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Pest management facing warming and chemical stresses: Multi-stress effects on the biological agent *Trichogramma oleae*

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HIGHLIGHTS

- Warm conditions increase the survival of a moth pest exposed to copper sulfates
- Copper sulfates and temperature impact development and reproduction of a moth pest
- Multi-stress conditions affect the interaction between pest and biocontrol agent
- Warm conditions decrease *Trichogramma* control of a pest exposed to copper sulfates

GRAPHICAL ABSTRACT
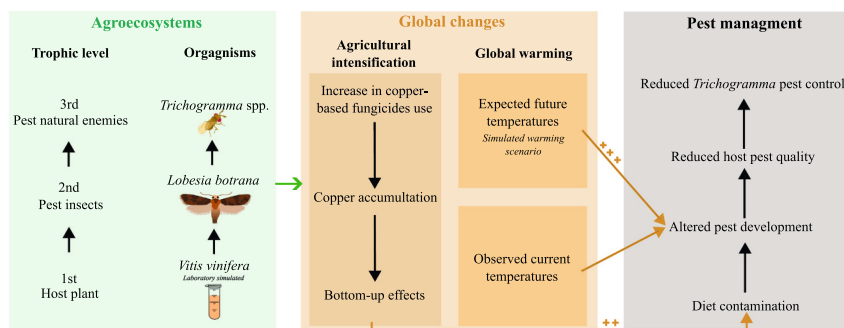
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ABSTRACT

Global change is affecting plant-insect interactions in agroecosystems and can have dramatic consequences on yields when causing non-targeted pest outbreaks and threatening the use of pest natural enemies for biocontrol. The vineyard agroecosystem is an interesting system to study multi-stress conditions: on the one hand, agricultural intensification comes with high inputs of copper-based fungicides and, on the other hand, temperatures are rising due to climate change. We investigated interactive and bottom-up effects of both temperature increase and copper-based fungicides exposure on the important Lepidopteran vineyard pest *Lobesia botrana* and its natural enemy, the oophagous parasitoid *Trichogramma oleae*. We exposed *L. botrana* larvae to three increasing copper sulfate concentrations under two fluctuating thermal regimes, one current and one future. Eggs produced by *L. botrana* were then exposed to *T. oleae*. Our results showed that the survival of *L. botrana*, was only reduced by the highest copper sulfate concentration and improved under the warmer regime. The development time of

L. botrana was strongly reduced by the warmer regime but increased with increasing copper sulfate concentrations, whereas pupal mass was reduced by both thermal regime and copper sulfate. *T. oleae* F1 emergence rate was reduced and their development time increased by combined effects of the warmer regime and increasing copper sulfate concentrations. Size, longevity and fecundity of *T. oleae* F1 decreased with high copper sulfate concentrations. These effects on the moth pest and its natural enemy are probably the result of trade-offs between the survival and the development of *L. botrana* facing multi-stress conditions and implicate potential consequences for future biological pest control. Our study supplies valuable data on how the interaction between pests and biological control agents is affected by multi-stress conditions.

1. Introduction

Global changes in agroecosystems are driving various stress in the development of organisms since these changes encompass evolutions in cultural practices and environmental modifications (Hanaček and Rodríguez-Labajos, 2018; IPCC, 2023). Agroecosystems are impacted by policy-lead changes made to ensure food sovereignty in terms of pesticide and amendment use, which can often have harmful consequences on the environment and human health (Rani et al., 2021). Pesticides pose another threat as some of the active substances accumulate in the soils and can thus have long-term effects on agroecosystems, even after the end of their use (Sharma et al., 2020). In addition, agroecosystems face new sets of climatic conditions that could affect yields, associated to an increase in mean temperature and the frequency of extreme climatic events expected in the coming decades (Vogel et al., 2019; Tripathy et al., 2023). Global changes thus threaten agricultural production by modifying biotic and abiotic conditions in the agroecosystems and also by reducing photosynthesis and hence crop yields (Mathur et al., 2014; Ostberg et al., 2018). Agricultural yields could also be reduced by global changes as they can cause alterations of the relationships between plants and insects, such as with beneficial insects like pollinators (Sponsler et al., 2019; Freimuth et al., 2022) or with pest insects damaging crops (Zidon et al., 2016).

Studies have shown that insects can have various responses to multi-stress conditions (i.e., increase in temperature and exposure to contaminants) (Pallarés et al., 2017; Lupi et al., 2021). A population decline has been observed in insects in the last decades, particularly caused by extensive use of pesticides and fertilizers (Møller et al., 2021; Brühl et al., 2021). The lack of specificity of pesticides (Rani et al., 2021) is causing side-effects on non-targeted insects (Palma-Onetto et al., 2021; Serrão et al., 2022). Insects are indeed threatened by lethal and sometimes sublethal effects of pesticides resulting to the death of most of their population or affecting the development, behavior or reproduction of each individual exposed to the contaminants (Desneux et al., 2007; Martinou et al., 2014). The use of pesticides could both affect pest insects, thus reducing their damage on the crops and affect pest natural enemies, thus reducing their capacity to protect crops from pests (Martinou et al., 2014; Di et al., 2016; Guedes et al., 2016). Alongside the effects of contaminants, insects could be affected by increase in temperatures expected in the coming years, as they are all ectothermic organisms (Estay et al., 2014; Ma et al., 2021a). Temperature increase can have various effects on insect species depending on their spatial and temporal distribution (Harvey et al., 2020), as well as their thermal plasticity (Rodrigues and Beldade, 2020; Mirth et al., 2021). In agroecosystems, higher temperatures can for example shorten the development time of insects, allowing the development of an additional generation in the year (Zidon et al., 2016). Today, the combined effects between global warming and exposure to contaminants can hardly be predicted as species responses to multi-stress conditions seem to vary among insects (Lehmann et al., 2020; Ahmad et al., 2023), showing either deleterious combined effects (Verheyen and Stoks, 2019; Silva et al., 2024), or an increased tolerance to pesticides under warming conditions (Ma et al., 2021b; Iltis et al., 2022). There is thus an increasing interest in studying the interactive and bottom-up effects of global warming and agriculture intensification on biocontrol services

(Han et al., 2019).

Understanding multi-stress effects on pests in agroecosystems is a key challenge for the coming decades, especially when biocontrol methods are used to protect crops, as an additive trophic level could complexify the effects of multi-stress conditions on the pest population. The ability of natural enemies to control pests' population could indeed be reduced under multi-stress conditions (Ahmad et al., 2023) as it has already been shown for different parasitoid species (Thiéry et al., 2018; Di Giovanni et al., 2022). As ectothermic organisms, parasitoid species are known to be strongly dependent on the temperature for their development, reproduction and size (Firake and Khan, 2014; Chen et al., 2021). Although endoparasitoids may be protected from external stress until they are released or emerged from their hosts, such strong effects of temperature when they emerge could lessen their ability to locate, and then reach and parasitize pests (Kroder et al., 2007). Parasitoids could also be threatened by the intensive use of pesticides in agroecosystems, which have been shown to reduce natural predation and parasitism (Fontes et al., 2018; Pennington et al., 2018). In addition, parasitoids, and more particularly oophagous parasitoids, strongly depends on the quality of their hosts for their development (Moreau et al., 2009; Moghaddassi et al., 2019), which can also be affected by a global warming scenario (Iltis et al., 2020). Although previous studies have shown a decrease in fecundity and fertility in pest insects exposed to pesticides (Irigaray et al., 2010; Smagghe et al., 2019), their effects on intrinsic egg quality (e.g., transfer of pesticide residues into the eggs, egg composition) have poorly been investigated. Parasitoids could thus face the consequences of stresses the parental generation of their hosts have faced, such as temperature (Iltis et al., 2020; Spahn and Lill, 2022), or pesticide exposition (Nusillard et al., 2024). To date, very little research has been carried out to study the combined effects of increased temperatures and exposure to contaminants on the natural enemies that control pest insects.

In this context, vineyards show an interesting set of multi-stress conditions for their pest insects and their natural enemies (Reineke and Thiéry, 2016). As a perennial crop, vineyards and their regular pests are threatened by the evolution of the climatic conditions under which they grow and could be subjected to a further increase of pest pressure (Santillán et al., 2019; Ioriatti et al., 2023). Indeed, temperature increase expected for the coming decades may favor the dispersal of one of its principal pest insect in Europe, the grapevine moth *Lobesia botrana* (Denis and Schiffermüller, 1775) (Lepidoptera: Tortricidae) further North, hence affecting a larger part of the vineyards, probably with an additional pest generation per year (Martín-Vertedor et al., 2010; Gutierrez et al., 2018; Deutsch et al., 2018). Vineyards also face an increase of pollution resulting from decades of pesticide use such as Bordeaux mixture (copper-sulfate and lime) to reduce the high pressure of cryptogamic diseases (Dagostin et al., 2011; Gessler et al., 2011). Such pesticide, one of the most heavily and historically used, contains a heavy metal, copper, which role can be contrasted. On the one hand, its properties as an oligo-element make it essential for the development of the plants and their hosts (Coates and Costa-Paiva, 2020; Zehra et al., 2020). On the other hand, copper is known to accumulate in the soils of agroecosystems where copper-based pesticides are used, like vineyards (Mirlean et al., 2007; Mackie et al., 2012; Hummes et al., 2019; Pesce et al., 2024). In the European Union, copper-based fungicides are

authorized in organic viticulture (European Commission, 2018) where they can account for up to 98 % of the total pesticide use, while in conventional viticulture, they account up to 85 % of the total pesticide use (ANSES, 2022). The extensive use of copper-based fungicides is the main cause of copper accumulation in vineyard soils and can reach concentrations of 250 mg.kg^{-1} of copper in most vineyards (Brun et al., 1998; SOGREAH, 2007; Andrivon et al., 2019), and up to 1200 mg.kg^{-1} in the soils of highly polluted vineyards (Mirlean et al., 2007; Mackie et al., 2012; Hummes et al., 2019), whereas the concentration of copper in untreated soils usually reaches 30 mg.kg^{-1} (Brun et al., 1998). This accumulation of copper in the soil can lead to multi-stress conditions for all the trophic network in the majority of vineyards worldwide for the whole trophic network as it has been shown to accumulate in all organs of the plants, even the fructiferous organs on which pest develop (up to 100 mg.kg^{-1} copper in grapes in some vineyards) (Lai et al., 2010; Miotto et al., 2014). Elevated concentrations of copper can be toxic to most insects (Michaud and Grant, 2003; Bernardes et al., 2021). Interestingly, some studies have shown that increasing temperatures can improve pest tolerance to copper-based fungicides (Vogelweith and Thiéry, 2018; Iltis et al., 2022). Thus, multi-stress conditions could lead to unpredictable effects on the vineyards such as increasing damage on grapes due to a higher pest pressure in warmer scenario. It is then essential to understand the tri-trophic interaction of grape, pests and pests' natural enemies facing climate changes and pesticide pollution to predict the efficiency of biocontrol methods in the future (Castex et al., 2018; Han et al., 2019).

In this study, we assessed the consequences of the accumulation of the most used pesticide in vineyards, Bordeaux mixture, on a *Trichogramma* species' ability to control the moth pest *L. botrana*, when the most pest is exposed to a global warming scenario. *Trichogramma oleae* (Voegelé and Pointel, 1979) (Hymenoptera: Trichogrammatidae) was chosen as it is a thelytokous species known to parasitize several moth species and to be efficient facing various temperatures (Ksentini et al.,

2011). A previous study have shown that a warming scenario may increase *L. botrana* tolerance to Bordeaux mixture contained in its diet in terms of survival and development (Iltis et al., 2022). Another previous study, using fourteen increasing constant temperatures from 12 °C to 34 °C on *L. botrana* showed that the development time of eggs, larvae and pupae decreased with increasing temperatures from 12 °C to an optimum of about 28 °C and then increased at higher temperatures (Briere and Pracros, 1998). We thus hypothesized that the survival and development of *L. botrana* will be improved under the future regime compared to the current regime when facing increasing copper sulfate concentrations. However, an increased tolerance to a copper-based pollutant could also suggest trade-off mechanisms to detoxify copper sulfates, which could lead to a lessened reproductive success of the moth, thus reducing the quality of their eggs that *T. oleae* could parasitize. A previous study has indeed shown that host exposition to copper could lead to reduced development and reproductive success of an ectoparasitic wasp species *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) (Ye et al., 2009). Consequences of accumulated copper sulfates in the diet of moth pests have also been observed in *Trichogramma* species used as biological control agents, but only at the highest concentration (Nusillard et al., 2024). We therefore hypothesized that *T. oleae* would show reduced development, survival and reproductive success when the parental generation of its hosts was exposed to the highest multi-stress conditions (highest temperature and concentration of copper sulfates).

2. Materials and methods

2.1. Host eggs production

The overall procedure used in this study is schematized in Fig. 1. All pest insects originated from a laboratory-reared population of *L. botrana* at Geisenheim University, Geisenheim, Germany, whose genetic
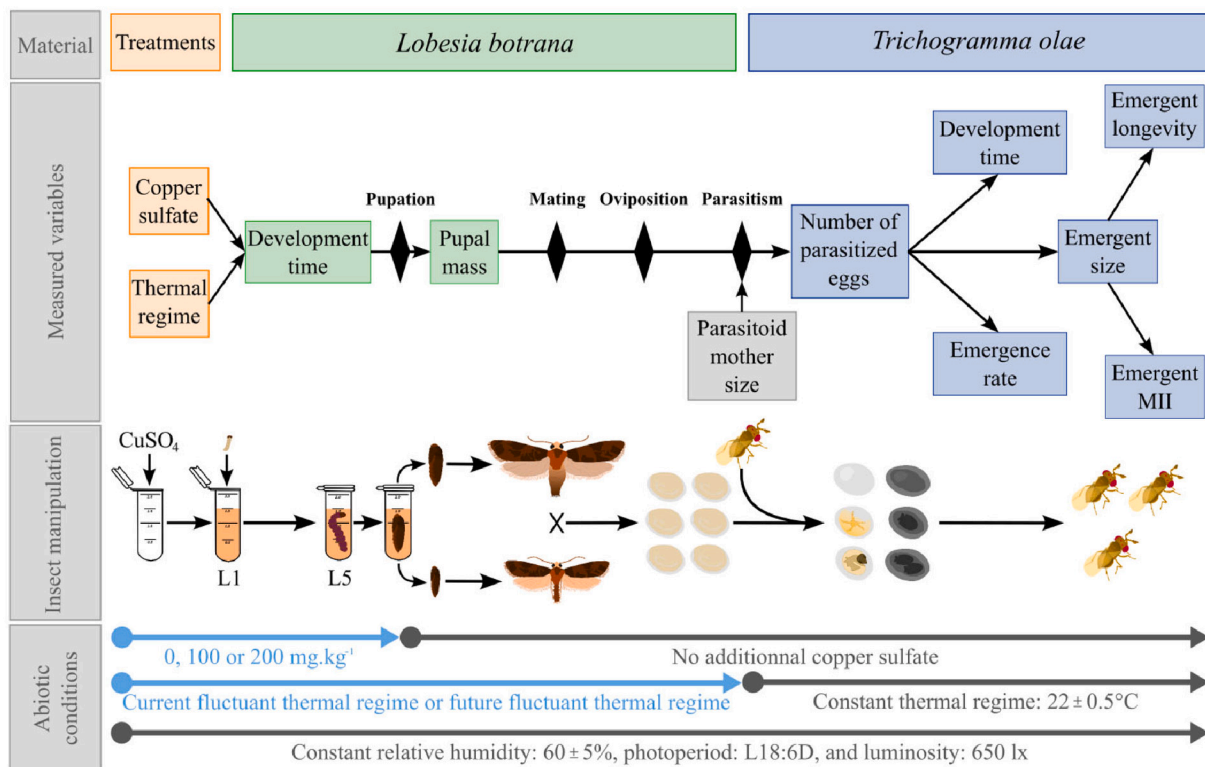

Fig. 1. General schematized procedure. Green (for *L. botrana*) and blue (for *T. oleae*) rectangles indicate measured variables. Blue arrows show the duration of exposure to copper sulfates and thermal regimes. The symbols L1 and L5 correspond to the first and the fifth instars of *L. botrana*. The Maternal Investment Index (MII) was calculated as reflecting the reproductive potential of a female *T. olea*: (number of oocytes × mean volume of oocyte)/10,000.

diversity was maintained by the supplementation of wild individuals or individuals reared in other laboratories (Becker et al., 2022). Around one thousand pupae were extracted from their pupation papers then put into a cylindrical cage (28 × 25 cm in diameter) under standardized conditions (temperature: 22 ± 0.5 °C, relative humidity: 60 ± 5 %, photoperiod: L18:6D, and luminosity: 650 lx) (Nusillard et al., 2024). Two days after the emergence of the first adults, oviposition plastic sheets (PET square of 10 cm^2) were suspended in the cage and changed daily. Oviposition sheets carrying *L. botrana* eggs were then isolated under the same standardized conditions as the cages. After hatching, *L. botrana* larvae (<24 h old) were gently isolated with a brush and placed single in 2 ml pierced-lid Eppendorf tubes containing 1.5 ml of standardized nutrient media for *L. botrana* rearing (composition for 1000 ml: 1000 ml water, 15 g agar, 84.6 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-hydroxybezoate and 5 ml 95 % ethanol) (Thiéry and Moreau, 2005; Muller, 2016). Eppendorf tubes containing one of the three copper sulfate concentrations, added into the diet and homogenized before solidification, and a single *L. botrana* larva were equally distributed between two thermal regimes in two climatic chambers (Memmert HPP260eco, Schwabach, Germany) programmed either with a current or an expected future thermal regime (see below) ($n = 298$ for 0 mg.kg^{-1}, $n = 332$ for 100 mg.kg^{-1}, $n = 557$ for 200 mg.kg^{-1} of copper sulfate for the current regime and $n = 301$ for 0 mg.kg^{-1}, $n = 335$ for 100 mg.kg^{-1}, n = 557 for 200 mg.kg^{-1} of copper sulfate for the future regime). Larval deposition for each treatment was performed proportionally over five consecutive days to minimize the potential effects of hatching days.

In order to study the effects of copper-based fungicides, Bordeaux Mixture (20 % copper content in the form of copper sulfate CuSO$_4$, Bouillie Bordelaise Caffaro WG, Phyteurop) was chosen, as it is one of the most widely used fungicides in vineyards against downy mildew. The lethal dose of copper sulfate for moths or their natural enemies in vineyards has not, to our knowledge, been evaluated in any previous study. Three copper sulfate concentrations were chosen to encompass observable and estimated copper sulfate concentrations in grapes, from a control treatment with distilled water (0 mg.kg^{-1}) to high copper sulfate concentrations of 100 mg.kg^{-1} observed in grapes (Lai et al., 2010; Miotto et al., 2014), and hypothetical 200 mg.kg^{-1} encompassing a wide range of possible concentrations accumulated in grapes in highly treated vineyards (Mirlean et al., 2007; Mackie et al., 2012; Hummes et al., 2019).

Larvae were reared inside two climatic chambers (Memmert HPP260eco, Schwabach, Germany), programmed with thermal regimes designed to be representative of the observed field temperature average from mid-July to mid-August between 1995 and 2014 (current regime, average 20.78 °C, fluctuating between 16 °C and 25 °C), as well as the projected field temperature average on the same period between 2081 and 2100 (future regime, average 27.59 °C, fluctuating between 22 °C and 33 °C) in Burgundian vineyards (Longvic-Dijon weather station, 47.27°N; 5.09°E; altitude = 219 m). These projections were based on linear hourly fluctuation models derived from the average of 18 CMIP6 general circulation model downscaled and debiased (see method in Zito et al., 2021) with the highest-risk scenario outlined by the IPCC (SSP5–8.5) (Fig. S1) (IPCC, 2023). The abiotic conditions inside the climatic chambers were controlled (temperature: ± 0.1 °C, relative humidity: 60 ± 0.1 %, photoperiod: L16:8D, luminosity: 650 lx) and monitored directly on the climatic chambers and after extraction of the data on a computer.

After all the larvae were deposited, each Eppendorf tube was monitored daily for pupation. Pupae were gently extracted from their cocoons with fine flexible forceps, weighed (± 0.1 mg, Pioneer PA214C, OHAUS, Greifensee, Switzerland), isolated in glass tubes (100 × 15 mm in diameter) sealed with moistened cotton, then stored under the same thermal regimes as for their larval development and monitored daily until emergence. Each moth mating carried out under the same thermal conditions as for the development of both males and females. Each mating started at dusk in new glass tubes (100 × 15 mm) sealed with moistened cotton and left free for 24 h between a single two-day-old female and a single three-day-old male, both from the same copper and thermal treatment. Mated females were then isolated in their glass tubes under the same thermal regime as for their development and the number of their eggs laid on the inner surface of the glass tube were monitored every morning. As the classical procedure using both *L. botrana* and *Trichogramma* species, only the tubes containing >30 eggs laid in <24 h were used for parasitism experiments (Moreau et al., 2009; Pizzol et al., 2012; Iltis et al., 2020; Nusillard et al., 2024).

2.2. Parasitism experiments

Following the protocol of Nusillard et al. (2024), female *T. oleae* parasitoids, originating from a thelytokous mass-reared population, were continuously reared in the laboratory on adhesive strips (6 × 1 cm with the last centimeter folded) coated over with alternative host eggs of *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae). After the first emergence, adhesive strips with parasitized host eggs were isolated in new glass tubes every day to use only <24 h old parasitoids for the experiments. A single female parasitoid was introduced in each tube containing *L. botrana* eggs for six hours under standardized conditions (temperature: 22 ± 0.5 °C, relative humidity: 60 ± 5 % and luminosity: 650 lx) (Moreno et al., 2009; Iltis et al., 2020; Nusillard et al., 2024). After parasitism, female *T. oleae* were isolated in 1 ml Eppendorf tubes and frozen, their hind tibia length were then measured using 40× amplification stereomicroscope (Stemi 508, Zeiss, France) and a measurement software (± 0.01 μm, Motic Image Plus, MoticEurope, Spain) (Olson and Andow, 1998; Farahani et al., 2016; Iltis et al., 2020). A total number of 237 parasitized oviposition tubes were analyzed ($n = 47$ for 0 mg.kg^{-1}, $n = 42$ for 100 mg.kg^{-1}, $n = 41$ for 200 mg.kg^{-1} of copper sulfate for the current regime and $n = 37$ for 0 mg.kg^{-1}, n = 37 for 100 mg.kg^{-1}, $n = 33$ for 200 mg.kg^{-1} of copper sulfate for the future regime).

Each oviposition tube exposed to a female *T. oleae* was isolated under standardized climatic conditions (temperature: 22 ± 0.5 °C, relative humidity: 60 ± 5 %, photoperiod: L18:6D and luminosity: 650 lx). After the minimum of four days necessary for parasitized eggs to blacken due to melanization (Hutchison et al., 1990), oviposition tubes containing parasitized host eggs were monitored every day. Emergence of F1 parasitoids from their host eggs was monitored for each parasitized egg

Table 1
Results of the generalized linear models (GLM and GLMM) after a backward selection on the development of *L. botrana*. Significant results ($p < 0.05$) are shown in bold. The letters indicate the nature of the statistical test performed before type II ANOVAs ([a] GLM with binomial distribution; [b] GLMM with Poisson distribution).

	Survival rate [a]		Development time [b]		Pupal mass [b]	
	Test value	*p*	Test value	*p*	Test value	*p*
Thermal regime	$\chi^2_1 = 7.182$	**0.007**	$\chi^2_1 = 1732.195$	**<0.001**	$\chi^2_1 = 83.546$	**<0.001**
Copper sulfate concentration	$\chi^2_2 = 48.522$	**<0.001**	$\chi^2_2 = 136.630$	**<0.001**	$\chi^2_2 = 11.650$	**0.003**
Thermal regime*Copper	$\chi^2_2 = 14.496$	**<0.001**	$\chi^2_2 = 22.995$	**<0.001**	$\chi^2_2 = 2.014$	0.365
Sex	–	–	$\chi^2_1 = 73.057$	**<0.001**	$\chi^2_1 = 557.380$	**<0.001**
Development time	–	–	–	–	$\chi^2_1 = 55.552$	**<0.001**

twice a day (at 8 am and 6 pm). Oviposition tubes were monitored for 20 consecutive days, long enough for all eggs to hatch (Moreau et al., 2009; Moreno et al., 2009). As described by Nusillard et al. (2024), after 20 days, each oviposition tube was observed with a stereomicroscope (Stemi 508, Zeiss, Göttingen, Germany) to count each egg category: (1) number of unparasitized eggs, (2) number of parasitized eggs showing an emergence, (3) number of parasitized eggs showing no emergence, (4) number of unfertilized eggs showing an undifferentiated yellow vitellus, and (5) the number of unparasitized *L. botrana* eggs that hatched. Emergence rate was evaluated as the ratio of the number of parasitized eggs showing an emergence and the total number of parasitized eggs (Nusillard et al., 2024). As super-parasitism dimly occurred (13 % of all parasitized eggs), each super-parasitized egg was counted as one parasitized egg and each super-parasitized egg showing one or more emergence was counted as one egg showing an emergence.

2.3. Measures on emerging parasitoids

Up to three randomly selected F1 parasitoids (all females) that emerged during the first 24 h of emergence of each tube were immediately frozen for later dissection. F1 parasitoids were dissected to count their oocytes and up to five randomly selected oocytes were measured using fine forceps under a 40× amplification stereomicroscope (Stemi 508, Zeiss, France) and a measurement software (± 0.01 µm, Motic Image Plus, MoticEurope, Spain). The oocyte size was estimated by the volume of an ellipsoid using the formula: $V = (4/3) \times (\pi \times a \times b^2)$, where a is the semi-axe of the length of the oocyte and b the radius of the larger part of the oocytes (Martel et al., 2011). We calculated a Maternal Investment Index (MII), reflecting the reproductive potential of a female: (number of oocytes × mean volume of oocyte)/10,000 (Durocher-Granger et al., 2011). The hind tibia length of dissected females was also measured as an indicator of their size using 40× amplification stereomicroscope (Stemi 508, Zeiss, France) and a measurement software (± 0.01 µm, Motic Image Plus, MoticEurope, Spain) (Olson and Andow, 1998; Farahani et al., 2016; Iltis et al., 2020; Nusillard et al., 2024). Up to three other randomly selected F1 parasitoids (all females) that emerged during the first 24 h of emergence of each tube were isolated for longevity monitoring. The longevity assessment of emerging F1 parasitoids was conducted in new glass tubes containing a small drop of honey under standardized conditions (temperature: 22 ± 0.5 °C, relative humidity: 60 ± 5 %, photoperiod: L18:6D, and luminosity: 650 lx). The survival of these emerging F1 parasitoids was monitored twice a day (at 8 am and 6 pm) (Doyon and Boivin, 2005; Moreau et al., 2009). Once parasitoids were observed dead, the size of their hind tibia was measured as described before.

2.4. Statistical analyses

All statistical analyses were performed using the R software version 4.2.3 (R Core Team, 2023). Effects of the thermal and chemical treatments as well as their interaction on the larval survival and the mating success of adult *L. botrana* were analyzed using a Generalized Linear Model (GLM) according to a binomial distribution. Analyses of the effects of thermal and chemical treatments, and their interaction on the development of the host parental generation of *L. botrana* and the development of emerging parasitoids were analyzed using Generalized Linear Mixed Models (GLMM, package 'lme4') (Bates et al., 2015) according to Poisson distributions with a log link function and binomial distributions with a logit link function for the emergence rate, with oviposition tube identifications as random effects. Prior to analyses, a stepwise backward selection was performed to select significant variables ($p \leq 0.05$), from the full set of covariates presented in Tables 1, 2, S1 and S2, that best explain each response variable. This procedure was conducted using the package 'MuMIn' (Bartoń, 2023). We evaluated the variance inflation factor (function vif, package 'car') (Fox and Wiesberg, 2019) and adapted the models to shun multicollinearity in the analyses.

Table 2

Results of the generalized linear mixed models (GLMM) after a backward selection on *T. oleae* parasitism performances. Significant results ($p < 0.05$) are shown in bold. The letters indicate the nature of the statistical test performed before type II ANOVAs ([a] GLMM with Poisson distribution; [b] GLMM with binomial distribution; [c] GLMM with negative binomial distribution; [d] GLMM with lognormal Poisson distribution).

	Number of parasitized eggs [a]		Emergence rate [b]		Development time [a]		Emergent size [c]		Longevity [d]		Maternal Investment Index [d]	
	Test value	p	Test value	p	Test value	p	Test value	p	Test value	p	Test value	p
Thermal regime	$\chi^2_1 = 4.299$	**0.038**	$\chi^2_1 = 35.298$	**<0.001**	$\chi^2_1 = 50.295$	**<0.001**	$\chi^2_2 = 0.083$	0.773	$\chi^2_2 = 1.413$	0.265	$\chi^2_2 = 0.000$	0.995
Copper sulfate concentration	$\chi^2_2 = 0.637$	0.727	$\chi^2_2 = 9.389$	**0.009**	$\chi^2_2 = 51.475$	**<0.001**	$\chi^2_2 = 6.931$	**0.031**	$\chi^2_2 = 24.805$	**<0.001**	$\chi^2_2 = 8.444$	**0.015**
Thermal regime*Copper	–	–	$\chi^2_2 = 17.041$	**<0.001**	$\chi^2_2 = 6.418$	**0.040**	$\chi^2_2 = 1.127$	0.569	$\chi^2_2 = 2.654$	0.265	$\chi^2_2 = 7.931$	**0.019**
Parasitoid mothers' size	$\chi^2_1 = 47.967$	**<0.001**	$\chi^2_1 = 0.036$	0.849	–	–	$\chi^2_2 = 0.093$	0.761	–	–	–	–
Number of parasitized eggs	–	–	–	–	–	–	$\chi^2_1 = 13.799$	**<0.001**	–	–	–	–
Emergent size	–	–	–	–	–	–	–	–	$\chi^2_1 = 6.697$	**0.010**	$\chi^2_1 = 120.569$	**<0.001**

To avoid overdispersion while performing GLMMs, we applied Poisson lognormal distributions to analyze the longevity and MII of emerging parasitoids and a negative binomial distribution for the size of emerging parasitoids. Tests and *p*-values were obtained after type II ANOVAs applied on the different models.

3. Results

3.1. Host development

Both copper treatments and thermal regimes, as well as their inter-action affected the survival rate of developing *L. botrana* larvae and pupae (Table 1). Under the future regime *L. botrana* showed an almost constant survival rate whatever the copper sulfate concentration (69 % for 0 mg.kg^{-1}, 71 % for 100 mg.kg^{-1} and 64 % for 200 mg.kg^{-1}) but slightly above the mean survival rate of *L. botrana* under the current regime (74 % for 0 mg.kg^{-1}, 70 % for 100 mg.kg^{-1} and 51 % for 200 mg.kg^{-1}). However, the highest concentration of copper sulfates reduced the survival significantly more under the current regime than under the future regime. The development duration of adult *L. botrana* (from hatching to emergence) was significantly reduced for both males and females under the future regime, whereas copper sulfate treatments significantly increased the development time of *L. botrana* (Table 1). This increase of duration at the highest concentration is more marked under the future regime (Fig. 2a). The pupal mass of *L. botrana* males and females was significantly lower with an increase in copper sulfate con-centrations and under the future thermal regime (Table 1, Fig. 2b). In addition, individuals taking longer to fully develop showed higher pupal mass (*r* = 0.16, intercept = 0.38, slope = 0.04, Table 1). *Lobesia botrana* males showed shorter development times and reduced masses compared to females (Table 1, Fig. 2a, b). The proportion of fertilized eggs laid by *L. botrana* females was reduced by the future thermal regime (Table S1, Fig. S2a). The proportion of hatched eggs was significantly reduced by both the future thermal regime and increasing copper sulfate concen-trations (Table S1, Fig. S2b).

3.2. Parasitism and parasitoid development

The total number of parasitized eggs was impacted by the thermal regime under which the host parental generation developed (Table 2), fewer eggs were parasitized when they were coming from a female *L. botrana* raised under the future thermal regime (Fig. 3a). Copper sulfates had no effect on the total number of parasitized eggs (Table 2). Larger *T. oleae* females parasitized significantly more eggs than smaller female (*r* = 0.48, intercept = −13.65, slope = 0.18, Table 2). The emergence rate was impacted both by the copper sulfate treatments and the thermal regime as well as their interaction (Table 2). The emergence rate was significantly reduced with the highest copper sulfate concen-tration only when associated with the future thermal regime (Fig. 3b). The development time was impacted by chemical, thermal treatments, and their interaction (Table 2). Overall, the development time signifi-cantly increased with increasing copper sulfate concentrations associ-ated with the current regime, whereas the development time only increased for the highest concentration when associated with the future regime (Fig. 3c). The size of emerging parasitoids was reduced when they came from eggs laid by *L. botrana* females exposed to the highest concentration of copper sulfates whatever the thermal regimes (Table 2, Fig. 3d). The size of emerging parasitoids was positively correlated to the total number of eggs their mother parasitized (*r* = 0.19, intercept = 137.89, slope = 0.42, Table 2). The longevity of emerging parasitoids was strongly reduced with increasing copper sulfate concentrations, regardless of the thermal regime faced by the host parental generation (Table 2, Fig. 3e). The MII was affected and significantly reduced by the highest copper sulfate concentration, the strength of this effect was greater when the host eggs came from *L. botrana* females reared under the future thermal regime (Table 2, Fig. 3f). Similar effects were found on the mean number and volume of oocytes per *T. olea* F1 used to calculate the MII (Table S2, Fig. S3a, b). Both the longevity and the MII of the emerging parasitoids were positively correlated with their size (*r* = 0.20, intercept = −43.469, slope = 2.31 for the longevity and *r* = 0.59, intercept = −1762.52, slope = 15.96 for the MII, Table 2).

4. Discussion

In this study, we investigated the effects of a temperature increase and copper sulfate consumption on *Trichogramma oleae* through its host *L. botrana* exposed to these treatments during its larval development. Our results showed that *L. botrana* is negatively impacted by these multi-
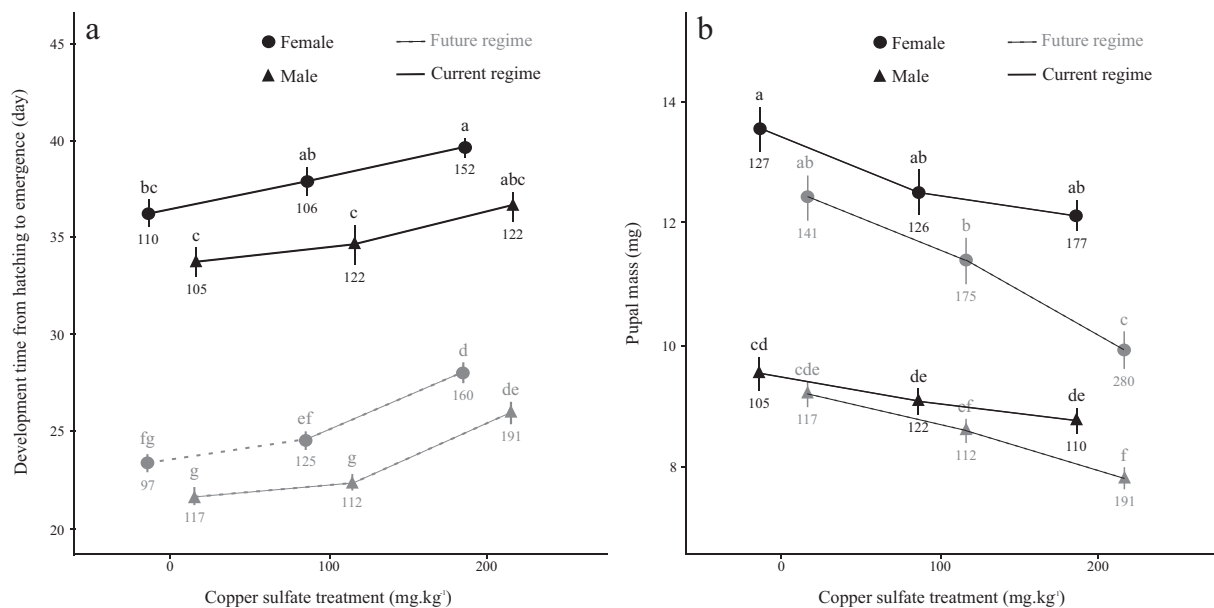

Fig. 2. Effects of copper sulfate treatments between thermal regimes (grey and black) (a) on the development time of *L. botrana* males (triangle) and females (circle) from hatching to emergence and (b) on their pupal mass. Values correspond to the means ±95 % confidence interval. Low case letters above confidence intervals indicate significantly different statistical groups resulting from Dunn tests with Bonferroni adjustments. Numbers below confidence intervals indicate sample sizes.
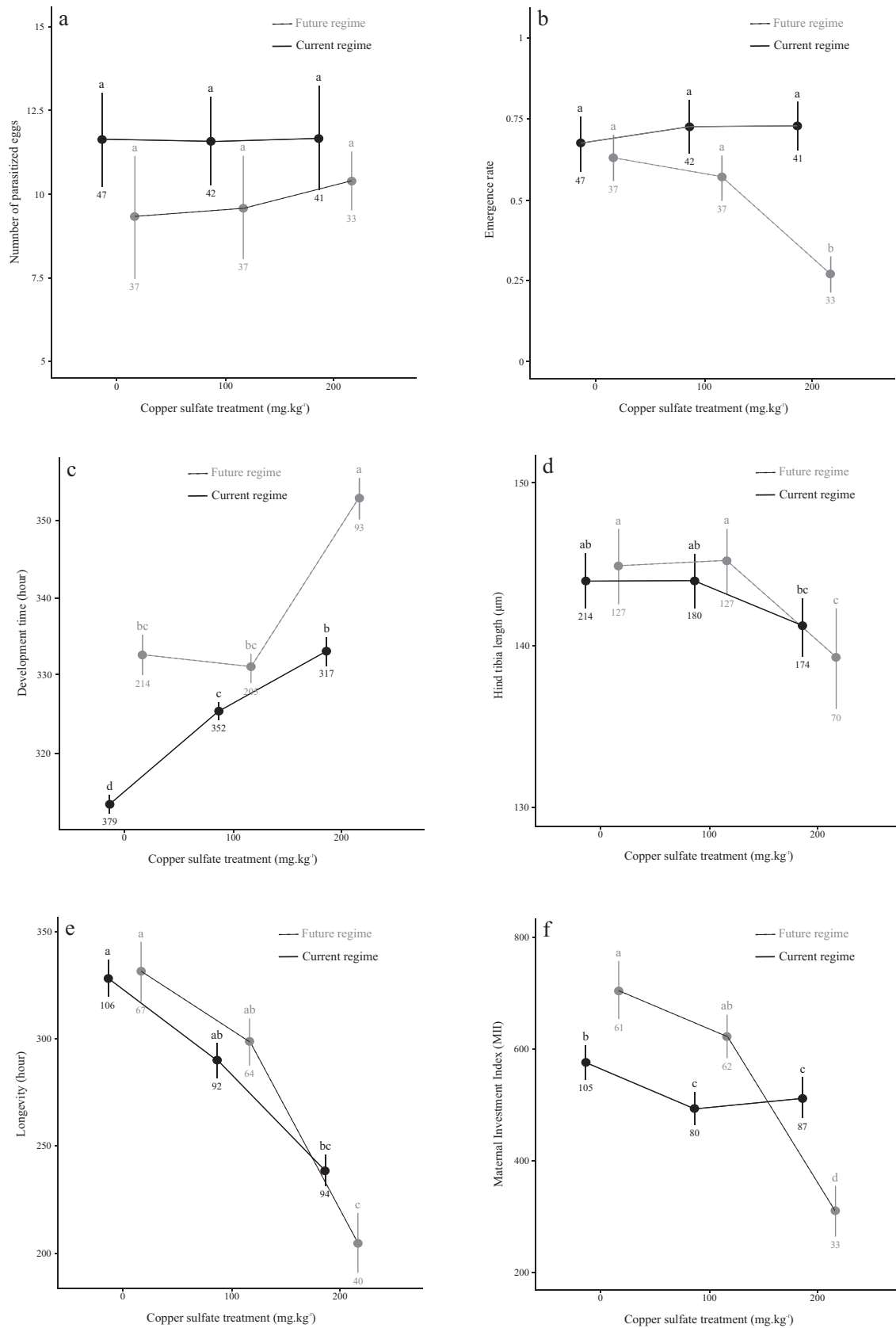
Fig. 3. Effects of copper sulfate treatments between thermal regimes (a) on the total number of eggs parasitized by *T. oleae*, (b) the emergence rate, (c) the development time, (d) the size of emerging parasitoids, (e) the longevity and (f) the Maternal Investment Index (MII) of F1 *T. oleae*. Values correspond to the means ±95 % confidence interval. Low case letters above confidence intervals indicate significantly different statistical groups resulting from Dunn tests with Bonferroni adjustments. Numbers below confidence intervals indicate sample sizes.

stress conditions experienced as larvae, which led us to reject our first hypothesis of an increased tolerance of *L. botrana* under the future regime compared to the current regime. We also found that high copper sulfate concentrations faced by the host parental generation had deleterious effects on the survival, development and fecundity of *T. oleae* F1, which confirms our second hypothesis. These negative effects of high copper sulfate concentrations were generally stronger when the host parental generation developed under the future thermal regime.

When exposed to high copper sulfate concentrations in their diet *L. botrana* larvae show an increased mortality but their survival rate is higher under the future thermal regime than under the current thermal regime. However, larvae that have survived showed an increased development time and a reduced pupal mass when exposed to high copper sulfate concentrations. We can suggest a toxic effect of copper sulfates at the highest concentration. Previous studies have indeed shown that exposition to copper sulfate can be harmful at high concentration to insects (Bernardes et al., 2021; Garinie et al., 2024) but can also improve their development at low copper concentrations following hormetic responses (Iltis et al., 2022; Cutler et al., 2022). We hypothesize that *L. botrana* larvae consuming copper sulfates in their diet used costly detoxification mechanisms, such as copper inactivation (Tibbett et al., 2021), storage (Hopkin, 1990) or excretion (Pigino et al., 2005), and would have paid for such inactivation. This could have reduced the energy available for their larval development but also for oocyte quality produced during the mature stages. It has been shown that increased temperature can improve detoxification proteins' activity in aphids (Durak et al., 2021). Thus, detoxification mechanisms against copper sulfates in *L. botrana* larvae may have been enhanced, which could explain their higher survival rate compared to larvae that developed under the current thermal regime. The relationship between temperature and insect development has been strongly documented, suggesting an optimal temperature for an insect species to develop (Régnière et al., 2012; Du Plessis et al., 2020). In our study, increases in temperature have led to reduced pupal mass and development time in *L. botrana*, which is consistent with the results of a previous study (Iltis et al., 2022). Additionally, increasing copper sulfate concentrations in the diet led to increased development time of *L. botrana* larvae and reduced pupal mass, which is in line with previous studies (Iltis et al., 2022; Nusillard et al., 2024). Our results may either indicate an increased tolerance to the fungicide under the warmer regime or a stronger effect of temperatures on the development of *L. botrana* larvae than copper sulfate treatments. The quality of the eggs laid by *L. botrana* females seemed to be lower when the parental generation faced these multi-stress conditions, as the proportion of fertilized eggs and the proportion of hatched eggs were both reduced coming from the warmer thermal regime. In the same way, the proportion of hatched eggs was reduced at the highest copper sulfate concentration. Our results suggest that facing multi-stress conditions (increase in temperature and exposure to a chemical), *L. botrana* could have redirected a greater part of their energy from their food to detoxify copper sulfates, reducing their energy inputs for their development and reproduction. Host eggs laid by female moths coming from the highest stress conditions could be of inferior quality for the development of *T. oleae* F1.

The negative effects of copper sulfates consumption by the host on the longevity, size and Maternal Investment Index (MII) of *T. oleae* F1, which were particularly strong in host eggs coming from the warmer regime, could be related to the potentially reduced concentrations of proteins, lipids, and glycogen in the vitellus of *L. botrana* eggs. Previous studies have shown that insect parents exposed to stress conditions such as temperature variations and poor quality diet lay eggs of a poorer quality, which impacted their offspring or natural enemies (Geister et al., 2009; Moghaddassi et al., 2019). Reduced concentrations of proteins, triglycerides, and glycogen contained host eggs of *E. kuehniella* have for example lead to reduced fecundity and longevity of emerging F1 *Trichogramma brassicae* (Bezdenko, 1968) (Hymenoptera: Trichogrammatidae) (Farahani et al., 2016). Further studies are necessary to

precisely assess the energy (e.g., lipids, proteins, glycogen, carbohydrates) contained in the eggs of *L. botrana* exposed to multi-stress conditions.

We found that *T. oleae* F1 developing in host eggs coming from *L. botrana* females exposed to multi-stress conditions (high temperatures and high copper sulfate concentrations) had a reduced emergence rate, an increase development time, survived less long and had a reduced potential fecundity (reduced MII). The increased development time and mortality in host eggs from the warmer thermal regime and highest copper sulfate concentrations could be due to improved host eggs defense mechanisms against parasitoids, such as immunity, which is triggered by *Trichogramma* parasitism (Abdel-Latief and Hilker, 2008). Phenoloxidase activity is one of the key elements of insects' immune defenses at all stages of development and it relies on copper for its regulation (Cerenius and Söderhäll, 2004; González-Santoyo and Córdoba-Aguilar, 2012). Its activity is increased with higher temperature (Catalán et al., 2012) and showed hormetic responses when facing different copper concentrations in previous studies (van Ooik et al., 2008; Iltis et al., 2022). These previous studies were caried out on larvae, which could have used detoxification mechanism against high copper sulfate concentrations, potentially leading to copper concentrations increasing phenoloxidase activity. This increase in immunity could have been transferred into the eggs following a transgenerational immune priming (TGIP). TGIP usually consists in an investment of the parents to prepare the offspring for defense against pathogens and parasites to which the parental generation was exposed (Vilcinskas, 2021). Transgenerational immune priming has been shown for *Manduca sexta* (Linnaeus, 1763) (Lepidoptera: Sphingidae) reducing the emergence rate of *Trichogramma evanescens* (Westwood, 1833) (Hymenoptera: Trichogrammatidae) following an increase in phenoloxidase activity (Trauer-Kizilelma and Hilker, 2015), and has been suggested for other species (Pölkki et al., 2012; Triggs and Knell, 2012).

Our results overall suggest that the chemical and thermal stress conditions to which *L. botrana* were exposed could have triggered trade-offs between their survival, development and physiology leading to a lower reproductive success, subsequently affecting the development, longevity, and fecundity of *T. oleae* parasitizing their eggs. This may result in a reduced ability of *T. oleae* to control the moth pest population that have been exposed to multiple stress conditions, potentially compromising future biological control by *Trichogramma* releases in vineyards. These multi-stress conditions for the host pest could have long-lasting effects on the population of its natural enemies, such as reduction in their natural population or even their complete disappearance in certain areas. In the context of Integrated Pest Management, the prolonged effects of multi-stress conditions would probably lead to an increase in the frequency and volume of *T. oleae* releases to control moth pests. Facing low copper sulfate concentrations accumulated in vineyards, pest population outbreaks could increase due to hormetic effects on their physiology (Iltis et al., 2022; Silva et al., 2024). Additionally, pest populations could benefit from the negative effects of pesticides on natural predation and parasitism (Fontes et al., 2018; Pennington et al., 2018). However, at high copper sulfate concentrations that strongly affect pests, estimating the dynamics of insect pest populations could be much more complex, especially with the expected temperature increase in the coming decades. Our results highlight the importance of implementing different thermal regimes in the study of ecotoxicological effects of pesticides on the development and reproduction of pests as hosts for biological control agents, even more so as extreme climate episodes are expected to become more frequent in the coming decades (Vogel et al., 2019; Harvey et al., 2020; Ma et al., 2021a; Tripathy et al., 2023). It is therefore crucial to conduct complementary studies to better understand how the interaction between pests and biological control agents is affected by the multi-stress conditions in agroecosystems.

5. Conclusions

This study focuses on two components of global change, agricultural intensification and climate change, which affect pests and consequently, the biological control agents used against them. The effects of the accumulation of a widely used copper-based fungicide on a biological control agent used against one of the most common vineyard pests in Europe were studied under a simulated global warming scenario. The results showed that copper sulfate contained in the diet of the moth pest *L. botrana* had lethal and sublethal effects on the moth pest and led to changes in the development, survival and reproduction of its natural enemies. *The* detoxification mechanisms of *L. botrana*, which are likely to be enhanced by the future thermal scenario, may be responsible for its prolonged development time, reduced pupal mass and reduced reproductive performance. The combined effects of temperature increase and copper sulfate exposition may have led to host eggs of poor nutritive quality for *T. oleae* F1, which reduced their longevity, potential fecundity and size. These conditions under which the parental generation of host eggs developed, probably led to host eggs having an improved immunity, which increased the development time and mortality of *T. oleae* in host eggs. The future of biological control methods in vineyards is still uncertain as the evolutions in biotic and abiotic conditions expected in the coming decades can have various impacts the interaction between pests and their natural enemies. Transitions to organic viticulture could for instance lead to an increased use of copper-based fungicides. Further studies will hence be necessary to better understand which precise biological mechanisms are impacted by these multistress conditions.

CRediT authorship contribution statement

William Nusillard: Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Tessie Garinie:** Validation, Methodology, Formal analysis. **Yann Lelièvre:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Sébastien Zito:** Writing – review & editing, Resources, Methodology. **Christine Becker:** Writing – review & editing, Resources. **Denis Thiéry:** Writing – review & editing, Conceptualization. **Jacques Frandon:** Writing – review & editing, Resources. **Jérôme Moreau:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, 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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.174709.

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