The impact of temperature on insecticide sensitivity depends on transgenerational effects

Thibaut Bagni, David Siaussat, Annick Maria, Philippe Couzi, Martine Maïbèche, Manuel Massot

The impact of temperature on insecticide sensitivity depends on transgenerational effects

Thibaut Bagni, David Siaussat, Annick Maria, Philippe Couzi, Martine Maïbèche, and Manuel Massot*

Sorbonne Université, CNRS, INRAe, Institut d'Ecologie et des Sciences de l'Environnement de Paris, iEES-Paris, F-75005, Paris, France

*Corresponding author: manuel.massot@sorbonne-universite.fr

ABSTRACT

The combined effects of insecticides and temperature are increasingly being studied because species are expected to change their responses to insecticides with climate warming. As recently highlighted, the impact of temperature on insecticide sensitivity might be influenced by the environment experienced by the previous generation. However, a pioneering study that showed this transgenerational effect in the mosquito *Culex pipiens* needs to be confirmed because two other studies did not show similar results. Here, we performed an experiment on the moth *Spodoptera littoralis* to test this hypothesis. We analysed reaction norms among experimental families to test transgenerational effects, *i.e.*, the variation in the response of families to the combined effects of the insecticide chlorpyrifos and developmental temperature. Reaction norm analyses revealed that the responses of the families to chlorpyrifos and temperature differed for developmental time and larval survival, two key parameters in *S. littoralis*. Crucially, for larval survival, a family effect influenced the impact of temperature on chlorpyrifos sensitivity. This finding confirms the pioneering study on *C. pipiens* that showed transgenerational effects on the combined effects of insecticides and temperature. This result also highlights that transgenerational plasticity can be important to consider in ecotoxicology.

Keywords: Pesticide, climate warming, transgenerational plasticity, reaction norm, mortality, development.

1. INTRODUCTION

Ecotoxicologists are increasingly concerned about the combined effects of insecticides and temperature on species (Massot et al., 2021; Meng et al., 2022a, 2022b, 2020; Noyes and Lema, 2015). The focus on this interaction between temperature and insecticides has occurred for two reasons. First, greater insecticide use as a result of the current climate change is likely because the impact of warming on insect pests is predicted to augment crop production losses (Deutsch et al., 2018). Second, temperature can influence species responses to insecticides in multiple ways: (*i*) the uptake and excretion of insecticides are often positively related to temperature (Hooper et al., 2013; Noyes et al., 2009), particularly in ectotherms such as insects (Hooper et al., 2013); (*ii*) temperature can alter the biotransformation of insecticides, with a change in their toxicity (Hooper et al., 2013; Noyes et al., 2009); (*iii*) the activity of detoxification enzymes likely depends on temperature as in other enzymes (Angilletta, 2009; Kingsolver, 2009); and (*iv*) species resistance to insecticides might evolve with climate warming (Fournier-Level et al., 2016).

As recently highlighted, the interaction between temperature and insecticides might depend on the environmental effects experienced by the previous generation (Tran et al., 2018). In the mosquito *Culex pipiens*, the effect of warming on mortality caused by the insecticide chlorpyrifos disappeared in the offspring of parents that suffered from warming and the insecticide (Tran et al., 2018). However, this parental effect on the interaction between temperature and insecticides was not found in two other studies (Massot et al., 2021; Meng et al., 2022a). The environmental effects experienced by the previous generation are defined as transgenerational plasticity (Salinas and Munch, 2012; Shama et al., 2014). Transgenerational plasticity describes a variation in offspring plasticity among parents that experienced different

environments (Shama et al., 2014). Transgenerational plasticity can be inferred from studies testing the same environmental factors on offspring and parental generations (*e.g.*, Tran et al., 2018) or from studies testing different environmental factors between the two generations (*e.g.*, Bashey, 2006). Here, we aimed to test the influence of transgenerational plasticity on the interaction between temperature and insecticide in *Spodoptera littoralis*, a model insect for which we have already shown other transgenerational effects (Bagni et al., 2020; Massot et al., 2021).

In one study, we showed transgenerational plasticity on the sensitivity of *S. littoralis* to the insecticide chlorpyrifos, namely, an effect of maternal size. The toxicity of chlorpyrifos was higher in the offspring of larger mothers (Bagni et al., 2020). In a second study, we showed transgenerational plasticity on insecticide and thermal sensitivities of *S. littoralis*, namely, variation in larval survival and developmental time among families for their response to temperature and the insecticide deltamethrin (Massot et al., 2021). This study illustrated the benefit of performing reaction norm analyses to test the variation in the responses of families to toxicants, an approach advised for use more in ecotoxicology (Massot et al., 2021). In the current study, we tested the hypothesis of transgenerational plasticity on the impact of temperature on insecticide sensitivity (Fig. 1). This experiment is based on reaction norm analyses among experimental families as in Massot et al. (2021), but it tested the insecticide chlorpyrifos rather than deltamethrin. We expected different results because the pyrethroid insecticide deltamethrin is less toxic at elevated temperatures (Riskallah, 1984), while the organophosphorus insecticide chlorpyrifos is more toxic at elevated temperatures (Lydy et al., 1999; Meng et al., 2020; Tran et al., 2018). In particular, higher temperatures enhance the biotransformation of chlorpyrifos to more toxic metabolites (Lydy et al., 1999). Although chlorpyrifos was recently banned in some countries, its use is still very common worldwide

(Rahman et al., 2021). Its use is also common against *S. littoralis* (Dewer et al., 2016), this species being a major crop pest (CABI, 2020).

The study is based on a split-plot design crossing chlorpyrifos and temperature treatments. Temperatures of 23, 25, 27 and 29 °C were applied during all development stages. We tested experimental effects on larval survival, pupal survival, development duration, and pupal body mass. Projection models based on life cycle modelling were also used to estimate the effects on the population growth rate. Reproductive parameters were not studied because they minimally impact the population growth rate of *S. littoralis* at temperatures between 23 and 29 °C (Massot et al., 2021). Key analyses of the study were tests of the variation in the response of experimental families for the temperature-induced increase in chlorpyrifos toxicity, *i.e.*, the analyses that questioned transgenerational plasticity of the interaction between temperature and insecticide.

2. MATERIALS AND METHODS

2.1. Experimental design

We performed the study on a laboratory strain maintained at 23 °C and 60-70 % relative humidity, with a 16:8 light/dark cycle, and using a semiartificial food (Hinks and Byers, 1976). We produced experimental clutches following the methodology detailed in Massot et al. (2021). We collected clutches from 19th March to 11th April 2018. This short period was chosen to discard the possibility that a genetic evolution in our rearing leads to effects of the date of collection of the clutches. Therefore, we expected effects of the date of clutch collection related only to transgenerational plasticity as found in Massot et al. (2021). We used a split-plot design to test the response of clutches to temperature and chlorpyrifos (*i.e.*, reaction norms). Clutches collected were divided into two parts to rear them at 23 or 25 °C,

and at 27 or 29 °C (Fig. 2). We chose these close temperatures from 23 to 29 °C because our previous study on deltamethrin insecticide showed that differences of 2 °C within this thermal range were sufficient to induce variation in the response of families to temperature in *S. littoralis* (Massot et al., 2021). We also chose these temperatures because they are near the thermal optimum of *S. littoralis*, and responses to temperature are better estimated near the thermal optima of species (Johnson et al., 2015). The thermal optimum of *S. littoralis* was previously estimated at 29 °C (Massot et al., 2021). We used environmental test chambers (Panasonic MLR-352H, France) to control temperatures independently of relative humidity, maintained at 70 %.

We applied the chlorpyrifos treatments when experimental larvae were at the 4^{th} instar. Each of the half-clutches was divided into three treatment groups of fifteen larvae (Fig. 2). The test solutions with chlorpyrifos (45395, Sigma Pestanal, France) were prepared by serial dilutions in ethanol and hexane. We used hexane because chlorpyrifos has a low solubility in ethanol. The treatment solutions with chlorpyrifos were compared to a control solution of 10 % ethanol and 90 % hexane (ratio with the highest quantity of ethanol used in our chlorpyrifos solutions). The control solution had no toxicity or a very low toxicity in the studied species (Massot et al., 2021). The treatment solutions had chlorpyrifos concentrations of 31 and 44 mg/L. The chlorpyrifos concentration of 44 mg/L is close to the LC_{50} defined in a previous study, with a larval mortality of 58% (Bagni et al., 2020). We applied 0.5 µL of the treatment solutions to the head of the larvae (Hamilton 25 µL syringe with Hamilton dispenser). Mortality was checked 48 hours after the treatment, at pupation, and at adult emergence. Our survey was performed on 2295 larvae reared in 153 experimental boxes. At pupation, we identified the sex of surviving larvae and we weighed two males and two females per experimental box. Developmental time was measured by the duration between the chlorpyrifos treatment and adult emergence.

2.2. Data analyses

Table S1 in Appendix A provides the sample sizes per temperature and chlorpyrifos treatment for each of the tested parameters: larval survival 48 h posttreatment, larval survival > 3 days posttreatment, pupal survival, developmental time and pupal body mass. Following the approach detailed in Massot et al. (2021), all parameters were analysed in two steps. First, we tested the responses to temperature, chlorpyrifos, and their interaction. Second, we analysed the reaction norms of the clutches to temperature and chlorpyrifos. Reaction norm analyses were based on a comparison of statistical models with no clutch variation, total variation among clutches, and variation in the date of clutch collection. Total variation among clutches was modelled with our identification number of clutches. Variation in the date was modelled with the day or week of collection of clutches (clutches were collected at 11 days of three weeks). Models were compared with Akaike information criterion (White and Burnham, 1999). When we found a variation among clutches, the variation in the responses of the clutches to temperature and chlorpyrifos was examined with the tests on the interactions Temperature x Clutch, Chlorpyrifos x Clutch, and Temperature x Chlorpyrifos x Clutch.

We used logistic models to analyse the binomial variables (larval survival 48 h posttreatment, larval survival > 3 days posttreatment, and pupal survival). For developmental time and pupal body mass, we performed an analysis of variance in our test of temperature and chlorpyrifos effects. Reaction norms were tested with generalized linear models to include random factors (clutch and date factors) in mixed-effects linear models. Residuals of the models were examined for normality and homogeneity of variances. We had to perform logistic models for developmental time because these conditions were not met (even using logarithmic transformation). We discretized developmental time with regard to the median values of each temperature treatment to maintain variation in analyses. Indeed, the overlapping between the

developmental times of temperature treatments was very limited. The 99 % confidence interval of the developmental time was 27.9-28.4 days at 23 °C (*n* = 349), 22.3-22.7 days at 25 °C (*n* = 391), 19.4-19.7 days at 27 °C (*n* = 421), and 17.9-18.2 days at 29 °C (*n* = 328). We carried out statistical analyses with JMP software (JMP Pro 15, SAS Institute Inc., Cary, NC), and we simplified models by stepwise removal of terms with $P > 0.10$.

2.3. Life cycle modelling

We followed the methodology detailed in Massot et al. (2021) to estimate the multiplication rate at the population level (Caswell, 2001). In summary, the life cycle of *S. littoralis* was modelled with an age-structured matrix where each age class was one day, and the asymptotic population growth rate was estimated by the dominant eigenvalue of the matrix. This matrix model was parameterised with the estimates obtained in the study for larval survival 48 h posttreatment, larval survival > 3 days posttreatment, pupal survival, duration between the 4th larval instar and pupation, duration of pupal period, and sex-ratio. Other parameters were fixed with estimates obtained in previous studies (Malbert-Colas et al., 2020; Massot et al., 2021). Table S2 provides the values used in the models. The modelling was conducted using the program ULM (Legendre and Clobert, 1995).

3. RESULTS

3.1. Effects of temperature and chlorpyrifos

We first investigated the influence of temperature and chlorpyrifos on larval survival between the chlorpyrifos treatment (at the 4th larval instar) and pupation. Larval survival rates were decreased by chlorpyrifos 48 hours after treatment (Fig. 3A) for both experimental concentrations ($X^2_1 = 11.2$, $P = 0.001$ for 31 mg/L *versus* the control group; $X^2_1 = 99.3$, $P <$

0.001 for 44 mg/L *versus* the control group). If this chlorpyrifos effect did not significantly differ between temperatures for the concentration of 31 mg/L ($X^2_3 = 6.6$, $P = 0.086$ for Chlorpyrifos x Temperature interaction), then it was stronger at 29 °C for the concentration of 44 mg/L (Fig. 3A, $X^2_3 = 8.7$, $P = 0.033$ for Chlorpyrifos x Temperature interaction). We also observed an overall effect of temperature, with larval survival rates decreasing with the warmest temperatures ($X^2_3 = 39.2$, $P < 0.001$; Fig. 3A).

For the larval survival rates estimated between the third day after the chlorpyrifos treatment and pupation (Fig. 3B), we detected a marginally significant Chlorpyrifos x Temperature interaction in our comparison between the concentration of 31 mg/L and the control group ($X^2_1 = 7.6$, $P = 0.056$) and a significant Chlorpyrifos x Temperature interaction in our comparison between the concentration of 44 mg/L and the control group ($X^2_1 = 9.3$, $P = 0.026$). When compared to those of the control group, the larval survival rates of the chlorpyrifos treatments tended to be lower at the highest temperatures (Fig. 3B). However, this response was small compared to the quick response after the first 48 hours (comparison with Fig. 3A).

The chlorpyrifos treatments did not affect female pupal survival ($X^2_1 = 1.2$, $P = 0.28$ for 31 mg/L *versus* the control group; $X^2_1 = 0.8$, $P = 0.38$ for 44 mg/L *versus* the control group) and male pupal survival ($X^2_1 = 1.9$, $P = 0.16$ for 31 mg/L *versus* the control group; $X^2_1 = 0.3$, $P = 0.56$ for 44 mg/L *versus* the control group). Moreover, the Chlorpyrifos x Temperature interaction was not significant in females ($X^2_3 = 2.5$, $P = 0.47$ for 31 mg/L *versus* the control group; $X^2_3 = 1.8$, $P = 0.62$ for 44 mg/L *versus* the control group) or males ($X^2_3 = 3.2$, $P = 0.37$ for 31 mg/L *versus* the control group; $X^2_3 = 0.9$, $P = 0.83$ for 44 mg/L *versus* the control group). Pupal survival rates responded only to temperature, in females ($X^2_3 = 10.7$, $P = 0.014$ for 31 mg/L *versus* the control group; $X^2_3 = 12.9$, $P = 0.005$ for 44 mg/L *versus* the control group) and males ($X^2_3 = 24.1$, $P < 0.001$ for 31 mg/L *versus* the control group; $X^2_3 = 9.0$, $P =$

0.030 for 44 mg/L *versus* the control group). Overall, pupal survival rates were smaller at the lowest developmental temperature of 23 °C (Fig. 3C and D).

A dramatic shortening of development was observed at the warmest temperatures in females and males (Fig. 4A and B). The 99 % confidence intervals of the developmental time did not overlap between the four studied temperatures (see values in Materials and Methods). When compared to the control group, the chlorpyrifos treatments did not affect the developmental time of females ($X^2_1 = 0.2$, $P = 0.69$ for 31 mg/L; $X^2_1 < 0.1$, $P = 0.85$ for 44 mg/L) or the developmental time of males at the chlorpyrifos concentration of 44 mg/L ($X^2_1 = 2.6$, $P = 0.11$). The developmental time of males differed significantly between the chlorpyrifos concentration of 31 mg/L and the control group ($X^2_1 = 5.4$, $P = 0.021$ for 31 mg/L), but this difference corresponded only to a very slight lengthening of their development with chlorpyrifos (Fig. 4B). Moreover, the developmental time of males did not significantly differ between the chlorpyrifos concentrations of 31 and 44 mg/L ($X^2_1 = 0.3$, $P = 0.56$). The Chlorpyrifos x Temperature interaction was not significant in females ($X^2_3 = 6.0$, $P = 0.11$ for 31 mg/L; $X^2_3 = 4.1$, $P = 0.25$ for 44 mg/L) or males ($X^2_3 = 1.2$, $P = 0.75$ for 31 mg/L; $X^2_3 = 4.7$, $P = 0.19$ for 44 mg/L). Males had a slower development than females, except at the highest temperature (Wilcoxon tests: $P<0.001$ at 23, 25 and 27°C, $P=0.198$ at 29°C).

Figure 4 shows an overall decrease in pupal body mass at elevated temperatures in females ($F_{3,194} = 41.4$, $P < 0.001$ for 31 mg/L; $F_{3,190} = 31.9$, $P < 0.001$ for 44 mg/L) and males ($F_{3,199} = 17.2$, $P < 0.001$ for 31 mg/L; $F_{3,192} = 18.6$, $P < 0.001$ for 44 mg/L). When compared to the control group, the chlorpyrifos treatment with the highest concentration of 44 mg/L led to a higher body mass of females ($F_{1,190} = 15.4$, $P < 0.001$; Fig. 4C) and males ($F_{1,192} = 5.9$, $P = 0.016$; Fig. 4D). The lowest chlorpyrifos concentration of 31 mg/L did not induce a significant increase in body mass in females ($F_{1,194} = 3.5$, $P = 0.062$) or males ($F_{1,198} = 0.4$, $P = 0.54$). The Chlorpyrifos x Temperature interaction was not significant in females ($F_{3,191} = 0.2$, $P =$

0.87 for 31 mg/L; $F_{3,187} = 0.7$, $P = 0.58$ for 44 mg/L) or males ($F_{3,195} = 0.2$, $P = 0.87$ for 31 mg/L; $F_{3,189} = 1.0$, $P = 0.40$ for 44 mg/L).

3.2. Reaction norm analyses

We studied the variation among clutches and in the response of clutches to temperature and chlorpyrifos (reaction norms) as allowed by our split-plot experimental design (Fig. 2). Variation among clutches was tested for clutches reared at 23 or 25 °C, and clutches reared at 27 or 29 °C. We first compared statistical models with no clutch variation, total variation among clutches (modelled with the identification number of clutches), and variation in the day or week of the collection of clutches (see Table S3 for the comparison of models). We found that developmental time varied among clutches for males and females, and for the two thermal ranges. The week of collection of clutches better fit the data than other clutch variations for developmental times under rearing temperatures of 23 and 25 °C. The day of collection of clutches better fit the data for developmental times under temperatures of 27 and 29 °C. Under rearing temperatures of 27 and 29 °C, larval survival 48 hours after chlorpyrifos treatment was also dependent on the date of collection of experimental clutches (with equivalent models for the date modelled as the day or week). For all these cases where a variation among clutches was found, we tested the variation in the response of clutches to temperature and chlorpyrifos. These reaction norms were significant for larval survival and developmental time under temperatures of 27 and 29 °C (Table 1). The interaction Temperature x Chlorpyrifos x Date of clutch collection (modelled as the day or week) was significant for larval survival 48 h posttreatment. Variation in the response of clutches to temperature was detected for the developmental time of males and females, and variation in the response of clutches to chlorpyrifos was detected for the developmental time of females.

3.3. Projection models

The responses of the studied parameters were integrated into demographic models. We simulated the twelve scenarios that combined experimental temperatures and chlorpyrifos treatments (see Table S2 for the values used to fix parameters). Overall, the multiplication rate of the simulated populations increases as the temperature increases (Fig. 5). This response is particularly strong between 23 and 25 °C. Chlorpyrifos decreases the multiplication rate as expected, particularly at the highest concentration of 44 mg/L. The strongest decrease in the multiplication rate with a concentration of 44 mg/L occurs at 29 °C, which highlights the impact of the significant Chlorpyrifos x Temperature interaction on the larval survival rates 48 hours after the chlorpyrifos treatment (Fig. 3A).

4. DISCUSSION

The main aim of the study was to test the influence of transgenerational plasticity on the impact of temperature on insecticide sensitivity (Fig. 1). The analyses of reaction norms showed variation in the response of clutches to temperature and chlorpyrifos. These significant reaction norms of the clutches were revealed for larval survival and developmental time (Table 1). Larval survival and developmental time are critical parameters, particularly in *S. littoralis*, in which they strongly influence the population multiplication rate (Massot et al., 2021). The key result of the study is the significant interaction between chlorpyrifos, temperature and the date of clutch collection for larval survival, indicating that the offspring response to the interaction between insecticide and temperature differed among experimental families, showing transgenerational plasticity for the impact of temperature on chlorpyrifos toxicity. Before discussing this main finding, the overall effects of temperature and chlorpyrifos are discussed.

4.1. Effects of temperature and chlorpyrifos

As advised in studies on the thermal biology of species in the context of climate change, we tested the influence of temperature on multiple parameters, considered responses to small temperature variations to take into account the non-linearity of most thermal responses, tested interactive effects, and controlled for transgenerational effects (Mordecai et al., 2019; Sinclair et al., 2016). We found that the increase in temperature decreased larval survival 48 hours after the chlorpyrifos treatment (Fig. 3A), increased pupal survival rates of females and males (Fig. 3C and D), strongly reduced developmental time of females and males (Fig. 4A and B), and decreased pupal body mass of females and males (Fig. 4C and D). These various responses to temperature were expected because the characteristics of ectotherms are almost all sensitive to temperature (Angilletta, 2009). Interestingly, we observed here that small variations of only 2° C were sufficient to induce noticeable responses. In particular, a variation between 27 and 29° C was sufficient to lead to significant reaction norms of the clutches (significant interactions with the temperature factor in Table 1). Moreover, we found a dramatic shortening of developmental time between 23 and 25 °C (Fig. 4A and B). This strong response can be explained by the positive effect of temperature on the metabolic rate of insects (Deutsch et al., 2018). For *S. littoralis*, which can complete two to seven generations per year (Khafagi et al., 2016), the dramatic shortening of developmental time with a small temperature variation is an important threat because it will likely increase the number of generations per year for this pest in the context of climate change (Altermatt, 2010).

Another interesting finding is the varying responses among the studied parameters. The warmest temperatures were beneficial for pupal survival and developmental time but detrimental for larval survival 48 hours after the chlorpyrifos treatment and pupal body mass. These opposite responses show the importance of studying the effects of temperature on multiple parameters (Mordecai et al., 2019; Sinclair et al., 2016) and specifically show that

the thermal optimum differs among parameters in *S. littoralis*. Moreover, the upper thermal limit of this species is close to 30°C: *S. littoralis* cannot reproduce at a temperature of 30 °C, and this temperature also causes low pupal survival (Sidibé and Laugé, 1977). The latter response is in contrast to the high pupal survival rates observed at 29 °C (Fig. 3C and D, and Massot et al., 2021). The temperature of 29 °C also led to the highest multiplication rate in the control group without chlorpyrifos (Fig. 5), as also observed in a previous study (Massot et al., 2021). Thus, the thermal optimum in *S. littoralis* is very close to its upper thermal limit. The dramatic shortening of developmental time at the highest temperatures is the main reason for the thermal optimum at 29 °C (Fig. 4A and B): the developmental time between 23 and 29 °C was shorten by 35.2 % in females and 36.9 % in males, values very close to previous estimates (36.1 % in females and 36.7 % in males) obtained in Massot et al. (2021). The faster development of *S. littoralis* at the highest temperatures was detrimental to pupal body mass (Fig. 4C and D) as in many ectotherms (Atkinson, 1996).

Larval survival 48 hours after treatment was decreased by the two experimental concentrations of chlorpyrifos (Fig. 3A). Chlorpyrifos also tended to decrease larval survival between 3 days posttreatment and pupation at the highest temperatures (Fig. 3B). The pupal survival and developmental time of females and males were not sensitive to chlorpyrifos, except maybe for a slight lengthening of male development. However, the highest chlorpyrifos concentration of 44 mg/L increased pupal body mass in both sexes (Fig. 4C and D). As an organophosphorus insecticide, chlorpyrifos is expected to be more toxic at elevated temperatures (Lydy et al., 1999; Meng et al., 2020; Tran et al., 2018), except when elevated temperatures induce a faster insecticide degradation (Meng et al. 2022b). This synergistic interaction between chlorpyrifos and temperature can be caused by changes in metabolism, the activity of detoxification enzymes, and/or biotransformation processes (Hooper et al., 2013; Noyes et al., 2009). In our study, chlorpyrifos and temperature acted synergistically on

larval survival 48 hours after treatment with a significant Chlorpyrifos x Temperature interaction at a concentration of 44 mg/L. Indeed, chlorpyrifos toxicity was stronger at 29 °C (Fig. 3A), as also illustrated by its impact on the population multiplication rate (Fig. 5).

4.2. Transgenerational plasticity for the impact of temperature on chlorpyrifos toxicity

The environmental effects experienced by the previous generation might influence the interaction between insecticides and temperature. This scenario was shown in one study on the mosquito *Culex pipiens* with the loss of the effect of temperature on mortality caused by chlorpyrifos in the offspring of parents that suffered from warming and chlorpyrifos (Tran et al., 2018). However, other studies did not find such a transgenerational effect on the interaction between insecticides and temperature (Massot et al., 2021; Meng et al., 2022a). Our reaction norm analyses tested whether the impact of temperature on chlorpyrifos toxicity depended on transgenerational effects. Transgenerational effects were inferred from the variation in the responses of clutches as in Massot et al. (2021). In particular, we tested the responses of clutches in function of their date of collection (Day and Week effects of the Tables 1 and S3). Indeed, the date of the collection of clutches was previously shown to parsimoniously describe the variation in the responses of clutches (Massot et al., 2021). The experiment was performed on clutches collected during a short period (from 19th March to 11th April 2018) to discard the possibility that the variation in the responses of clutches among dates was caused by a genetic evolution in our laboratory rearing.

Variation in the responses of clutches among dates was found for larval survival and developmental time (Table S3), and analyses of the reaction norms revealed that the clutches responded differently to temperature and chlorpyrifos (Table 1). This variation among clutches in *S. littoralis* was also detected in a study that tested the impact of temperature on the toxicity of another insecticide, the pyrethroid insecticide deltamethrin (Massot et al.,

2021). The significant reaction norms to chlorpyrifos (significant interactions with the chlorpyrifos factor in Table 1) shows that the sensitivity of larvae to chlorpyrifos differed among the clutches. The finding of this transgenerational plasticity reinforces the results of a previous study that showed a relationship between maternal body mass and offspring sensitivity to chlorpyrifos in *S. littoralis* (Bagni et al., 2020). The significant reaction norms to temperature (significant interactions with the temperature factor in Table 1) suggest also a variation in the thermal optimum of the clutches.

The variation among our experimental clutches might reflect a transgenerational plasticity induced by epigenetic changes (DNA methylation, histone change). Unfortunately, our data did not allow to investigate epigenetic factors (Brevik et al., 2018; Latzel, 2015). Alternatively, variation among clutches might have been embryo changes (energy storage, size, hormones,...) (Bernardo, 1996; Latzel, 2015). Maternal environment or characteristics often induce such changes (Moore et al., 2019). These maternal effects are the main component of transgenerational plasticity (Salinas and Munch, 2012; Shama et al., 2014) and are well documented in insects (Mousseau and Dingle, 1991). In *S. littoralis*, we previously showed a relationship between maternal body mass and the sensitivity to the insecticide chlorpyrifos. The mortality caused by chlorpyrifos was higher in larvae of larger mothers (Bagni et al., 2020).

4.3. Conclusions

The key result of our study is the significant interaction between chlorpyrifos, temperature and clutches for larval survival (Table 1). This result shows that the offspring response to the interaction between insecticide and temperature differed among families, and consequently provides evidence of transgenerational plasticity for the impact of temperature on chlorpyrifos toxicity. Because two studies did not show the influence of transgenerational effects on the

interaction between insecticide and temperature (Massot et al., 2021; Meng et al., 2022a), this result is an important confirmation of the pioneering study of Tran et al. (2018), who also showed the sensitivity of the interaction between insecticide and temperature to transgenerational effects. Furthermore, our results illustrate that transgenerational effects can be important to consider in ecotoxicology, as highlighted by other recent studies (Brevik et al., 2018; Massot et al., 2021; Meng et al., 2022a). Transgenerational effects are important to consider as a lagged source of individual heterogeneity (Benton et al., 2006) and their study could benefit the management of pest species and risk assessment procedures on non-target species (Müller, 2018).

ACKNOWLEDGEMENTS

We acknowledge funding from the Région Ile-de-France (*Réseau de Recherche sur le Développement Soutenable*, DIM R2DS 2014) and the CNRS (*Centre National de la Recherche Scientifique*, PEPS FaiDoRA).

REFERENCES

Altermatt, F., 2010. Climatic warming increases voltinism in European butterflies and moths. Proc. R. Soc. B. 277, 1281–1287.

Angilletta, M.J., 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford Univ. Press, Oxford.

Atkinson, D., 1996. Ectotherm life-history responses to developmental temperature, in: Johnston, I.A., Bennett, A.F. (Eds.), Animals and Temperature: Phenotypic and Evolutionary Adaptation. Cambridge Univ. Press, pp. 183–204.

Bagni, T., Siaussat, D., Maria, A., Couzi, P., Maïbèche, M., Massot, M., 2020. A maternal effect influences sensitivity to chlorpyrifos pesticide in the pest moth *Spodoptera*

littoralis. Ecotoxicol. Environ. Saf. 204, 111052. https://doi.org/10.1016/j.ecoenv.2020.111052

Bashey, F., 2006. Cross-generational environmental effects and the evolution of offspring size in the Trinidadian guppy *Poecilia reticulata*. Evolution 60, 348–361. http://dx.doi.org.inee.bib.cnrs.fr/10.1111/j.0014-3820.2006.tb01111.x

Benton, T.G., Plaistow, S.J., Coulson, T.N., 2006. Complex population dynamics and complex causation: devils, details and demography. Proc. R. Soc. B. 273, 1173–1181. https://doi.org/10.1098/rspb.2006.3495

Bernardo, J., 1996. Maternal effects in animal ecology. Am. Zool. 36, 83–105. https://doi.org/10.1093/icb/36.2.83

Brevik, K., Lindström, L., McKay, S.D., Chen, Y.H., 2018. Transgenerational effects of insecticides - implications for rapid pest evolution in agroecosystems. Curr. Opin. Insect. Sci. 26, 34–40. https://doi.org/10.1016/j.cois.2017.12.007

CABI, 2020. *Spodoptera littoralis* (cotton leafworm). In: Invasive Species Compendium. Wallingford, UK: CAB International.

Caswell, H., 2001. Matrix population models: Construction, analysis, and interpretation, 2nd ed. Sinauer Associates Inc., Sunderland, Massachusetts, USA.

Deutsch, C.A., Tewksbury, J.J., Tigchelaar, M., Battisti, D.S., Merrill, S.C., Huey, R.B., Naylor, R.L., 2018. Increase in crop losses to insect pests in a warming climate. Science 361, 916–919. https://doi.org/10.1126/science.aat3466

Dewer, Y., Pottier, M.-A., Lalouette, L., Maria, A., Dacher, M., Belzunces, L.P., Kairo, G., Renault, D., Maibeche, M., Siaussat, D., 2016. Behavioral and metabolic effects of sublethal doses of two insecticides, chlorpyrifos and methomyl, in the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Environ. Sci. Pollut. Res. 23, 3086–3096. https://doi.org/10.1007/s11356-015-5710-1

Fournier-Level, A., Neumann-Mondlak, A., Good, R.T., Green, L.M., Schmidt, J.M., Robin, C., 2016. Behavioural response to combined insecticide and temperature stress in natural populations of *Drosophila melanogaster*. J. Evol. Biol. 29, 1030–44. https://doi.org/10.1111/jeb.12844

Hinks, C.F., Byers, J.R., 1976. Biosystematics of the genus *Euxoa* (Lepidoptera: Noctuidae): V. Rearing procedures, and life cycles of 36 species. Can. Entomol. 108, 1345–1357. https://doi.org/10.4039/Ent1081345-12

Hooper, M.J., Ankley, G.T., Cristol, D.A., Maryoung, L.A., Noyes, P.D., Pinkerton, K.E., 2013. Interactions between chemical and climate stressors: A role for mechanistic toxicology in assessing climate change risks. Environ. Toxicol. Chem. 32, 32–48. https://doi.org/10.1002/etc.2043

Johnson, L.R., Ben-Horin, T., Lafferty, K.D., McNally, A., Mordecai, E., Paaijmans, K.P., Pawar, S., Ryan, S.J., 2015. Understanding uncertainty in temperature effects on vector-borne disease: a Bayesian approach. Ecology 96, 203–213. https://doi.org/10.1890/13-1964.1

Khafagi, W.E., Hegazi, E.M., Aamer, N.A., 2016. Effects of temperature on the development, food consumption and utilization parameters of the last two larval instars of *Spodoptera littoralis* (Boisd.). J. Agric. Sci. Food Sci. Technol. 2, 93–99.

Kingsolver, J.G., 2009. The well-temperatured biologist. Am. Nat. 174, 755–768. https://doi.org/10.1086/648310

Latzel, V., 2015. Pitfalls in ecological research: Transgenerational effects. Folia Geobot. 50, 75–85. https://doi.org/10.1007/s12224-015-9208-x

Legendre, S., Clobert, J., 1995. ULM, a software for conservation and evolutionary biologists. J. Appl. Stat. 22, 817–834. https://doi.org/10.1080/02664769524649

Lydy, M.J., Belden, J.B., Ternes, M.A., 1999. Effects of temperature on the toxicity of m-parathion, chlorpyrifos, and pentachlorobenzene to *Chironomus tentans*. Arch. Environ. Contam. Toxicol. 37, 542–547. https://doi.org/10.1007/s002449900550

Malbert-Colas, A., Drozdz, T., Massot, M., Bagni, T., Chertemps, T., Maria, A., Maïbèche, M., Siaussat, D., 2020. Effects of low concentrations of deltamethrin are dependent on developmental stages and sexes in the pest moth *Spodoptera littoralis*. Environ. Sci. Pollut. Res. 27, 41893-41901. https://doi.org/10.1007/s11356-020-10181-9

Massot, M., Bagni, T., Maria, A., Couzi, P., Drozdz, T., Malbert-Colas, A., Maïbèche, M., Siaussat, D., 2021. Combined influences of transgenerational effects, temperature and insecticide on the moth *Spodoptera littoralis*. Environ. Pollut. 289, 117889. https://doi.org/10.1016/j.envpol.2021.117889

Meng, S., Delnat, V., Stoks, R., 2022a. Multigenerational effects modify the tolerance of mosquito larvae to chlorpyrifos but not to a heat spike and do not change their synergism. Environ. Pollut. 292, 118333. https://doi.org/10.1016/j.envpol.2021.118333

Meng, S., Delnat, V., Stoks, R., 2020. The exposure order strongly modifies how a heat spike increases pesticide toxicity. Environ. Sci. Technol. 54, 11476–11484. https://doi.org/10.1021/acs.est.0c03848

Meng, S., Tran, T.T., Van Dinh, K., Delnat, V., Stoks, R., 2022b. Acute warming increases pesticide toxicity more than transgenerational warming by reducing the energy budget. Sci. Total Environ. 805, 150373. https://doi.org/10.1016/j.scitotenv.2021.150373

Moore, M.P., Whiteman, H.H., Martin, R.A., 2019. A mother's legacy: the strength of maternal effects in animal populations. Ecol. Lett. 22, 1620–1628. https://doi.org/10.1111/ele.13351

Mordecai, E.A., Caldwell, J.M., Grossman, M.K., Lippi, C.A., Johnson, L.R., Neira, M., Rohr, J.R., Ryan, S.J., Savage, V., Shocket, M.S., Sippy, R., Stewart Ibarra, A.M., Thomas, M.B., Villena, O., 2019. Thermal biology of mosquito- borne disease. Ecol. Lett. 22, 1690–1708. https://doi.org/10.1111/ele.13335

Mousseau, T.A., Dingle, H., 1991. Maternal effects in insect life histories. Annu. Rev. Entomol. 36, 511–534. https://doi.org/10.1146/annurev.en.36.010191.002455

Müller, C., 2018. Impacts of sublethal insecticide exposure on insects - Facts and knowledge gaps. Basic Appl Ecol 30, 1–10. https://doi.org/10.1016/j.baae.2018.05.001

Noyes, P.D., Lema, S.C., 2015. Forecasting the impacts of chemical pollution and climate change interactions on the health of wildlife. Curr. Zool. 61, 669–689. https://doi.org/10.1093/czoolo/61.4.669

Noyes, P.D., McElwee, M.K., Miller, H.D., Clark, B.W., Van Tiem, L.A., Walcott, K.C., Erwin, K.N., Levin, E.D., 2009. The toxicology of climate change: Environmental contaminants in a warming world. Environ. Int. 35, 971–986. https://doi.org/10.1016/j.envint.2009.02.006

Rahman, H.U., Asghar, W., Nazir, W., Sandhu, M.A., Ahmed, A., Khalid, N., 2021. A comprehensive review on chlorpyrifos toxicity with special reference to endocrine disruption: Evidence of mechanisms, exposures and mitigation strategies. Sci. Total Environ. 755, 142649. https://doi.org/10.1016/j.scitotenv.2020.142649

Riskallah, M.R., 1984. Influence of posttreatment temperature on the toxicity of pyrethroid insecticides to susceptible and resistant larvae of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.). Experientia 40, 188–190. https://doi.org/10.1007/BF01963592

Salinas, S., Munch, S.B., 2012. Thermal legacies: transgenerational effects of temperature on growth in a vertebrate: Thermal transgenerational plasticity. Ecol. Lett. 15, 159–163. https://doi.org/10.1111/j.1461-0248.2011.01721.x

Shama, L.N.S., Strobel, A., Mark, F.C., Wegner, K.M., 2014. Transgenerational plasticity in marine sticklebacks: maternal effects mediate impacts of a warming ocean. Funct. Ecol. 28, 1482–1493. https://doi.org/10.1111/1365-2435.12280

Sidibé, B., Laugé, G., 1977. Incidence des thermopériodes et de températures constantes sur quelques critères biologiques de *Spodoptera littoralis* Boisduval [Lepidoptera Noctuidae]. Ann. Soc. Entomol. Fr. 13, 369–379.

Sinclair, B.J., Marshall, K.E., Sewell, M.A., Levesque, D.L., Willett, C.S., Slotsbo, S., Dong, Y., Harley, C.D.G., Marshall, D.J., Helmuth, B.S., Huey, R.B., 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? Ecol. Lett. 19, 1372–1385. https://doi.org/10.1111/ele.12686

Tran, T.T., Janssens, L., Dinh, K.V., Stoks, R., 2018. Transgenerational interactions between pesticide exposure and warming in a vector mosquito. Evol. Appl. 11, 906–917. https://doi.org/10.1111/eva.12605

White, G.C., Burnham, K.P., 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46, 120–139.

Table 1. Response of clutches to temperature and chlorpyrifos. The clutch factor (C_{factor}) tested for each variable is the one that better fit the data (see Table S3). The C_{factor} Day or Week tested variation in the day or week of the collection of clutches, respectively. Significant effects are reported in bold.

	C_{factor}	Temperature x Chlorpyrifos x C_{factor}	Temperature x C_{factor}	Chlorpyrifos x C_{factor}
Temperatures of 23 and 25 °C				
Female developmental time	Week	X^2_2=1.3 P=0.524	X^2_1=0.8 P=0.381	X^2_4=1.7 P=0.793
Male developmental time	Week	X^2_2=1.3 P=0.528	X^2_1=1.5 P=0.225	X^2_4=8.2 P=0.084
Temperatures of 27 and 29 °C				
Larval survival 48 h posttreatment	Day	**X^2_{10}=23.0 P=0.011**	X^2_5=9.4 P=0.094	X^2_{10}=16.1 P=0.098
Larval survival 48 h posttreatment	Week	**X^2_4=9.7 P=0.047**	X^2_2=5.8 P=0.054	**X^2_4=10.3 P=0.035**
Female developmental time	Day	X^2_9=11.8 P=0.223	**X^2_5=34.2 P<0.001**	**X^2_{10}=30.0 P<0.001**
Male developmental time	Day	X^2_{10}=9.0 P=0.532	**X^2_5=51.6 P<0.001**	X^2_{10}=15.8 P=0.107

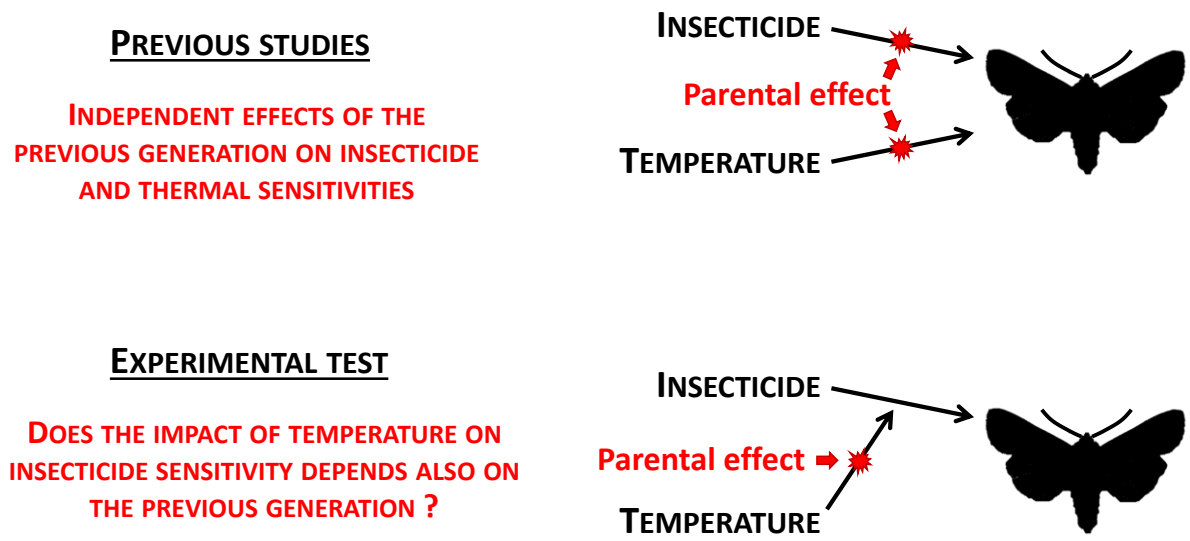

Fig. 1. Conceptual framework of the study. Previous studies (top scenario) showed transgenerational plasticity on insecticide and thermal sensitivities of *S. littoralis* (Bagni et al., 2020; Massot et al., 2021). Our experimental study (bottom scenario) aims to test transgenerational plasticity on the impact of temperature on insecticide sensitivity.

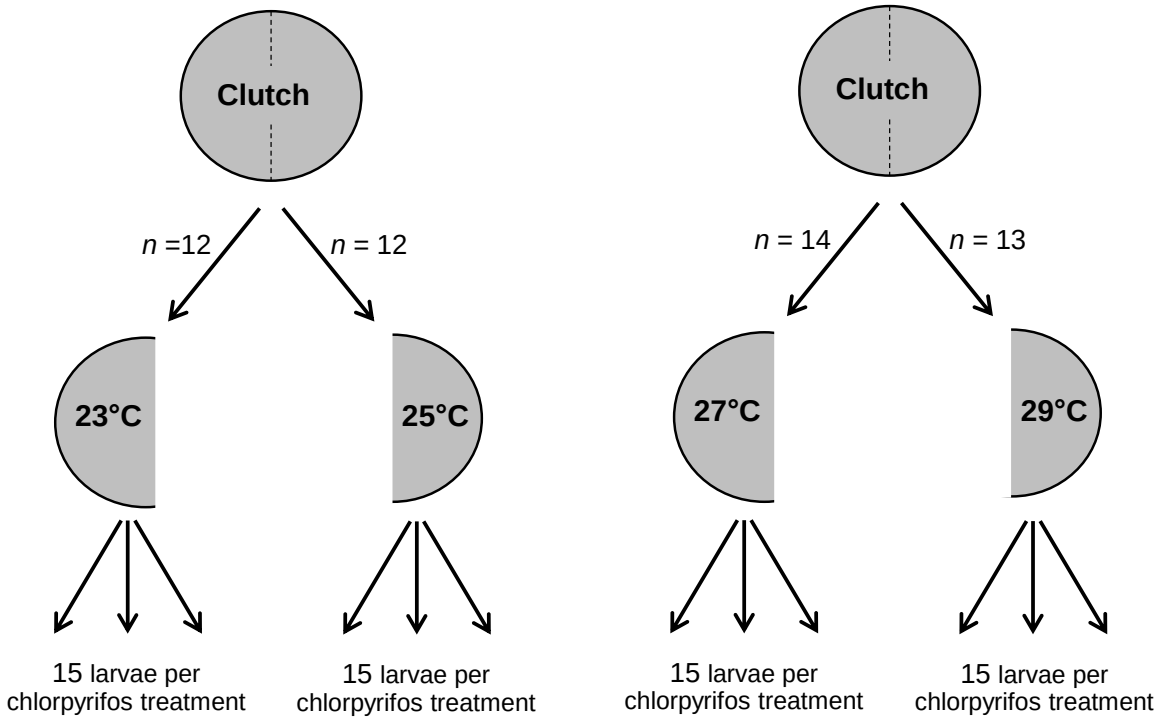

Fig. 2. Split-plot experimental design. Clutches were divided to rear them under two temperatures. These half-clutches were divided into three insecticide groups (two chlorpyrifos concentrations and one control solution) when larvae were at the 4th instar. The rearing temperature of larvae was the same before and after the insecticide treatment. The influence of transgenerational effects on the combined effects of temperature and chlorpyrifos was tested from the variation in the responses of clutches.

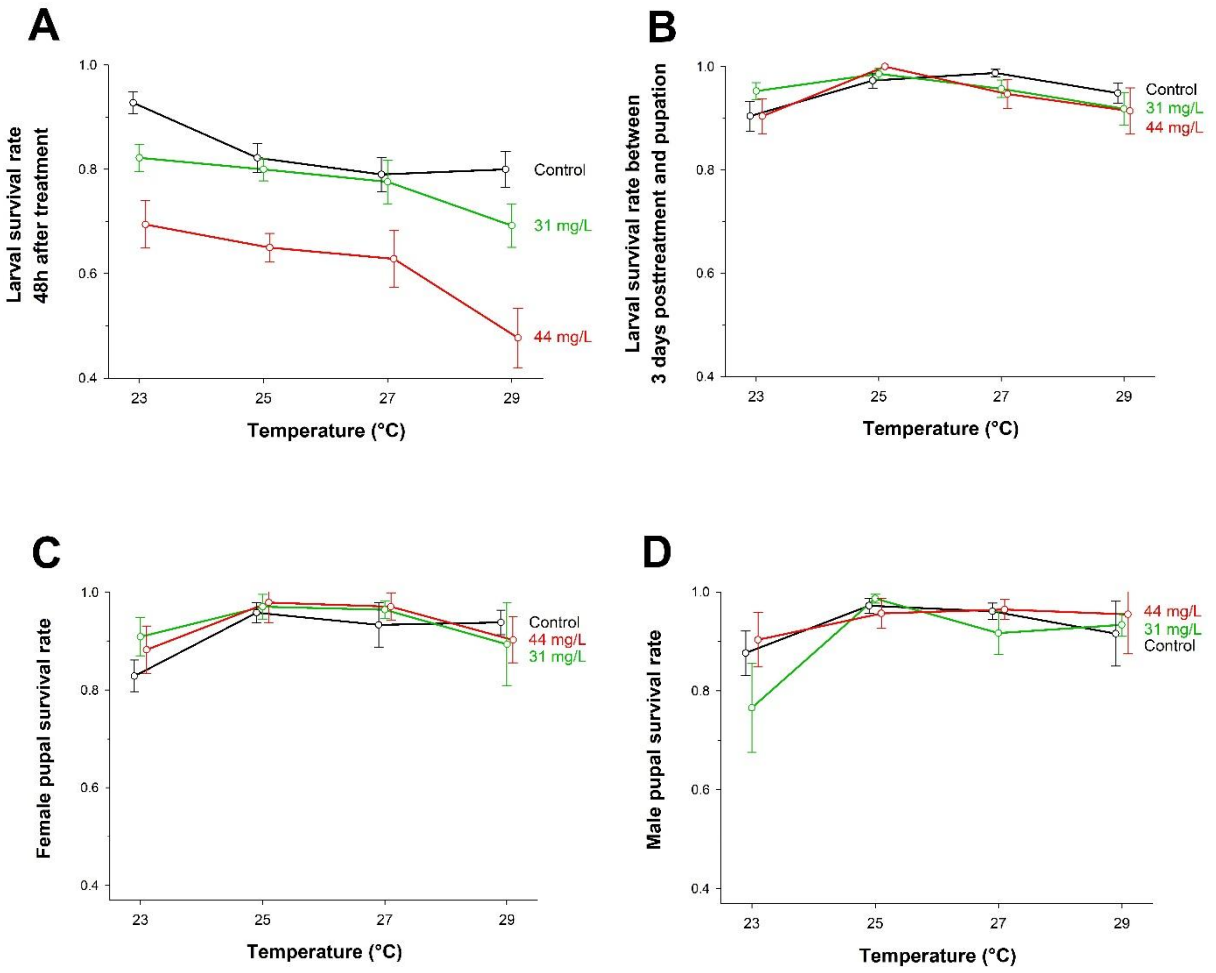

Fig. 3. Variation in survival between temperature and chlorpyrifos treatments. A: Larval survival rate 48 h after the chlorpyrifos treatment, B: larval survival rate between the 3rd day after the chlorpyrifos treatment and pupation, C: female pupal survival rate, D: male pupal survival rate. Error bars are s.e.m. between clutches.

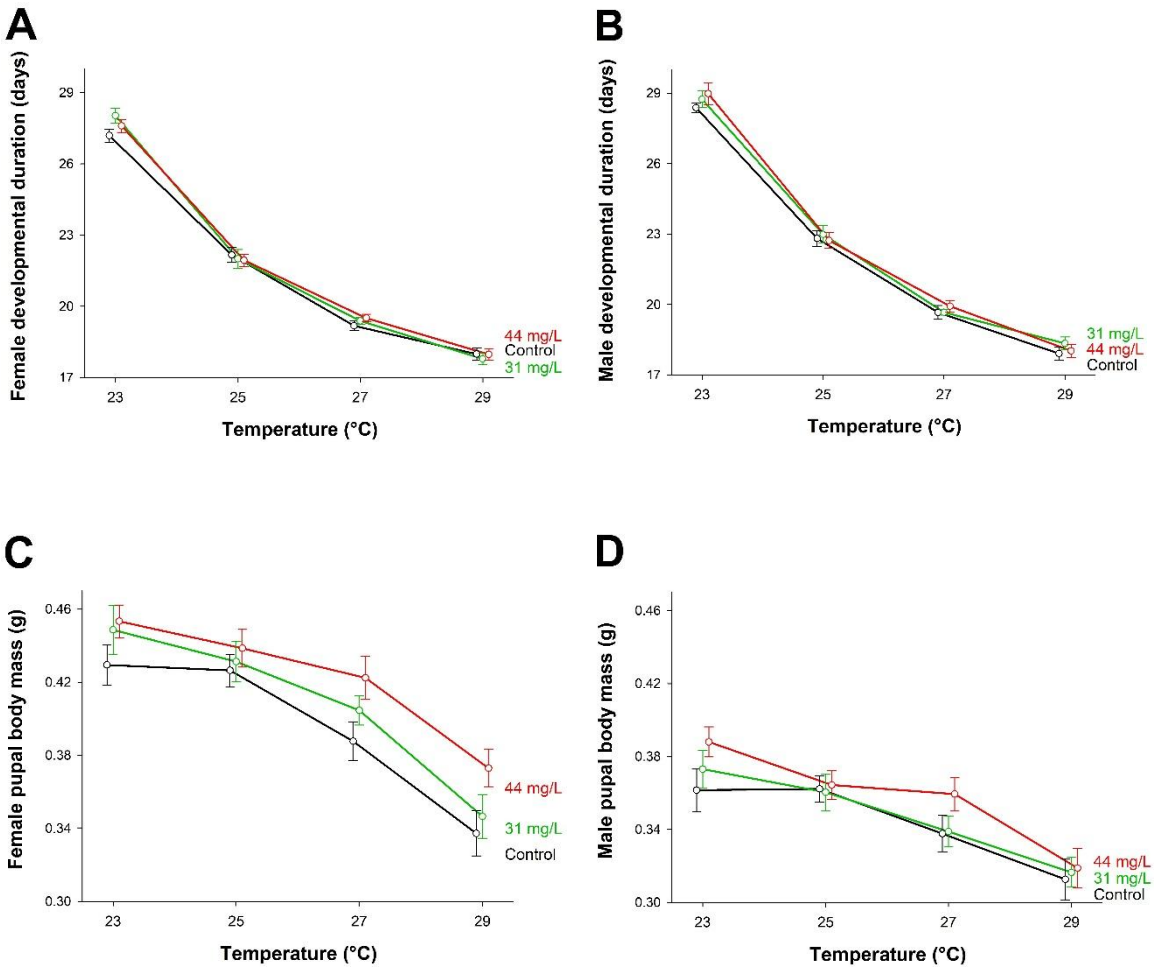

Fig. 4. Variation in developmental time and body mass between temperature and chlorpyrifos treatments. A: Time between the chlorpyrifos treatment (4th larval instar) and adult female emergence, B: time between the chlorpyrifos treatment and adult male emergence, C: female pupal body mass, D: male pupal body mass. Error bars are s.e.m. between clutches.

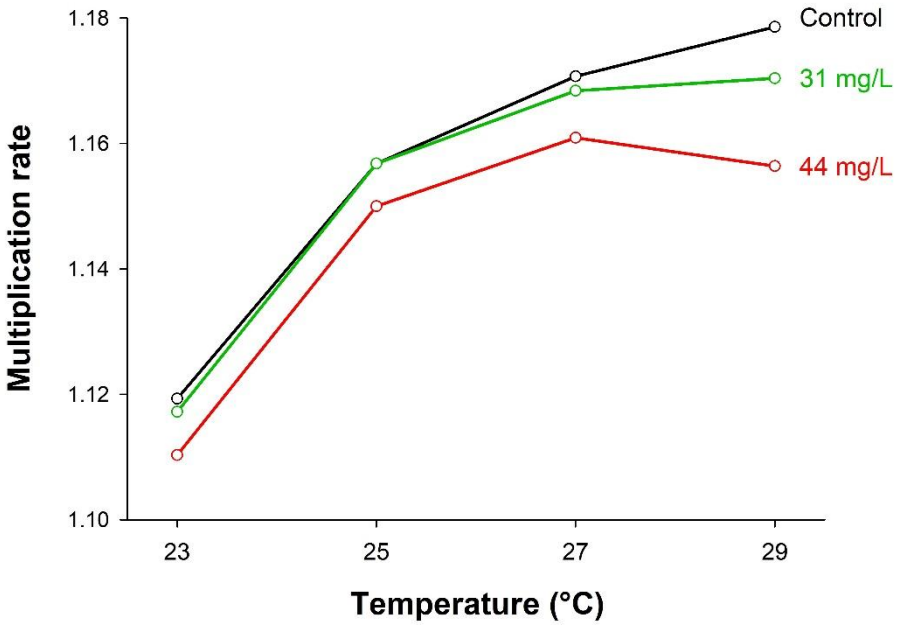

Fig. 5. The multiplication rate of simulated populations as a function of temperature and chlorpyrifos.

APPENDIX A

The impact of temperature on insecticide sensitivity depends on transgenerational effects

Thibaut Bagni, David Siaussat, Annick Maria, Philippe Couzi, Martine Maïbèche, and Manuel Massot

Sorbonne Université, CNRS, INRAe, Institut d'Ecologie et des Sciences de l'Environnement de Paris, iEES-Paris, F-75005, Paris, France

* To whom correspondence may be addressed:

Manuel Massot, iEES-Paris, F-75005, Paris, France; e-mail: manuel.massot@sorbonne-universite.fr

Table S1: Sample sizes

Table S1a. Sample sizes per temperature for control groups without chlorpyrifos.

	23°C	25°C	27°C	29°C
Larval survival 48 h posttreatment	180	180	210	195
Larval survival > 3days posttreatment	167	148	164	156
Female pupal survival	70	72	60	65
Male pupal survival	81	72	102	83
Female developmental time	58	69	56	61
Male developmental time	71	70	98	76
Female pupal body mass	24	24	26	25
Male pupal body mass	24	24	28	26

Table S1b. Sample sizes per temperature for groups with 31 mg/L of chlorpyrifos.

	23°C	25°C	27°C	29°C
Larval survival 48 h posttreatment	180	180	210	195
Larval survival > 3days posttreatment	148	144	163	135
Female pupal survival	77	68	84	47
Male pupal survival	64	74	72	75
Female developmental time	70	66	81	42
Male developmental time	49	73	66	70
Female pupal body mass	24	24	28	24
Male pupal body mass	24	24	28	25

Table S1c. Sample sizes per temperature for groups with 44 mg/L of chlorpyrifos.

	23°C	25°C	27°C	29°C
Larval survival 48 h posttreatment	180	180	210	195
Larval survival > 3days posttreatment	125	117	132	93
Female pupal survival	51	48	68	41
Male pupal survival	62	69	56	44
Female developmental time	45	47	66	37
Male developmental time	56	66	54	42
Female pupal body mass	24	24	26	22
Male pupal body mass	23	24	25	23

Table S2: Values used to fix parameters in matrix models

Table S2a. Estimates of the parameters used in matrix models for control groups without chlorpyrifos. For the parameters indicated with *, estimates were obtained in a previous study (Massot et al., 2021) that used the four developmental temperatures. For the reproductive parameters indicated with **, we used estimates obtained in another study (Malbert-Colas et al., 2020) from a control group of adults reared under 23 °C (however, the influence of reproductive parameters on the multiplication rate of *S. littoralis* is much smaller than the influence of the parameters we estimated under the four developmental temperatures - Massot et al., 2021).

Developmental temperature		23°C	25°C	27°C	29°C
Duration of incubation period (days)	*	4	3	2	2
Duration 1st - 4th instars (days)	*	9	8	7	7
Duration 4th instar - pupation (days)		13	11	11	9
Duration of pupal period (days)		15	11	9	9
Survival from 1st to 4th instars (%)	*	68.0	81.1	68.2	63.9
Larval survival 48 h posttreatment (%)		92.8	82.2	79.0	80.0
Larval survival > 3days posttreatment (%)		90.4	97.3	98.8	94.9
Pupal survival (%)		85.4	96.5	95.1	92.6
Sex-ratio (% of females)		46.4	50.0	37.0	43.9
Adult female survival until laying (%)	**	92.8	92.8	92.8	92.8
Laying success after mating (%)	**	78.3	78.3	78.3	78.3
Number of eggs laid	**	371	371	371	371
Hatching success (%)	**	71.3	71.3	71.3	71.3

Table S2b. Estimates of the parameters used in matrix models with 31 mg/L of chlorpyrifos. The values for other parameters are reported in Table S2a.

Developmental temperature	23°C	25°C	27°C	29°C
Duration 4th instar - pupation (days)	13	11	11	9
Duration of pupal period (days)	15	11	9	9
Larval survival 48 h posttreatment (%)	82.2	80.0	77.6	69.2
Larval survival > 3days posttreatment (%)	95.3	98.6	95.7	91.9
Pupal survival (%)	84.4	97.9	94.2	90.3
Sex-ratio (% of females)	54.6	47.9	53.8	38.5

Table S2c. Estimates of the parameters used in matrix models with 44 mg/L of chlorpyrifos. The values for other parameters are reported in Table S2a.

Developmental temperature	23°C	25°C	27°C	29°C
Duration 4th instar - pupation (days)	14	11	11	10
Duration of pupal period (days)	15	11	9	8
Larval survival 48 h posttreatment (%)	69.4	65.0	62.9	47.7
Larval survival > 3days posttreatment (%)	90.4	100.0	94.7	91.4
Pupal survival (%)	89.4	96.6	96.0	92.9
Sex-ratio (% of females)	45.1	41.0	54.8	48.2

Table S3: Statistical modelling of variation among clutches

Table S3. Tests of the variation among clutches reared at 23 and 25 °C, or at 27 and 29 °C. Statistical modelling was performed with no clutch variation (M_0 models), total variation among clutches (Clutch), and variation in the day or week of the collection of clutches (Day, Week). All models included temperature, chlorpyrifos treatments, Temperature x Chlorpyrifos interaction, and all interactions with the clutch factor tested. Table reports AICc, *i.e.*, Akaike Information Criterion corrected for small sample size (White & Burnham, 1999). The lower the AICc value is, the more appropriate the model to fit the data. The lowest AICc values, comparable with values that do not differ by more than two, are reported in bold. Logistic analyses were used for all parameters, except for pupal body mass analysed with generalized linear models.

	Models			
	M_0	Clutch	Day	Week
Temperatures of 23 and 25 °C				
Larval survival 48 h posttreatment	**1077.2**	1245.2	1167.1	1092.6
Larval survival > 3days posttreatment	**310.9**	491.7	406.4	331.5
Female pupal survival	**212.9**	466.7	308.1	227.1
Male pupal survival	**235.4**	462.9	318.3	248.2
Female developmental time	402.3	542.4	441.8	**393.8**
Male developmental time	539.6	647.2	554.4	**536.5**
Female pupal body mass	**- 447.9**	- 393.0	- 393.7	- 389.3
Male pupal body mass	**- 506.8**	- 452.6	- 449.9	- 445.4
Temperatures of 27 and 29 °C				
Larval survival 48 h posttreatment	1433.9	1455.8	**1419.3**	**1419.2**
Larval survival > 3days posttreatment	**340.1**	432.4	367.5	352.4
Female pupal survival	**173.7**	330.3	198.2	180.7
Male pupal survival	**205.5**	329.3	230.5	207.8
Female developmental time	468.9	489.5	**393.4**	431.9
Male developmental time	566.1	504.2	**454.4**	518.4
Female pupal body mass	**- 490.9**	- 439.9	- 430.1	- 430.5
Male pupal body mass	**- 543.1**	- 498.4	- 498.0	- 491.5