



Stage-specific effects of a fungicide and global warming on copper accumulation and development in a major vineyard insect pest

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ABSTRACT

The use of copper-based fungicides in agroecosystems has resulted in copper accumulation in soils, increasing its uptake by plants and its transfer along the trophic chain. While fungicides are effective to control fungal diseases, they can also impact non-target organisms such as insect pests that feed on copper-contaminated vegetation. This copper exposure can impair developmental and reproductive processes. In addition, global warming alters the functioning of agroecosystems through rising temperatures and shifting precipitation patterns, and by influencing the distribution and abundance of insect pests, as well as their sensitivity to contaminants. Elevated temperatures may mitigate some of adverse effects of copper by enhancing pest survival. However, the influence of temperature on copper bioaccumulation in insects remain poorly investigated, complicating predictions of pest population dynamics. This study investigates copper accumulation in the vineyard pest *Lobesia botrana* across developmental stages and increasing copper exposure concentrations under current and projected climate conditions. We evaluated the combined effects of copper and climate warming on development time, larval mass, and head-capsule width. Our results showed that insect copper concentrations increased in response to rising external copper levels, but declined over time through life stages, suggesting internal regulation. High copper concentrations combined with warming increased copper accumulation. Copper exposure delayed development and reduced head-capsule width, while warming accelerated growth and increased larval mass. Overall, global warming may enhance larval performance while promoting copper accumulation in *L. botrana*, potentially affecting copper transfer across trophic levels and undermining biological control in vineyards.

1. Introduction

Copper-based fungicides are widely used in agriculture to control fungal diseases affecting crops, particularly in vineyards (Komárek et al., 2010). Although synthetic organic fungicides are prohibited under European organic viticulture regulations, copper-based fungicides remain authorized and are widely used for effective disease control (European Commission, 2019). Despite their widespread use, copper-based products — comprising compounds like copper oxide (Cu₂O), copper hydroxide (Cu(OH)₂), copper oxychloride (3Cu(OH)₂·CuCl₂), and copper sulphate (CuSO₄) — raise significant environmental concerns (Burandt

et al., 2024; Karimi et al., 2021; Komárek et al., 2010). The persistence of copper in soils contributes to its gradual accumulation and poses a long-term environmental contamination risk, especially in older vineyard systems (Mackie et al., 2012; Pesce et al., 2025; Ruyters et al., 2013). Additionally, differences in national regulations may lead to uneven patterns of copper accumulation in soils. For example, the United States does not impose a strict limit but rather requires justification and documentation for repeated applications (USDA, 2022), whereas Chile has no national limits on copper use (Neaman et al., 2024; SAG, 2017). In Europe, the use of copper-based fungicides is regulated both in terms of application rates — with a maximum of 28 kg.ha⁻¹ over

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seven years (European Commission, 2018) — and residue levels, with a limit of 50 mg.kg⁻¹ of copper in grape berries at harvest (European Food Safety Authority, 2018). However, fluctuations around the permitted maximal residue levels are often observed during the growing season and at harvest time, depending on geographical context, local agricultural practices (e.g., fungicide application frequency and grape variety), and meteorological conditions promoting successive leaching of copper (Komárek et al., 2010; Mackie et al., 2012). Consequently, the long-term sustainability of copper as the active ingredient in fungicides is now a growing global concern.

Although copper accumulation in soils has been extensively investigated (Ballabio et al., 2018; Neaman et al., 2024; Vázquez-Blanco et al., 2022), the ecotoxicological impact of copper use on non-target organisms such as phytophagous insect pests has received limited attention. Pests are primarily exposed to copper via consumption of contaminated plant tissues or through contact with residues resulting from foliar fungicide applications (Hummel et al., 2019; Tibbett et al., 2021). Copper ingestion can disrupt cellular processes, induce oxidative stress, and alter multiple life-history traits of insect species (Burandt et al., 2024; Garinie et al., 2024; Komárek et al., 2010; Stevenson et al., 2013). For instance, many studies have shown that copper exposure may: (i) increase the development time of the European grapevine moth *Lobesia botrana* (Garinie et al., 2024; Iltis et al., 2022) and the parasitoid *Trichogramma oleae* (Nusillard et al., 2024b); (ii) reduce the body weight of the Lepidoptera *Limnora dispar* (Gintenreiter et al., 1993) and the honeybee *Apis mellifera* L. (Di et al., 2016); (iii) decrease the fecundity of the ladybeetle *Olla v-nigrum* (Michaud and Grant, 2003); and (iv) alter the reproductive organs of mosquitoes (Jeanrenaud et al., 2020; Ye et al., 2009). Despite its toxicity at high levels, copper is also an essential micronutrient for insects, with moderate exposure shown to stimulate immune responses, resulting in complex dose-dependent physiological responses (Coates and Costa-Paiva, 2020; Iltis et al., 2022). However, when copper uptake exceeds an organism's capacity to detoxify or excrete it, copper accumulates in insect tissues and may lead to physiological damages and/or impair vital functions at high concentrations (Cheruiyot et al., 2013; Kazimirová and Ortel, 2000; Ye et al., 2009). Some insect species progressively accumulate metals, whereas others actively regulate their internal metal concentrations after initial uptake (Tibbett et al., 2021). A few insect species exhibit hyperregulation, maintaining stable internal metal levels despite varying exposure conditions and over extended periods (Tibbett et al., 2021). Most studies assessing copper accumulation have focused on a single life stage, generally using adult insects or late-instar larvae (Cheruiyot et al., 2013; Crawford et al., 1996; Lindqvist, 1992). However, this approach fails to account for the physiological changes occurring during the different developmental stages, including shifts in detoxification capacity, metabolic rate, and excretion efficiency (Gekiére, 2025). Indeed, accumulation patterns may affect not only pest survival but also the survival of their natural enemies (e.g., predators and parasitoids) (Nusillard et al., 2024b; Ye et al., 2009). These natural enemies often target specific life stages (e.g., eggs, particular instar larvae, or pupae), and may ingest sublethal doses of copper by consuming contaminated hosts (Heraty, 2017; Thiéry et al., 2018). A thorough understanding of copper accumulation dynamics across developmental stages in insects, especially phytophagous pests, is critical for accurately predicting their resilience and assessing their contribution to contaminant transfer through the trophic chain.

Additionally, climate change may expand the insect pests' geographic distribution and increase their number of annual generations (i.e., voltinism) as temperatures rise (Bebber et al., 2013; Reineke and Thiéry, 2016). Increased temperatures accelerate insect developmental rates and metabolic processes, leading to higher contaminant uptake rates and modifying physiological responses to environmental contaminants such as copper (Holmstrup et al., 2010; Hooper et al., 2013). Warmer conditions are typically associated with higher contaminant uptake rates in organisms due to an increased metabolic rate (Camp and

Buchwalter, 2016; Hooper et al., 2013). However, elevated temperatures can also lead to higher elimination and/or detoxification rates in the organism, potentially mitigating some toxic effects (Harwood et al., 2009; Lydy et al., 1999). For instance, although warmer temperatures accelerate the development time of the moth pest *Lobesia botrana* (Denis & Schiffermüller, Lepidoptera: Tortricidae) and increase its copper tolerance by enhancing its survival and immunity, they may also worsen copper's negative effects on reproduction (Garinie et al., 2025a; Iltis et al., 2022). These results highlight the complexity of organisms' responses to copper and temperature and the need to understand their combined effects. Nevertheless, the influence of elevated temperatures on copper accumulation in insect tissues remains unclear. Addressing this gap is critical for anticipating copper exposure risks and guiding pest management strategies in a changing environment.

In this study, we investigated the accumulation of copper across the life stages of the European grapevine moth *L. botrana*, a major insect pest, and examined its effects on key developmental life-history traits under both current and projected future climatic scenarios. This species feeds directly on contaminated grape tissues throughout its larval development (Benelli et al., 2023). It is also sensitive to copper and temperature stresses, making it a relevant model for examining dose-dependent responses and climatic interactions (Garinie et al., 2024; Garinie et al., 2025a,b; Iltis et al., 2022). In this context, we exposed *L. botrana* to a realistic gradient of Bordeaux mixture (CuSO₄) concentrations under current and projected climatic conditions (SSP5-8.5 scenario for late 21st-century summers in Burgundy) under laboratory conditions. The objectives of this study were to (i) quantify copper concentrations across larval and adult stages, and (ii) evaluate the combined effects of copper exposure and temperature on development time, body mass, and head-capsule width.

2. Materials and methods

2.1. Insect rearing

Lobesia botrana moths were provided by an inbred stock maintained at the INRAE, Villenave d'Ornon, France. To preserve genetic diversity, the insect stock was regularly supplemented with wild individuals collected from nearby vineyards (Vogelweith et al., 2011). Moths were reared under controlled conditions (22 ± 1°C, 60 ± 10 % RH, photoperiod: L16:D8) in cages, inside which females laid eggs on suspended waxed paper strips. Egg-carrying waxed paper strips were transferred into ventilated plastic boxes and maintained until hatching. Newly hatched larvae (<12 h old) were carefully collected with a paintbrush and individually placed in 2 mL microtubes containing 1.5 mL of semi-artificial diet, prepared as described by Thiéry and Moreau (2005) [composition for approximately 1 kg: 1000 mL of water, 15 g of agar, 86.6 g of corn flour, 41.3 g of wheat germ, 45.5 g of beer yeast, 6 g of ascorbic acid, 3.4 g of Wesson salt mixture, 128 mg of pyrimethanil, 2.7 g of benzoic acid, 2.8 g of methyl 4-hydroxybenzoate, and 5 mL of 95 % ethanol]. Lids of the tubes were pierced with a needle to allow air circulation.

2.2. Copper-based fungicide exposure

To simulate oral exposure to a copper-based fungicide used in vineyard environments, Bordeaux mixture (20 % copper content, BB Caffaro WG) was incorporated into the larval diet at three concentrations: 25 mg.kg⁻¹, 50 mg.kg⁻¹, and 100 mg.kg⁻¹. A control was also included (0 mg.kg⁻¹). These concentrations were selected based on copper concentrations reported in vineyard agroecosystems worldwide (Angelova et al., 1999; Brun et al., 2001; Cuq et al., 2020; Hummel et al., 2019; Lai et al., 2010; Mackie et al., 2012; Miotto et al., 2014). Copper-contaminated diets were prepared by adding 10 mL of distilled water containing the appropriate concentration of Bordeaux mixture to the semi-artificial diet, ensuring final concentrations of 25, 50, and

100 mg of copper per kg of medium. The control diet (0 mg.kg⁻¹) was prepared by adding 10 mL of distilled water without Bordeaux mixture. All prepared diets were thoroughly mixed to ensure homogeneous copper distribution before being aliquoted into microtubes for individual larvae.

2.3. Climatic conditions

In order to assess the response of *L. botrana* under current and future climatic conditions, individuals exposed to different copper concentrations were placed in controlled environment chambers (Memmert HPP260eco, Schwabach, Germany). Two chambers were programmed to simulate both current and projected end-of-century climatic conditions in Burgundy (Eastern France), based on meteorological data from the Longvic-Dijon weather station (47.27°N, 5.09°E; altitude: 219 m). The period from 15th July to 15th August was selected because it corresponds to the second generation of *L. botrana*, which overlaps with the grapevine's flowering and fruit-setting stages, a critical period for pest control to prevent damage to the developing berries (Benelli et al., 2023). The current climatic scenario was modelled using 30-day hourly averages from the 2002–2021 period, to represent the average diurnal cycle of temperature (Fig. 1). Future climatic conditions were derived from the mean output of 18 CMIP6 GCM (General Circulation Model) runs with the SSP5–8.5 scenario, representing the highest greenhouse gas emission pathway (Calvin et al., 2023). The models were down-scaled and corrected using a quantile mapping method, which was calibrated using data from the Dijon weather station (see Zito, 2021 for method details). To minimize condensation of the nutrient medium inside the microtubes due to large daily humidity fluctuations predicted in the initial models, relative humidity was set at a constant 69% for the current climatic regime and 59% for the future regime, reflecting respective daily means (Fig. 1). Temperature was maintained within ± 0.1 °C and the relative humidity within ± 5 %, in the climatic chambers, with conditions continuously monitored throughout the experiment. Lighting was set to a 16:8-hour light–dark photoperiod, with gradual transitions in the first and last hour of light, at 33 % and 66 % intensity to mimic sunrise and sunset. Illumination was provided by a mix of cool white (6500 K) and warm white (2700 K) light sources, delivering a maximum intensity of 2000 lux.

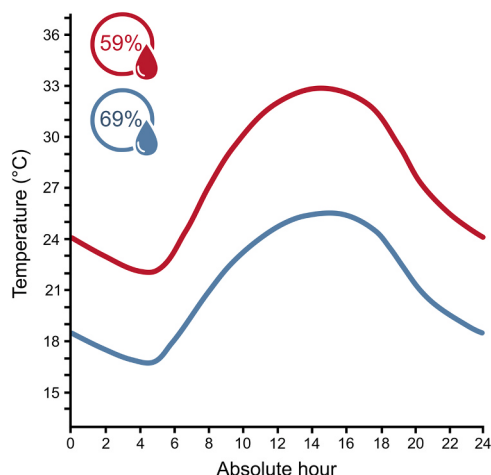


Fig. 1. Climatic scenarios were programmed in the two experimental chambers. Blue and red curves represent current and projected future climatic conditions, respectively. For each scenario, temperature (°C) is shown as daily fluctuations over time. Relative humidity is displayed in the top left corner, with blue and red indicating current and future scenarios, respectively.

2.4. General procedure

A total of 3112 newly hatched larvae (<12 h old) were used in all experiments. They were deposited over three-day periods each week for three consecutive weeks. Larvae were randomly assigned to one of eight experimental groups, representing a full factorial design with four copper concentrations and two climatic conditions. *Lobesia botrana* undergoes five larval stages, which can be identified by the size of the head-capsule. This anatomical feature grows in discontinuous stages and remains stable within each instar (Delbac et al., 2010). To avoid potential stage misclassification caused by environmental stressors such as copper or temperature, the first experiment (experiment 1) focused on measuring head-capsule width across all larval instars. This experiment also included developmental metrics (i.e., body mass and development time), based on a total sample of 931 larvae and 471 adults. The second experiment (experiment 2) aimed to quantify copper content in the body tissues of *L. botrana* at the 3rd and 5th larval instars, as well as in newly emerged males and females (<12 h post-emergence). Since head-capsule width was characterized in experiment 1 across all larval instars and treatment conditions, it was subsequently used in experiment 2 to ensure accurate identification of the 3rd and 5th larval instars prior to copper content analysis. In total, 644 individuals were sampled for copper quantification, including 3rd and 5th instar larvae and adults.

2.5. Experiment 1: head-capsule width measurement and development monitoring

The first experiment enabled detailed tracking of *L. botrana* development and longevity, including (i) larval head-capsule width, (ii) larval and pupal mass, and (iii) development time across life stages from egg hatching to adult emergence, and longevity of adults. Each day, from day 1 until the end of the larval stage (i.e., until pupation), four larvae per condition (32 larvae in total) were delicately sampled using a fine brush, weighed (± 0.01 mg), and anesthetized by placing them at -20 °C for 30 sec to measure the head-capsule width. Head-capsule widths were measured as the distance between the most distant lateral sides of the margins, following the method described by Delbac et al. (2010). Measurements were recorded using a $40\times$ magnification stereomicroscope (Stemi 508, Zeiss, France) coupled with ZEN 2 (blue edition) software (Carl Zeiss Microscopy GmbH, Germany) and using a calibration slide (Motic, MoticEurope, Spain) with an accuracy of ± 0.1 μ m. To avoid developmental bias resulting from the stress manipulation, the sampled and measured larvae were sacrificed and not used in subsequent experiments. Larvae that had not yet been collected were checked daily until pupation. Each pupa was collected, weighed, and placed in a glass tube (70 $\times$ 9 mm diameter) for monitoring until emergence. At emergence, the sex of adults was determined based on abdominal morphology. Adults were examined checked daily to assess longevity.

2.6. Experiment 2: quantification of body copper content

The second experiment aimed to quantify the copper content of body tissues in *L. botrana* at the 3rd and 5th larval instar, as well as in newly emerged adult males and females. Accurate identification of larval instars was ensured by measuring head-capsule width prior to sampling. Following identification, larvae were delicately cleaned with a fine brush to remove any residual artificial medium from their bodies, transferred to microtubes, and weighed. Adults, already isolated in clean tubes without medium, were directly transferred to microtubes and weighed without further cleaning. All samples were immediately frozen in liquid nitrogen and kept at -80 °C until copper quantification. Analyses were performed at the PEA²t platform of the Chrono-environment laboratory (Université Marie et Louis Pasteur, France).

Sample preparation. Copper (Cu) was extracted from Cu-contaminated *L. botrana* body tissues by mineralisation of dried matter (DM) samples (mass range: 0.050–7.620 mg) using an acid-oxidant

mixture (300 μL HNO_3 and 100 μL H_2O_2 ; Optima™ for ultra-traces, Fisher Scientific) in closed tubes placed in a block digestion system (DigiPREP, SCP Sciences, Courtaboeuf, France). The temperature was gradually increased to 100 °C over a total digestion time of 265 min. Ultrapure water (Synergy Model, Millipore, 18.2 M Ω -cm) was then added to reach a final volume of 15 mL, followed by filtration through a 1 μm filter prior to analysis.

Measurements of trace metal concentrations. ^{65}Cu analysis were conducted using inductively coupled plasma mass spectrometry (ICP-MS, iCAP RQ model, ThermoFisher Scientific, Courtaboeuf, France). Final concentrations are expressed as $\mu\text{g}\cdot\text{g}^{-1}$ dry mass. Measurement accuracy was verified using certified reference material: lobster hepatopancreas (TORT-3, National Research Council of Canada). Recovery percentages between certified values and average measured values were considered satisfactory if between 75 % and 125 % (USEPA, 2000). Average recoveries were 75 ± 6 % ($n = 3$). Repeatability of all sample measurements ($n = 3$) was monitored by checking relative standard deviation values (< 20 %). Quality controls were performed during measurements (every 10 samples) using control solutions and internal standard (^{70}Ga) to verify absence of drift. Blanks (i.e., acid-oxidant mixture) and certified reference materials were prepared and analysed following the same procedure as the samples. Detection (DL; median + 3-fold the standard deviation of the blanks, $n = 9$ blanks) and quantification (QL; median + 10-fold the standard deviation of the blanks, $n = 9$ blanks) limits were 0.068 and 0.323 mg Cu/kg DM, respectively.

2.7. Statistical analysis

To evaluate the effects of copper exposure and climatic conditions on *L. botrana* development and copper accumulation, statistical models were first applied across all life stages to assess overall trends, followed by separate analyses for each larval instar as well as males and females. Each model included one of the following response variables: head-capsule width, mass, development time, or copper content. Linear models (LMs) were used when residuals followed a normal distribution, and logarithmic transformations were applied when necessary to improve residual normality and model fit. When normality assumption was not met, generalized linear models (GzLMs) were employed, testing different distribution families (Poisson, quasi-Poisson, Gamma, and negative binomial) to best fit the data. The fixed explanatory variables were copper concentration exposure, climatic scenarios, and their interaction. Copper concentration (0, 25, 50, and 100 $\text{mg}\cdot\text{kg}^{-1}$) and climatic scenarios (current and future) were considered as categorical factors. Given the prior expectation that body mass affects head-capsule width and development time (Garinie et al., 2024; Mo et al., 2013), body mass was included as a covariate in the models. When significant effects were detected, *post-hoc* comparisons were conducted using the *emmeans* package (Lenth, 2025) to determine specific differences between copper concentrations and climatic scenarios within each life stage, with *p-values* adjusted using the Tukey method. GzLMs were performed using the MASS package (Venables and Ripley, 2002). All statistical analyses were performed using R software (version 4.3.2, R Core Team, 2023).

3. Results

3.1. Development time across life stages

Development time and longevity from egg hatching to adult death were significantly influenced by copper exposures and climatic conditions (Table 1, Fig. 2). Firstly, development time and longevity decreased significantly under future climatic conditions compared to

current ones for all life stages and levels of copper exposure, except for the 1st larval instar, exposed to 50 $\text{mg}\cdot\text{kg}^{-1}$ (GzLM quasi-Poisson, $F_{1,1396} = 1454.1$, $p < 0.001$). From the 1st larval instar to the adult death, development time and longevity under future conditions, compared to current conditions was reduced by 8.3 days (-34 %) in unexposed larvae (Fig. 2). Exposure to copper significantly increased development time and longevity at the highest tested concentration compared to the control concentration (Fig. 2; GzLM quasi-Poisson, $F_{3,1397} = 13.8$, $p < 0.001$), extending it by approximately 5 days ($+17$ %) under current conditions and 3.4 days ($+18$ %) under future conditions. Body mass was also positively associated with development time and longevity, with heavier individuals developing more slowly ($\beta = 0.003$, GzLM quasi-Poisson, $F_{1,1390} = 4.9$, $p = 0.026$). However, none of the interaction terms tested in the overall model significantly influenced development time and longevity. Specifically, the full model, which included all life stages and both sexes, revealed no significant interactions between copper exposure and climatic conditions (GzLM quasi-Poisson, $F_{3,1387} = 2.4$, $p = 0.068$), copper exposure and developmental stage or sex ($F_{15,1372} = 1.6$, $p = 0.079$), or climatic conditions and developmental stage or sex ($F_{5,1367} = 2.0$, $p = 0.072$). These results show that the effects of copper exposure and climate conditions on development time and longevity are consistent across life stages and sexes, with no significant stage-specific or sex-specific interaction effects observed.

3.2. Larval growth: larval head-capsule width and mass of larvae and pupae

Head-capsule width was significantly influenced by larval instar (GzLM, quasi-Poisson, $F_{4,874} = 11,122.1$, $p < 0.001$), copper concentration exposure (GzLM, quasi-Poisson, $F_{3,879} = 71.1$, $p < 0.001$), climatic conditions (GzLM, quasi-Poisson, $F_{1,878} = 163.7$, $p < 0.001$) and larval mass (GzLM quasi-Poisson, $F_{1,873} = 29.89$, $p < 0.001$). Copper exposure had a significant negative effect on head-capsule width, with a decrease observed in later larval instars (3rd to 5th larval instars) exposed to the highest copper concentration (100 $\text{mg}\cdot\text{kg}^{-1}$) (Tables 2–3). Larvae at 3rd and 4th larval instar exhibited significantly larger head-capsule widths at 25 $\text{mg}\cdot\text{kg}^{-1}$ copper compared to 100 $\text{mg}\cdot\text{kg}^{-1}$, while no significant difference was found between the control (0 $\text{mg}\cdot\text{kg}^{-1}$) and the 100 $\text{mg}\cdot\text{kg}^{-1}$ treatment (Table 2). Climatic conditions significantly increased head-capsule width, and the significant interaction between climatic conditions and instar (GzLM, quasi-Poisson, $F_{4,854} = 4.22$, $p = 0.002$) shows that the increase in head-capsule width under future climate conditions, relative to current conditions, varied across larval instars. Specifically, *post-hoc* comparisons showed an increase in head-capsule width exclusively in 2nd and 3rd larval instars (Table 2). Head-capsule width was positively associated with larval mass ($\beta = 0.00331 \pm 0.00058$ SE, $t = 5.69$, $p < 0.001$), larger individuals had wider head-capsules depending on the larval instar (Table 3).

Larval mass was not significantly affected by copper exposure (GzLM, quasi-Poisson distribution, $F_{3,879} = 46.91$, $p = 0.139$). However, under future climatic conditions, larval mass was significantly greater than under current conditions (Table 4; GzLM, quasi-Poisson distribution, $F_{1,878} = 118.31$, $p < 0.001$). In addition, the relationship between larval mass and development time showed that larvae reared under future climatic conditions tended to reach higher masses in a shorter time than those reared under current conditions (Fig. 3). This pattern was consistent across all copper exposure concentrations, with copper-exposed individuals experiencing a longer development time without significant loss of mass (Fig. 3). Finally, pupal mass was significantly reduced by copper exposure but increased by future climatic conditions

Table 1
Statistical results of variables influencing development time across larval instars, pupae, male and female adults.

Larval instar	Copper (Cu)		Climatic Scenario (CS)		Interaction (Cu:CS)		Mass	
	Test value	p	Test value	p	Test value	p	Test value	p
L1 ^a	$\chi^2_{3,168} = 4.79$	0.188	$\chi^2_{1,167} = 475.47$	< 0.001	$\chi^2_{1,163} = 0.31$	0.958	$\chi^2_{1,166} = 46.03$	< 0.001
L2 ^b	$F_{3,133} = 7.13$	< 0.001	$F_{1,133} = 140.82$	< 0.001	$F_{3,133} = 1.48$	0.222	$F_{1,133} = 53.35$	< 0.001
L3 ^b	$F_{3,132} = 10.31$	< 0.001	$F_{1,132} = 184.72$	< 0.001	$F_{3,132} = 1.60$	0.193	$F_{1,132} = 40.07$	< 0.001
L4 ^b	$F_{3,168} = 30.46$	< 0.001	$F_{1,168} = 256.96$	< 0.001	$F_{3,168} = 1.99$	0.118	$F_{1,168} = 9.78$	0.002
L5 ^a	$\chi^2_{3,247} = 13.94$	0.003	$\chi^2_{1,246} = 125.12$	< 0.001	$\chi^2_{3,242} = 1.33$	0.723	$\chi^2_{1,245} = 2.31$	0.129
Pupe ^a	$\chi^2_{3,514} = 16.73$	< 0.001	$\chi^2_{1,513} = 479.75$	< 0.001	$\chi^2_{3,509} = 1.26$	0.739	$\chi^2_{1,512} = 0.96$	0.328
Female ^c	$\chi^2_{3,217} = 0.21$	0.004	$\chi^2_{1,216} = 10.44$	< 0.001	$\chi^2_{3,212} = 0.06$	0.233	$\chi^2_{1,215} = 0.16$	< 0.001
Male ^c	$\chi^2_{3,240} = 0.02$	0.018	$\chi^2_{1,239} = 9.35$	< 0.001	$\chi^2_{3,235} = 0.02$	0.679	$\chi^2_{1,238} = 0.09$	0.002

Significant effects ($p < 0.05$) are given in bold. For each measured trait, the statistical test and distribution family that best fit the data are shown by superscript letters (a, b or c).

- ^a Generalized linear model, Poisson distribution.
^b Linear model, log-transformation
^c Generalized linear model, Gamma distribution.

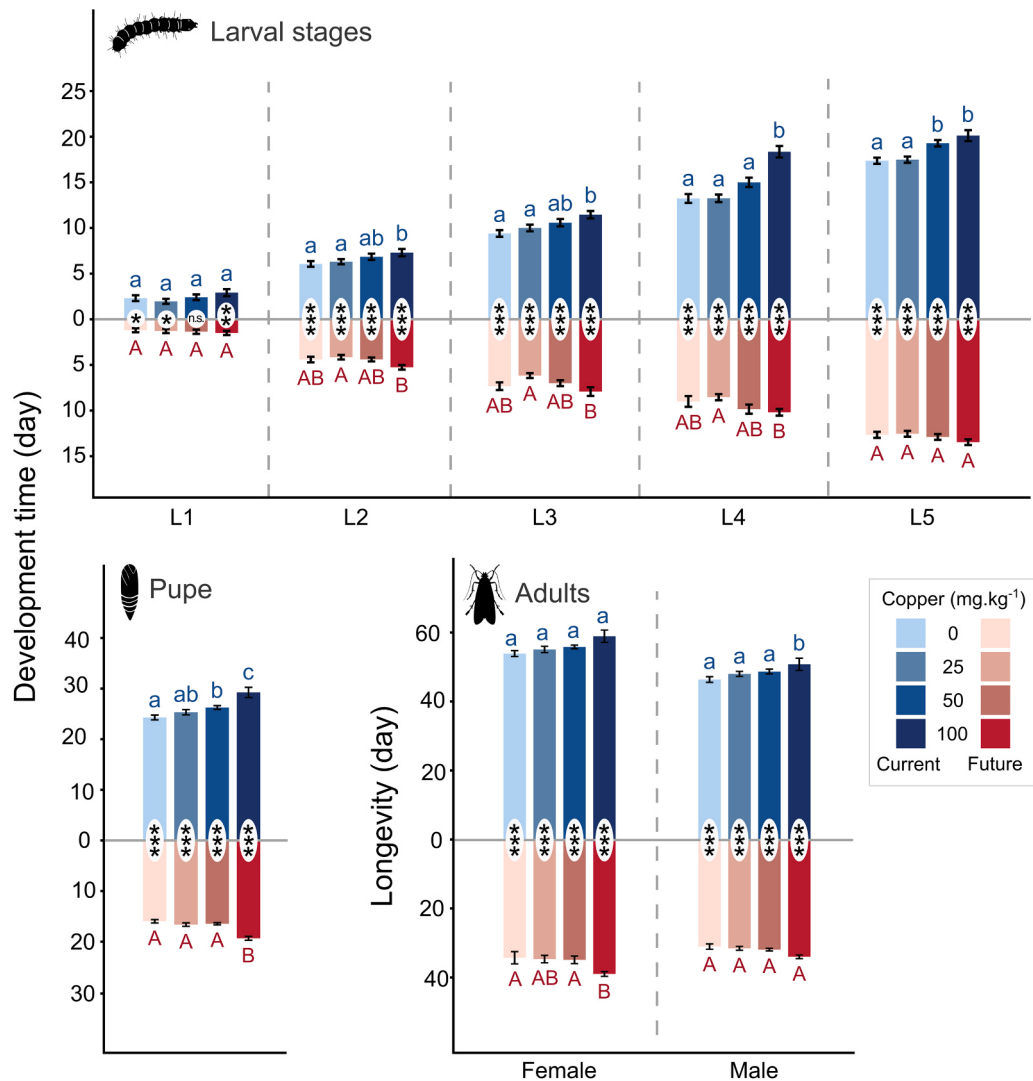


Fig. 2. Development time and longevity of *L. botrana* from egg hatching to adult death across larval (L1, L2, L3, L4, L5), pupal, and adult stages for both sexes, facing different copper exposure concentrations (0, 25, 50, or 100 mg.kg⁻¹) and climatic conditions (blue and red for current and future climatic scenarios, respectively). Lowercase blue and uppercase red letters indicate significant differences between copper concentrations under current and future climatic scenarios, respectively ($p < 0.05$, *post-hoc* test). Asterisks highlight significant differences between climatic conditions for each copper exposure condition (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s. = non-significant).

Table 2

Head-capsule widths (mean $\pm$ SD, μm) of larvae exposed to different copper concentrations (0, 25, 50, and 100 mg.kg^{-1}) under current and future climatic scenarios. L1 to L5 correspond to larval instars. Lowercase and uppercase letters indicate significant differences ($p < 0.05$) between copper exposure concentrations under current and future climatic conditions (within the same column), respectively. Asterisks indicate significant differences between climatic conditions for each copper exposure concentration.

Copper exposure (mg.kg^{-1})	Climatic scenario	Head capsule width (μm) across larval instars				
		L1	L2	L3	L4	L5
0	Current	203.8 $\pm$ 6.7 a	287.3 $\pm$ 12.3 a	416.3 $\pm$ 26.7 a	580.2 $\pm$ 29.9 ab	817.3 $\pm$ 33.9 a
	Future	205.3 $\pm$ 7.6 A	282.5 $\pm$ 16.0 A	408.9 $\pm$ 18.1 AB	577.1 $\pm$ 39.8 AB	808.0 $\pm$ 48.8 A
25	Current	206.6 $\pm$ 6.1 a	281.8 $\pm$ 10.8 a*	406.6 $\pm$ 16.1 a*	594.1 $\pm$ 38.1 a	803.5 $\pm$ 61.3 a
	Future	208.6 $\pm$ 5.0 A	308.7 $\pm$ 48.8 A*	426.5 $\pm$ 26.9 A*	590.8 $\pm$ 35.3 A	806.9 $\pm$ 50.0 A
50	Current	204.7 $\pm$ 8.7 a	276.0 $\pm$ 26.8 a	400.3 $\pm$ 19.2 a*	580.8 $\pm$ 29.3 ab	799.5 $\pm$ 33.0 a
	Future	208.5 $\pm$ 8.1 A	292.6 $\pm$ 30.5 A	418.3 $\pm$ 35.3 A*	592.9 $\pm$ 64.8 A	787.6 $\pm$ 73.1 A
100	Current	205.3 $\pm$ 6.8 a	279.2 $\pm$ 13.8 a	398.2 $\pm$ 22.1 b	560.7 $\pm$ 44.4 b	782.9 $\pm$ 66.7 b
	Future	206.2 $\pm$ 4.4 A	294.3 $\pm$ 39.0 A	392.9 $\pm$ 18.9 B	562.3 $\pm$ 34.4 B	775.6 $\pm$ 53.8 B

Table 3

Statistical results for variables influencing head-capsule width across larval instars.

Larval instar	Copper (Cu)		Climatic Scenario (CS)		Interaction (Cu:CS)		Mass	
	Test value	p	Test value	p	Test value	p	Test value	p
L1 ^a	$F_{3,163} = 1.87$	0.137	$F_{1,163} = 3.43$	0.066	$F_{3,163} = 1.00$	0.396	$F_{1,163} < 0.001$	0.987
L2 ^b	$F_{3,138} = 0.75$	0.523	$F_{1,137} = 10.68$	0.001	$F_{3,133} = 1.46$	0.228	$F_{1,136} = 22.29$	< 0.001
L3 ^a	$F_{3,132} = 4.52$	0.005	$F_{1,132} = 2.15$	0.145	$F_{3,132} = 3.19$	0.026	$F_{1,132} = 1.65$	0.201
L4 ^b	$F_{3,173} = 5.34$	0.002	$F_{1,172} = 0.16$	0.687	$F_{3,133} = 0.29$	0.835	$F_{1,171} = 1.71$	0.192
L5 ^b	$F_{3,247} = 4.85$	0.003	$F_{1,246} = 0.90$	0.343	$F_{3,242} = 0.14$	0.932	$F_{1,245} = 19.91$	< 0.001

Significant effects ($p < 0.05$) are given in bold. For each measured trait, the statistical test and distribution family that best fit the data are shown by superscript letters (a or b).

^a Linear model

^b Generalized linear model, quasi-Poisson distribution.

Table 4

Larval and pupal mass (mean $\pm$ SD, mg) of individuals exposed to different copper concentrations under current and future climatic scenarios. L1 to L5 correspond to larval instars. Values in bold with asterisks highlight significant differences ($p < 0.05$) between climatic conditions for each copper exposure condition. Lowercase and uppercase letters are shown when there are significant differences between copper exposure concentrations under current and future climate conditions respectively.

Copper exposure (mg.kg^{-1})	Climatic scenario	Mass (mg)					
		L1	L2	L3	L4	L5	Pupa
0	Current	0.06 $\pm$ 0.03	0.28 $\pm$ 0.10	0.98 $\pm$ 26.7*	3.87 $\pm$ 2.0	13.98 $\pm$ 6.0	10.86 $\pm$ 2.3 a
	Future	0.07 $\pm$ 0.4	0.29 $\pm$ 0.14	1.49 $\pm$ 0.97*	4.88 $\pm$ 2.57	15.51 $\pm$ 6.2	11.50 $\pm$ 2.4 A
25	Current	0.07 $\pm$ 0.03	0.24 $\pm$ 0.11*	1.08 $\pm$ 0.54	4.58 $\pm$ 2.39	13.09 $\pm$ 5.39*	10.49 $\pm$ 2.0 ab*
	Future	0.08 $\pm$ 0.03	0.43 $\pm$ 0.30*	1.10 $\pm$ 0.48	4.17 $\pm$ 1.83	16.42 $\pm$ 5.74*	11.50 $\pm$ 2.4 A*
50	Current	0.07 $\pm$ 0.03	0.28 $\pm$ 0.11	0.97 $\pm$ 0.40*	4.70 $\pm$ 2.09	13.90 $\pm$ 4.76	10.64 $\pm$ 2.0 ab
	Future	0.06 $\pm$ 0.04	0.31 $\pm$ 0.14	1.41 $\pm$ 0.52*	5.53 $\pm$ 1.95	15.67 $\pm$ 5.82	10.97 $\pm$ 2.5 A
100	Current	0.07 $\pm$ 0.03	0.23 $\pm$ 0.11*	0.95 $\pm$ 0.35	3.69 $\pm$ 1.43	12.51 $\pm$ 4.64	9.77 $\pm$ 2.1 b
	Future	0.07 $\pm$ 0.03	0.39 $\pm$ 0.35*	1.14 $\pm$ 0.54	4.22 $\pm$ 1.92	14.62 $\pm$ 5.27	9.86 $\pm$ 2.3 B

(GzLM, quasi-Poisson distribution, $F_{3,514} = 6.27$, $p < 0.001$; $F_{1,513} = 7.33$, $p = 0.007$), with no significant interaction ($F_{3,510} = 0.94$, $p = 0.419$) (Table 4).

3.3. Body copper content

The copper content in individuals was significantly influenced by copper exposure, climatic conditions, developmental stage, and sex, as well as by several interactions between these factors. Copper accumulation significantly increased with increasing copper exposure concentrations across all studied groups (i.e., 3rd and 5th larval instars, female and male adults) (Fig. 4; NB GzLM, $\text{LR}_{3,640} = 747.8$, $p < 0.001$). A significant interaction between copper exposure and climatic conditions

was observed (NB GzLM, $\text{LR}_{1,639} = 4.7$, $p = 0.030$), indicating that future climatic conditions enhanced copper accumulation at specific copper exposure concentrations (Fig. 4). Developmental stage and sex also had a significant effect on copper content (negative binomial, GzLM, $\text{LR}_{9,624} = 91.8$, $p < 0.001$). Later developmental stages (i.e., 5th instar larvae, female and male adults) showed significantly lower copper concentrations than 3rd instar larvae under most conditions (Fig. 4). However, at elevated copper concentrations (50 and 100 mg.kg^{-1}), an increase in the amount of accumulated copper was observed in these later stages (Fig. 4).

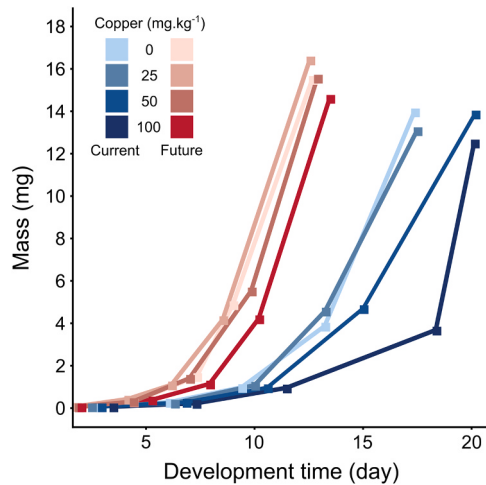


Fig. 3. Mean larval mass at each developmental stage under varying copper exposure concentrations and climatic scenarios. Blue curves represent current climatic conditions, while red curves represent future conditions. Color intensity corresponds to copper concentration, with darker shades indicating higher exposure levels.

4. Discussion

4.1. Copper accumulation and developmental responses

Our results highlighted a dose-dependent accumulation of copper during the developmental stages of *L. botrana*, with body concentrations increasing in response to higher exposure to copper. This metal accumulation in response to environmental exposure has already been described in other terrestrial and aquatic organisms (Cheruiyot et al., 2013; Mebane et al., 2020). Interestingly, we observed a decrease in body copper concentration between the third and the fifth larval instars despite continuous ingestion of contaminated diet. This suggests that physiological regulation of copper may occur over time through mechanisms such as controlled uptake, sequestration, or excretion. In addition, copper accumulation was lower in adults compared with fifth-instar larvae. Adults exposed to the fungicide accumulated significantly less copper than larvae subjected to the same treatments, suggesting the activation of detoxification or excretion mechanisms during metamorphosis. This hypothesis is further supported by the observed reduction in pupal mass under copper exposure, which may indicate the occurrence of energetically costly detoxification processes during pupation (Janssens et al., 2009). Several mechanisms regulating heavy metals in insects have already been reported (Gekière, 2025). Recent studies on silkworm and housefly larvae have highlighted the role of gut microbiota in metal sequestration and precipitation, reducing metal bioavailability and facilitating excretion (Chen et al., 2023; Wang et al., 2023; Yin et al., 2023). Additionally, metallothioneins, metal-binding proteins, transport copper out of cells for excretion through fecal

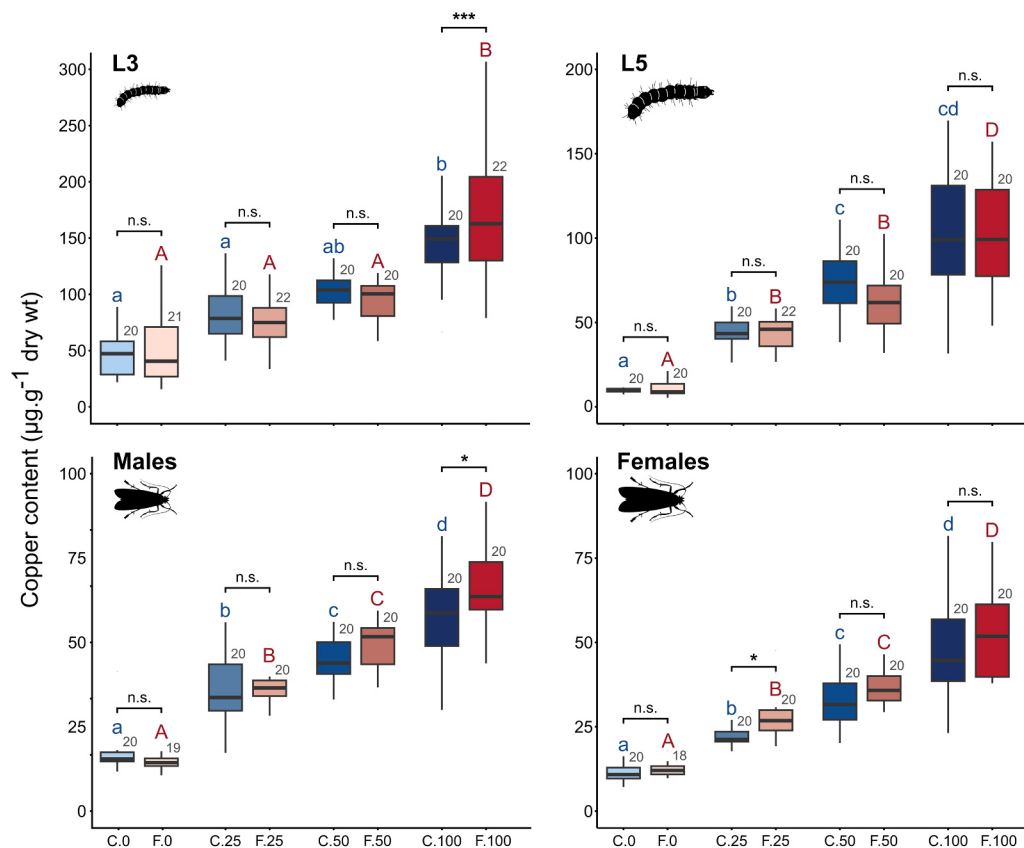


Fig. 4. Body content of copper ($\mu\text{g.g}^{-1}$ dry weight) in *Lobesia botrana* at different developmental stages after ingesting a copper-contaminated diet (0, 25, 50, or 100 mg of copper per kg of medium) during larval development. Blue boxes represent individuals reared under current climatic conditions, while red boxes represent those reared under future climatic conditions. Lowercase blue and uppercase red letters indicate significant differences in copper accumulation among exposure concentrations within current and future climatic conditions, respectively ($p < 0.05$, *post-hoc* test). Asterisks highlight significant differences between climatic conditions for each copper exposure concentration (** $p < 0.001$, * $p < 0.05$, n.s. = non-significant). Grey numbers above the boxes refer to the number of measured individuals.

matter, larval molting processes, and the shedding of pupal exuviae during metamorphosis (Gekière, 2025; Lindqvist and Block, 1995; Neff and Dharmarajan, 2021; Vásquez-Procopio et al., 2020). However, this reduction in copper levels throughout life stages may also reflect changes in body composition during development, as variations in tissue composition can impact metal storage capacity (Hopkin, 1989; Janssens et al., 2009). Although body copper concentration decreases along the life stages of *L. botrana*, the mechanisms underlying copper detoxification remains uncertain. Further studies are needed to better understand and quantify the contribution of symbionts, metallothioneins, and other potential physiological adaptations in managing metal stress.

Regarding temperature effects, we identified a significant interaction between copper exposure and temperature, with elevated temperatures amplifying copper accumulation, especially at higher exposure levels. This suggests that rising temperatures may enhance copper uptake, potentially by influencing metabolic rates or modifying excretory functions. Similar temperature-mediated increases in contaminant accumulation have been observed in aquatic insects such as *Isonychia bicolor* (Walker, Ephemeroptera: Isonychiidae), where pesticide uptake was accelerated under elevated thermal conditions (Camp and Buchwalter, 2016). While these findings highlight the capacity of *L. botrana* to regulate copper levels during development, the physiological consequences of copper accumulation nevertheless adversely affected insect development.

4.2. Effects of copper exposure on development

Beyond its accumulation, copper exposure induced notable sublethal effects on *L. botrana* development, including both temporal and morphological disruptions. As previously reported (Garinie et al., 2024), we observed a prolonged development time positively correlated with increasing copper concentrations. This developmental delay was consistent across all larval instars and persisted into the adult stage, indicating that copper interferes with physiological processes throughout the insect's ontogeny.

Interestingly, while larval mass did not differ significantly between treatments, copper-exposed individuals required more time to reach equivalent mass. This suggests that growth potential is preserved, but development time is slowed, likely reflecting an energetic trade-off wherein metabolic resources are reallocated from growth to detoxification and stress responses (Gillis et al., 2002; Karouna-Renier and Zehr, 2003; Servia et al., 2006). In contrast, head-capsule width was significantly reduced by copper exposure. The observed decoupling between body mass and morphological structure may reflect a shift in energetic allocation. We hypothesize that larvae favor biomass accumulation, potentially to preserve energy reserves, at the expense of structural investment such as head-capsule width. Moreover, since head-capsule size is a hormonally regulated trait closely linked to molting cycles, its reduction may also indicate endocrine disruption (Grossniklaus-Bürgin et al., 1998; Pinto et al., 2021). Copper exposure could interfere with endocrine function by disrupting signaling pathways mediated by ecdysteroids or juvenile hormone, potentially altering both molt timing and morphological development (Jones et al., 1981; Pinder et al., 1999). Furthermore, a substantial decrease in pupal mass was observed in response to copper exposure. This stage is highly energy-demanding, and stress accumulated during larval development can reduce the reserves needed for both successful metamorphosis and future reproductive output, especially in capital breeders, where pupal mass is strongly correlated with fecundity (Cheng Zhu et al., (2002); Muller et al., (2015)). Overall, these findings indicate a delayed and stage-specific response to copper exposure, with potential long-term impacts on key life-history traits such as fecundity and survival.

4.3. Effects of future climatic conditions on development

Under a projected climatic scenario, the development of *L. botrana*

was accelerated by approximately 34 % compared to that under current conditions. Larvae developed faster and reached greater body mass in a shorter period. Head-capsule width was significantly larger in the 2nd and 3rd instars under warmer temperatures, suggesting enhanced somatic growth. These results suggest that elevated temperatures may bring individuals closer to their thermal optimum for growth (Denny, 2017; Sinclair et al., 2016). At first glance, this acceleration in development, and the increase in larval body mass may appear beneficial for *L. botrana*. However, previous studies have shown that warmer conditions can impair larval immunity and reduce key reproductive parameters, including female fecundity, fertility, mating success, and male sperm quality (Garinie et al., 2025a; Iltis et al., 2020, 2018). These apparently contradictory effects may reflect thermal stress. Even if temperatures remain within the species' tolerance range, chronic exposure to elevated temperatures can cause physiological stress, leading to a shift in developmental strategy (González-Tokman et al., 2020). Specifically, resources may be reallocated towards rapid growth and early maturation, potentially at the cost of immune defense, reproductive capacity, or lifespan. In this context, faster development and larger size do not necessarily confer long-term survival or reproductive advantages (Angilletta et al., 2004; Zera and Harshman, 2001). Instead, they may represent a trade-off shaped by thermal stress. Overall, while warming appears to enhance early developmental traits in *L. botrana*, it may also impose hidden physiological costs that limit adult performance, highlighting the importance of considering not only short-term growth benefits, but also the broader life-history consequences of climate change.

4.4. Multi-stress effects of copper contamination and temperature: implications for pest management

Copper exposure and elevated temperature exerted antagonistic effects on larval development time in *L. botrana*. While future climatic conditions significantly accelerated development, copper exposure caused significant delays. The absence of a significant interaction between climatic conditions and copper exposure indicates that their effects on development time act independently. Taken together, these findings imply that under future warming conditions, the accelerated development may partially offset the delays induced by copper exposure observed under current conditions. This could potentially lead to faster development, increased voltinism, and broader geographic distribution (Reineke and Thiéry, 2016). However, pest pressure associated with increased voltinism may be mitigated by other factors. For instance, earlier grape harvests and reduced larval development time could decrease the window during which larval pests cause damage (Castex et al., 2023; Martín-Vertedor et al., 2010; Reineke and Thiéry, 2016). Conversely, warming may also enhance the pest's physiological tolerance to contaminants. Iltis et al. (2022) reported that elevated temperatures may increase copper tolerance in *L. botrana*, with low copper doses improving larval survival and immunity (i.e., a hormetic effect). A similar hormetic pattern was observed in the present study, in which head-capsule width increased at intermediate copper concentrations under future climatic conditions. However, this effect was limited to the 3rd and 4th instars, highlighting the stage-specific nature of hormetic responses and reinforcing the need to assess sublethal effects of contaminants across all life stages. It is important to note that, even when moderate exposure enhances certain traits, the accumulation of copper remains a potential ecological risk, particularly because of its persistence into later life stages. Our results confirmed that copper accumulates in *L. botrana*, with measurable concentrations persisting until the adult stage despite potential excretion mechanisms. This bioaccumulation raises concerns about trophic transfer, as natural enemies such as parasitoids that target eggs, larvae, or pupae may ingest sublethal doses of copper when feeding on contaminated hosts (Thiéry et al., 2018). Previous studies have reported adverse effects of copper on parasitoid performance and survival (Nusillard et al., 2024b, 2024a; Ye

et al., 2009). Furthermore, reducing applications of copper-based fungicides applications has been shown to improve biological control efficiency in vineyard systems (Pennington et al., 2018). These findings emphasize the necessity for integrated pest management strategies to consider the multi-stress effects of climate warming and copper contamination. The possibility that bioaccumulation compromises biological control efficacy highlights the need to limit unintended ecological impacts by adopting more sustainable vineyard disease management practices.

5. Conclusion

This study provides new insights into copper accumulation in a phytophagous insect and its effects across developmental stages. The moth pest *L. botrana* exhibited a copper accumulation-regulation strategy. Body copper concentration increased with exposure level but appeared to be regulated during development to mitigate toxicity. Copper exposure delayed development and reduced head-capsule width, while elevated temperatures accelerated growth and increased body mass. Under future climatic conditions, intermediate larval instars (i.e., 2nd and 3rd) developed larger head-capsules. Individuals exposed to low copper concentrations (25 mg.kg⁻¹) tended to exhibit an increase of the head-capsule width. Additionally, under high copper concentrations, warming increased the internal copper content of insects, suggesting altered bioaccumulation dynamics. Overall, our findings suggest that global warming may enhance larval performance while sustaining, or even promoting, copper accumulation in the major vineyard pest *L. botrana*. Such changes could increase copper transfer through trophic chain and compromise the efficiency of biological control strategies in viticultural ecosystems.

CRedit authorship contribution statement

Jérôme Moreau: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Denis Thiéry:** Writing – review & editing, Resources. **Yann Lelièvre:** Writing – review & editing, Visualization, Formal analysis. **Nadia Crini:** Writing – review & editing, Investigation, Data curation. **William Nusillard:** Writing – review & editing, Visualization, Formal analysis. **Tessie Garinie:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

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

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Data availability

Raw data are available on the dat@UBFC repository (doi: 10.25666/DATAUBFC-2025-06-02).

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