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1 **Determination of the baseline susceptibility of European populations of *Cydia***  
2 ***pomonella* (Lepidoptera: Tortricidae) to chlorantraniliprole and the role of**  
3 **cytochrome P450 monooxygenases.**

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14 Short title: Baseline susceptibility of *Cydia pomonella* to chlorantraniliprole

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## Abstract

21 The codling moth, *Cydia pomonella*, (L.) (Lepidoptera: Tortricidae) is the key pest on  
22 pome fruit and walnut orchards worldwide. Its resistance to available insecticides has  
23 been widely reported. Chlorantraniliprole is an anthranilic diamide that was introduced  
24 in European countries in 2008-2009 and acts by activating the insect's ryanodine  
25 receptors.

26 The aims of this study were to determine the baseline susceptibility of European  
27 populations of *C. pomonella* to chlorantraniliprole, to establish the discriminant  
28 concentrations (DC) to check the possible development of resistance, and to know the  
29 role of cytochrome P450 monooxygenases (P450) in the possible susceptibility decrease  
30 of field populations to the insecticide.

31 Ten field populations from Spain along with others were used to calculate the baseline  
32 response of larvae to chlorantraniliprole incorporated into the diet. A pooled probit line  
33 was calculated and three DC were established: 0.3 mg a.i./kg (close to the LC<sub>50</sub>), 1.0 mg  
34 a.i./kg (close to the LC<sub>90</sub>), and 10 mg a.i./kg diets (three-fold the LC<sub>99</sub>). The DC were  
35 used to test the susceptibility of 27 field populations from France, Germany, Hungary,  
36 Italy and Spain. The corrected mortality observed in all cases ranged within the expected  
37 interval, even with Spanish populations that showed between 12.1% and 100.0% of  
38 individuals with high P450 activity. However, the mortality caused by the DC<sub>0.3</sub>  
39 decreased as the mean P450 activity increased. Field populations resistant to other  
40 insecticides were susceptible to chlorantraniliprole. The determined baseline codling  
41 moth susceptibility is a valuable reference for tracking possible future alterations in the  
42 efficacy of the insecticide.

43 KEY WORDS: Codling moth, chlorantraniliprole, baseline, resistance monitoring

## Introduction

45 The codling moth (CM), *Cydia pomonella*, (L.) (Lepidoptera: Tortricidae) is the key pest  
46 on pome fruit and walnut orchards in almost all the areas where these crops are cultivated.  
47 At present, its control is mostly achieved by a combination of pesticides and mating  
48 disruption (Witzgall et al., 2008; Ioriatti & Lucchi 2016). The existence of CM  
49 insecticide-resistant populations is a widespread problem in many pome fruit production  
50 areas in the world, and it concerns 10 out of the 11 insecticide modes of action available  
51 to control this pest, depending on the country (IRAC Codling Moth WG, 2013), which  
52 are: 1A – carbamates, 1B – organophosphates, 3A – pyrethroids, 4A – neonicotinoids, 5  
53 – spinosyns, 6 – avermectins, 7B – phenoxy-ethyl carbamates, 15 – benzoylureas, 18 –  
54 diacylhydrazines and 22A oxadiazines (Sauphanor et al., 1998, 2000; Dunley and Welter,  
55 2000; Reuveny & Cohen, 2004; Fuentes-Contreras et al., 2007; Ioriatti et al., 2007; Stará  
56 & Kocourek, 2007; Knight, 2010; Rodríguez et al., 2010, 2011, 2012; Voudouris et al.,  
57 2011; Grigg-McGuffin et al., 2015; Iscy and Ay, 2017). Codling moth resistance to  
58 insecticides is mainly metabolic, due to three enzymatic complexes (cytochrome P450  
59 monooxygenases (P450), glutathione transferases (GST) and esterases (EST))  
60 ([www.ircac-online.org](http://www.ircac-online.org)), but mutations in the insecticide target site protein have also been  
61 detected (the acetylcholinesterase (*AChE*) mutation, only reported in the fruit growing  
62 area of Lleida (Spain) (Cassanelli et al., 2006), and the knockdown resistance (*kdr*)  
63 mutation (Brun-Barale et al., (2005)). For example, when compared to non-chemically  
64 treated orchards, an increase of the frequency of resistant codling moth individuals has  
65 been detected in many chemically treated orchards in the tree fruit area of Lleida, and  
66 enhanced P450 activity was the main enzymatic mechanism involved (Rodríguez et al.,  
67 2010, 2011; Bosch et al., 2016). However, enhanced GST and EST activity was also  
68 detected (Rodríguez et al., 2011), as the presence of *AChE* and *kdr* mutations (Bosch et

69 al., 2014). Metabolic cross-resistance is a big concern in any management resistance  
70 strategy, which may occur either at the level of interaction with the various chemical  
71 families or at a geographical level (Dunley and Welter, 2000; Reyes et al., 2007;  
72 Voudouris et al., 2011; IRAC Codling Moth WG, 2013). New pesticides with new modes  
73 of action and with an environmentally safe toxicological profile are then necessary to  
74 control resistant CM populations.

75 Chlorantraniliprole (Rynaxypyr®) and cyantraniliprole (Cyazypyr®), developed by  
76 DuPont (USA), are anthranilic diamides whose mode of action has been classified in the  
77 new insecticide group 28 (ryanodine receptor modulator) within the IRAC (Insecticide  
78 Resistance Action Committee) mode of action classification scheme (Nauen, 2006). By  
79 activating the insect ryanodine receptors (RyRs), they stimulate the release and  
80 depletion of intracellular calcium stores from the sarcoplasmic reticulum of muscle  
81 cells, causing impaired muscle regulation, paralysis and, ultimately, death (Cordova et  
82 al., 2006). Chlorantraniliprole acts primarily by ingestion and by contact on the larvae  
83 of chewing pests. Newly hatched larvae from treated eggs die when eating the chorion  
84 at emergence. It has extremely broad-spectrum effectiveness within the insect order  
85 Lepidoptera and some Coleoptera, Diptera and Isoptera, acting in a broad range of crops  
86 and showing very low mammalian toxicity and high selectivity to non-target arthropods  
87 (DuPont™, 2008; Lahm et al, 2009). Chlorantraniliprole is active on the codling moth,  
88 and it has been registered in European countries since 2008. Due to the potential of *C.*  
89 *pomonella* to develop resistance to insecticides, it is necessary to establish a baseline  
90 susceptibility database before or early after the introduction of a new insecticide to the  
91 market (Roditakis et al., 2013). The database has to include data from a wide  
92 geographical area, to know the natural variability of the response to the chemical in

93 field populations, and it has to consider the state of the insecticide resistance in every  
94 area.

95 The aims of this study were to determine the baseline susceptibility of European codling  
96 moth populations to chlorantraniliprole, to establish the discriminant concentrations  
97 (DC), to check the possible development of resistance in the field, and to understand the  
98 possible role of P450 in the lack of susceptibility of field populations to this insecticide.

99

100

## **Material and methods**

101

### *Experimental protocol outline*

102 In our study, the concentration – response to chlorantraniliprole lines (mortality vs.  
103 concentration) by ingestion were first determined for eight Spanish field populations  
104 collected from 2007 to 2009. These data along with others obtained from populations in  
105 Italy, France, Netherlands and Belgium and tested since 2005 in Italy were used by  
106 DuPont® to calculate the Discriminant Concentrations (DC). Values close to the LC<sub>50</sub>,  
107 LC<sub>90</sub>, and to three times the LC<sub>99</sub> were chosen as the DC. These concentrations were then  
108 applied to 27 European populations collected from 2008 to 2015 to test their  
109 susceptibility. Two more concentration – response lines were later determined for two  
110 more Spanish populations collected in 2010. Their DC results were within the range of  
111 the ones calculated previously and all the probit line data were pooled to calculate a  
112 common probit line. Additionally, the probit line of chlorantraniliprole was also  
113 calculated for a Spanish susceptible strain (S-Spain) whose response to several  
114 insecticides is well known. Finally, the activity of P450 was measured for 12 field  
115 populations and for the laboratory susceptible strain.

116

118 The list of the CM field populations used in the bioassays is shown in Table 1. The 10  
119 Spanish populations used to adjust probit lines were collected from 2007 to 2010 in the  
120 Ebro Valley pome fruit production area (Catalonia and Aragon, NE of Spain) when the  
121 product was not registered. The 27 populations tested with the DC were collected from  
122 2008 to 2015 in the pome fruit growing areas of France, Germany, Hungary, Italy, and  
123 Spain. They were collected before or after the registration of chlorantraniliprole, or from  
124 orchards that had not been sprayed with it.

125 Except in one case (Jun 3-63 (09), that was collected from an abandoned orchard), the  
126 Spanish and most of the rest of the European field populations originated from  
127 commercial IPM orchards. The pest management strategy with chlorantraniliprole was  
128 the same in all countries, once the product was registered: only two applications per  
129 season on one single generation, preferable first generation. Most of the CM field  
130 populations were collected as diapausing larvae using corrugated cardboards installed in  
131 the field from July–August until October, but in some cases injured apples were collected  
132 or corrugated cardboards were installed in the field to obtain non diapausing larvae or  
133 both. When it was needed, the populations were reared until their second generation (F2)  
134 to have enough progeny to carry out the bioassays (Table 1).

135 The susceptible CM strain, S-Spain, was collected from an abandoned apple orchard in  
136 Lleida in 1992, and it has been reared since then using a semi-artificial dehydrated apple  
137 diet at the joint IRTA and UdL laboratory (Lleida, Spain). Its response to many  
138 insecticides, its P450, GST and EST enzymatic activity and the presence of the *AChE*  
139 mutation are well known (Rodríguez et al., 2010; Rodríguez et al., 2011; Bosch et al.,  
140 2014).

141 The P450 activity in CM adults was determined for 12 field populations from the Ebro  
142 Valley (Spain). Codling moth adults from the first flight were captured from seven  
143 orchards in pheromone traps in 2009 and 2010 (Table 1). As for the other five populations  
144 collected in 2010 and 2011, the P450 activity was measured on adults emerging from the  
145 collected diapausing larvae.

146 *Insecticide and bioassays experimental procedure*

147 Chlorantraniliprole was used as DPX-E2Y45 20SC (Coragen® 20SC, DuPont de  
148 Nemours France SAS, Nambenheim, France) and a Stonefly Premix® (Stonefly Industries  
149 LTD) lyophilized diet was used as feeding substrate in the bioassays, following the IRAC  
150 susceptibility test method 017 ([www.illac-online.org](http://www.illac-online.org)) To complement the diet, CD  
151 International BA-128 multiwell plastic trays (each well of 15.9 mm diameter and 15.9  
152 mm deep) and lids were used. Codling moth neonate larvae (less than 24-h old) were  
153 exposed to chlorantraniliprole in diet incorporated assays.

154 To calculate the baseline probit lines 20 grams of diet were mixed with 60 g of the  
155 insecticide solution at given concentrations ranging from 0.01 to 1.0 mg a.i./kg of diet at  
156 approximately 3x series dilution. Given the results, a second series was performed to get  
157 a better fitting of the probit line. Distilled water was used as a solvent. Each well was  
158 filled with 0.5 g of treated diet, and the diet was pressed to be evenly distributed across  
159 the bottom. One neonate larvae per well was placed on the treated diet. At least three  
160 replications of 16 larvae were tested per each concentration. The trays were incubated at  
161  $22 \pm 1$  °C, 16:8 h (L:D) and 45% humidity. Larval mortality was assessed after four days.  
162 Larvae were considered dead when they did not move after a light touch with a brush or  
163 when they were moribund. Moribund larvae were those visibly affected and significantly  
164 different from normal ones; when they were probed and flipped on their back, the larva  
165 could not flip back right-side up, or, when it was able to do it, it did so with uncoordinated



166 and slow movements. Only data in which control mortality was <20% were analyzed.  
167 Missing larvae were subtracted from the initial number.  
168 Once the DC were calculated, to test the susceptibility of 27 European field populations  
169 the same procedure was followed.

#### 170 *P450 enzymatic activity*

171 The adult P450 activity was determined in the susceptible strain S-Spain and in 12 field  
172 populations (Table 1) with an *in vivo* protocol (Rodríguez et al. 2012) using 7-ethoxy-  
173 coumarin-O-deethylation (ECOD) in a black microplate of 96 wells. The dissected  
174 abdomens of the adults were placed individually in a well containing 100 µL of phosphate  
175 buffer (50 mM, pH 7.2) and 7-ethoxycoumarin (0.4 mM). After 4 h of incubation at 30  
176 °C, the reaction was stopped by adding 100 µL of glycine buffer (pH 10.4, 10<sup>-4</sup> M) with  
177 ethanol (v/v). Before the incubation, a minimum of 10% of the wells of each microplate  
178 were used as controls and received the glycine buffer to stop the reaction. The ECOD  
179 activity was measured by fluorescence with a 380 nm excitation filter and 465 nm  
180 emission filters and was expressed as pg of 7-ethoxycoumarin (7OH).insect<sup>-1</sup>.min<sup>-1</sup>.

#### 181 *Data analysis*

182 To calculate the baseline a probit analysis using the program POLO Plus (LeOra  
183 Software, 1987) was performed, and the LC<sub>50</sub>, the LC<sub>90</sub> and their 95% fiducial limits were  
184 calculated. Two LC<sub>50</sub> were considered significantly different when their fiducial limits  
185 did not overlap (Robertson *et al.* 2007). The resistant ratio (RR) relative to the most  
186 susceptible field population (RR-F<sub>50</sub>) and the resistance ratio relative to the susceptible  
187 laboratory strain (RR-L<sub>50</sub>) were calculated for the LC<sub>50</sub>. Values close to the LC<sub>50</sub>, to the  
188 LC<sub>90</sub>, and to three times the LC<sub>99</sub> were chosen as the DC. The highest DC was expected  
189 to kill approximately 100% of larvae of the susceptible population, but a smaller  
190 percentage when applied to a resistant population (ffrench-Constant and Roush, 1990).

191 To evaluate the susceptibility of the European field populations the corrected mortality  
192 produced by the DC was calculated using Abbott's formula (Abbott, 1925), being the  
193 correction factor the mortality produced by the control treatment (water). The RR of each  
194 population for every DC was calculated by dividing the mortality of the most susceptible  
195 field population by the mortality produced in every field population strain (RR-F<sub>0.3</sub>, RR-  
196 F<sub>1.0</sub> and RR-F<sub>10.0</sub>). Mean  $\pm$  SEM of the corrected mortality produced by the DCs and the  
197 coefficient of variation (CV) across all the field populations to evaluate the dispersion of  
198 the data were calculated.

199 The frequency of P450-resistant individuals in every CM field population analysed was  
200 compared to the susceptible S-Spain using a Pearson chi-square ( $\chi^2$ ) test. Moths were  
201 classified as resistant if their P450 enzyme activity exceeded the highest activity value  
202 corresponding to 90% of S-Spain individuals (Reyes et al., 2007). Regression lines  
203 between the mean P450 activity (pg 7OH insect<sup>-1</sup> min<sup>-1</sup>) and the LC<sub>50</sub>, and between the  
204 P450 activity and the mortality produced by the DC<sub>0.3</sub> for each population were  
205 calculated. Only the orchards with more than 20 adults analyzed were taken into account  
206 for the regression lines.

207

208

## Results

209 The results of the probit analysis and the RR are shown in Table 2. The LC<sub>50</sub> values for  
210 the CM field populations ranged from 0.161 to 0.446 mg a.i./kg diet. Some significant  
211 differences were found among the field populations, namely Torref 15-3 (SP) (08), Bal  
212 2-480 (SP) (08) and SAS (SP) (09) were significantly less susceptible than five field  
213 populations, and Riud (SP) (10) was significantly more susceptible than four field  
214 populations. One population, Torreg 11-160 (SP) (08), showed no significant differences

215 with any of the other field populations. Jun 3-63 (SP) (09), from an abandoned orchard,  
216 showed no significant differences with six of the ten tested field populations. The RR-  
217  $F_{50}$ , calculated comparing each  $LC_{50}$  value with the  $LC_{50}$  value of the most susceptible  
218 field population (Riud (SP) 10, 0.161 mg a.i./kg diet), ranged from 1.1 to 2.8.. The  
219 laboratory susceptible strain, S-Spain, was significantly more susceptible to  
220 chlorantraniliprole than any field population and it was not included in the calculation of  
221 the pooled LCs values. The RR- $L_{50}$ , calculated comparing each  $LC_{50}$  value with the  $LC_{50}$   
222 value of the laboratory strain (S-Spain, 0.086 mg a.i./kg diet) ranged from 1.9 to 5.2. SAS  
223 (SP) (09) had a high slope ( $4.860 \pm 0.557$ ) suggesting a more homogeneous response than  
224 the other populations and a narrow concentration range between the  $LC_{50}$  and  $LC_{90}$ . The  
225 same happened with Riud (SP) (10), S-Spain and Tam (SP) (10) that had parallel slopes  
226 to SAS (SP) (09) ( $\chi^2 = 3.34$ , d.f. = 3,  $P < 0.343$ ). Torreg 11-160 (SP) (08) had the lowest  
227 slope ( $1.371 \pm 0.168$ ) compared to the next smallest one, Torref 15-3 (SP) (08) ( $\chi^2 = 4.80$ ,  
228 d.f. = 1,  $P < 0.028$ ). Due to its low slope Torreg 11-160 (SP) (08) had a very wide interval  
229 of concentrations between  $LC_{90}$  and  $LC_{50}$  and thus, had high intrapopulation variability  
230 and it did not present significant differences with any other field population. The  $LC_{90}$   
231 values for the CM field populations ranged from 0.339 to 2.788 mg a.i./kg diet.

232 The  $LC_{50}$  value of the pooled data was 0.250 mg a.i./ kg of diet, the  $LC_{90}$  was 0.888 mg  
233 a.i./ kg of diet and the  $LC_{99}$  was 3.323 mg a.i./ kg of diet. These results are according to  
234 the DC selected to test the susceptibility of CM field populations to chlorantraniliprole:  
235 0.3 and 1.0 mg a.i./kg diets, approximately the  $LC_{50}$  and the  $LC_{90}$ , respectively, and 10  
236 mg a.i./kg diet which corresponded to three-fold the  $LC_{99}$  value of the pooled data probit  
237 line results.

238 Table 3 shows the corrected mortality and the resistance ratio relative to the most  
239 susceptible field population (RR-Fs) obtained with each DC for the European CM field

240 populations. All the DC applied produced a 100% of mortality on the susceptible  
241 population S-Spain, as expected according to its probit line results (Table 2). Feeding the  
242 European field populations larvae with concentrations of 0.3, 1.0 and 10.0 mg a.i./kg diet  
243 produced mean corrected mortalities of 70.82%, 96.44% and 99.85%, respectively. The  
244 CV were low for 0.3 mg a.i./kg diet (31.17), and very low for 1.0 (7.21) and 10.0 mg  
245 a.i./kg diets (0.52). The RR-F<sub>0.3</sub> ranged between 1.1 and 4.8, and between 1.0 and 1.5 for  
246 the other two DC.

247 The P450 enzymatic activity of CM adults and the relative frequency of resistant  
248 individuals (those whose P450 activity was higher than 23.92 pg 7OH adult<sup>-1</sup> min<sup>-1</sup>) are  
249 shown in Table 4. The mean P450 activity ranged from 10.52 to 74.61 pg 7OH insect<sup>-1</sup>  
250 min<sup>-1</sup>, and the frequency of resistant individuals in the field populations ranged from  
251 12.1% to 100.0% (populations Jun 3-63 (SP) (09) and Balaguer 2-480 (SP) (08) for both  
252 variables, respectively). The variance of the frequency of resistant insects is strongly  
253 explained by the P450 mean activity of the field populations ( $R^2 = 95.32$ ), which implies  
254 that there are few individuals with a very high resistance level that strongly influence the  
255 mean P450 value (Figure 1). Although data were restricted to four field populations, the  
256 regression line between the frequency of resistant insects and the LC<sub>50</sub> values showed a  
257 high coefficient of determination ( $R^2 = 89.77$ , Figure 2). When the regression line was  
258 adjusted between the P450 mean activity and the mortality produced by the DC<sub>0.3</sub>, the  
259 coefficient of determination was lower than in the other cases, but it explained the 69.82%  
260 of the variance (Figure 3). The regression lines obtained with the higher DCs (1.0 mg  
261 a.i./kg of diet and 10.0 mg a.i./kg of diet) hadn't good adjustments due to the high  
262 mortalities obtained in all the field populations treated.

263

264

## Discussion

265

266 To determine the DC, populations collected before the registration of chlorantraniliprole  
267 were used. The first European countries to get the product registered on pome fruits  
268 were Romania in 2008 and Italy in 2009, and in Spain the product was registered in  
269 2011. The CM Spanish field populations showed a high and relatively uniform  
270 susceptibility to chlorantraniliprole. This is likely because of the RR relative to the most  
271 susceptible population (RR-F<sub>50</sub>) was lower than 2.8, even though significant differences  
272 on the LC<sub>50</sub>s were observed among populations (Mota-Sanchez et al., 2002). All the  
273 field populations, even the abandoned orchard Jun 3-63 (SP) (09), were significantly  
274 less susceptible to chlorantraniliprole than the laboratory strain, S-Spain. French-  
275 Constant and Roush (1990) stated that susceptible strains held for long periods in the  
276 laboratory may bear little resemblance to susceptible strains currently found in the field.  
277 Thus, it would be more useful to determine the appropriate DC on the basis of field  
278 strains before wide commercial introduction of the specific pesticide. Therefore, to  
279 establish a useful baseline to know the natural response variability to the insecticide in  
280 the field populations, the laboratory susceptible strain was not included in the pooled  
281 data to determine the DCs. Nevertheless, once the product is applied in the field,  
282 susceptibility of insect populations might reduce over time due to potential resistance  
283 evolution (French-Constant and Roush, 1990). In this case, susceptible strains can be  
284 helpful as an invariable mortality reference point, which are commonly used (Zheng et  
285 al., 2011; Lai et al., 2011; da Silva, 2012; Caballero et al., 2013).

286 The LC<sub>50</sub> values obtained varied between 0.086 mg a.i./kg of diet (S-Spain) and 0.446  
287 mg a.i./kg of diet (Torref 15-3 (SP) (08)), and are equivalent to 0.110 mg a.i./L and 0.59  
288 mg a.i./L, respectively. The same insecticide diet incorporation bioassay obtained an  
289 LC<sub>50</sub> value range in different laboratory susceptible strains of different species of

290 Lepidoptera: 0.014 mg a.i./L in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)  
291 (Lai et al., 2011) and 0.28 mg a.i./L in *Choristoneura rosaceana* (Harris) (Lepidoptera:  
292 Tortricidae) (Sial and Brunner, 2012). Different species respond in a different way to  
293 the same insecticide, independently of the body mass differences. For example, CM is  
294 heavier than *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) but  
295 females of CM were 115 times more susceptible to thiacloprid than females of *L.*  
296 *botrana* were (Navarro et al., 2017). In the laboratory, where the larval feeding behavior  
297 is conditioned, these different responses are mainly due to that different species may  
298 have different metabolic procedures or detoxification methods (Rodríguez et al., 2012).  
299 The highest resistance ratio relative to the laboratory strain of S-Spain (RR-L<sub>50</sub>) was 5.2  
300 (Table 2) which is a low value compared to the highest value observed for Chinese *S.*  
301 *exigua* field populations (17.1, Lai et al., 2011) in which chlorantraniliprole was either  
302 briefly introduced or, in some cases, never used against. As found our study, narrow  
303 variations in the LC<sub>50</sub> values were also found in some other Lepidoptera species: *Tuta*  
304 *absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Campos et al., 2015), *Plutella*  
305 *xylostella* (Linnaeus) (Lepidoptera: Plutellidae) (Wang et al., 2010) or *Cnaphalocrocis*  
306 *medinalis* (Güenée) (Lepidoptera: Pyralidae) (Zheng et al., 2011).

307 The mortality caused by the DC<sub>0.3</sub> (concentration close to the LC<sub>50</sub> of the pooled field  
308 population) on European field populations also showed a low variability among  
309 populations, with a mean mortality of 70.82 %, and a CV equal to 31.17 (Table 3). The  
310 RR-F<sub>0.3</sub> obtained were similar to the RR-F<sub>50</sub>, demonstrating its reliability in comparing  
311 populations. The DC<sub>1.0</sub> produced a mean mortality of 96.44%, and all the mortalities were  
312 higher than 85.3% except in the French population, Isle SLS (FR) (13), where it was  
313 67.7%. The highest DC tested, 10 mg a.i./kg of diet, caused 100.0% mortality in all the  
314 field populations except in Isle SLS (FR) (13) and Malpartit (SP) (09), suggesting the

315 presence of a small proportion of resistant individuals in these two populations, but  
316 generally supporting the lack of resistance to chlorantraniliprole in the field. Malpartit  
317 (SP) (09) was collected before the introduction of the product in the marked but the  
318 populations had a very high mean P450 activity (Table 4), what can play a role in the  
319 detoxification of the active ingredient, as is discussed later. Eleven European field  
320 populations were collected after registration of the product, from 2012 onwards, and these  
321 populations proved as susceptible as the previously tested Spanish populations. Some of  
322 them were collected over more than a year, as in: Le Thor (two years), Isle SLS (three  
323 years) and Noves P (five consecutive years). The mortality obtained with Isle SLS with  
324 the DC of 0.3 mg a.i./kg of diet decreased over the years from 89.1% in 2009 to 38.1%  
325 in 2013. This population was from a location with very high pest pressure and where trials  
326 with Rynaxypyr were done over several years, so, the loss of efficacy of the product may  
327 be due to the presence of resistant individuals in the population. Nevertheless, the field  
328 population, Noves, showed a similar level of mortality for all the years, ranging between  
329 69.6% to 93.3%, except in 2012, when the efficacy of the DC<sub>0.3</sub> decrease to 20.8%  
330 (obtaining a RR<sub>0.3</sub> of 4.8). In these field populations, the DC<sub>1.0</sub> and DC<sub>10.0</sub> reached 100.0%  
331 of mortality suggesting an error in the assay or an unexplained variation in the mortality  
332 obtained, something that can occur in unexposed field populations (Sawicki, 1987) and  
333 demonstrating the utility of using more than one DC.

334 Widespread resistance of CM field populations from the Ebro Valley area (NE of Spain)  
335 has been demonstrated, mainly to azynphos-methyl and other OP, lambda-cyhalothrin  
336 and methoxyfenozide, among other active ingredients (Rodríguez et al., 2010;  
337 Rodríguez et al., 2011). Insecticide bioassays (Bosch et al., 2017) were done with some  
338 of the Spanish field populations tested with chlorantraniliprole. Compared with the  
339 susceptible population S-Spain, PuigverdB (11) was resistant to the pyrethroid lambda-

340 cyhalothrin (RR = 15.4), and PuigverdC (11) was resistant to lambda-cyhalothrin (RR =  
341 872.0), methoxyfenozide (RR = 14.6) and thiacloprid (RR = 11.2), besides being  
342 tolerant ( $2 < RR < 10$ ) to other active ingredients such as chlorpyrifos-ethyl and  
343 spinetoram. Tam (10) was susceptible to all the tested active ingredients, and SAS (11)  
344 and Le Thor (12) were tolerant to different active ingredients but with RRs always  
345 lower than 4.4. None of these populations were resistant to chlorantraniliprole, with  
346 SAS (11) the only one that had a RR slightly higher than 2 (RR = 2.2). Despite being  
347 multi-resistant populations, PuigverdB (11) and PuigverdC (11) responded to the DCs  
348 of chlorantraniliprole with high mortalities, at the same or a higher level than the rest of  
349 the tested populations (Table 3). These results showed the absence of cross-resistance  
350 among chlorantraniliprole and lambda-cyhalothrin, methoxyfenozide and thiacloprid in  
351 these field populations suggesting that the resistant mechanisms involved do not affect  
352 the proper activity of the product. This lack of cross-resistance was also found in *P.*  
353 *xylostella* field and laboratory selected populations (Wang et al., 2010), *Spodoptera*  
354 *litura* (Fabricius) (Lepidoptera: Tortricidae) (Sang et al., 2016) and with  
355 cyantraniliprole, another anthranilic diamide, in selected resistant populations of  
356 *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Grávalos et al., 2015).  
357 Therefore, a high correlation between the two anthranilic diamides was found in *T.*  
358 *absoluta* (Campos et al., 2015) and in *S. litura* (Sang et al., 2016); and with  
359 flubendiamide, a phthalic diamide, in *P. xylostella* although this active ingredient had  
360 never been used (Wang et al., 2013). Chlorantraniliprole is the only diamide currently  
361 registered for pest control in pome fruits, but formulated products of cyantraniliprole are  
362 under development to control a cross-spectrum of chewing and sucking pests from the  
363 insect orders Hemiptera, Lepidoptera and Coleoptera (Selvy et al., 2013). They have  
364 obtained promising results in the control of aphid pests with no evidence of cross-



365 resistance with other aphid insecticides (Foster et al., 2012). In the case of using both  
366 active ingredients in the same crop to control different pests, an intensive check to  
367 detect an increase in the resistance levels would be necessary due to the possible cross-  
368 resistance between IRAC Group 28 products, together with an accurate resistance  
369 management strategy combining its use.

370 With reference to enzymatic detoxification mechanisms, synergism assays in *S. litura*  
371 (Su et al., 2012), *S. exigua* (Lai et al., 2011) and *P. xylostella* (Wang et al., 2010)  
372 demonstrated that P450, EST and GST were not the main mechanisms involved in  
373 chlorantraniliprole resistance and neither were they involved in the cyantraniliprole  
374 resistance of *S. litura* in China (Sang et al., 2016). However, in *T. absoluta*, Campos et  
375 al. (2015) found a moderate correlation between the cytochrome-P450-monoxygenases  
376 activity (P450) and a susceptibility to chlorantraniliprole and cyantraniliprole. Sial et al.  
377 (2011) found that EST could be involved in the detoxification of chlorantraniliprole in a  
378 resistant selected laboratory population of *C. rosaceana*; and Cao et al. (2010) found  
379 that there was an increase of EST and GST activity in chlorantraniliprole treated insects  
380 of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). The main enzymatic  
381 mechanism for insecticide detoxification of the Spanish CM field populations is P450  
382 (Rodríguez et al., 2010; Rodríguez et al., 2011), and in the studied populations, the  
383 frequency of P450 resistant insects present in the field explained the 69.72% of the  
384 mortality obtained with the lower chlorantraniliprole DC (0.3 mg a.i./kg of diet). The  
385 coefficient of determination was higher when the  $LC_{50}$  was used instead of the mortality  
386 of the  $DC_{0.3}$  ( $R^2 = 89.77$ ); however, we considered that five points (the laboratory and  
387 four field populations) to adjust a regression line are too few to draw any conclusion.  
388 Despite these results, due to the high efficacy of the product in the tested field

389 populations, P450 seems not to be the main mechanism implied in the detoxification of  
390 the product although it may have a certain role in it.

391 *C. pomonella* is a key fruit pest that has extensively demonstrated its ability to develop  
392 resistance to most of the registered insecticides. Chlorantraniliprole, a new reduced risk  
393 insecticide that can control a wide range of lepidopteran pests, has proved its high  
394 efficacy in European field populations by obtaining low RR and variability when the LC  
395 were calculated and DC tested. The efficacy of the product in this assay not only has  
396 shown the natural variability in the response concentration-mortality in a broad  
397 geographical area, but also the lack of cross-resistance of the product with other  
398 commonly used insecticides in Spanish field populations, such as lambda-cyhalothrin,  
399 methoxyfenozide or thiacloprid. Nevertheless, it seems there is a relationship between  
400 the frequency of resistant individuals due to high P450 enzymatic activity and the  
401 mortality produced by the approximate LC<sub>50</sub> used as a DC. As many insecticides can  
402 induce an P450 enzymatic activity increase, the use of a strict resistance management  
403 strategy would be necessary to maintain the efficacy of the product for a long time  
404 (<http://www.irac-online.org>). In fact, this strategy has been considered from the  
405 beginning by DuPont which recommend a restricted number of applications per season  
406 on the same generation, within spray programs that include other effective insecticides  
407 with different modes of action. The CM baseline susceptibility data established provides  
408 a valuable reference for tracking possible future alterations in the efficacy of the  
409 product.

410

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**Table 1.** Population name, origin, year of collection, state of the insects collected (DP = diapausing) and generation tested of the field codling moth populations treated with chlorantraniliprole to obtain either the probit line (Probit) or the mortality produced by the discriminant concentrations (DC). The P450 activity was calculated for the populations reported in column: P450 activity.

| Population              | Country / County              | Collection year | Insect collection state | Tested laboratory generation | Bioassay done | P450 activity |
|-------------------------|-------------------------------|-----------------|-------------------------|------------------------------|---------------|---------------|
| S-Spain                 | Spain / Catalunya             |                 |                         |                              | Probit        | Yes           |
| Bal 2-371 (SP) (07)     | Spain / Catalunya             | 2007            | DP larvae               | F1                           | Probit        |               |
| Jun 14-16 (SP) (08)     | Spain / Catalunya             | 2008            | Non DP & DP larvae *    | F2                           | Probit        | Yes           |
| Cal-4 (SP) (08)         | Spain / Aragón                | 2008            | Non DP & DP larvae      | F2                           | Probit        |               |
| Torref 15-3 (SP) (08)   | Spain / Catalunya             | 2008            | Non DP & DP larvae *    | F2                           | Probit        | Yes           |
| Torreg 11-160 (SP) (08) | Spain / Catalunya             | 2008            | Non DP & DP larvae      | F2                           | Probit        |               |
| Bal 2-480 (SP) (08)     | Spain / Catalunya             | 2008            | Non DP & DP larvae *    | F2                           | Probit        | Yes           |
| SAS (SP) (09)           | Spain / Catalunya             | 2009            | DP larvae               | F2                           | Probit        |               |
| Jun 3-63 (SP) (09)      | Spain / Catalunya             | 2009            | Non DP & DP larvae *    | F1                           | Probit        | Yes           |
| Tamarite (SP) (10)      | Spain / Aragón                | 2010            | Non DP & DP larvae      | F1                           | Probit        | Yes           |
| Riud (SP) (10)          | Spain / Catalunya             | 2010            | DP larvae               | F1                           | Probit        |               |
| Torreg 11-166 (SP) (09) | Spain / Catalunya             | 2008            | DP larvae *             | F2                           | DC            | Yes           |
| La Almunia (SP) (09)    | Spain / Aragón                | 2009            | DP larvae               | F1                           | DC            |               |
| La AlmuniaG (SP) (09)   | Spain / Aragón                | 2009            | DP larvae               | F2                           | DC            |               |
| Tossal (SP) (09)        | Spain / Catalunya             | 2009            | Non DP & DP larvae *    | F2                           | DC            | Yes           |
| Malpartit (SP) (09)     | Spain / Catalunya             | 2009            | Non DP & DP larvae *    | F1                           | DC            | Yes           |
| Vauvert (FR) (09)       | France / Languedoc-Roussillon | 2009            | DP larvae               | F2                           | DC            |               |
| Isle SLS (FR) (09)      | France / Vaucluse             | 2009            | DP larvae               | F2                           | DC            |               |
| Nedel Market (HU)(09)   | Hungary / Bács-Kiskun         | 2009            | DP larvae               | F2                           | DC            |               |

\*Adults caught in pheromone traps

**Table 1 cont.** Population name, origin, year of collection, state of the insects collected (DP = diapausing) and generation tested of the field codling moth populations treated with chlorantraniliprole to obtain either the probit line (Probit) or the mortality produced by the discriminant concentrations (DC). The P450 activity was calculated for the populations reported in column P450 activity.

| Population           | Country / County         | Collection year | Insect collection state | Tested laboratory generation | Bioassay done | P450 activity |
|----------------------|--------------------------|-----------------|-------------------------|------------------------------|---------------|---------------|
| Aseleben (DE) (09)   | Germany / Sachsen Anhalt | 2009            | DP larvae               | F1                           | DC            |               |
| Isle SIS (FR) (10)   | France / Vaucluse        | 2010            | DP larvae               | F1                           | DC            |               |
| Noves P (FR) (10)    | France / Provence-A-C.A. | 2010            | DP larvae               | F1                           | DC            |               |
| Albalate (SP) (11)   | Spain / Aragón           | 2011            | Non DP & DP larvae      | F2                           | DC            | Yes           |
| PuigvertC (SP) (11)  | Spain / Catalunya        | 2011            | DP larvae               | F1                           | DC            | Yes           |
| PuigvertB (SP) (11)  | Spain / Catalunya        | 2011            | DP larvae               | F1                           | DC            | Yes           |
| SAS (SP) (11)        | Spain / Catalunya        | 2011            | DP larvae               | F1                           | DC            | Yes           |
| Noves P (FR) (11)    | France / Provence-A-C.A. | 2011            | DP larvae               | F1                           | DC            |               |
| Noves P (FR) (12)    | France / Provence-A-C.A. | 2012            | DP larvae               | F1                           | DC            |               |
| Le Thor (FR) (12)    | France / Provence-A-C.A. | 2012            | DP larvae               | F1                           | DC            |               |
| Isle SLS (FR) (13)   | France / Vaucluse        | 2013            | DP larvae               | F2                           | DC            |               |
| Noves P (FR) (13)    | France / Provence-A-C.A. | 2013            | DP larvae               | F2                           | DC            |               |
| Noves P (FR) (14)    | France / Provence-A-C.A. | 2014            | DP larvae               | F1                           | DC            |               |
| Lumpiaque (SP) (15)  | Spain / Aragón           | 2015            | DP larvae               | F1                           | DC            |               |
| Salillas (SP) (15)   | Spain / Aragón           | 2015            | DP larvae               | F1                           | DC            |               |
| Le Thor (FR) (15)    | France / Provence-A-C.A. | 2015            | DP larvae               | F1                           | DC            |               |
| Meckenheim (DE) (15) | Germany                  | 2015            | DP larvae               | F1                           | DC            |               |
| Orsingen (DE) (15)   | Germany                  | 2015            | DP larvae               | F1                           | DC            |               |
| Ravenna (IT) (15)    | Italy                    | 2015            | DP larvae               | F1                           | DC            |               |

\*Adults caught in pheromone traps

**Table 2.** Baseline susceptibility of *Cydia pomonella* Spanish field collected populations to chlorantraniliprole. n = number of individuals tested. RR-F<sub>50</sub> = resistance ratio calculated by dividing the LC<sub>50</sub> of the strain tested by the LC<sub>50</sub> of the most susceptible field population (Riud (SP) (10)). RR-L<sub>50</sub> = resistance ratio calculated by dividing the LC<sub>50</sub> of the strain tested by the LC<sub>50</sub> of the susceptible laboratory population (S-Spain). Values of the LC are mg a.i./kg diet. LC<sub>50</sub> followed by the same letter are not significantly different (LC<sub>50</sub> are considered significantly different when their CI do not overlap).

| Population (year)       | n    | Probit analyses parameters |               |                  |             |                  |              |                     |        | RR-F <sub>50</sub> | RR-L <sub>50</sub> |
|-------------------------|------|----------------------------|---------------|------------------|-------------|------------------|--------------|---------------------|--------|--------------------|--------------------|
|                         |      | Intercept                  | Slope ± SE    | LC <sub>50</sub> | CI 95%      | LC <sub>90</sub> | CI 95%       | χ <sup>2</sup> (dF) | P*     |                    |                    |
| S-Spain                 | 577  | 3.555                      | 3.345 ± 0.252 | 0.087            | 0.079-0.095 | 0.209            | 0.182-0.249  | 0.36 (4)            | > 0.05 | -                  | -                  |
| Bal 2-371 (SP) (07)     | 275  | 1.606                      | 2.220 ± 0.396 | 0.189ab          | 0.124-0.254 | 0.714            | 0.488-1.502  | 0.71 (4)            | > 0.05 | 1.2                | 2.2                |
| Jun 14-16 (SP) (08)     | 572  | 1.102                      | 2.183 ± 0.260 | 0.313bc          | 0.220-0.426 | 1.209            | 0.786-2.861  | 5.09 (6)            | > 0.05 | 1.9                | 3.6                |
| Cal-4 (SP) (08)         | 612  | 1.974                      | 2.800 ± 0.269 | 0.197ab          | 0.160-0.243 | 0.566            | 0.427-0.867  | 8.03 (6)            | > 0.05 | 1.2                | 2.3                |
| Torref 15-3 (SP) (08)   | 400  | 0.684                      | 1.954 ± 0.260 | 0.446c           | 0.309-0.665 | 2.021            | 1.173-6.365  | 5.63 (5)            | > 0.05 | 2.8                | 5.2                |
| Torreg 11-160 (SP) (08) | 262  | 0.671                      | 1.371 ± 0.168 | 0.324abc         | 0.182-0.592 | 2.788            | 1.302-11.396 | 9.31 (3)            | < 0.05 | 2.0                | 3.8                |
| Bal 2-480 (SP) (08)     | 436  | 0.809                      | 2.079 ± 0.248 | 0.408c           | 0.312-0.536 | 1.687            | 1.114-3.531  | 5.70 (5)            | < 0.05 | 2.5                | 4.7                |
| SAS (SP) (09)           | 380  | 2.235                      | 4.860 ± 0.557 | 0.347c           | 0.251-0.431 | 0.636            | 0.503-1.051  | 37.47 (4)           | < 0.05 | 2.2                | 4.0                |
| Jun 3-63 (SP) (09)      | 283  | 2.041                      | 2.722 ± 0.317 | 0.178ab          | 0.137-0.230 | 0.526            | 0.383-0.849  | 4.22 (3)            | > 0.05 | 1.1                | 2.1                |
| Tam (SP) (10)           | 684  | 2.624                      | 3.748 ± 0.486 | 0.199ab          | 0.162-0.248 | 0.438            | 0.332-0.749  | 4.99 (4)            | > 0.05 | 1.2                | 2.2                |
| Riud (SP) (10)          | 554  | 3.139                      | 3.951 ± 0.337 | 0.161a           | 0.136-0.185 | 0.339            | 0.258-0.431  | 11.42 (5)           | < 0.05 | 1.0                | 1.9                |
| Pooled                  | 4458 | 1.401                      | 2.324 ± 0.087 | 0.250            | 0.225-0.376 | 0.888            | 0.763-1.068  | 186.73 (50)         | < 0.05 | 1.6                | 2.9                |

\*The p-value >0.05 indicates that the observed bioassay data are not significantly different from the expected data for the probit model.

**Table 3.** Susceptibility, expressed as corrected mortality (%), of *Cydia pomonella* European field collected populations to discriminant concentrations of chlorantraniliprole (0.3, 1.0 and 10.0 mg a.i./kg diet). RR-F = resistance ratio for every DC was calculated by dividing the mortality of the most susceptible field population (Meckenheim (DE) (15)) by the mortality produced in every field population strain. Mean  $\pm$  SEM of the corrected mortality produced by the DCs and coefficient of variability (CV) across all the field populations were calculated. Numbers in brackets are the number of individuals tested.

| Population              | Control mortality (%) | Discriminant concentration (mg a.i./kg diet) |                     |            |                     |            |                      |
|-------------------------|-----------------------|--|---------------------|------------|---------------------|------------|----------------------|
|                         |                       | 0.3  | RR-F <sub>0.3</sub> | 1.0        | RR-F <sub>1.0</sub> | 10.0       | RR-F <sub>10.0</sub> |
| S-Spain                 |                       | 100.0 (44)                                   |                     | 100.0 (47) |                     | 100.0 (48) |                      |
| Torreg 11-166 (SP) (08) | 4.2 (48)              | 44.3 (45)                                    | 2.3                 | 97.8 (48)  | 1.0                 | 100.0 (69) | 1.0                  |
| La Almunia (SP) (09)    | 0.0 (50)              | 78.6 (47)                                    | 1.3                 | 95.7 (47)  | 1.0                 | 100.0 (46) | 1.0                  |
| La Almunia G (SP) (09)  | 2.5 (40)              | 36.9 (53)                                    | 2.7                 | 97.1 (52)  | 1.0                 | 100.0 (68) | 1.0                  |
| Tossal (SP) (09)        | 6.7 (45)              | 35.4 (48)                                    | 2.8                 | 97.7 (44)  | 1.0                 | 100.0 (48) | 1.0                  |
| Malpartit (SP) (09)     | 2.4 (42)              | 51.0 (46)                                    | 2.0                 | 93.6 (16)  | 1.1                 | 97.8 (46)  | 1.0                  |
| Vauvert (FR) (09)       | 0.0 (48)              | 54.2 (48)                                    | 1.8                 | 85.3 (44)  | 1.2                 | 100.0 (49) | 1.0                  |
| Isle SLS (FR) (09)      | 0.0 (48)              | 89.1 (46)                                    | 1.1                 | 100.0 (47) | 1.0                 | 100.0 (47) | 1.0                  |
| Nedel Market (HU) (09)  | 6.1 (50)              | 93.3 (48)                                    | 1.1                 | 96.5 (51)  | 1.0                 | -          | -                    |
| Aseleben (DE) (09)      | 1.6 (64)              | 90.9 (59)                                    | 1.1                 | 100.0 (60) | 1.0                 | 100.0 (64) | 1.0                  |
| Isle SIS (FR) (10)      | 2.1 (48)              | 48.9 (40)                                    | 2.0                 | 98.0 (50)  | 1.0                 | 100.0 (48) | 1.0                  |
| Noves P (FR) (10)       | 2.0 (49)              | 69.6 (47)                                    | 1.4                 | 100.0 (48) | 1.0                 | 100.0 (45) | 1.0                  |
| Albalate (SP) (11)      | 0.0 (48)              | 69.4 (48)                                    | 1.4                 | 100.0 (48) | 1.0                 | 100.0 (48) | 1.0                  |
| PuigvertC (SP) (11)     | 8.3 (48)              | 81.4 (47)                                    | 1.2                 | 93.0 (47)  | 1.1                 | 100.0 (48) | 1.0                  |
| PuigvertB (SP) (11)     | 14.6 (48)             | 95.0 (47)                                    | 1.1                 | 100.0 (47) | 1.0                 | 100.0 (48) | 1.0                  |

**Table 3 cont.** Susceptibility, expressed as corrected mortality (%), of *Cydia pomonella* European field collected populations to discriminant concentrations of chlorantraniliprole (0.3, 1.0 and 10.0 mg a.i./kg diet). RR-F = resistance ratio for every DC was calculated by dividing the mortality of the most susceptible field population (Meckenheim (DE) (15)) by the mortality produced in every field population strain. Mean  $\pm$  SEM of the corrected mortality produced by the DCs and coefficient of variability (CV) across all the field populations were calculated. Numbers in brackets are the number of individuals tested.

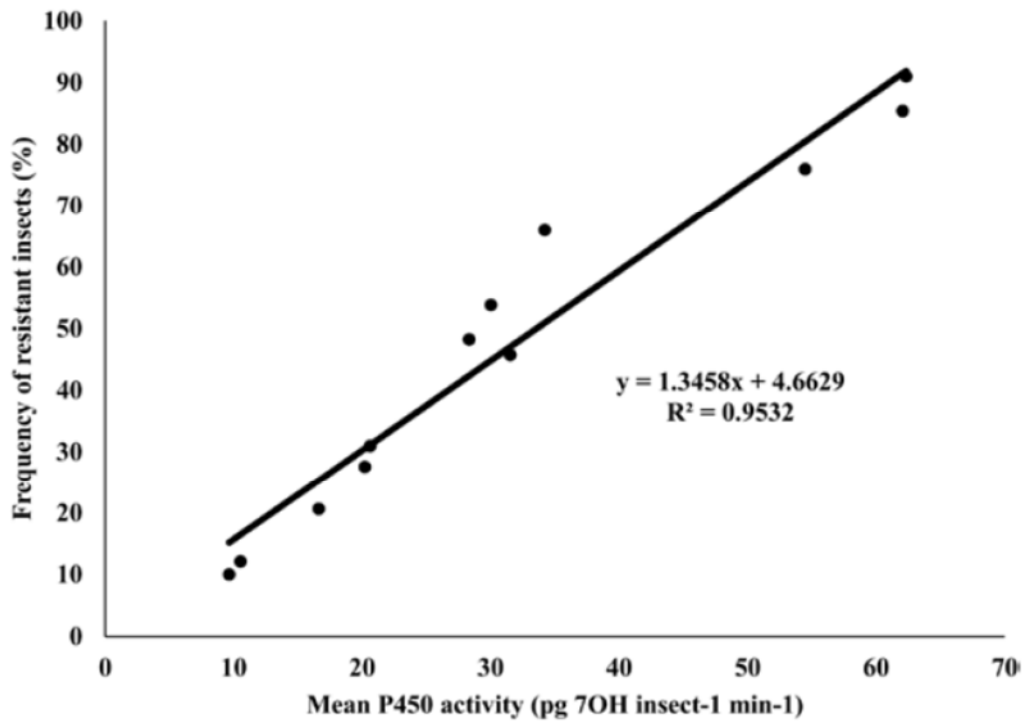
| Population          | Control mortality (%) | Discriminant concentration (mg a.i./kg diet) |                     |                  |                     |                  |                      |
|---------------------|-----------------------|--|---------------------|------------------|---------------------|------------------|----------------------|
|                     |                       | 0.3  | RR-F <sub>0.3</sub> | 1.0              | RR-F <sub>1.0</sub> | 10.0             | RR-F <sub>10.0</sub> |
| SAS (SP) (11)       | 0.0 (48)              | 82.5 (48)                                    | 1.2                 | 100.0 (48)       | 1.0                 | 100.0 (48)       | 1.0                  |
| Noves P (FR) (11)   | 4.7 (64)              | 79.9 (47)                                    | 1.3                 | 100.0 (63)       | 1.0                 | 100.0 (46)       | 1.0                  |
| Noves P (FR) (12)   | 0.0 (62)              | 20.8 (48)                                    | 4.8                 | 100.0 (48)       | 1.0                 | 100.0 (48)       | 1.0                  |
| Le Thor (FR) (12)   | 4.7 (64)              | 75.4 (47)                                    | 1.3                 | 100.0 (63)       | 1.0                 | 100.0 (46)       | 1.0                  |
| Isle SLS (FR) (13)  | 2.1 (48)              | 38.1 (61)                                    | 2.6                 | 67.7 (79)        | 1.5                 | 98,9 (95)        | 1.0                  |
| Noves P (FR) (13)   | 0.0 (48)              | 82.2 (45)                                    | 1.2                 | 100.0 (47)       | 1.0                 | 100.0 (48)       | 1.0                  |
| Noves P (FR) (14)   | 0.0 (48)              | 93.3 (45)                                    | 1.1                 | 100.0 (33)       | 1.0                 |                  |                      |
| Lumpiaque (SP) (15) | 4.3 (82)              | 77.8 (63)                                    | 1.3                 | 87.2 (122)       | 1.1                 | 100.0 (45)       | 1.0                  |
| Salillas (SP) (15)  | 0.0 (27)              | 72.3 (47)                                    | 1.4                 | 100.0 (55)       | 1.0                 | 100.0 (55)       | 1.0                  |
| Le Thor (FR) (15)   | 0.0 (64)              | 64.8 (88)                                    | 1.5                 | 94.3 (138)       | 1.1                 |                  |                      |
| Meckenheim (DE)(15) | 8.3 (48)              | 100.0 (53)                                   | 1.0                 | 100.0 (45)       | 1.0                 | 100.0 (48)       | 1.0                  |
| Orsingen (DE) (15)  | 0.0 (16)              | 93.2 (44)                                    | 1.1                 | 100.0 (45)       | 1.0                 |                  |                      |
| Ravenna (IT) (15)   | 4.2 (96)              | 93.7 (95)                                    | 1.1                 | 100.0 (94)       | 1.0                 |                  |                      |
| Mean $\pm$ SEM      |                       | 70.82 $\pm$ 4.25                             |                     | 96.44 $\pm$ 1.34 |                     | 99.85 $\pm$ 0.11 |                      |
| CV                  |                       | 31.17  |                     | 7.21             |                     | 0.52             |                      |



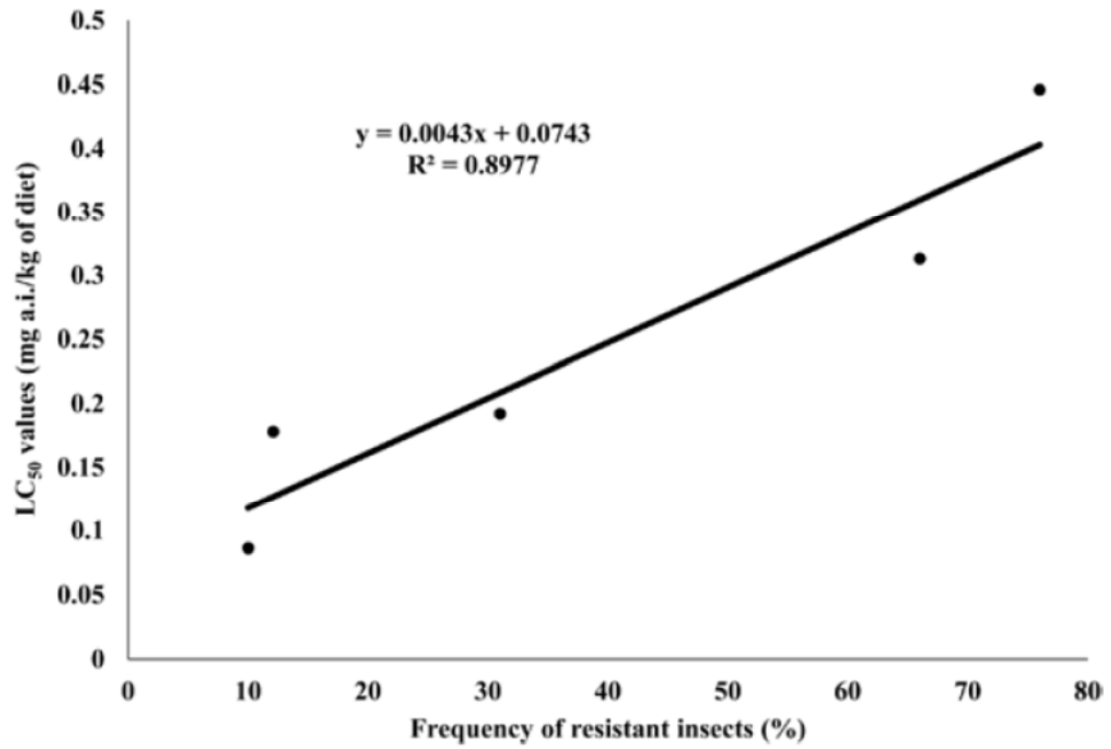
**Table 4.** Enzymatic P450 activity, expressed as frequency of resistant insects (%) and mean activity  $\pm$  SEM (pg 7OH $\cdot$ insect<sup>-1</sup> $\cdot$ min<sup>-1</sup>), of *Cydia pomonella* field adults. The value of the P450 threshold obtained using the S-Spain population was 23.92 pg 7OH $\cdot$ insect<sup>-1</sup> $\cdot$ min<sup>-1</sup>. The frequency of resistant individuals was compared using a Pearson  $\chi^2$  test (df = 1; \*p = 0.05; \*\*p = 0.01; \*\*\*p = 0.001).

| Population (year)  | n   | Resistance frequency (%) | Mean P450 activity $\pm$ SEM | LC <sub>50</sub> | DC <sub>0.3</sub> mortality (%) |
|--------------------|-----|--------------------------|------------------------------|------------------|---------------------------------|
| S-Spain            | 223 | 10.0                     | 9.64 $\pm$ 0.74              | 0.086            | 100.0                           |
| Jun 14-16 (08)     | 47  | 66.0***                  | 34.20 $\pm$ 3.18             | 0.313            | 51.8                            |
| Torref 15-3 (08)   | 125 | 76.0***                  | 54.47 $\pm$ 2.95             | 0.446            | 34.2                            |
| Bal 2-480 (08)     | 8   | 100.0***                 | 74.61 $\pm$ 9.57             | 0.408            | 49.3                            |
| Jun 3-63 (09)      | 107 | 12.1n.s.                 | 10.52 $\pm$ 0.98             | 0.178            | 83.0                            |
| Torreg 11-166 (09) | 48  | 45.8***                  | 31.52 $\pm$ 4.50             | -                | 44.3                            |
| Tossal (09)        | 41  | 85.4***                  | 62.05 $\pm$ 6.24             | -                | 35.4                            |
| Malpartit (09)     | 82  | 91.0***                  | 62.34 $\pm$ 3.87             | -                | 51.0                            |
| Tam (10)           | 29  | 31.0**                   | 20.60 $\pm$ 2.73             | 0.192            | 75.6                            |
| SAS (11)           | 29  | 20.7n.s.                 | 16.60 $\pm$ 2.68             | -                | 82.5                            |
| Albalate (11)      | 23  | 27.6***                  | 20.20 $\pm$ 3.23             | -                | 69.4                            |
| PuigverdC (11)     | 39  | 53.9***                  | 30.00 $\pm$ 3.34             | -                | 81.4                            |
| PuigverdB (11)     | 29  | 48.3***                  | 28.31 $\pm$ 3.24             | -                | 81.4                            |

**Figure 1.** The frequency of resistant codling moth adults as explained by the mean enzymatic P450 activity in 11 Spanish field populations and the laboratory strain S-Spain.



**Figure 2.** The LC<sub>50</sub> of chlorantraniliprole for four Spanish field populations and the laboratory strain S-Spain as explained by the frequency of resistant codling moth adults.



**Figure 3.** The mortality caused by the chlorantraniliprole DC<sub>0.3</sub> on larvae of 11 Spanish field populations and the laboratory strain S-Spain as explained by the mean enzymatic P450 activity in adults.

