Evaluation of the susceptibility to emamectin benzoate and lambda cyhalothrin in European populations of Cydia pomonella (L.) (Lepidoptera: Tortricidae)

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Abstract

The codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) is one of the key pests of pome fruit and walnut in almost all growing regions of the world and has developed resistance to several insecticides. In this study, the susceptibilities of 15 codling moth populations to emamectin benzoate and 9 populations to lambda cyhalothrin collected in five European countries were measured by standard diet incorporation bioassays. Variation in susceptibility was observed among populations, with LC_{50} values ranging from 0.0017 to 0.0119 mg a.i./kg diet for emamectin benzoate and from 0.033 to 0.292 mg a.i./kg diet for lambda cyhalothrin. Our results revealed only small variations in emamectin benzoate susceptibility between populations, indicating no selection of resistance to this active ingredient. Even though a wider range of responses was detected for lambda cyhalothrin, our results also suggest that populations remain susceptible to this insecticide. Based on pooled LC_{95} evaluations, we propose the use of concentrations 0.02 mg a.i./kg of diet for emamectin benzoate and 0.60 mg a.i./kg of diet for lambda cyhalothrin in order to discriminate between resistant and susceptible individuals.

Keywords Cydia pomonella, codling moth, resistance monitoring, insecticide resistance, emamectin benzoate, lambda cyhalothrin

Introduction

The codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) is one of the key pests of pome fruit and walnut in almost all growing regions of the world. Codling moth has a high damage potential and can cause total yield losses (Geier 1964, MacLellan 1976). Although non-chemical control methods such as mating disruption, insect-proof nets and *Cydia pomonella* granulosis virus (CpGV) are available, codling moth management mostly relies on chemical insecticides. The long-term use of pesticides has selected populations resistant to insecticides with different modes of action, including neurotoxic insecticides, insect growth regulators and granuloviruses (Bosch et al. 2018a).

Selection of resistant populations of codling moth can be dated back to the late 1920s, when resistance to arsenate insecticides was reported in the USA (Hough 1928). Since 1990, there have been reports of codling moth populations resistant to several insecticidal classes, including neonicotinoids, benzoylureas, macrocyclic lactones and older compounds such as pyrethroids or organophosphates (Reyes et al. 2007). Pesticide resistance is a major threat to pest control and causes control failures in numerous cropping systems worldwide, thus proactive resistance management is recommended to maximize the lifespan of pesticides. Monitoring pest susceptibility can provide the basis to understand the status of insecticide resistance and to develop a successful strategy to delay its occurrence (Roditakis et al. 2013).

Emamectin benzoate derives from a fermentation product of the soil microorganism *Streptomyces avermitilis* (Burg et al. 1979). According to the IRAC Mode of Action (MoA) classification, it is included in group 6 acting as an allosteric activator of glutamate-gated chloride channels in the insect nervous system. Emamectin benzoate shows translaminar movement through the leaf blade and has a residual activity on leaf-chewing lepidopteran species. However, residues on foliage surfaces are rapidly broken-down by sunlight (Feely et al. 1992) and contact activity on beneficial arthropods is limited to short periods (Depalo et al. 2017). This makes the use of emamectin benzoate particularly suited for control of lepidopteran pests in IPM strategies.

Emamectin benzoate was registered in 2000 for codling moth control in Europe and other countries (Ioriatti et al. 2009). To our knowledge no resistance of tortricid moths was previously recorded to emamectin benzoate. However, a few cases have been reported for other lepidoptera such as *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) from Pakistan (Ishtiaq et al. 2014, Ahmad et al. 2018) and China (Che et al. 2015) and *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) from China (Pu et al. 2010).

Lambda cyhalothrin is a pyrethroid insecticide, i.e. a synthetic chemical analogue of pyrethrins found in chrysanthemums (i.e. *Chrysanthemum cinerariaefolium* and *C. coccineum*). Pyrethroids have been widely used since the 1970s on many crops to control insect pests, including Hemiptera, Coleoptera and Lepidoptera. The use of pyrethroid insecticides has increased in recent years, probably due to the withdrawal of the organophosphate chlorpyrifos (He et al. 2008, Hites 2021). Since commercialization, pyrethroids have been largely used all over the world to control codling moth, thus favoring the selection of resistant strains to this class of insecticides (Soleño et al. 2008, Soleño et al. 2019). Lambda cyhalothrin has been available for the control of codling moth since the 1980s (Roush et al. 1990) and populations resistant to lambda cyhalothrin have been detected since 2000 in Europe (Bosch et al. 2018b), Northeastern China (Wei et al. 2020) and North America (Mota-Sanchez et al. 2008).

In orchards where chemicals are widely used to decrease pest pressure and crop rotation is not possible, insecticide resistance management (IRM) is crucial (Sparks et al. 2015). Regular monitoring is the core of IRM and is essential to to manage insecticide resistance. Therefore, the susceptibility of pests should be regularly assessed when any insecticide is used extensively. When an a.i. is recommended for pests with known problems of resistance, proactive resistance monitoring should be performed even before product commercialization.

In this 4-year survey we examined the susceptibility of 15 codling moth populations to emamectin benzoate and 9 populations to lambda-cyhalothrin across five European countries. The aims of the study were to (1) estimate lethal concentrations (LC_{50} and LC_{95}) for each population to both insecticides; (2) check the occurrence of a potential shift in the responses of codling moth populations to emamectin benzoate and lambda cyhalothrin; (3) provide an overall European measure of susceptibility to these active ingredients for tracking possible future alterations in the efficacy of the products.

Materials and methods

Insects

Codling moth populations were collected by means of cardboard trunk traps as mature diapausing larvae from commercial orchards during 2015-2019. Populations tested against emamectin benzoate were collected in from five EU Countries: Italy (7 populations), France (5), Belgium (1), Spain (1) and Poland (1). Populations used for the assays with lambda cyhalothrin were collected in France (4), Italy (4) and Belgium (1) (Table 1). None of the growers where collections were made had reported any failure in controlling codling moth using insecticides.

Diapausing larvae were reared to adulthood and allowed to mate. Eggs were collected and newly hatched larvae (F1) were used for the bioassays. Some populations were reared for one or two generations to obtain enough offspring to complete bioassays for LC_{50} estimation.

The susceptible population was collected from an abandoned apple orchard in Lleida (Spain) in 1992. It has since been continuously reared in the laboratory without exposure to pesticides. This population was kindly provided by the Institute for Food and Agricultural Research and Technology and University of Lleida (Spain).

Insecticides

Commercial formulation of emamectin benzoate (Affirm Opti® with 0.95% w/w = 9.5 g. active ingredient (a.i.)/kg formulated product) and of lambda cyhalothrin (Karate Zeon® 9.48% =100 g/L) were provided by Syngenta Crop Protection (Basel, Switzerland). The insecticides were mixed with distilled water to make up the serial dilutions needed for bioassays. Distilled water only was used for control groups.

Bioassays

Standard diet incorporated bioassays were carried out on newly hatched larvae (< 24 h) following the IRAC susceptibility test method 017 (www.irac-online.org). The meridic artificial diet Stonefly *Heliothis* Premix (Stonefly Industries Ltd., Bryan, TX) was used throughout the experiments. To study the concentration-response curves, serial dilution of formulated emamectin benzoate and lambda cyhalothrin were prepared and used to knead the diet. The final a.i. concentrations into the diet ranged from 0.0005 to 0.1 mg a.i./kg for emamectin benzoate and 0.01 and 10 mg a.i./kg for lambda cyhalothrin.

Experiments were carried out in 2-mL well plates, filling each well with approximately 0.5 g of diet, which was gently pressed to evenly fill the bottom of the well. A single newly hatched larva was placed in each well. The plates were then sealed with transparent ventilated adhesive lids to prevent escaping of larvae. Plates were incubated in rearing chambers at 25 ± 1 °C, 50 ± 10% RH and 16:8 (L:D) h photoperiod.

Mortality was scored four days after placement of larvae in the wells. Larvae were considered dead if not reacting to the touch with a fine brush. When larvae were visibly affected and unable to upright themself when flipped on their back, they were scored as moribund. Dead and moribund larvae were both considered as observed responses. Larvae that could not be found at mortality checks were not included in the number of tested subjects.

For each codling moth population, six evenly spaced concentrations of the insecticides were used along with a negative control to calculate preliminary LC values. Emamectin benzoate was tested at 0.001, 0.003, 0.01, 0.03, 0.1, 0.3 mg/kg; Lambda-cyhalothrin was tested at 0.01, 0.03, 0.10, 0.30, 1, 3 mg/kg. The preliminary results were used to optimize a second (and in some cases a third) set of concentrations more precisely aiming at final LC estimation. These results were used to optimize a second (and in some cases a third) set of concentrations more precisely aiming at final LC estimation. A total of 48 codling moth larvae were tested for each concentration.

Statistical analysis

Probit regressions including intercept, slope, LC_{50}, LC_{95} and associated 95% confidence limits (CLs) were run using IBM SPSS Statistics ver. 23 (Chicago, IL, USA). If Pearson goodness-of-fit test detected significant deviation for probit models, a heterogeneity factor was used in the calculation of 95% confidence limits (CLs). Natural response rate was included as a parameter in each probit regression to account for mortality in the control groups of each population. The proportion of dead larvae in the controls was used as the initial value to estimate the natural response rate in each model (https://www.ibm.com/docs/en/SSLVMB_28.0.0/pdf/IBM_SPSS_Regression.pdf).

The ratio test was used to compare LCs of different populations (Depalo et al. 2016, Robertson et al. 2017).

Resistance ratios (RR) were calculated comparing each LC_{50} of any tested population to the LC_{50} of the most susceptible field population (RR-F), to the LC_{50} of the laboratory colony (RR-L) and to the pooled LC_{50} values (RR-P).

Results

Emamectin benzoate

Only limited variation in susceptibility to emamectin benzoate was detected among the 15 field-collected populations, with LC_{50} values ranging from 0.0017 mg a.i./kg diet for Vaucluse (France) to 0.0119 mg a.i./kg diet for Villanova de Belpuig (Spain). The LC_{95} values for the codling moth field populations ranged from 0.0071 to 0.0361 mg a.i./kg diet (Table 2).

The LC_{50} of the colony reared in the laboratory since 1992 (for up to 100 generations) was 0.0068 (0.0040-0.0106) mg a.i./kg diet, and it was not the most susceptible of the populations tested. Indeed, based on the results of LC ratio test, the LC_{50}s of populations from Vaucluse and Le Pont de Cè were significantly lower than the laboratory strain. The pooled LC_{50} and LC_{95} values were 0.0050 mg a.i./kg diet and 0.0213 mg a.i./kg diet, respectively.

The RR-Fs (compared to the most sensible field population) ranged from 1.2 to 7.0, RR-Ls (compared to the laboratory population) ranged from 0.3 to 1.8, RR-Ps (compared to the pooled LC50 values) ranged from 0.3 to 2.4. All populations can be considered susceptible to emamectin benzoate as all RRs were below tenfold, providing no indication of resistance (Table 2).

Lambda cyhalothrin

A small range of variation was recorded among 8 out of the 9 field populations tested with lambda cyhalothrin. The LC_{50} values of these eight susceptible populations ranged from 0.033 mg a.i./kg diet for Le Ponts de Cè (France) to 0.292 mg a.i./kg diet for Grisolles (France). The LC_{95} values ranged from 0.104 to 2.91 mg a.i./kg diet (Table 3). The value of LC_{50} of laboratory colony was 0.049 (0.044-0.055) mg a.i./kg diet, and the laboratory population was not the most susceptible.

The LC_{50}s of populations from Baricella, Ghibullo and Le Pont de Cè were not significantly different from the laboratory population, while LC_{50}s of the other populations were significantly higher than the laboratory strain.

The pooled LC_{50} and LC_{95} calculated excluding data of the L'Isle sur la Sorgue were 0.080 mg a.i./kg diet and 0.594 mg a.i./kg diet, respectively. Among the susceptible populations, the RR-Fs ranged from 1.4 to 8.8, RR-Ls ranged from 0.7 to 6.0, and RR-Ps ranged from 0.4 to 3.7. The Isle sur la Sorgue population had RR values of 17.1 when compared to the most susceptible population, 11.5 when compared to the laboratory colony and of 7.1 when compared to the pooled LC_{50} (Table 3).

Discussion

Only small variations in emamectin benzoate susceptibility were observed between populations of codling moth collected across a wide geographic range in Europe, indicating no selection of resistance to this active ingredient. These results are in agreement with the findings of the only other study on emamectin benzoate against codling moth in Spain (Bosch et al. 2018b). Earlier studies conducted either by surface-treated diet (Wu et al. 2015) or leaf/fruit-dip bioassays (Ioriatti et al. 2009) recorded LC_{50} values ranging between 0.016 mg/L and 0.026 mg/L. Lethal concentrations in our bioassays were three to five folds lower in comparison with the values reported by other authors. The differences were likely due to our use of a diet-incorporated bioassay, which involves continuous exposure of larvae to insecticides in the diet, which may have led to lower LC values than surface assays for all active ingredients.

Emamectin benzoate was registered nearly 20 years ago, but little evidence of resistance has been recorded to date on lepidopteran pests. A six fold decrease in susceptibility to emamectin benzoate compared to a laboratory susceptible strain originally obtained from an isolated abandoned apple orchard was found in *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) collected in apple and cherry orchards in USA (Hafez et al. 2018). Contrasting results have been reported for noctuid moths. In major cropping regions of eastern Australia, no resistant population was detected for *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), a pest species which is considered prone to the selection of insecticide resistance (Bird et al. 2017), whereas high levels of resistance were reported for *S. exigua* (Hübner) (Lepidoptera: Noctuidae) in

Pakistan (Ahmad et al. 2018). A European survey on emamectin benzoate resistance in *Tuta absoluta* (Lepidoptera: Gelechiidae) reported resistance factors ranging from susceptibility levels (twofold and fourfold) to low resistance levels (15- to 16-fold) (Roditakis et al. 2018).

In this study, a wider range of responses was detected for lambda-cyhalothrin than for emamectin benzoate in codling moth populations across Europe. Three out of four French populations were considered tolerant, including L'Isle sur la Sorgue, which was also above the arbitrary limit to be ranked as resistant (i.e. with an LC_{50} nearly 11-fold higher than the susceptible population). Codling moth populations resistant to lambda-cyhalothrin have been detected in Spain since 2011 (Rodríguez et al. 2011, Bosch et al. 2018b). High levels of lambda-cyhalothrin resistance were also found in codling moth larvae collected in the US (Mota-Sanchez et al. 2008) and more recently in apple and pear orchards throughout Argentina (Soleño et al. 2019) and China (Wei et al. 2020). Because of the long term and widespread use of lambda cyhalothrin and the chances of cross resistance with other widely used pyrethroids, the detection of codling moth populations resistant to lambda cyhalothrin was expected, but only two population from south France showed a RR L similar to resistant populations collected in North America, which had six- and tenfold resistance to lambda-cyhalothrin (Mota-Sanchez et al. 2008). Although most codling moth populations remained susceptible to lambda cyhalothrin in this study, in contrast with resistance already documented in Spain, Argentina, and China (Rodríguez et al. 2011, Soleño et al. 2019, Wei et al. 2020), the decrease of susceptibly in codling moth collected in southern France is concerning. Therefore, proactive resistance monitoring in codling moth field populations in Europe is important to help to implement insecticide resistance management strategies for this key pest.

The determination of diagnostic (or discriminant) concentrations to separate resistant from susceptible individuals of a given insecticide is highly valuable because it can speed up resistance monitoring without the need to establish concentration-response curves for each field population.

The appropriateness of a laboratory-susceptible strains as reference point for determining changes in susceptibility in field populations over time has been questioned (Roush et al. 1990), as susceptible colonies isolated and reared for a long time in the laboratory may not resemble susceptible strains actually occurring in the fields (ffrench-Constant et al. 1990). The susceptibility of a range of geographically diverse field populations can also be used to set diagnostic concentrations rather than a poorly representative response of an inbred laboratory-susceptible strain (Sawicki et al. 1987). Therefore, using our estimates of the pooled LC_{95}, we propose the use of 0.021 mg a.i./kg and 0.59 mg a.i./kg of diet for emamectin benzoate and lambda cyhalothrin, respectively, to discriminate between resistant and susceptible individuals.

Although non-chemical control methods are available, codling moth management still relies on insecticides in most of the regions where this pest occur. Therefore, IRM strategies implementing rotation of insecticides with different MoA is paramount. Unfortunately, proper rotation of active ingredients is often hampered by pressures of the food chains in favor of a small number of active ingredients identified in residues. Stringent market requirements in terms of number of active substances and their residue levels in fruits at harvest drove farmers toward the use of broad-spectrum insecticides such as pyrethroids (Bosch 2018b). The severe residue requirements claimed by the commercial market which are stronger than the legal requirements should be reduced, in order to provide growers with active ingredients with different mode of actions, to promote rotation of insecticides and to develop a resistance management strategy in IPM programs (Ju et al. 2021).The susceptibility data for emamectin benzoate lambda-cyhalothrin established in this study can be a useful tool for tracking future changes in the susceptibility of codling moth populations to these widely used active ingredients, thus avoiding a possible decline in effectiveness in controlling *C. pomonella*. Nevertheless, further sampling for proactive resistance management will be required for improving the control of this pest.

References

Ahmad, M., A. Farid and M. Saeed (2018). "Resistance to new insecticides and their synergism in Spodoptera exigua (Lepidoptera: Noctuidae) from Pakistan." Crop Protection **107**: 79-86.

Bird, L. J., L. J. Drynan and P. W. Walker (2017). "The Use of F2 Screening for Detection of Resistance to Emamectin Benzoate, Chlorantraniliprole, and Indoxacarb in Australian Populations of Helicoverpa armigera (Lepidoptera: Noctuidae)." Journal of Economic Entomology **110**(2): 651-659.

Bosch, D., J. Avilla, S. Musleh and M. A. Rodríguez (2018a). "Target-site mutations (AChE and kdr), and PSMO activity in codling moth (Cydia pomonella (L.) (Lepidoptera: Tortricidae)) populations from Spain." Pesticide Biochemistry and Physiology **146**: 52-62.

Bosch, D., M. A. Rodríguez and J. Avilla (2018b). "Monitoring resistance of Cydia pomonella (L.) Spanish field populations to new chemical insecticides and the mechanisms involved." Pest Management Science **74**(4): 933-943.

Burg, R. W., B. M. Miller, E. E. Baker, J. Birnbaum, S. A. Currie, R. Hartman, Y.-L. Kong, R. L. Monaghan, G. Olson, I. Putter, J. B. Tunac, H. Wallick, E. O. Stapley, R. Oiwa and S. Ōmura (1979). "Avermectins, New Family of Potent Anthelmintic Agents: Producing Organism and Fermentation." Antimicrobial Agents and Chemotherapy **15**(3): 361-367.

Che, W., J. Huang, F. Guan, Y. Wu and Y. Yang (2015). "Cross-resistance and Inheritance of Resistance to Emamectin Benzoate in Spodoptera exigua (Lepidoptera: Noctuidae)." Journal of Economic Entomology **108**(4): 2015-2020.

Depalo, L., A. Lanzoni, A. Masetti, E. Pasqualini and G. Burgio (2017). "Lethal and Sub-lethal Effects of Four Insecticides on the Aphidophagous Coccinellid Adalia bipunctata (Coleoptera: Coccinellidae)." Journal of Economic Entomology **110**(6): 2662-2671.

Depalo, L., A. Masetti, J. Avilla, D. Bosch and E. Pasqualini (2016). "Toxicity and residual activity of spinetoram to neonate larvae of Grapholita molesta (Busck) and Cydia pomonella (L.) (Lepidoptera: Tortricidae): Semi-field and laboratory trials." Crop Protection **89**: 32-37.

Feely, W. F., L. S. Crouch, B. H. Arison, W. J. VandenHeuvel, L. F. Colwell and P. G. Wislocki (1992). "Photodegradation of 4''-(epimethylamino)-4''-deoxyavermectin B1a thin films on glass." Journal of Agricultural and Food Chemistry **40**(4): 691-696.

ffrench-Constant, R. H. and R. T. Roush (1990). Resistance Detection and Documentation: The Relative Roles of Pesticidal and Biochemical Assays. Pesticide Resistance in Arthropods. R. T. Roush and B. E. Tabashnik. Boston, MA, Springer US**:** 4-38.

Geier, P.W. (1964). "Population dynamics of codling moth, cydia pomonella (L) (Tortricidae), in the Australian Capital Territory." Australian Journal of Zoology **12**, 381-416.

Hafez, A. M., D. Mota-Sanchez, L. J. Gut and J. C. Wise (2018). "Choristoneura rosaceana (Lepidoptera: Tortricidae) Resistance to Insecticides in Michigan Apple and Cherry Orchards." Journal of Economic Entomology **112**(2): 812-817.

He, L.-M., J. Troiano, A. Wang and K. Goh (2008). Environmental Chemistry, Ecotoxicity, and Fate of Lambda-Cyhalothrin. Reviews of Environmental Contamination and Toxicology. D. M. Whitacre. New York, NY, Springer New York**:** 71-91.

Hites, R. A. (2021). "The Rise and Fall of Chlorpyrifos in the United States." Environmental Science & Technology **55**(3): 1354-1358.

Hough, W. S. (1928). "Relative Resistance to Arsenical Poisoning of two Codling Moth Strains." Journal of Economic Entomology **21**(2): 325-329.

Ioriatti, C., G. Anfora, G. Angeli, S. Civolani, S. Schmidt and E. Pasqualini (2009). "Toxicity of emamectin benzoate to Cydia pomonella (L.) and Cydia molesta (Busck) (Lepidoptera: Tortricidae): laboratory and field tests." Pest Management Science **65**(3): 306-312.

Ishtiaq, M., M. Razaq, M. A. Saleem, F. Anjum, M. Noor ul Ane, A. M. Raza and D. J. Wright (2014). "Stability, cross-resistance and fitness costs of resistance to emamectin benzoate in a re-selected field population of the beet armyworm, Spodoptera exigua (Lepidoptera: Noctuidae)." Crop Protection **65**: 227-231.

Ju, D., D. Mota-Sanchez, E. Fuentes-Contreras, Y. L. Zhang, X. Q. Wang and X. Q. Yang (2021). "Insecticide resistance in the Cydia pomonella (L): Global status, mechanisms, and research directions." Pesticide Biochemistry and Physiology **178:** 104925.

MacLellan, C. (1976). "Suppression of codling moth (Lepidoptera: Olethreutidae) by sex pheromone trapping of males. " The Canadian Entomologist **108** (10): 1037-1040.

Mota-Sanchez, D., J. C. Wise, R. V. Poppen, L. J. Gut and R. M. Hollingworth (2008). "Resistance of codling moth, Cydia pomonella (L.) (Lepidoptera: Tortricidae), larvae in Michigan to insecticides with different modes of action and the impact on field residual activity." Pest Management Science **64**(9): 881-890.

Pu, X., Y. Yang, S. Wu and Y. Wu (2010). "Characterisation of abamectin resistance in a field-evolved multiresistant population of Plutella xylostella." Pest Management Science **66**(4): 371-378.

Reyes, M., P. Franck, P.-J. Charmillot, C. Ioriatti, J. Olivares, E. Pasqualini and B. Sauphanor (2007). "Diversity of insecticide resistance mechanisms and spectrum in European populations of the codling moth, Cydia pomonella." Pest Management Science **63**(9): 890-902.

Roditakis, E., C. Skarmoutsou and M. Staurakaki (2013). "Toxicity of insecticides to populations of tomato borer Tuta absoluta (Meyrick) from Greece." Pest Management Science **69**(7): 834-840.

Roditakis, E., E. Vasakis, L. García-Vidal, M. del Rosario Martínez-Aguirre, J. L. Rison, M. O. Haxaire-Lutun, R. Nauen, A. Tsagkarakou and P. Bielza (2018). "A four-year survey on insecticide resistance and likelihood of chemical control failure for tomato leaf miner Tuta absoluta in the European/Asian region." Journal of Pest Science **91**(1): 421-435.

Rodríguez, M. A., T. Marques, D. Bosch and J. Avilla (2011). "Assessment of insecticide resistance in eggs and neonate larvae of Cydia pomonella (Lepidoptera: Tortricidae)." Pesticide Biochemistry and Physiology **100**(2): 151-159.

Robertson, J., Jones, M. M., Olguin, E., & Alberts, B. (2017). Bioassays with Arthropods (3rd ed.). CRC Press. 194 pp.

Roush, R. and J. C. Daly (1990). The Role of Population Genetics in Resistance Research and Management. Pesticide Resistance in Arthropods. T. B. E. Roush R.T. Boston MA, Springer Science & Business Media: 97-152.

Sawicki, R. M. and I. Denholm (1987). "Management of resistance to pesticides in cotton pests." Tropical Pest Management **33**(4): 262-272.

Soleño, J., L. Anguiano, A. P. de D'Angelo, L. Cichón, D. Fernández and C. Montagna (2008). "Toxicological and biochemical response to azinphos-methyl in Cydia pomonella L. (Lepidoptera: Tortricidae) among orchards from the Argentinian Patagonia." Pest Management Science **64**(9): 964-970.

Soleño, J., L. B. Parra-Morales, L. Cichón, S. Garrido, N. Guiñazú and C. M. Montagna (2019). "Occurrence of pyrethroid resistance mutation in Cydia pomonella (Lepidoptera: Tortricidae) throughout Argentina." Bulletin of Entomological Research **110**(2): 201-206.

Sparks, T. C. and R. Nauen (2015). "IRAC: Mode of action classification and insecticide resistance management." Pesticide Biochemistry and Physiology **121**: 122-128.

Wei, Z.-H., M. Liu, C. Hu and X.-Q. Yang (2020). "Overexpression of Glutathione S-Transferase Genes in Field λ-Cyhalothrin-Resistant Population of Cydia pomonella: Reference Gene Selection and Expression Analysis." Journal of Agricultural and Food Chemistry **68**(21): 5825-5834.

Wu, Z.-W., Y.-L. Zhang and S.-Q. Shang (2015). "Effectiveness of 12 Insecticides to a Laboratory Population of <i>Cydia pomonella</i> (Lepidoptera: Tortricidae) Newly Established in China." Journal of Economic Entomology **108**(3): 1271-1278, 1278.

Table 1. Details of the population tested. Each population was named with the name of the closest village on the map.

Country	City	Region	Collection date	Crop	Coordinates	Insecticides tested
France	L'Isle sur la Sorgue	Provence	09/2015	Apple	43°54'07"N 5°03'25"E	Emamectin benzoate Lambda cyhalothrin
France	Meauzac	Occitane	10/2015		44°05'15.0"N 1°14'09.0"E	Emamectin benzoate
France	Grisolles	Occitane	10/2017	Apple	43°48'18.2"N 1°17'12.9"E	Emamectin benzoate Lambda cyhalothrin
France	Le Ponts de Cè	Pays de la Loire	09/2018	Apple	47°26'16.8"N 0°32'24.0"W	Emamectin benzoate Lambda cyhalothrin
France	Vaucluse	Provence	09/2019	Apple	43°54'26.0"N 5°03'26.0"E	Emamectin benzoate Lambda cyhalothrin
Italy	Ghibullo	Emilia Romagna	09/2015		44°20'30.51"N 12°08'48.24"E	Emamectin benzoate Lambda cyhalothrin
Italy	Ravenna	Emilia Romagna	10/2015		44°25'59.6"N 12°11'55.8"E	Emamectin benzoate
Italy	Belfiore	Veneto	10/2016	Apple	45°22'46.2"N 11°13'23.4"E	Emamectin benzoate
Italy	Palù	Veneto	11/2016	Apple	45°19'36.5"N 11°09'45.8"E	Emamectin benzoate
Italy	Pieve di Cento	Emilia Romagna	10/2017	Pear	44°43'40.0"N 11°19'24.5"E	Emamectin benzoate Lambda cyhalothrin
Italy	Baricella	Emilia Romagna	10/2017	Pear	44°38'37.0"N 11°33'00.1"E	Emamectin benzoate Lambda cyhalothrin
Italy	Bovolone	Veneto	11/2017	Apple	45°15'57.6"N 11°08'55.8"E	Emamectin benzoate Lambda cyhalothrin
Poland	Grójec	Mazowiecke	12/2015		51°45'56.1"N 20°46'37.1"E	Emamectin benzoate
Spain	Villanova de Belpuig	Catalunya	09/2016		41°37'06.6"N 0°57'08.7"E	Emamectin benzoate
Belgium	Ciney	Wallonie	11/2017		50° 16' 36"N 5° 09' 57"E	Emamectin benzoate Lambda cyhalothrin

Table 2. Results of the probit analysis and the RRs of populations tested with emamectin benzoate.

Population	n	Intercept ± SE	Slope ± SE	LC_{50} (mg/kg)	95%CL	LC_{95} (mg/kg)	(95%CL)	χ^2 (df)	p	RR F	RR L	RR P
Baricella IT	989	7.65 ±0.65	3.41 ±0.30	0.0057	0.0044-0.0070	0.0172	0.0127-0.0311	55.30 (15)	<0.001	3.4	0.8	1.1
Belfiore IT	667	6.47 ±0.61	2.74 ±0.27	0.0043	0.0035-0.0051	0.0173	0.0139-0.0232	4.68 (9)	0.86	2.5	0.6	0.9
Bovolone IT	669	6.87 ±0.60	3.21 ±0.29	0.0083	0.0043-0.0149	0.0271	0.0151-0.273	94,38 (9)	<0.001	4.9	1.2	1.7
Ciney BE	665	9.06 ±0.97	3.37±0.43	0.0036	0.0017-0.0050	0.0101	0.0072-0.0264	46.02 (9)	<0.001	2.1	0.5	0.7
Ghibullo IT	663	7.49 ±0.79	3.06 ±0.34	0.0036	0.0029-0.0042	0.0123	0.0101-0.0164	12.88 (9)	0.17	2.1	0.5	0.7
Grisolles FR	997	6.60 ±0.50	2.91 ±0.23	0.0054	0.0019-0.0092	0.0198	0.0110-0.2340	256.31 (15)	<0.001	3.2	0.8	1.1
Grójec PL	656	12.25 ±1.3	5.37 ±0.58	0.0052	0.0047-0.0058	0.0106	0.0092-0.0128	6.28 (9)	0.71	3.1	0.8	1.0
L'Isle Sorgue FR	652	7.91 ±0.78	3.63 ±0.36	0.0066	0.0058-0.0075	0.0187	0.0154-0.0247	15.61 (9)	0.08	3.9	1.0	1.3
Lab colony	702	6.77 ±0.6	3.12 ±0.28	0.0068	0.0040-0.0106	0.0228	0.0136-0.0955	73.45 (10)	<0.001	4.0	1.0	1.4
Le Ponts de Cè FR	648	8.32 ±0,75	3.10 ±0.30	0.0021	0.0011-0.0032	0.0071	0.0047-0.0140	31.20 (9)	<0.001	1.2	0.3	0.4
Meuzac FR	945	5.82 ±0.51	2.56 ±0.25	0.0056	0.0009-0.0105	0.0244	0.0127-0.4830	180.27 (14)	<0.001	3.3	0.8	1.1
Palù IT	668	9.01 ±0.98	4.07 ±0.45	0.0061	0.0045-0.0079	0.0186	0.0136-0.0329	29.08 (9)	<0.001	3.6	0.9	1.2
Pieve di Cento IT	333	6.67 ±0.91	3.35 ±0.46	0.0102	0.0082-0.0124	0.0316	0.0239-0.0491	2.60 (3)	0.46	6.0	1.5	2.0
Ravenna IT	598	6.63 ±0.83	2.97 ±0.34	0.0059	0.0046-0.0071	0.0210	0.0162-0.0311	9.58 (9)	0.39	3.5	0.9	1.2
Vaucluse FR	651	6,84 ±0.87	2.47 ±0.40	0.0017	0.0008-0.0026	0.0078	0.0058-0.0109	7.47 (9)	0.59	1.0	0.3	0.3
Villanova de B. ES	666	6.55 ±0.71	3.40 ±0.40	0.0119	0.0089-0.0145	0.0361	0.0298-0.0479	15.91 (9)	0.07	7.0	1.8	2.4
Pooled	11169	6.00 ±0.15	2.61 ±0.068	0.0050	0.0043-0.0056	0.0213	0.0181-0.0263					

Table 3. Results of the probit analysis and the RRs of populations tested with lambda cyhalothrin.

Population	n	Intercept (± SE)	Slope (± SE)	LC_{50} (mg/kg)	(95%CL)	LC_{95} (mg/kg)	(95%CL)	χ2 (df)	p	RR F	RR L	RR P
Baricella IT	661	4.30 ±0.47	3.22 ±0.39	0.046	0.039-0.053	0.150	0.122-0.204	8.76 (9)	0.46	1.4	0.9	0.6
Bovolone IT	664	2.17 ±0.24	3.14 ±0.35	0.204	0.176-0.234	0.682	0.543-0.953	8.68 (9)	0.47	6.2	4.2	2.6
Ciney BE	710	3.70 ±0.35	3.71±0.37	0.100	0.088-0.113	0.279	0.234-0.335	15.73 (10)	0.11	3.0	2.0	1.3
Ghibullo IT	661	3.94 ±0.45	2.93 ±0.38	0.045	0.036-0.054	0.166	0.131-0.236	10.14 (9)	0.34	1.4	0.9	0.6
Grisolles FR	661	0.88 ±0.09	1.65 ±0.22	0.292	0.192-0.391	2.910	2.020-5.160	12.28 (9)	0.33	8.8	6.0	3.7
L'Isle sur la Sorgue FR	1089	0.48 ±0.07	1.92 ±0.14	0.565	0.411-0.747	4.070	2.650-7.880	50.51 (17)	<0.001	17.1	11.5	7.1
Lab colony	642	6.62 ±0.76	5.07 ±0.60	0.049	0.044-0.055	0.104	0.089-0.131	1.96 (9)	0.99	1.5	1.0	0.6
Le Ponts de Cè FR	614	2.97 ±0.26	2.00 ±0.23	0.033	0.005-0.068	0.218	0.111-0.009	39.42 (9)	<0.001	1.0	0.7	0.4
Pieve di Cento IT	665	4.41 ±0.54	4.77 ±0.63	0.119	0.103-0.133	0.263	0.225-0.335	7.13 (9)	0.62	3.6	2.4	1.5
Vaucluse FR	666	1.45 ±0.13	1.91 ±0.21	0.174	0.128-0.222	1.260	0.930-1.940	9.20 (9)	0.42	5.3	3.6	2.2
Pooled	5944	2.07±0.06	1.89 ±0.07	0.080	0.058-0.103	0.594	0.443-0.896					

Highlights

The susceptibility to two insecticides was tested in many codling moth populations.

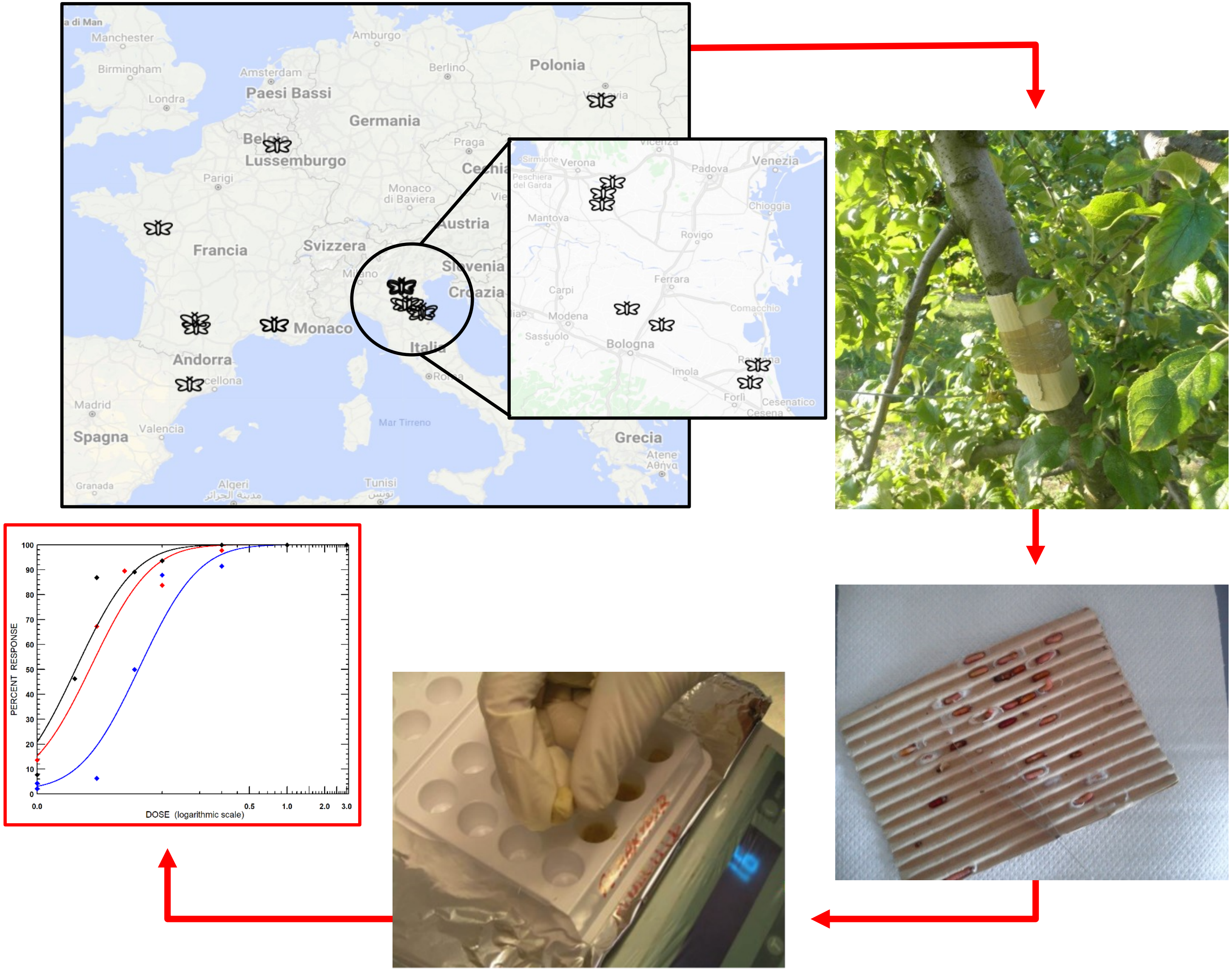
Small range of responses and no resistant strains were found for emamectin benzoate.

A wider variability and a resistant population were detected for lambda cyhalothrin.

0.021 mg a.i./kg is suggested as discriminant concentration for emamectin benzoate.

A discriminant concentration of 0.60 mg a.i./kg is proposed for lambda cyhalothrin.

Graphical Abstract



Graphical abstract Caption

The susceptibility of wild populations of the codling moth *Cydia pomonella* (L.) to emamectin benzoate and lambda-cyhalothrin, was tested by diet incorporation bioassays.

Results revealed small variations in emamectin benzoate susceptibility between all the populations, while a wider range of responses was detected for lambda cyhalothrin.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: