010	F1	Synergists	Ecological
Linyola	<i>Spain / Catalunya</i>	2010	F2	Bioassays	IPM
Paradet	<i>Spain / Catalunya</i>	2010	F2	Bioassays	IPM
SAS	<i>Spain / Catalunya</i>	2011	F2	Bioassays	IPM
Mir7/84	<i>Spain / Catalunya</i>	2011	F1	Bioassays	IPM
Poalbud	<i>Spain / Catalunya</i>	2011	F2	Bioassays	IPM
La Almunia	<i>Spain / Aragón</i>	2010	F2	Bioassays	IPM
Tamarite	<i>Spain / Aragón</i>	2010	F1 / F2	Synergists / bioassays	IPM
ADC	<i>Spain / Aragón</i>	2011	F1	Bioassays	IPM
AstAb	<i>Spain / Asturias</i>	2012	F1	Bioassays	Cider production
AstN	<i>Spain / Asturias</i>	2012	F1 / F2	Bioassays	Cider production
AstC	<i>Spain / Asturias</i>	2012	F1 / F2	Bioassays	Cider production
Le Thor	<i>France / Provence-A.-C.A.</i>	2012	F2	Bioassays	IPM
Pomiers	<i>France / Provence-A.-C.A.</i>	2012	F2	Bioassays	IPM
Noves	<i>France / Provence-A.-C.A.</i>	2010	F1	Synergists	IPM

Table 2. Active ingredients and commercial products. The concentration (mg a.i./L) applied corresponds approximately to the LC<sub>90</sub> and LC<sub>50</sub> of the *C. pomonella* susceptible laboratory population for the insecticide efficacy and the synergist tests, respectively.

Active ingredient	Commercial product - Supplier	Assay	Concentration (mg a.i./L)
Chlorpyrifos-ethyl	Cuspide - 25 % - Comercial Química Massó, Spain	Insecticide bioassay Synergists	90.0
		bioassay	31.3
Lambda-cyhalothrin	Karate Zeon CS - 10 % - Singenta España S.A., Spain	Insecticide bioassay Synergists	0.5
		bioassay	0.1
Chlorantraniliprole	Coragen 20 SC - DuPont Ibérica S.L. , Spain	Insecticide bioassay Synergists	5.0
		bioassay	4.0
Emamectin	Affirm - 0,855 % - SG- Syngenta, Italy	Insecticide bioassay Synergists	0.6
		bioassay	0.3
Methoxyfenozide	Runner CS - 24 % - Dow AgroSciences Ibérica S.A., Spain	Insecticide bioassay	4.0
Tebufenozide	Mimic 2F CS - 24 % - Nisso Chemical Europe GMBH, Germany		
Thiacloprid	Calypso SC - 48 % - Bayer CropScience S.L., Spain	Insecticide bioassay	15.0
Indoxacarb	Steward - 30 % - WP - DuPont Ibérica, S.L., Spain	Insecticide bioassay	40.0
Spinosad	Spintor 480 SC - 48 % - Dow AgroScience, Spain	Insecticide bioassay	16.0
Spinetoram	Delegate - 25 % - Dow AgroSciences, France	Insecticide bioassay	1.0

Table 3. Corrected mortality (%) of insecticides at the diagnostic concentration, LC<sub>90</sub> (mg a.i./L), on *C. pomonella* neonate larvae of the susceptible strain and of field populations. Numbers in parentheses show the number of insects treated. RR = corrected mortality of S-Spain / corrected mortality of the field population. The mortality obtained was compared using  $\chi^2$  (df = 1; \*p = 0.05; \*\*p = 0.01; \*\*\*p = 0.001).

Popul- ation	Active ingredient (mg a.i./L)																			
	Chlorpyrif os-ethyl (90.0)		Lambda- cyhalothrin (0.5)		Methoxyfe nozide (5.0)		Tebufenoz ide (20.0)		Thiaclopri d (15.0)		Indoxacar b (40.0)		Spinosad (16.0)		Spinetora m (1.0)		Chlorantran iliprole (5.0)		Emamecti n (0.6)	
	C. Mort. (%)	R R	C. Mort. (%)	R R	C. Mort. (%)	R R	C. Mort. (%)	R R	C. Mort. (%)	R R	C. Mort. (%)	R R	C. Mort. (%)	R R	C. Mort. (%)	R R	C. Mort. (%)	RR	C. Mort. (%)	R R
S- Spain- 12	93.8 (33) a		87.2 (71) a		97.1 (36) a		86.4 (37) a		91 (35) a		97.1 (36) a		81.9 (44) a		88.1 (35) a		88.5 (71) a		95.7 (71) a	
S- Spain- 13	95.5 (90) a		93.7 (48) a		84.2 (96) b		97.9 (47) a		93.7 (48) a		95.8 (48) a		86.1 (95) a		88.2 (50) a		89.2 (48) a		86.6 (99) a	
S- Spain- 14	91.5 (87) a		93.3 (45) a		92.4 (46) a		89.2 (81) a				97.8 (47) a		86.6 (47) a		95.1 (45) a				88.9 (48) a	
SAS	100.0 (35) ns	0. 9	30.4 (26) ***	2.9	22.2 (36) ***	4. 4	75.0 (36) ns	1. 2	59.7 (34) **	1. 5	87.8 (34) ns	1. 1	94.4 (36) ns	0. 9	91.7 (36) ns	1. 0	40.6 (32) ***	2.2	70.6 (35) ***	1. 4
Puigve rdB	85.7 (36) ns	1. 1	5.7 (37) ***	15. 4	64.5 (70) ***	1. 5	97.1 (36) ns	0. 9	68.6 (72) **	1. 3	94.3 (71) ns	1. 0	97.1 (36) *	0. 8	69.8 (74) *	1. 3	82.9 (36) ns	1.1	94.3 (36) ns	1. 0
Poalbu d	85.6 (35) ns	1. 1	71.5 (32) ns	1.2	65.9 (35) ***	1. 5	97.2 (36) ns	0. 9	94.4 (36) ns	1. 0			100.0 (29) *	0. 8	100.0 (36) *	0. 9	85.1 (34) ns	1.0	97.2 (36) ns	1. 0

PuigverdC	42.4 (36) ***	2. 2	0.0 (35) ***	87 2.0	6.6 (35) ***	14 .6	68.0 (27) ns	1. 3	8.1 (33) ***	11 .2	75.8 (37) **	1. 3	86.1 (32) ns	1, 0	40.4 (30) ***	2. 2	60.1 (32) ***	1.5	93.9 (33) ns	1. 0
Mir7/84	72.0 (34) *	1. 3	0.6 (35) ***	14 8.1	6.1 (36) ***	15 .9	93.9 (34) ns	0. 9	36.4 (36) ***	2. 5	79.2 (32) *	1. 2	68.1 (37) ns	1. 2	96.0 (26) ns	0. 9	93.9 (35) ns	0.9	93.9 (36) ns	1. 0
Linyola	100.0 (48) ns	0. 9	82.7 (35) ns	1.1					77.2 (32) ns	1. 2						91.3 (36) ns	1.0	91.3 (39) ns	1. 0	
Tossal			18.8 (48) ***	5.0	17.6 (43) ***	5. 3										97.9 (48) n.s.	1.0	77.1 (48) ns	1. 2	
Paradet	100.0 (36) ns	0. 9	69.2 (35) *	1.3														100.0 (16) ns	1. 0	
ADC	100.0 (35) ns	0. 9	11.4 (35) ***	7.7	30.6 (36) ***	3. 2	97.2 (36) ns	0. 9	29.6 (34) ***	3. 1	66.7 (36) ***	1. 5	97.2 (36) *	0. 8	86.1 (36) ns	1. 0	62.1 (34) **	1.4	85.4 (34) ns	1. 1
Tamarite	88.6 (36) ns	1. 1	96.9 (33) ns	0.9					100.0 (16) ns	0. 9						88.6 (36) ns	1.0	91.4 (36) ns	1. 0	
La Almunia	88.6 (31) ns	1. 1							87.0 (37) ns	1. 0								94.2 (38) ns	1. 0	
AstAb	92.6 (72) ns	1. 0	100.0 (69) *	0.9					100.0 (36) ns	0. 9							100.0 (97) **	0.9	87.5 (39) ns	1. 0
AstN	84.7 (48) *	1. 1	98.0 (48) ns	1.0	63.7 (46) **	1. 3					97.8 (41) ns	1. 0			80.3 (46) ns	1. 1	85.5 (48) ns	1.0	82.5 (48) ns	1. 0
AstC	95.0 (91) ns	1. 0	100.0 (49) ns	0.9	79.8 (89) *	1. 2	95.3 (47) ns	0. 9	91.6 (25) ns	1. 0	99.0 (96) ns	1. 0	93.5 (45) ns	0. 9	92.2 (140) ns	1. 0	93.1 (45) ns	1.0	96.8 (96) ns	0. 9

Pompi ers	35.9 (47) ***	2.6	28.1 (38) ***	3. 0			53.5 (45) ***	1. 8	85.6 (49) ns	1. 1			85.7 (86) ns	1. 0	1.6	95.6 (47) ns	0. 9
Le Thor	53.7 (48) ***	1.7	56.4 (45) ***	1. 5	69.2 (39) ***	1. 4	70.1 (46) **	1. 3	98.2 (50) ns	1. 0	82.2 (44) ns	1. 0	76.0 (92) ns	1. 2	1.4	96.0 (48) ns	0. 9

Table 4. Corrected mortality (%) of insecticides at the diagnostic concentration,  $LC_{50}$  (mg a.i./L), on *C. pomonella* neonate larvae of the susceptible strain and of field populations. Numbers in parentheses show the number of insects treated. RR = corrected mortality of S-Spain / corrected mortality of the field population. The mortality obtained was compared using  $\chi^2$  (df = 1; \*p = 0.05; \*\*p = 0.01; \*\*\*p = 0.001).

Population	Insecticide (mg a.i./L)								
	Control	Chlorpyrifos- ethyl (31.3)		Lambda-cyhalothrin (0.1)		Emamectin (0.3)		Chlorantraniliprole (4.0)	
	Mort. (%)	C. Mort. (%)	RR	C. Mort. (%)	RR	C. Mort. (%)	RR	C. Mort. (%)	RR
<b>S-Spain-11</b>	3.79 (107)	56.56 (70)		69.07 (72)		55.57 (72)		69.69 (72)	
<b>PuigverdB</b>	2.78 (35)	54.29 (36) n.s.	1.0	0.00 (33) ***	62.8	66.75 (34) n.s.	0.8	80.04 (65) n.s.	0.9
<b>PuigvertC</b>	6.25 (48)	33.74 (34) *	1.7	23.72 (37) ***	2.9	85.19 (35) **	0.7	80.00 (46) n.s.	0.9
<b>Torregrossa</b>	11.11 (36)	43.75 (36) n.s.	1.2	46.88 (36) *	1.5	90.63 (36) ***	0.6		
<b>Coll</b>	5.56 (36)	67.65 (36) n.s.	0.8	85.29 (36) n.s.	0.8				
<b>Tossal</b>	0.00 (36)	75.00 (36) n.s.	0.7	3.03 (35) ***	22.8	97.22 (33) ***	0.6		
<b>Noves</b>	5.56 (36)	88.24 (36) ***	0.6	53.21 (36) n.s.	1.3				
<b>Tamarite</b>	2.78 (36)	54.29 (36) n.s.	1.0	58.10 (36) n.s.	1.2	62.86 (36) n.s.	0.9	62.86 (80) n.s.	1.1

Table 5. Effect of metabolic synergists on *C. pomonella* neonate larvae expressed as corrected mortality (%) of 4 insecticides at the diagnostic concentration, LC<sub>50</sub> (mg a.i./L) of the susceptible strain. Numbers in parentheses show the number of insects treated. SR = synergistic ratio = corrected mortality with synergist / corrected mortality without synergist. The mortality obtained was compared using  $\chi^2$  (df = 1; \*p = 0.05; \*\*p = 0.01; \*\*\*p = 0.001).

Population	Chl-e	PBO synergist		DEM synergist		DEF synergist	
		Chl-e + PBO	SR	Chl-e + DEM	SR	Chl-e + DEF	SR
<b>S-Spain</b>	56.6 (70)	45.5 (36) n.s.	0.8	48.3 (36) n.s.	0.9	46.1 (36) n.s.	0.8
<b>PuigverdB</b>	54.3 (36)	60.8 (35) n.s.	1.1	43.5 (58) n.s.	0.8	75.0 (37) n.s.	1.4
<b>PuigvertC</b>	33.7 (34)	27.0 (29) n.s.	0.8	26.3 (31) n.s.	0.8	52.1 (34) n.s.	1.5
<b>Torregrossa</b>	43.8 (36)	87.1 (36) ***	2.0	76.5 (36) **	1.7	79.4 (36) **	1.8
<b>Coll</b>	67.7 (36)	78.0 (36) n.s.	1.2	56.3 (36) n.s.	0.8	77.8 (36) n.s.	1.1
<b>Tossal</b>	75.0 (36)	60.0 (36) *	0.8				
<b>Tamarite</b>	54.3 (36)	62.9 (36) n.s.	1.2	69.7 (36) n.s.	1.3	56.3 (36) n.s.	1.0
<b>Noves</b>	88.2 (36)	97.2 (36) n.s.	1.1	100.0 (35) *	1.1	100.0 (34) *	1.1
	$\lambda$ -cyhal	$\lambda$ -cyhal + PBO	SR	$\lambda$ -cyhal + DEM	SR	$\lambda$ -cyhal + DEF	SR
<b>S-Spain</b>	69.1 (72)	62.5 (36) n.s.	0.9	65.5 (36) n.s.	0.9	62.9 (36) n.s.	0.9
<b>PuigverdB</b>	0.00 (33)	0.0 (34) n.s.	1.0	6.2 (35) n.s.			
<b>PuigvertC</b>	23.7 (37)	27.0 (29) n.s.	1.1				
<b>Torregrossa</b>	46.9 (36)	64.5 (36) n.s.	1.4	67.7 (36) n.s.	1.4	73.5 (36) *	1.6

<b>Coll</b>	85.3 (36)	74.9 (36) n.s.	0.9	93.8 (36) n.s.	1.1		
<b>Tossal</b>	3.0 (35)	51.4 (36) ***	17.1				
<b>Tamarite</b>	57.1 (34)	51.4 (34) n.s.	0.9	48.5 (36) n.s.	0.8	53.1 (36) n.s.	0.9
<b>Noves</b>	53.2 (34)	74.2 (34) n.s.	1.4	63.1 (34) n.s.	1.2		
	<b>Emam</b>	<b>Emam + PBO</b>	<b>SR</b>	<b>Emam + DEM</b>	<b>SR</b>	<b>Emam + DEF</b>	<b>SR</b>
<b>S-Spain</b>	55.6 (72)	48.9 (36) n.s.	0.9	48.3 (36) n.s.	0.9	49.4 (36) n.s.	0.9
<b>PuigverdB</b>	66.8 (34)	87.5 (33) *	1.3	97.1 (36) **	1.5	91.4 (36) *	1.4
<b>PuigvertC</b>	85.2 (35)	100.0 (12) n.s.	1.2	90.0 (34) n.s.	1.1		
<b>Torregrossa</b>	90.6 (36)	83.9 (36) n.s.	0.9				
<b>Tossal</b>	97.2 (33)	97.1 (36) n.s.	1.0				
<b>Tamarite</b>	62.9 (36)	65.7 (36)n.s.	1.0	100.0 (36) ***	1.6	100.0 (36) ***	1.6
	<b>Ryn</b>	<b>Ryn + PBO</b>	<b>SR</b>	<b>Ryn + DEM</b>	<b>SR</b>	<b>Ryn + DEF</b>	<b>SR</b>
<b>S-Spain</b>	69.7 (72)	59.1 (36) n.s.	0.8	62.1 (36) n.s.	0.9	59.6 (36) n.s.	0.9
<b>PuigverdB</b>	80.0 (65)	59.0 (56) *	0.7	50.1 (58) ***	0.6	83.8 (63) n.s.	1.0
<b>PuigvertC</b>	80.0 (46)	53.7 (51) **	0.7	71.4 (34) n.s.	0.9	71.5 (34) n.s.	0.9
<b>Tamarite</b>	62.9 (80)	42.9 (36) *	0.7	39.4 (36) *	0.6	53.1 (36) n.s.	0.8