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# Differential influence of temperature on the toxicity of three insecticides against the codling moth *Cydia pomonella* (L.) and two natural enemies

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## Research Article

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# Abstract

Insecticide toxicity may strongly vary with temperature, and interspecific differences have been commonly reported for this relationship. A differential influence of temperature on insecticide toxicity between pests and their natural enemies may have important consequences on biological control in a global warming context. This study aimed to investigate cross effects between temperature and three insecticides - i.e., chlorantraniliprole, emamectin and spinosad - on the mortality of a major pest in orchards, *Cydia pomonella* L., and two of its natural enemies in southern France, the predatory earwig *Forficula auricularia* L. and the introduced parasitoid *Mastrus ridens* Horstmann. We observed a decreased efficiency of emamectin and spinosad with increasing temperature on mortality of codling moth, while no influence of temperature on chlorantraniliprole efficacy was observed. Increasing temperatures increased the toxicity of all insecticides against *M. ridens* and only for emamectin on *F. auricularia*. This study provides essential insight to make recommendations for using these insecticides in combination with two natural enemies to control the codling moth in a warming world. Our results suggest that the use of spinosad may become sub-optimal under higher temperatures. In contrast, chlorantraniliprole should remain suitable under warmer climatic conditions to control *C. pomonella*, conserve *F. auricularia* and facilitate the establishment of *M. ridens*. For conservation biological control relying on *F. auricularia*, alternating use of emamectin during early spring, when its toxicity is the lowest on this natural enemy, and chlorantraniliprole during summer could limit resistance risks in codling moth populations and reduce the insecticides' impact on the populations of natural enemies.

## Introduction

Since the second half of the 19th century, with the first industrial revolution, climatic variations accelerated considerably, increasing greenhouse gas emissions, which are responsible for the warming of Earth's climate. All these climate changes induced by human activities are causing substantial changes in the balance of ecosystems, including agroecosystems, that are of great concern as they are essential for human existence.

Most studies agree that there will be an overall increase in crop losses with accelerated development of resistance to certain control methods, such as the use of insecticides in some species and thus increased damages. This possibility raises major questions about the sustainability of certain pest control methods. Moreover, frequent applications of insecticides to control agricultural pests induce side effects on human health, the environment, and beneficial organisms (Pajač et al. 2011; Koureas et al. 2012; Stehle and Schulz 2015). Due to climate change, their use is expected to increase significantly in the future, aggravating their impact and economic costs (Chen and McCarl 2001; Koleva and Schneider 2009). Several solutions have been developed to ensure a more sustainable agricultural transition. One of them is Integrated Pest Management (IPM) strategy which is a science-based, decision-making process that combines biological, cultural, physical, and chemical tools to identify, manage and reduce risk from pests in a way that minimises economic, health and environmental risks (Stark et al. 2007; Dara 2019).

In IPM strategy, natural enemies, whether they are imported, conserved, or supplemented in agroecosystems (Boivin 2001; Winkler et al. 2005), play a key role as biological control agents (Lacey and Unruh 2005). Despite their prominent role, natural enemies commonly provide incomplete pest control, and insecticides must be used sparingly as a complement (Stark et al. 2007). Nevertheless, most insecticides cause lethal and sublethal effects on natural enemies, thus indirectly reducing pest control (Desneux et al. 2007; Rajak et al. 2014). It is then important in IPM strategy to assess the impact of insecticides on natural enemies and to select active substances with a low impact on biocontrol agents.

Such ecotoxicological studies have been commonly performed, but very few attempted to assess the insecticides' impact on natural enemies in a climate change context, even though temperature influences their toxicity on several pests (Glunt et al. 2013), predators (Mansoor et al. 2015) or parasitoid species (Abbes et al. 2015). Contrasting results have been reported, depending on the insecticide class (Musser and Shelton 2005; Boina et al. 2009), the active substance itself and the insect species. For example, the toxicity of acetamiprid and chlorpyrifos increases with increasing temperature but decreases for  $\lambda$ -cyhalothrin and spinosad (Mansoor et al. 2015) in the predator *Chrysoperla carnea* Stephens (Neuroptera, Chrysopidae). Conversely,  $\lambda$ -cyhalothrin toxicity increases at high temperatures, while temperature does not influence spinosad toxicity in *Philodromus* spiders (Michalko and Košulič 2016). This species-dependency of temperature-mediated toxicity of insecticides may be especially problematic with global warming in IPM strategy if toxicity decreases with temperature for the pest but increases for natural enemies. We may thus expect pest outbreaks because of both a reduced efficiency of insecticides and a drop in predator and parasitoids populations. To our knowledge, and despite the potential influence of global warming, no study investigated the impact of temperature and insecticides on a pest species and its natural enemies.

The codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) is one of the most important insect pests in apple orchards worldwide, causing serious yield losses (Barnes 1991; Lacey and Unruh 2005). Species from several insect orders have been reported to be its natural enemies, including predatory Dermaptera, Neuroptera, Coleoptera and Heteroptera (Nikolic et al. 2018) and a diverse assemblage of Hymenopteran parasitoids (Athanasov et al. 1997; Cross et al. 1999; Mills 2005). However, their effectiveness in orchards is quite limited (Maalouy et al. 2013; Thorpe et al. 2016) and insecticides are commonly used to manage *C. pomonella* and mating disruption (Witzgall et al. 2008; Grigg-McGuffin et al. 2015; Ioriatti and Lucchi 2016). The intensive use of insecticides, associated with the multivoltinism and high fertility of the codling moth, has, however, led to the development of resistance toward several chemical groups of insecticides in wild populations (Sauphanor et al. 2000; Boivin et al. 2001; Bouvier et al. 2001; Brun-Barale et al. 2005). There is thus a need to improve alternative or complementary methods to chemical products for the management of this pest.

In this paper, we investigated the effect of temperature on the efficacy of three commonly used insecticides against *C. pomonella* and two natural enemies of this pest occurring in southern France, the predatory European earwig *Forficula auricularia* (L.) (Dermaptera: Forficulidae) and the parasitoid *Mastrus ridens* Horstmann (Hymenoptera: Ichneumonidae). *Forficula auricularia* is an abundant native

predator known to feed on codling moth eggs in apple orchards (Glen 1975; Unruh et al. 2016). It thus plays a key role in conservation biological control (CBC). *Mastrus ridens* have been introduced in several countries to control *C. pomonella* (Sandanayaka et al. 2018; Charles et al. 2019). It was introduced in France between 2018 and 2021 in the frame of a classical biological control (CIBC) programme against the codling moth aiming at the permanent establishment of this parasitoid to provide sustainable pest control (Borowiec et al. 2020). To optimise field practices, we tested insecticides that are commonly used against codling moth and are promising for use in conjunction with biological control strategies: two reduced-risk insecticides used in conventional apple orchards, emamectin and chlorantraniliprole (European Food Safety Authority 2012; Redmond and Potter 2017), and one insecticide approved in organic apple orchards, spinosad.

Our experiment was thus performed to investigate changes in insecticide toxicity on the codling moth and its natural enemies as temperature increases and discuss the relevance of these substances in IPM strategy relying on both methods to control *C. pomonella* in a warming world.

## Material And Methods

### 1. Insects

#### 1.1 *Cydia pomonella* L.

The laboratory strain of *C. pomonella* used for this study (later called *C. pomonella* strain NPP) originates from northern France and has been mass-reared in the laboratory since 1995. It is used on an industrial scale to produce Carpovirusine™ by Natural Plant Protection Firme, Pau, France. In our study, *C. pomonella* strain NPP was reared on an artificial diet (prepared according to Guennelon et al. 1981 recipe without formaldehyde) at INRAE PSH Avignon (France). The rearing temperature was of 23°C with a photoperiod of 16:8 h Light:Dark (L:D).

#### 1.2 *Mastrus ridens* Horstmann

*Mastrus ridens* were reared at INRAE ISA (Sophia Antipolis, France) in insect-proof cages. The rearing temperature was 23°C with a photoperiod of 16:8 h L:D. This strain results from a mix of several strains: laboratory stock from New Zealand (2015) and Chile (2016) and individuals collected in Kazakhstan (2018). Each cage contained 50 females and 35 males and was provided fresh honey and water daily. For reproduction, 50 to 60 overwintering codling moth cocoons were placed in cages every 3–4 days.

#### 1.3 *Forficula auricularia* L.

*Forficula auricularia* insects were sampled between March and June 2021 using traps cardboard sheets in peach and apple experimental orchards of INRAE PSH Avignon, southern France, where very few insecticides have been applied for the last 10 years. Fifty *F. auricularia* adults were placed in ventilated

boxes with an artificial diet (the same as *C. pomonella*) and cardboard sheets as shelters. The rearing temperature was 25°C with a photoperiod of 16:8 h L:D until experiments.

## 2. Insecticides and bioassays

### 2.1 Insecticides

The commercial formulations of emamectin benzoate (Affirm® 0.95%), spinosad (SUCCESS® 4 480 g/L) and chlorantraniliprole (CORAGEN® 200 g/L) were purchased respectively from Syngenta (France), Dow AgroSciences Distribution (France) and FMC Agricultural solutions (France). These products were used diluted in water for all the bioassays by ingestion. The dilutions were made separately according to the species (Tables 1, 2 and 3). The pure active substances of insecticides (chlorantraniliprole PESTANAL®, standard analytical purity  $\geq 95.0\%$ ; emamectine benzoate PESTANAL®, standard analytical purity  $\geq 85.0\%$ ; spinosad PESTANAL®, standard analytical purity  $\geq 95.0\%$ ) were purchased and used dissolved in acetone to perform all the bioassay by contact. For these bioassays by contact, one unique dose corresponding to the one used in the field (X dose) was used for each insecticide: 2 mg/L for emamectin, 0.096 mg/L for spinosad and 0.035 mg/L for chlorantraniliprole.

### 2.2 Exposure methods

The application method differs for each species depending on the targeted stage in the field. Codling moths are targeted at the larval stage and are more likely to be exposed through ingestion. The two natural enemy species used will be present in the field at the end of their development as larvae and adults for *F. auricularia* and only as adults for *M. ridens*. These two species can then be exposed *in natura* by ingesting contaminated food (fruits, nectar, eggs, larvae and others) or by contact with the surface of contaminated leaves and fruits.

### 2.3 Temperature ranges

The temperature range evaluated is based on (i) the IPCC warming forecasts projected for the 2005–2010 average summer temperatures in Avignon and (ii) the thermal optima of the different species.

(i) According to the 5-year data (2005–2010) of the meteorological station of Avignon (Station METAR/SYNOP 07563, 48 m.a.s.l., 43.95°N 4.82°E), the average maximum summer temperature was 30.13°C.

According to the IPCC 2021, climatologists predict an average warming of 1.8°C (best scenario) to 4.4°C (worst case) by 2100 (IPCC 2021).

These combined data allowed us to set the maximum temperature tested in this study at 35°C, which is consistent with the warming predictions in the Southern France region.

(ii) For natural enemies more sensitive to temperature changes, temperature selection was based on two publications (Kharboutli & Mack, 1993; Devotto et al. 2010) and unpublished experimental data. For

codling moths, unpublished experimental data on larval development at different temperatures allowed the selection of the four most suitable temperatures.

#### 2.4 *Cydia pomonella* toxicity bioassay by ingestion

After a few days of mating, eggs were collected, washed with a water-based solution of dishwashing liquid and bleach, and dried. Neonatal larvae were used for bioassays since it is the target of insecticide treatments. We assessed the influence of both temperature and insecticides on the mortality of *C. pomonella* larvae.

To perform these tests, 96 microwell plates containing approximately 150 µL of artificial diet (Stonefly diet Industries Ltd, Rochester, NY) per microwell were treated with different insecticide concentrations diluted in osmosed water (Table 1). Six µL of insecticide solution, or water for control, was deposited in each microwell on the diet surface. After 20 minutes of drying, newborn larvae were individually deposited, and microwells were closed with parafilm strips. Plates were then placed in climatic chambers at four different temperatures: 20°C, 25°C, 30°C and 35°C. The mortality rate was assessed at 105-degree-days, i.e., 10 days at 20°C, 7 days at 25°C, 5 days at 30°C and 4 days at 35°C, to homogenise the larval stages at the time of observation. A larva was considered dead when not responding to a probe with dissecting forceps. Missing larvae were removed from the data (< 1%). The corrected mortality was calculated using the Abbott formula (Abbott 1925). Each test consisted of 24 individuals per concentration, insecticide, and temperature and was replicated three times. To ensure that potential differences in toxicity with temperature were physiologically explained and not the consequences of recording mortality at different times according to temperature (i.e., 105-degree-days), we also performed three replicates with mortality observations at 5 days for all temperatures.

$$Corrected\ mortality = 100 \times \left( 1 - \frac{\left( 1 - \left( \frac{dead\ treated\ individuals}{total\ treated\ individuals} \right) \right)}{\left( 1 - \left( \frac{dead\ control\ individuals}{total\ control\ individuals} \right) \right)} \right)$$

Table 1

Concentrations of active insecticide substance used for bioassays on *Cydia pomonella* neonate larvae (for information, only doses in mg/L were converted in X dose corresponding to the field dose used in French orchards)

Insecticides	Concentrations (mg/L)					
Affirm® (emamectin)	0.02	0.03	0.05	0.08	0.14	0.22
	(X/100)	(X/67)	(X/40)	(X/25)	(X/14)	(X/9)
CORAGEN® (chlorantraniliprole)	0.3	0.53	0.95	1.69	3	
	(8.5X)	(15X)	(27X)	(48X)	(86X)	
SUCCESS 4® (spinosad)	0.05	0.15	0.45	1.34	4.01	12
	(X/2)	(1.5X)	(4.7X)	(14X)	(42X)	(125X)

## 2.5 *Mastrus ridens* toxicity bioassay

### 2.5.1. By ingestion

In this experiment, we evaluated the influence of both temperature and ingestion of insecticides on the mortality of *M. ridens*. Since only adults are exposed to insecticides in orchards (larvae are ectoparasitoids of cocoons), we used freshly (< 48 h old) emerged males of *M. ridens* for bioassays. To perform this experiment, little pieces of paper (1 cm<sup>2</sup>) were soaked with 15 µL of a honey/water solution (3/1) untreated for control or polluted with different insecticides concentration (Table 2). Soaked papers were placed in 10 mL glass vial caps. After the preparation of control and treated vials, five males were placed per vial, and the caps were only partially screwed to allow the air to pass through. Bioassays vials were placed in climatic chambers at 23°C, 28°C or 33°C. The mortality rate was assessed each day for 72 hours. Individuals were considered dead when not responding to a probe with dissecting forceps. Missing individuals were removed from the dataset. The corrected mortality was calculated using the Abbott formula (Abbott 1925), represented in section 2.4. Each test consisted of five individuals per dose, insecticide and temperature and was repeated four times.

Table 2

Concentrations of active insecticide substance used for ingestion bioassays on *Mastrus ridens*. Concentrations are given in mg/L and converted in X dose corresponding to the field dose used in French orchards.

Insecticides	Concentrations (mg/L and X dose)						
Affirm® (emamectin)	0.4	1	2	4	10	20	
	(X/5)	(X/2)	(X)	(2X)	(5X)	(10X)	
CORAGEN® (chlorantraniliprole)	0.018	0.035	0.07	0.175	0.35	1.75	3.5
	(X/2)	(X)	(2X)	(5X)	(10X)	(50X)	(100X)
SUCCESS 4® (spinosad)	0.02	0.05	0.096	0.2	0.48	0.96	
	(X/5)	(X/2)	(X)	(2X)	(5X)	(10X)	

### 2.5.2. By contact

In this experiment, we assessed the influence of temperature and insecticide exposure by contact on the mortality of males. Little pieces of paper (1 cm<sup>2</sup>) were soaked with 15 µL of an untreated honey/water solution (3/1) and placed in 10 mL glass vial caps. Glass vials were previously treated with 500 µL of acetone for control or with 500 µL of insecticide-polluted acetone (see insecticides section). Control and treated vials were gently laid down and rolled until complete evaporation of the solvent to homogenise the distribution of the solution throughout the vial's surface. After 2 hours of drying, five males were



placed per vial. Caps were partially screwed on the vials, so air would always pass through. Bioassays vials were placed in climatic chambers at 23°C, 28°C or 33°C. The mortality rate was assessed as in the previous experiment. Each test consisted of five individuals per dose, insecticide and temperature and was repeated four times.

## 2.6 Forficula auricularia toxicity bioassay

### 2.6.1. By ingestion

Since *F. auricularia* adults are more active than larvae and the most likely to be exposed to insecticides, we tested the influence of both temperature and ingestion of insecticides on their mortality. To perform this experiment, individual ventilated Petri dishes were filled with humidified sand, and Eppendorf tube caps were filled with the artificial diet (Guennelon et al. 1981 without formaldehyde). For each cap, a 6 µL volume of insecticide solution with different concentrations or no solution for control was deposited on the diet surface (Table 3). After 20 minutes of drying, one cap was placed on a single petri dish, and one individual of *F. auricularia* was introduced. Petri dishes were placed in climatic chambers at 23°C, 28°C or 33°C. The mortality was assessed each day at the same hour for five days. Individuals were considered dead when not responding to a probe with dissecting forceps. Missing individuals were removed from the data. The corrected mortality was calculated using the Abbott formula (Abbott 1925), represented in section 2.4. Each test consisted of one individual per dose, insecticide and temperature and was repeated 15 times per sex.

Table 3

Concentrations of active insecticide substance used for ingestion bioassays on *Forficula auricularia*. Concentrations are given in mg/L and converted in X dose corresponding to the field dose used in French orchards.

Insecticides	Concentrations (mg/L and X dose)				
Affirm® (emamectin)	4 (2X)	10 (5X)	20 (10X)	40 (20X)	100 (50X)
CORAGEN® (chlorantraniliprole)	3.5 (100X)				
SUCCESS 4® (spinosad)	0.096 (X)	0.2 (2X)	0.48 (5X)	0.96 (10X)	2 (20X)

### 2.6.2. By contact

We evaluated the influence of temperature and insecticide exposure by contact on the mortality of *F. auricularia* adults. Insecticides were used in the form of pure active substances dissolved in acetone. Petri dishes were treated with 115 µL of acetone for control or with 115 µL of insecticide-polluted acetone

(see insecticides section). Following application, the solvent was spread over the entire surface of the Petri dish, including the sides. After 30 minutes, five individuals of *F. auricularia* were placed in a single Petri dish, and the artificial diet (Guennelon et al. 1981 without formaldehyde) was provided. The Petri dishes were placed in climatic chambers at 23°C, 28°C or 33°C. The mortality rate was assessed as in the previous experiment. Each test consisted of five individuals per dose, insecticide and temperature and was repeated three times per sex.

### 3. Data analyses

Dose-response curves were fitted with the package *drc* using R software (Ritz et al. 2015; R Core Team 2020 version 4.0.2) to calculate the different LD<sub>50</sub> for each temperature and each insecticide. The *drm* function was used to calculate the different LD<sub>50</sub>, and the *EDcomp* function was used to compare each concentration and temperature evaluated.

For contact bioassays, corrected mortality was calculated using the Abbott regression (Abbott 1925), and differences in mortality between treatments, sexes (for *F. auricularia*) and temperatures were analysed using a Chi-square test.

## Results

### *Cydia pomonella*

Based on observations at 105-degree-days, the temperature decreased the toxicity of emamectin by 1.5, two and three times the dose of chemicals required to kill 50% of the larvae at 25°C ( $p = 0.01$ ), 30°C ( $p = 0.008$ ) and 35°C ( $p = 0.009$ ) respectively, compared to 20°C (Fig. 1A). Temperature also decreased the toxicity of Spinosad with fourth the dose of chemicals required to kill 50% of the larvae at 25°C, 30°C and 35°C compared to 20°C ( $p = 0.04$ ) (Fig. 1B). However, no significant difference was observed between the three higher temperatures ( $p > 0.3$ ) (Fig. 1B). Temperature did not significantly influence the mortality of codling moth larvae exposed to chlorantraniliprole ( $p > 0.6$ ) (Fig. 1C).

We observed similar results for mortality assessment at constant time, i.e., 5 days (Online Resource 1, Table 1). Thus, our results are due to differences in the influence of temperature on insecticides' toxicity and not by differences in reading time.

### *Mastrus ridens*

#### Toxicity by ingestion

Spinosad toxicity increased with increasing temperatures when *M. ridens* ingested the insecticide. Four times less product was needed to kill 50% of the individuals at 28°C ( $p = 0.008$ ) and six times less at 33°C ( $p = 0.009$ ) compared to 23°C (Fig. 2B). Similarly, chlorantraniliprole toxicity increased with increasing temperatures with eight and 15 times less product needed to kill 50% of the individuals at 28°C and 33°C respectively compared to 23°C ( $p = 0.04$ ) (Fig. 2C). For emamectin, a similar trend as for the other

insecticides was observed. However, differences between temperatures were not significant ( $p > 0.8$ ), probably because of a high variability occurring at 23°C (Fig. 2A).

## Toxicity by contact

Table 4  
Effects of temperature and insecticides at field dose (X dose) on the mortality of *Mastrus ridens* adults. Different letters indicate significant differences between treatments. (Chi-square  $\chi^2$  test).

Insecticide (X dose)	Corrected mortality (%)		
	23°C	28°C	33°C
emamectin	64.74 <sup>a</sup> ( $\chi^2 = 0$ )	100 <sup>b</sup> ( $\chi^2 = 8.17$ )	100 <sup>b</sup> ( $\chi^2 = 8.17$ )
spinosad	68.27 <sup>a</sup> ( $\chi^2 = 0$ )	100 <sup>b</sup> ( $\chi^2 = 6.97$ )	100 <sup>b</sup> ( $\chi^2 = 6.97$ )
chlorantraniliprole	100 ( $\chi^2 = 0.40$ )	100 ( $\chi^2 = 0.15$ )	100 ( $\chi^2 = 0.15$ )

Toxicity by contact of emamectin and spinosad increased significantly with temperature as it rose from less than 70% at 23°C to 100% mortality at 28°C and 33°C for both insecticides. The mortality reached 100% when chlorantraniliprole was applied, regardless of the temperature.

## Forficula auricularia

### Toxicity by ingestion

Emamectin toxicity increased at 28°C ( $p = 0.008$ ) and 33°C ( $p = 0.009$ ) but did not at 23°C (Fig. 3A) when earwigs ingested the insecticide. A similar trend was observed for spinosad, but differences between temperatures were not significant ( $p > 0.8$ ), probably because of a high variability occurring at 23°C (Fig. 3B). For chlorantraniliprole, no mortality occurred at the single dose tested (3.5 mg/L, corresponding to 100× the approved field rate), regardless of temperature.

### Toxicity by contact

We tested a single dose corresponding to the approved field dose of each insecticide substance. No mortality was observed for *F. auricularia* adults regardless of the insecticide or the temperature evaluated.

## Discussion

Climate change may differentially affect the efficiency of insecticide treatments against pests and beneficial insects through a wide range of factors in the field. This study first wants to assess the effect of temperature on three insecticide toxicity in the controlled condition in the laboratory to avoid the complex interactions between factors, including the behaviour or ecology of arthropods *in natura*. It provides a first step of whether the efficacy of these insecticides may change with the climate using the

example of a pest and two of its natural enemies used in IPM programmes. Indeed, the consequences of climate change on biological control caused by a differential influence of temperature on insecticide toxicity between pests and their natural enemies have never been considered, although such an influence is known to be species-specific. This topic is especially relevant for organic farming and IPM strategy, which rely on both natural enemies and insecticides. The worst scenario is that insecticide toxicity decreases with global warming for a pest but increases for its natural enemies.

In this study, we observed that higher temperature (i) decreased the toxicity of emamectin and spinosad but did not influence the toxicity of chlorantraniliprole against the pest *C. pomonella*, while it (ii) increased the toxicity of these three insecticides on the parasitoid, *M. ridens*, and (iii) increased the toxicity of emamectin on the predator, *F. auricularia*. Our results suggest a possible problem in future *C. pomonella* population control in the field because of a decreased efficiency of insecticides on the pest coupled with the opposite effect on natural enemies' populations.

Our results on *C. pomonella* agree with those reported for spinosad on another pest species, the cotton mealybug *Phenacoccus solenopsis* Tinsley (Mansoor et al. 2014). However, our results on *C. pomonella* contrast with those obtained on *P. solenopsis* (Mansoor et al. 2014) and *Plutella xylostella* L., on which toxicity of emamectin increased with temperature as for chlorantraniliprole on *P. xylostella* (Li et al. 2004; Teja et al. 2018). Indeed, interspecific differences in temperature-mediated insecticides' toxicity between pests have been frequently reported (Boina et al. 2009), although underlying mechanisms still have to be investigated.

Compared to pests, few studies have been conducted on natural enemies. Still, increased toxicity at high temperatures was reported for chlorantraniliprole on the parasitoid *Bracon nigricans* Szépligeti (Abbes et al. 2015), as we observed for *M. ridens* when the substance was ingested. However, in our study, spinosad toxicity increased with temperature for *M. ridens*. At the same time, it was not influenced by temperature in *B. nigricans* (Abbes et al. 2015) and decreased with increasing temperature in the lacewing *Chrysoperla carnea* Stephens (Mansoor et al. 2015). The same interspecific differences for pests were also observed for natural enemies.

The positive relationship between toxicity and temperature observed for the three substances in *M. ridens* and emamectin ingested by *F. auricularia* may result from several mechanisms that should be investigated in further studies. First, high temperature may influence detoxification enzymes (Yan et al. 2013; Zhang et al. 2015; Liu et al. 2017) or Heat Shock Protein (Ge et al. 2013; Su et al. 2018) activities and expression. Second, metabolic rate increases with temperature (Brown et al. 2004), consequently increasing food consumption, insect locomotion (Gillooly 2001; Medrzycki et al. 2010), and substance penetration in the insect's body (Boina et al. 2009).

This study was conducted in a simplified system where insects are "forced" into contamination. *In natura*, other complex behavioural or ecological parameters might limit contact between insects and insecticide. For example, *F. XXXuricularia* is a nocturnal species that hunt at dusk, which could allow them to avoid

diurnal substances spraying (Vancassel 1973). Moreover, some parasitoid species also avoid hosts resistant to some insecticides to minimise contamination (Alfaro-Tapia et al. 2021).

Whether the two natural enemies ingested the insecticides or were exposed by contact, *M. ridens* was more sensitive to substances than *F. auricularia*. The three insecticides caused 100% mortality in *M. ridens* above 28°C at the authorised field dose, while no mortality was detected in *F. auricularia*. This interspecific difference may be due to differences in the composition or thickness of their cuticle (Fernandes et al. 2010) and/or insecticide penetration, depending on the affinity between the cuticle and the substance (Leite et al. 1998). The smaller size of *M. ridens* compared to *F. auricularia* may also account for this difference, as the specific target area of the insecticides decreases with increasing body size, resulting in reduced insecticide exposure (Picanco et al. 1997; Bacci et al. 2007).

Such differences may also explain that toxicity was mediated by temperature for the three insecticides for *M. ridens*, while it was only true for emamectin in *F. auricularia*. These interspecific differences in the relationship between temperature and insecticides' toxicity underline the need to study specifically each pest-natural enemy's system. This approach would allow for selecting insecticides that will be the most reliable with global warming. Ideally, insecticides used in IPM programmes and organic agriculture should be less toxic to natural enemies than to target insect pests (Zhao et al. 2012) and remain harmless as temperature increases.

From our results, spinosad was as toxic to the codling moth as it was to the European earwig and was even more toxic to *M. ridens*, regardless of the temperature. It was also more toxic to natural enemies than emamectin and chlorantraniliprole, considering the tiny dose needed to kill 50% of parasitoids compared to the two other insecticides. Lethal and sublethal effects of spinosad and other spinosyns on beneficial arthropods have been previously reported in several studies (Biondi et al. 2012; Abbes et al. 2015), and yet, this product is authorised and widely used in organic farming while emamectin and chlorantraniliprole are not (Biondi et al. 2012). Moreover, our results indicate that higher temperatures emphasise differences in spinosad toxicity between the two trophic levels. Among the insecticides evaluated, it is likely to cause problems managing codling moth populations under high-temperature conditions. The combination of spinosad with strategies of regulation based on natural enemies should be avoided. Its approval for organic production may raise questions in the future considering its increased toxicity on some beneficial organisms as temperature increases.

Surprisingly, chlorantraniliprole may be the most promising insecticide in a warming world for strategies relying partly on biological control by conservation. The toxicity of this substance indeed remained very effective at high temperatures against *C. pomonella* while it did not cause any mortality on *F. auricularia*, regardless of temperature or exposure method. Moreover, it was relatively safe against *M. ridens* when parasitoids ingested the substance. Despite the product's increased toxicity at high temperatures, the lethal doses for 50% of individuals remained high (equivalent to 10–50× the recommended field dose). However, the substance caused 100% mortality during contact for *M. ridens*. Thus, this substance may be

appropriate for classical biological control relying on the introduction of parasitic wasps, although field experiments should confirm our laboratory observations.

For both natural enemies, the LD<sub>50</sub> remained very high at low temperatures for emamectin, indicating that a high quantity of this insecticide should be applied to kill 50% of the individuals. These results are consistent with the literature on other natural enemies for this substance (Argentine et al. 2002; Khan et al. 2018). Emamectin has been recommended in IPM strategies for a long time because of its low toxicity on beneficial organisms, high selectivity for pests and rapid environmental degradation (Argentine et al. 2002; López et al. 2010). However, we observed that its toxicity increased rapidly with temperature for *F. auricularia* and *M. ridens* and decreased for the codling moth. In this sense, its efficacy may decrease with global warming despite being the most effective insecticide against *C. pomonella* among the three tested in terms of effective doses at high temperatures.

Contrary to chlorantraniliprole, emamectin toxicity on *F. auricularia* increased strongly with temperature. Alternating the use of these insecticides in conservation biological control, with preferential use of emamectin during the colder periods (e.g., early spring) and the use of chlorantraniliprole (e.g., during the summer) could limit the risks of resistance appearance in pest's populations while limiting the impact of chemical substances on the European earwig. However, its use in CBC programmes involving the introduction of *M. ridens* should be avoided considering its toxicity at high temperatures.

The present study highlights opposite cross effects of temperature and insecticides (spinosad, emamectin, chlorantraniliprole) on *C. pomonella* and two of its natural enemies, one exotic (*M. ridens*) and one native (*F. auricularia*) in southern France. According to the future climate change predictions, we recommend using these insecticides to preserve natural enemies associated with *C. pomonella*. The use of spinosad should be avoided, while emamectin can be used in conjunction with the conservation of *F. auricularia*. Only chlorantraniliprole appears suitable for controlling *C. pomonella*, conserving *F. auricularia* and establishing *M. ridens*. The present study remains a simplified but essential tool to understand the cross effects of temperature and three insecticides on the target pest and two of its natural enemies, allowing us to propose an adaptation of strategies relying on insecticides in apple orchards in a changing climate. We focused on the mortality of natural enemies, but the effects of sublethal exposures, which affect insects' behaviour and life history traits, should also be investigated since they may be critical to the long-term conservation and/or establishment of natural enemies (Saber 2011; Poorjavad et al. 2014).

## Declarations

### Ethical Approval

Not applicable.

### Competing interests

The authors have no relevant financial or non-financial interests to disclose.

## Authors' Contributions

Perrin M participated in the design of the study, performed the bioassays, performed the statistical analysis and drafted the manuscript. Borowiec N participated in the design and coordination of the study and helped to draft the manuscript. Moiroux J and Siegwart M conceived of the study, and participated in its design and coordination and helped to draft the manuscript. Delattre T helped to draft the manuscript. Thaon M helped to performed the bioassays. All authors read and approved the final manuscript.

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## Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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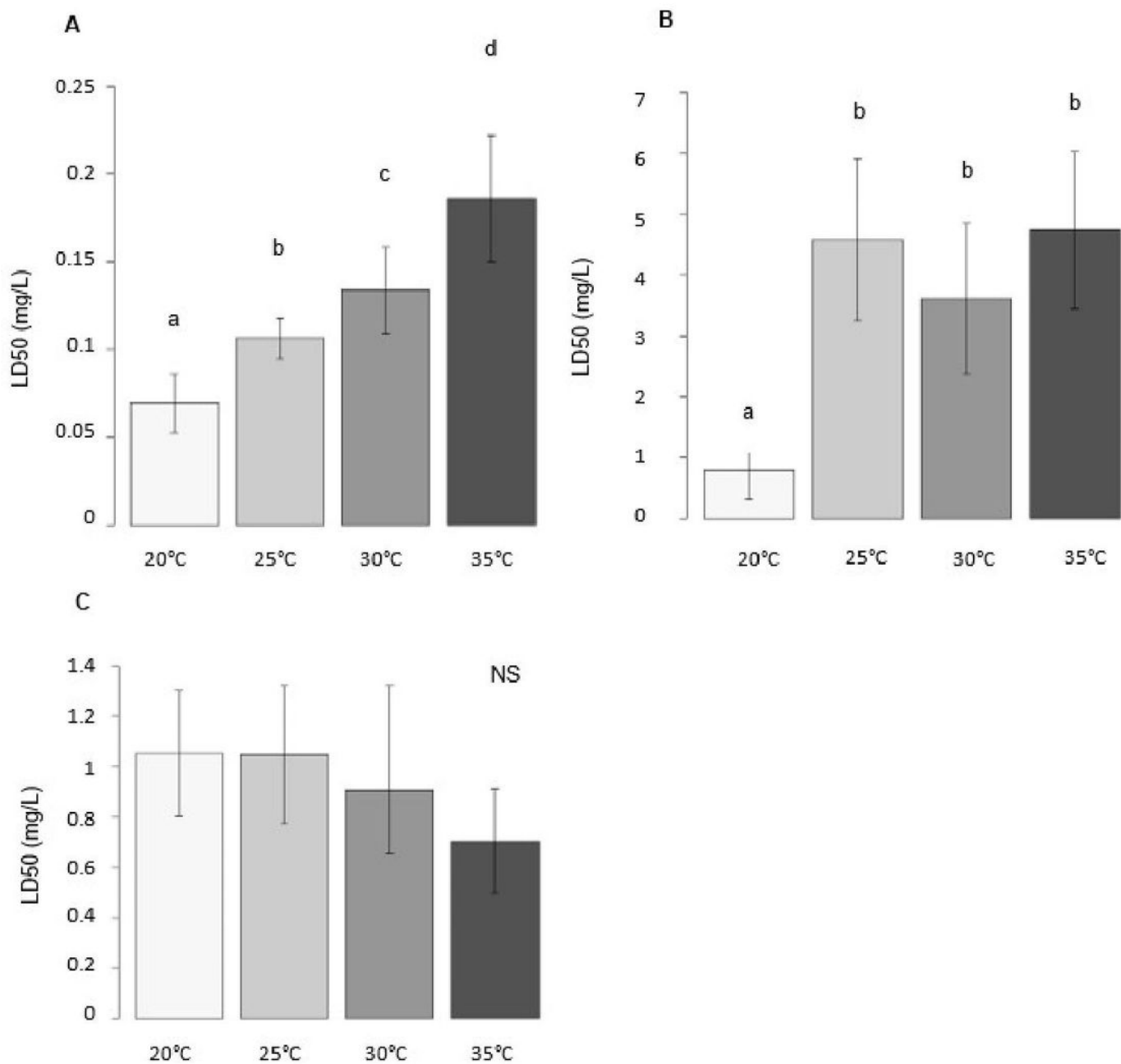
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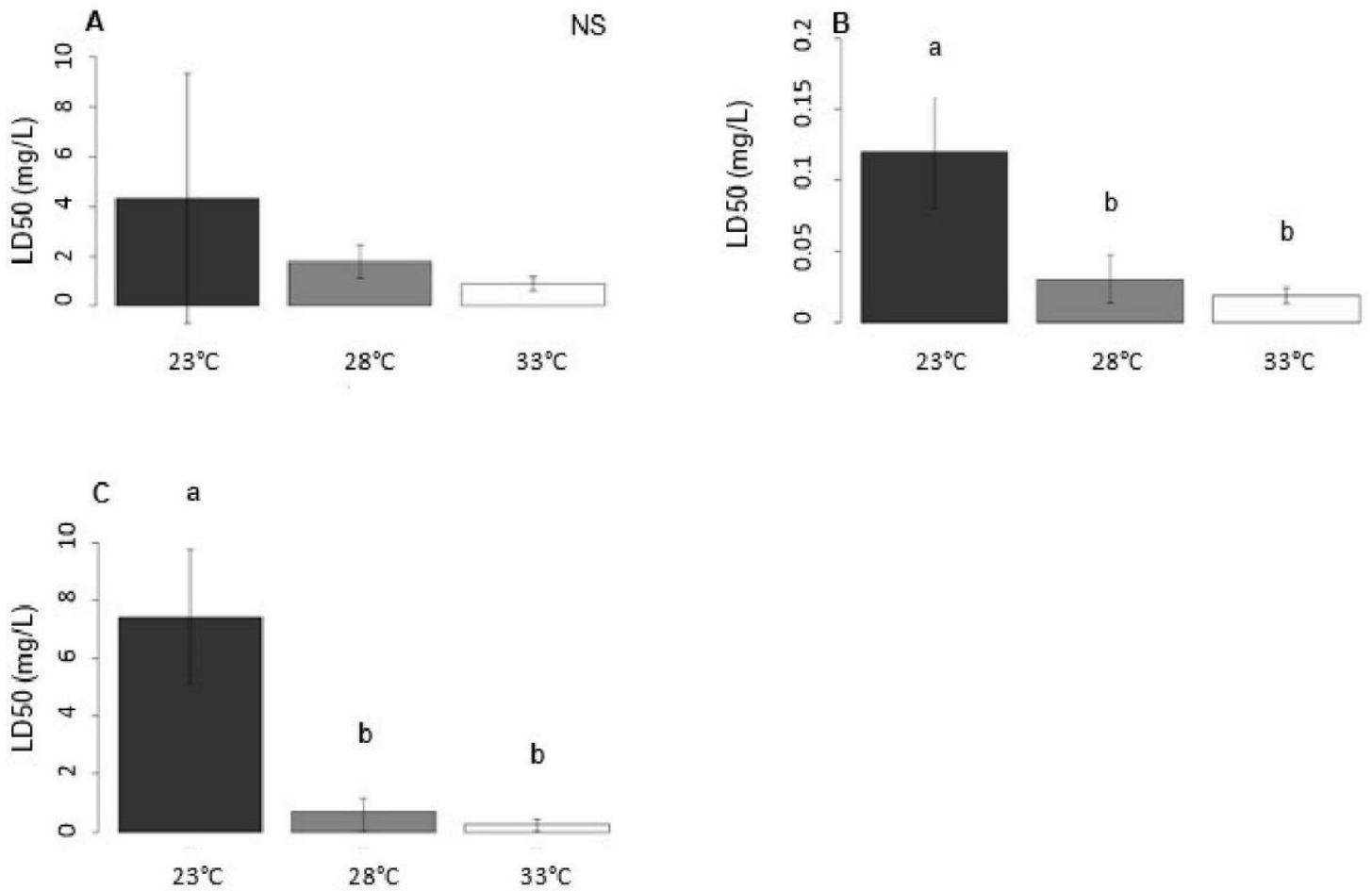
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# Figures



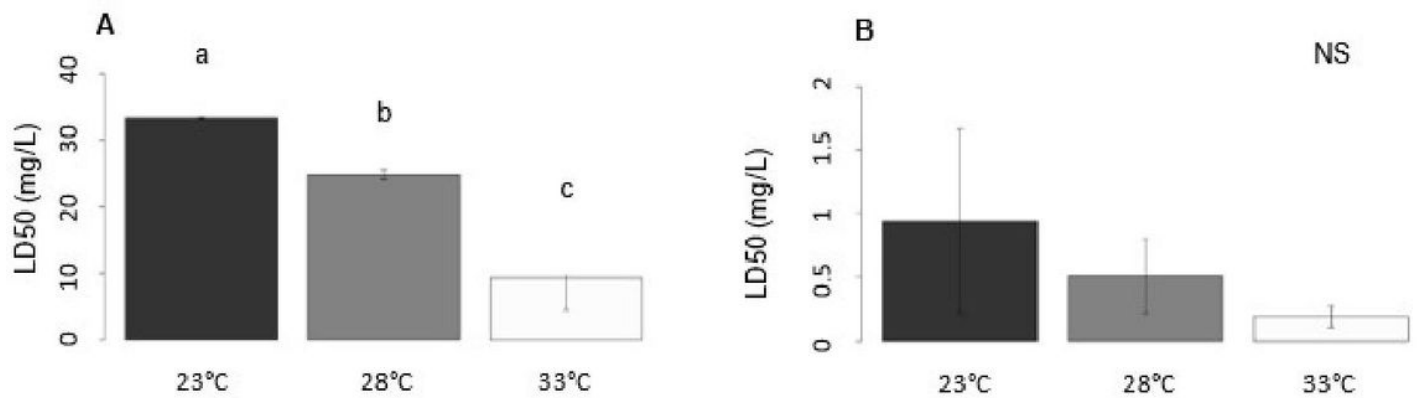
**Figure 1**

Effects of temperature on doses of emamectin (A), spinosad (B) and chlorantraniliprole (C) needed to kill 50% (LD<sub>50</sub>) of codling moth neonatal larvae. Different letters indicate significant differences between temperatures. (EDcomp,  $\alpha = 0.05$ ).



**Figure 2**

Effects of temperature on doses of emamectin (**A**), spinosad (**B**) and chlorantraniliprole (**C**) needed to kill 50% ( $LD_{50}$ ) of *Mastrus ridens* adults. Different letters indicate significant differences between treatments. (EDcomp,  $\alpha = 0.05$ ).



**Figure 3**

Effects of temperature on doses of emamectin (**A**) and spinosad (**B**) needed to kill 50% (LD<sub>50</sub>) of *Forficula auricularia* adults. Results for males and females were pooled since there were no significant differences between the sexes. Different letters indicate significant differences between treatments. (EDcomp,  $\alpha = 0.05$ ).

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [ESM1.docx](#)