



Warming increases tolerance of an insect pest to fungicide exposure through temperature-mediated hormesis

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Abstract

Pest management strategies relying on agrochemicals could be altered by climate change, because of the temperature-dependent toxicity of the compound involved. Many studies have explored the response of targeted pests to pesticide and temperature. Pesticides are seldom strictly selective and also affect nontarget pests. Surprisingly, the way temperature may shape these side effects of pesticides remains overlooked, limiting our understanding of the net impacts of future chemical treatments on the overall damage induced by different pests. We investigated how temperature modulates the response of a major grape insect pest (the tortricid moth *Lobesia botrana*) to a copper-based fungicide. We examined the lethal (larval survival) and sublethal (larval development, pupal mass, immune parameters) effects of exposure to different concentrations of copper in larval food. We found that copper concentration had negative linear effects on larval development and pupal mass. In addition, copper concentration had biphasic curvilinear effects on total phenoloxidase activity, which is indicative of hormesis (stimulation and inhibition of insect performance at low and high copper concentrations, respectively). Temperature stimulated development, while compromising immunity (total phenoloxidase activity). Significant interaction between copper concentration and temperature was detected for larval survival and phenoloxidase activity: warmer conditions improved pest tolerance to copper through temperature-driven hormesis (larval survival) or by shifting the hormesis-related peak of performance toward higher copper concentrations (phenoloxidase activity). This combination of simple and interactive effects could propagate to populations, communities and agroecosystem, with implications for future management of viticultural pests.

Keywords Climate change · Copper · European grapevine moth · Fungicide · Hormesis · Viticulture

Key message

- Temperature affects the toxicity of pesticides for targeted pests and nontarget auxiliaries.
- Less is known about the thermal modulation of pesticide toxicity for nontarget pests.
- We studied the biological response of an insect pest to a fungicide (copper) and temperature.
- We found additive and interactive effects of the two stressors on insect larval performance.
- Temperature improved the insect tolerance to copper in terms of survival and immunity.

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Introduction

Pesticides can affect exposed organisms by inducing short-term mortality, due to acute toxicity (i.e., lethal effects), and by altering several life-history traits for individuals that survived the chemical stress (i.e., sublethal effects). In insects, sublethal effects have been documented for a broad range of physiological (e.g., development, reproduction, immunity) and behavioral traits (e.g., learning, orientation, feeding, oviposition) (Desneux et al. 2007). The biological impacts of pesticides can be understood as a dose-dependent balance between lethal and sublethal effects, as illustrated by the concept of hormesis (Cutler and Guedes 2017). This term describes a reversed response of a given performance trait to low (stimulation) and high doses (inhibition) of pesticide and has been observed for several biological endpoints including survival, growth, reproduction and immunity (Calabrese and Blain 2005, 2011; Cutler 2013; Cutler and Guedes 2017).

Most pesticides are seldom selective and, apart from targeted pests, may affect several organisms (termed as nontarget fauna) in treated agroecosystems, including auxiliary biota (pollinators, soil engineers, natural enemies) and other pests not targeted by the chemical treatment (Hardin et al. 1995; Biondi et al. 2012; Guedes et al. 2016). The collateral effects of broad-spectrum pesticides on nontarget organisms may have important undesired consequences at the community and agroecosystem levels. This includes a decline in the services provided by beneficial arthropods that contribute toward maintaining agricultural productivity (e.g., pollination, natural pest control), as well as secondary pest outbreaks if pesticide spreading benefits these noxious species (Brittain et al. 2010; Guedes and Cutler 2014; Guedes et al. 2016; Hill et al. 2017). Therefore, the agricultural and ecotoxicological risks of pesticide use have to be evaluated by balancing the benefits and drawbacks associated with these pest management strategies (Zhao et al. 2011; Gill and Garg 2014).

Temperature influences the physiological processes involved in the detoxification and excretion of harmful compounds, such that the impacts of chemicals on organisms are largely temperature-dependent (Noyes et al. 2009; Holmstrup et al. 2010; Laskowski et al. 2010; Hooper et al. 2013). Several studies have reported the thermal modulation of pesticide toxicity (lethal and sublethal effects) for both targeted pests (Musser and Shelton 2005; Yu et al. 2012; Li et al. 2014) and nontarget auxiliaries, such as predators (Ricupero et al. 2020) and parasitoids (Abbes et al. 2015). From an applied perspective, such findings imply that climate change may have the potential to alter the benefits/costs balance of pesticide use within the agricultural context, through temperature-driven modulation

of the susceptibility of the different organisms exposed to the active ingredient (Delcour et al. 2015; Matzrafi 2019). In this regard, there is surprisingly little detailed knowledge about the thermal modulation of pesticide side effects on nontarget pests, which may also determine the efficiency and ecological impacts of future pesticide treatments by affecting the overall herbivory pressure exerted on agricultural commodities under warmed climates.

Viticulture relies on intensive fungicide schedule to control downy mildew epidemics that may cause considerable yield losses in susceptible cultivars if left untreated (Gessler et al. 2011; Pertot et al. 2017). Hence, fungicides can account from up to 96% of pesticides used in vineyards (Bereswill et al. 2012), and the largest amounts of these fungicides are represented by copper-containing products sprayed for more than 150 years and at rates up to 80 kg ha⁻¹.year⁻¹ (Dagostin et al. 2011; Mackie et al. 2012; Lamichhane et al. 2018). Copper fungicides are disruptive for arthropod communities present in vineyards and can benefit grape insect pests through relaxation of top-down control exerted by their natural enemies (Pennington et al. 2018; Vogelweith and Thiéry 2018). For instance, an increased number of successive fungicide applications was found to exacerbate the damage caused to berries by caterpillars of the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae) through adverse effects on its egg predators (e.g. ants) (Pennington et al. 2018). Of note, this tortricid moth is already recognized as a primary grapevine pest worldwide with respect to its densities (up to 20–30 larvae per bunch in heavily infested vineyards) and associated levels of economic injury (13.3–27% yield losses over a four-year period in Mediterranean vineyards) (Moschos 2006; Delbac and Thiéry 2016; Thiéry et al. 2018). This indicates that copper as a major contaminant in the agroecosystem could make this insect pest even more problematic for grape industry, but additional data on the direct and indirect influences of fungicides on *L. botrana* life history would be required to ascertain this statement. This is especially true within the context of anthropogenic climate change that may modify the deleterious impacts of the moth on grape stock (Reineke and Thiéry 2016; Gutierrez et al. 2017). Filling this knowledge gap should improve our understanding of the potential net benefits associated with future copper spraying within the viticultural context.

The European grapevine moth is a multivoltine species with non-obligatory diapause, able to fulfill between two and five generations during growing season depending on latitude. Grape damage are caused by larvae feeding upon vine fructiferous organs (floral buds, ripening and mature berries) and alternative Mediterranean host plants (e.g., *Rosmarinus officinalis*, *Daphne gnidium*) (Thiéry and Moreau 2005). Adults are non-feeding and rely on nutritional resources stored as larvae for their reproductive output, which may

reach up to 80–100 eggs for field-collected females (Savopoulou-Soultani et al. 1990; Muller et al. 2016). The main reported natural enemies of this phytophagous insect are predators (arthropods, birds and bats) and parasitoids, which may specifically parasitize eggs (Trichogrammatidae) and larvae (e.g., Ichneumonidae, Braconidae, Tachinidae) (Thiéry et al. 2018). Parasitism rates as high as 90% were locally reported for *L. botrana* larvae, suggesting that parasitoids probably act as the main natural agents of biological control of this pest in vineyards (Moreau et al. 2010). In response to this significant parasitism pressure, *L. botrana* larvae have evolved a defensive system largely relying on an important investment in the immune component (Vogelweith et al. 2014; Iltis et al. 2018).

We intended to assess the lethal and sublethal effects of a copper-based fungicide used extensively worldwide on *L. botrana* larvae under warming conditions associated with climate change scenario. Considering the dual role of copper (as an oligo-element and a heavy metal), we expect a concentration-dependent hormetic response (stimulation at low concentrations, inhibition at high concentrations) of many traits to copper treatment, as demonstrated by previous experimental investigations in insect models (Servia et al. 2006; van Ooik et al. 2008; Baghban et al. 2014). Additionally, provided that larval daily body mass gain (i.e., larval growth rate) increases alongside rearing temperature in *L. botrana* (Iltis et al. 2018), we conjecture that copper intake and accumulation in insect tissues should be higher in warmer conditions. For this reason, the insect should be increasingly sensitive to the amount of copper contained in its food as temperature rises (i.e., the copper concentrations for which maximal insect performance is observed should be lower in warmer conditions).

Materials and methods

Insect rearing and larvae collection

All insects originated from a laboratory-reared *L. botrana* population maintained diapause-free since 1998 (INRAe, Villenave d'Ornon, France). This insect stock was regularly supplemented with wild individuals collected in nearby vineyards to preserve genetic diversity. Preceding the experiments, the population was grown under standardized conditions of temperature (20 ± 0.5 °C), light (photoperiod of L17:D6 and 1 h of dusk, luminosity: 650 lx during photophase and 100 lx at dusk), and relative humidity ($60 \pm 5\%$). Eggs were collected via bands of waxed paper hung inside the rearing cages, transferred to plastic boxes and placed under a daily moistened paper towel to limit egg mortality caused by desiccation.

Neonate larvae (age < 12 h) were collected in large numbers ($n = 1905$ individuals) for the purpose of the experiments. They were delicately manipulated with a soft paintbrush to be isolated into microtubes with pierced cap and filled with 1.5 ml of artificial medium used for the laboratory rearing of this species (composition for 1000 ml: 1000 ml water, 15 g agar, 86.6 g corn flour, 41.3 g wheat germ, 45.5 g beer yeast, 6 g ascorbic acid, 3.4 g mineral salt, 128 mg pyrimethanil, 2.7 g benzoic acid, 2.8 g methyl 4-hydroxybenzoate and 5 ml 95% ethanol) (Thiéry and Moreau 2005).

Diet composition

Larvae were exposed to one of the four copper concentrations through feeding on artificial media elaborated according to the original recipe previously described and supplemented with either 10 ml of distilled water (control) or 10 ml of Bordeaux mixture (20% copper content, RSR Dispers NC, UPL) diluted in distilled water to obtain the final desired copper concentrations of 25, 50 and 75 mg.kg⁻¹. Bordeaux mixture is a derivative of copper in the form of copper sulfates (CuSO₄) and is widely used in viticultural areas for its antifungal properties (Mackie et al. 2012). Although the control is not completely copper-free (because of the presence of mineral salts and plant material as potential copper sources), its copper concentration can be deemed negligible (closer to 0 mg.kg⁻¹) in comparison with the three other copper concentrations included in this study. We chose to contaminate the larval food with Bordeaux mixture instead of pure copper to mimic an ecologically realistic insect exposure to the chemical in terms of formulation, exposure route (ingestion) and concentrations.

The different copper treatments were selected to reflect variations in field-relevant copper concentrations found in the skin and pulp of grape berries consumed by *L. botrana*. In Europe, the maximal residue levels (MRLs) of copper compounds in grape berries at harvest is set at 50.mg kg⁻¹ (EFSA 2018). However, during the summer period and grape ripening, fluctuations around the permitted MRL threshold can be observed in relation to geographical context, local agricultural policies (amounts and frequencies of fungicide application, cultivated grape varieties), meteorological conditions (causing successive copper leachings) and grape physiology (Mackie et al. 2012). For instance, copper concentrations ranging from less than 10.mg kg⁻¹ to more than 100.mg kg⁻¹ have been quantified in treated berries collected from several vineyards in Europe and elsewhere (Angelova et al. 1999; García-Esparza et al. 2006; Lai et al. 2010; Miotto et al. 2014). We did not examine insect response to copper concentrations higher than 75 mg.kg⁻¹ because these values remain less commonly observed in field conditions.

Thermal regimes

Larvae were reared inside cooled incubators (ST 2/2 BASIC, Pol-Eko Aparatura). Abiotic conditions inside these devices were controlled (temperature: ± 0.1 °C, relative humidity: $50 \pm 10\%$, photoperiod: L16:D8, luminosity: 650 lx) and monitored weekly with an independent data logger (Hobo, Onset Computer Corporation) to ensure they conformed with the regime programmed during the whole course of the experiments. Individuals were exposed to one of two realistic fluctuating thermal regimes (each constituted by six thermal segments of 4 h) designed to mimic the current and expected future thermal conditions (2081–2100 period) observed in Burgundian vineyards (Longvic-Dijon weather station, 47.27°N; 5.09°E; altitude = 219 m) under a local scenario of warming associated with climate change. A 20-year recording (1995–2014) of in situ meteorological data was used to build the ‘current regime,’ simulating the thermal conditions currently observed during one ‘mean’ summer day in the region of interest (Table 1). The ‘future regime’ was generated on the basis of fine-grained downscaled predictions provided by six climate simulations involving different climatic models (for more details, see Iltis et al. 2019) (Table 1).

General measurement procedure

Within each of the eight experimental blocks involved in the full factorial design of this study (four copper concentrations and two thermal regimes), newly hatched larvae were randomly split into two experimental groups because the array of traits included in this study could not have been measured on the same individuals. In one group, larvae were

Table 1 Temperature conditions (°C) for the two daily fluctuating regimes used in this study: the current regime and the future regime, each divided into six thermal segments of 4 h

	Current regime	Future regime
0–4 h	16.4	21.4
4–8 h	17.8	22.9
8–12 h	22.3	27.8
12–16 h	24.9	30.5
16–20 h	22.9	28.3
20–0 h	19.0	24.2
Mean temperature	20.5	25.8
Daily thermal range	8.5	9.1

Current regime was based on a large dataset of in situ recorded climatic conditions. Future regime was generated on the basis of six fine-grained climate simulations run with the most pessimistic scenario of greenhouse gas emission. Daily thermal range is defined as the difference between maximum and minimum daily temperatures

allowed to develop freely until pupation in order to record larval development time, pupal mass and larval survival. In another group, larvae were collected once they reached their fifth instar to quantify immune effectors (hemocyte load, activity of the phenoloxidase/prophenoloxidase system) expressed by this pest as a protective barrier against parasitoid attacks. Immune bioassays were conducted during the last larval instar as a way to evaluate the biological impacts of copper for individuals being exposed to this metal during their entire immature development. Moreover, measurements of immunity are usually performed at the end of larval development in *L. botrana* (Vogelweith et al. 2014; Iltis et al. 2018, 2019).

Larval development time, pupal mass and larval survival

Initially, this group involved a total of 1546 newly hatched larvae split among the experimental blocks (current regime: $n = 154$ for 0 mg.kg^{-1} , $n = 220$ for 25 mg.kg^{-1} , $n = 223$ for 50 mg.kg^{-1} , $n = 158$ for 75 mg.kg^{-1} ; future regime: $n = 151$ for 0 mg.kg^{-1} , $n = 223$ for 25 mg.kg^{-1} , $n = 232$ for 50 mg.kg^{-1} , $n = 185$ for 75 mg.kg^{-1}). For minimizing larval disturbance, daily monitoring of development started at a larval age of 14 days (for the future regime) and 21 days (for the current regime) because larvae are unlikely to reach pupation earlier given the thermal conditions experienced (Iltis et al. 2019). Larval development time was expressed in days as the time elapsed between egg hatching and pupation. Immediately following pupal transformation, chrysalises were weighed to the nearest 0.1 mg with a balance (Pioneer PA214C, OHAUS) to obtain pupal mass. We also calculated larval survival rates, expressed as the ratio between the number of pupae collected and the number of larvae initially deposited for each experimental block.

Immune parameters

Immune biochemical tests were conducted on a total of 359 fifth-instar larvae in the dedicated experimental blocks (current regime: $n = 42$ for 0 mg.kg^{-1} , $n = 45$ for 25 mg.kg^{-1} , $n = 44$ for 50 mg.kg^{-1} , $n = 44$ for 75 mg.kg^{-1} ; future regime: $n = 45$ for 0 mg.kg^{-1} , $n = 43$ for 25 mg.kg^{-1} , $n = 49$ for 50 mg.kg^{-1} , $n = 47$ for 75 mg.kg^{-1}). These tests focused on two immune parameters used to infer the susceptibility of this pest to its larval endoparasitoids: hemocyte load and activity of the phenoloxidase/prophenoloxidase system (Vogelweith et al. 2014; Iltis et al. 2018). Hemocytes are freely flowing cells able to form a multilayered cellular structure around a parasitoid egg during the encapsulation process. These immune cells release the phenoloxidase (PO), a copper-containing enzyme produced through cleavage of its inactive precursor (the prophenoloxidase, PPO).

The phenoloxidase/prophenoloxidase enzymatic system catalyzes a cascade of reactions leading to the synthesis of melanin (melanization) acting as a cytotoxic compound against the parasitoid egg (González-Santoyo and Córdoba-Aguilar 2012).

Fifth-instar larvae were chilled on ice for 15 min to facilitate handling, placed on a cold surface and delicately wounded in the dorsal part of the abdomen with a sterile entomological pin to collect a droplet of hemolymph (~ 1 µl) using a cold, sterile glass capillary (Hirschmann Laborgeräte). This hemolymph extract was immediately mixed with 20 µl of cold, filtered phosphate-buffered saline (PBS, 10 mM, pH 7.4) to obtain diluted hemolymph samples. Immediately following hemolymph collection, 10 µl of diluted hemolymph samples was pipetted into a Neubauer hemocytometer under a phase contrast microscope (Primo Star, Zeiss) at 400× magnification to conduct a standardized count of hemocyte load. The remaining 11 µl of diluted hemolymph samples were supplemented with 10 µl of PBS, and tubes were homogenized before being fixed in liquid nitrogen and stored at -27 °C for subsequent estimation of PO/PPO system activity. Pricked larvae were transferred at -27 °C to be euthanized and stored for latter measurements of larval body size, in an attempt to control for allometric effects on larval immunity. Larval body size was extrapolated through head capsule width (i.e., the distance between the most distant margins of the sclerotized capsule) using a stereomicroscope (Stemi 508, Zeiss) at 12.5× magnification.

The enzymatic activity of the PO/PPO system was assessed through spectrophotometric method formerly developed. We quantified the activity of naturally activated PO (i.e., PO activity) and the sum of PO activity and activity of proenzyme stock (i.e., total PO activity) (Vogelweith et al. 2013). The distinction between PO and total PO activity enables to quantify the levels of enzymatic activity expressed by larvae at a given time (PO activity) along with the maximal activity that might be potentially mobilized upon an actual infection (total PO activity). In addition, PO and total PO activities can be differentially affected by temperature in *L. botrana*, thereby providing different information about the thermal sensitivity of immune function in this species (Iltis et al. 2018, 2019, 2021). Diluted samples of hemolymph were centrifuged (4000 g, 15 min, 4 °C) before two supernatant aliquots (5 µl each) were collected and transferred into microplate wells. One of these two aliquots was mixed with 160 µl of diluted PBS solution (35 ml ultrapure water, 5 ml filtered PBS) and was reserved for PO activity measurement. The other was supplemented with 160 µl of chymotrypsin solution (35 ml ultrapure water, 5 ml filtered PBS, 2.45 mg trypsin) to quantify total PO activity. The reaction started after the addition of 20 µl of L-Dopa solution (40 ml ultrapure water, 160 mg L-Dopamine). It was left for

40 min at 30 °C in a microplate reader (Versamax, Molecular Devices). Absorbance curves were generated based on regular absorbance readings (every 15 s at 490 nm), before being analyzed using the SoftMax Pro software (version 4.0, Versamax, Molecular Devices). These analyses aimed to extract maximum enzymatic activity (V_{max}), approximated by the maximum slope of the absorbance curve during its starting linear phase. All immune measurements were reported to 1 µl of pure hemolymph for comparison purposes, and corrected for allometric effects through division by body size (head capsule width) (Vogelweith et al. 2013).

Statistical analyses

All statistical models encompassed the effects of the copper concentration (covariate), temperature regime (factor), and their interaction on the response traits measured. The influence of copper concentration on each response trait was conceptualized in two different ways, generating two candidate models further compared to select the one best suited to fit the data distribution. On the one side, it was modeled through linear terms only (linear function: $y = ax + b$), thereby yielding a monotone relationship between the trait investigated and copper concentration. On the other side, the copper effect was simulated using both linear and second order polynomial terms (quadratic function: $y = ax^2 + bx + c$). This latter equation is more relevant for modeling biphasic functions displaying a transient peak, thereby allowing for the proper identification of hormesis (Cedergreen et al. 2005; Deng et al. 2001). Following an AIC-based model selection procedure, we retained (i) the model with linear function for larval development time and pupal mass, and (ii) the model with quadratic function for larval survival and all three immune traits (hemocyte load, PO activity, total PO activity).

Data of larval development time and pupal mass did not require preliminary transformation to comply with the assumptions of parametric tests (normal distribution and homogeneity of variances) and were studied with analyses of covariance (ANCOVAs). Larval survival was compared between copper and temperature treatments with a generalized linear model (GLM) assuming a binomial error structure and a logit function. The three immune traits met the conditions required for parametric tests following different transformations (logarithm transformation for hemocyte load, square root transformation for PO and total PO activity). They were then processed through ANCOVAs. Parametric assumptions were ascertained through Shapiro–Wilk (normality) and Levene tests (homoscedasticity) conducted on the residuals of the fitted model.

All statistical analyses were performed with R software (version 4.1.0).

Results

Larval development time, pupal mass and larval survival

Larval development time was significantly modulated by copper concentration and thermal regime in an additive way (Table 2). Larval development was significantly slowed with an increasing amount of copper in larval food (positive linear relationship between larval development time and copper concentration in the two thermal regimes) (Fig. 1A). In addition, larvae developed significantly faster within the future regime (warmer conditions) (Fig. 1A). Pupal mass was only significantly affected by copper concentration (Table 2): it declined linearly with an increasing amount of copper in larval food (Fig. 1B). Larval survival was significantly affected by the interaction between copper concentration and thermal regime (Table 2). In the current regime, copper concentration tended to slightly decrease larval survival (Fig. 1C). In the future regime, a hormetic (curvilinear) effect of copper on larval survival was apparent: as copper concentration increased, survival rates rose to a maximal value reached for a copper concentration of 25 mg.kg⁻¹, and then progressively decreased (Fig. 1C). Accordingly, the highest differences between the two thermal regimes in terms of larval survival were observed at intermediate copper concentrations (25–50 mg.kg⁻¹): over that range of values, larvae were more likely to survive until pupation in the future regime (Fig. 1C).

Immune parameters

Hemocyte load was not significantly mediated by each of the variables included in the model (Table 2, Fig. 2A). PO activity was significantly affected by the interaction between copper concentration and thermal regime (Table 2). The hormetic effect of copper on PO activity was apparent in both regimes (Fig. 2B). Nonetheless, the peak of PO activity was reached for higher concentrations of copper in the future regime (c.a. 50 mg.kg⁻¹) in contrast to the current regime (c.a. 25 mg.kg⁻¹). Hence, when comparing the two thermal regimes, significant differences regarding this immune trait were only observed at low copper concentrations (0–25 mg.kg⁻¹), with larvae displaying lower PO activity in the future regime (Fig. 2B). Total PO activity was significantly impacted by the additive effects of copper concentration and thermal regime (Table 2). Maximal values of total PO activity were observed for intermediate copper concentrations (25–50 mg.kg⁻¹) in both of the two regimes, and larvae had reduced levels of total PO activity in the future regime (Fig. 2C).

Discussion

We performed this study to investigate how temperature (in a climate change context) may affect the balance of lethal and sublethal effects of a systemic fungicide on a major viticultural insect pest (the tortricid moth *L. botrana*). Exposing larvae to rising concentrations of copper via contaminated food resulted in negative linear effects (larval development, pupal mass) and hormetic curvilinear effects (total PO activity). Our simulation of local warming expedited larval development, but compromised immune function (total PO activity). More important, some traits were affected by the interaction between copper concentration and temperature, suggesting that copper toxicity for the insect pest is to some extent temperature-dependent. For larval survival, a

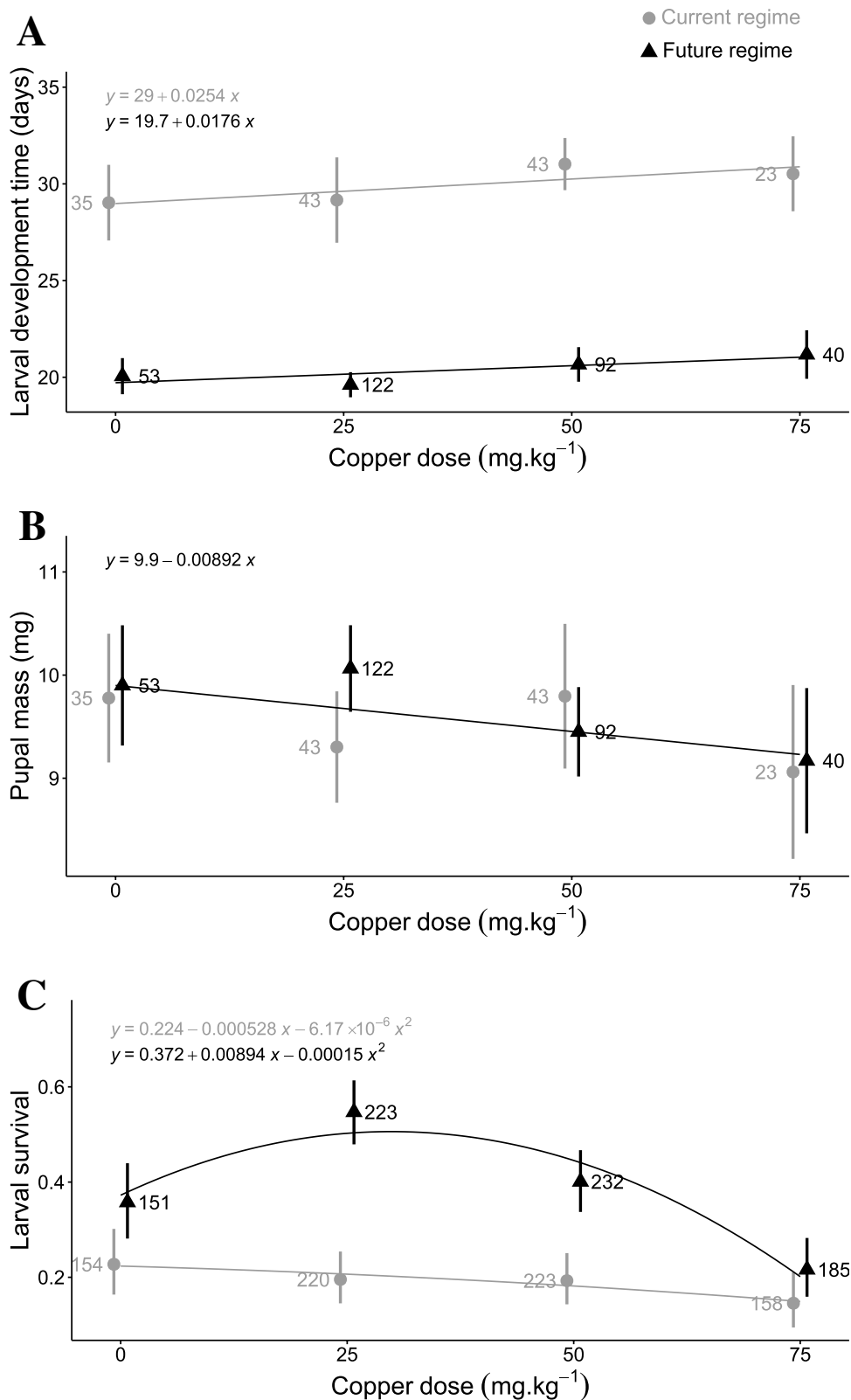
Table 2 Effects of copper concentration (0, 25, 50 or 75 mg.kg⁻¹), thermal regime (current regime or future regime) and their interaction on the traits measured in the two experimental groups

	Copper concentration		Thermal regime		Interaction	
	Test value	<i>P</i>	Test value	<i>P</i>	Test value	<i>P</i>
Larval development time ^a	$F_{1,447} = 6.64$	0.01	$F_{1,447} = 456$	< 0.001	$F_{1,447} = 0.24$	0.63
Pupal mass ^a	$F_{1,447} = 5.22$	0.02	$F_{1,447} = 0.96$	0.33	$F_{1,447} = 0.71$	0.40
Larval survival ^b	$\chi^2 = 34.0$	< 0.001	$\chi^2 = 80.5$	< 0.001	$\chi^2 = 10.4$	0.006
Hemocyte load ^a	$F_{2,353} = 1.25$	0.29	$F_{1,353} = 3.64$	0.06	$F_{2,353} = 2.94$	0.05
PO activity ^a	$F_{2,353} = 5.80$	0.003	$F_{1,353} = 22.9$	< 0.001	$F_{2,353} = 3.22$	0.04
Total PO activity ^a	$F_{2,353} = 5.50$	0.004	$F_{1,353} = 17.8$	< 0.001	$F_{2,353} = 0.67$	0.51

For larval development time and pupal mass, the effect of copper concentration was expressed as a linear function. For larval survival, hemocyte load, PO and total PO activities, it was modeled by a quadratic function (indicative of hormesis). Significant effects are evidenced by bold values

^aANCOVA ^bGLM with binomial error structure

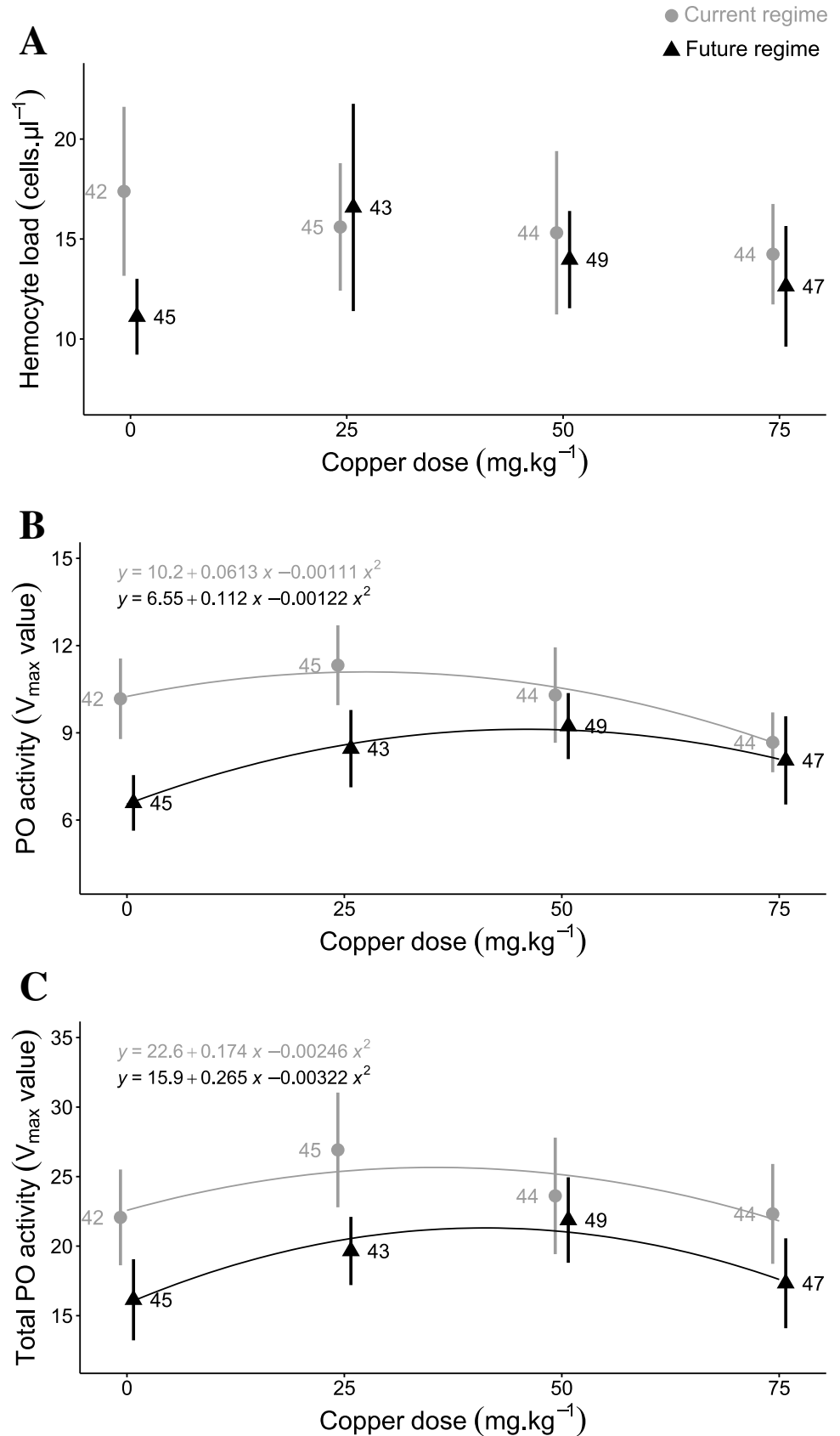
Fig. 1 Effects of copper concentration and thermal regime on: **A** larval development time (from egg hatching until pupation), **B** pupal mass and **C** larval survival (between egg hatching and pupation). Equations are given to describe significant copper effect for the two regimes (larval development time and pupal mass: linear regression; larval survival: polynomial regression with a quadratic function). For pupal mass, the parameters of the equation did not differ between the two thermal regimes, hence only one linear regression was plotted. Represented values are means and their 95% confidence intervals. Numbers associated with points refer to sample sizes



hormetic effect of copper was apparent only in the future regime. For PO activity, hormesis was observed in both thermal regimes, but warmer conditions in the future regime

shifted the hormesis-related peak of performance (maximal values of PO activity) toward higher copper concentrations.

Fig. 2 Effects of copper concentration and thermal regime on the three immune parameters inspected in fifth-instar *L. botrana* larvae: **A** hemocyte load, **B** PO activity and **C** total PO activity. All values were reported to one microliter of pure hemolymph and size-corrected through division by larval head capsule width. Equations are given to describe significant copper effect for the two regimes (polynomial regression with a quadratic function). Represented values are means and their 95% confidence intervals. Numbers associated with points refer to sample sizes



Congruent with our hypothesis, we found evidence for hormetic effects of copper on several larval traits—especially for immune parameters (PO and total PO activity)—but this response pattern was not consistently observed among life-history traits (e.g. a negative linear effect of copper on larval development and pupal mass). The transient peak in immunity observed for some copper concentrations could result from increased PO/PPO synthesis, because copper is a major component of the active site of PO and PPO (van Ooik et al. 2007; González-Santoyo and Córdoba-Aguilar 2012). These findings confirm the dual role of copper as a micronutrient and toxicant and highlight inter-trait variations in response to metal exposure, probably arising from a strong covariation (trade-offs) between those traits (van Ooik et al. 2007; Pölkki et al. 2014; Debecker and Stoks 2019). They also demonstrate that over a range of field realistic concentrations of a chemical, the description of dose–response might be best fitted by linear or hormetic model depending on the biological trait under investigation (Calabrese 2004; Cutler 2013; Guedes and Cutler 2014).

Further, by contrast to our hypothesis, we found that the presumed elevated food and copper intake observed under warmer conditions was associated with an increase (instead of an expected decrease) in pest's tolerance to copper. It thus could be proposed that temperature increased the efficacy of copper-handling mechanisms (e.g., distribution, sequestration, degradation) contributing toward maintaining copper homeostasis, which limited its toxicity for the organism (Pölkki et al. 2014; Op de Beeck et al. 2017). Another plausible explanation is that faster larval development in warmer conditions mechanistically reduced the duration of exposure to copper-contaminated food, which often positively correlates with a chemical treatment's toxicity (Connell et al. 2016). Biochemical quantification of copper accumulation in larval tissues should help assess the actual doses of copper assimilated by larvae and identify the physiological mechanisms underlying increased insect tolerance to copper in a warmer environment.

The pest physiological response to the stressors should determine its population dynamics and local abundance in agroecosystems affected by both copper pollution and warming. For instance, the accelerated larval development observed in the future regime should imply a shortened biological cycle and, subsequently, a higher number of *L. botrana* generations during growing season, as predicted by phenological models (Caffarra et al. 2012) and witnessed by field records (Martín-Vertedor et al. 2010). By increasing pest voltinism, climate change has the potential to exacerbate the herbivory pressure exerted by *L. botrana* on the viticultural output (Gutierrez et al. 2017; Castex et al. 2018). Higher pest virulence under a climate change scenario is further supported by our findings for larval survival, since we noted that warming triggered a hormetic response of this

trait (which culminated for a concentration of 25 mg.kg⁻¹) to copper concentration. This indicates that *L. botrana* larval abundance on infested bunches could benefit from copper treatments in the future, particularly at concentrations below the MRL.

However, larval settlement is also determined by adult reproductive success. Though our study did not include measures directly pertaining to the reproductive compartment, we reported that copper linearly decreased pupal mass, which may adversely impact female's fecundity since this reproductive trait is positively correlated with pupal mass in *L. botrana* (Iltis et al. 2020). Moreover, there is experimental evidence highlighting the negative impacts of warming on male's sperm quality and female's mating success in this species (Iltis et al. 2020). Though these potential adverse effects of copper and temperature on *L. botrana* reproduction might mitigate the abovementioned conclusions, it is worth noting that stressors applied in combination often yield interactive rather than simply additive biological effects, especially in a climate change context (Hooper et al. 2013; Kaunisto et al. 2016). Specifically, pesticide exposure and temperature can interact in non-easily foreseeable ways to modulate pest reproductive performance (Yu et al. 2012; Li et al. 2014). In the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae), for example, males and females benefited the most from a low-concentration pesticide treatment (in terms of reproductive performance) at relatively low and high temperatures (Yu et al. 2012). This example convincingly illustrates a temperature effect on the intensity of pesticide-driven hormesis that could drive in situ pest resurgence phenomena (i.e., an increase in pest abundance following pesticide application) (Yu et al. 2012). Considering such ecological and agricultural consequences of pesticide-by-temperature interaction, we argue for further investigations examining the combined impacts of these two stressors on *L. botrana* reproduction.

Variations in the abundance of *L. botrana* populations are also driven by interactions with organisms belonging to adjacent trophic levels within the tritrophic system (natural enemies and host plant), all being exposed to copper pollution as well. We found that the hormesis-related peak of PO activity was shifted toward higher copper concentrations when larvae were exposed to warmer conditions. This indicates that intense copper application could improve the pest's resistance to its parasitic fauna (especially during the larval stage) in a future climate, which may lead to a surge in pest damage to crops through relaxation of top-down regulatory control exerted by the upper trophic level. This is especially true considering the possible deleterious effects of copper on performance and populations of natural enemies (Vieira et al. 2001; Butler et al. 2009; Garcia et al. 2009; Bengochea et al. 2014), including predators of *L. botrana* eggs in the viticultural system (Pennington et al. 2018).

Interestingly, among these auxiliaries, oophagous parasitoids appear relatively tolerant to the contamination of host eggs by copper-based products. In *Trichogramma cordubensis* (Hymenoptera: Trichogrammatidae) for instance, application of copper sulfate during parasitoid immature stages had nonsignificant to slightly detrimental effect (depending on timing of exposure after egg parasitism) on parasitoid development, emergence rates and adult fecundity, suggesting the safe ecotoxicological profile of this chemical toward the beneficial insect (Vieira et al. 2001; Garcia et al. 2009). It could therefore be postulated that biological control relying on inundative release of egg-attacking parasitoids may prove increasingly important for the future regulation of *L. botrana* populations facing copper pollution and climate change. Additional data about the impacts of different copper concentrations on parasitism of *L. botrana* eggs would be required to ascertain this statement, especially if copper stimulate egg immunity in a similar temperature-dependent way as we evidenced for larvae (through direct exposure or transgenerational effects).

Conclusions

We demonstrated that thermal conditions can alter the response of a major insect pest to a commonly used fungicide through temperature-dependent hormetic effects. Of note, *L. botrana* is known to exacerbate plant susceptibility to fungal diseases through larval feeding on plant tissues (Pavan et al. 2014). Hence, copper treatments used for centuries could become counterproductive in future climatic conditions due to hormesis-related increase in the density of *L. botrana* populations that may facilitate epidemics of fungal pests, even at copper concentrations below the MRL. Careful consideration about the combined effects of copper and temperature on *L. botrana* life history and its associated ecological context should be incorporated in future strategies for the management of different viticultural pests.

Pesticide-induced hormesis is increasingly recognized as a crucial concept for the understanding of the ecotoxicological and agricultural risks associated with chemical control of arthropod pests (Cutler 2013; Guedes and Cutler 2014; Cutler and Guedes 2017). With our particular case example, we reported that hormetic responses to realistic concentrations of a chemical can occur for several key traits related to both population (larval survival) and trophic dynamics (immunity). Layered upon these trait-dependent conclusions, we also showed that hormetic responses can be shaped by environmental conditions, in the present case temperature (Yu et al. 2012). Extrapolating these laboratory-based conclusions to field conditions with practical reference to pest control decision-making is challenging due to the complex interaction between the different stressors. This strengthens

the need for multiple stressors experiments to develop our mechanistic and predictive understanding of insect response to climate change in multi-stress environments (Kaunisto et al. 2016). In this regard, one promising approach would be to integrate one supplementary level of trophic complexity by examining the role played by the host plant in the bottom-up regulation of pest response to the combined chemical and temperature stresses (Xie et al. 2011; Kleynhans et al. 2014). This is especially relevant for studying polyphagous pests like *L. botrana*, which is able to feed upon a large diversity of grape cultivars and alternative host plants, with demonstrated impacts on insect life history.

Author contributions

All authors conceived and designed the research. PH conducted the experiments. CI, PH and PL analyzed the data. CI, JM and PL led the writing of the manuscript. All authors significantly contributed to the intellectual content of the manuscript and gave their final approval for submission.

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Code availability R scripts used to analyze the data are available upon request.

Data availability Data involved in this study are available upon request.

Declarations

Conflict of interest The authors declare they have no conflict of interest.

Ethical approval All applicable institutional and/or national guidelines for the care and use of animals were followed.

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