Journal of Pest Science
https://doi.org/10.1007/s10340-023-01624-6

ORIGINAL PAPER

Heavy metals used as fungicide may positively affect *Trichogramma* species used as biocontrol agents in IPM programs

William Nusillard[1,2] · Tessie Garinie[2] · Yann Lelièvre[2] · Jérôme Moreau[2,3] · Denis Thiéry[4] · Géraldine Groussier[5] · Jacques Frandon[6] · Philippe Louâpre[2]

Received: 2 February 2023 / Revised: 18 March 2023 / Accepted: 13 April 2023

Abstract

The use of biological control agents in Integrated Pest Management programs has increased in the last decades, but may be affected by antagonistic effects generated by the accumulation of some pesticides and other chemicals. The objective of this study was to evaluate the performance of *Trichogramma cordubensis* (Hymenoptera: Trichogrammatidae), a candidate agent to control grapevine moths, when these beneficial insects are exposed to copper treatments through trophic accumulation. A generation of the host *Lobesia botrana* (Lepidoptera: Tortricidae) was reared on a diet with different concentrations of copper from Bordeaux mixture incorporated into the diet. The host eggs laid by these females were then exposed to female parasitoids. Our results showed that copper consumed by the host parental generation had no effects on the parasitism rate but had positive effects on the emergence rate and size of emerging parasitoids at the highest copper concentration. These effects of copper on parasitoids may be due to stress effects of copper on the host parental generation, linked with a trade-off between development and defenses leading to a reduced immunity in their host eggs. Another hypothesis may be that copper has been transferred into the host eggs at potentially beneficial concentrations for parasitoids due to detoxification mechanisms of the hosts' mothers facing a chemical stress. These positive effects of copper pesticides on parasitoids have been observed at the highest copper concentration, consistent with real exposure conditions in the field. This study thus highlights potential synergetic effects between pesticides and natural enemies.

Keywords *Trichogramma* · Pesticides · Non-target organisms · Copper · Biocontrol

Introduction

With the overall objective of maintaining yields, conventional agriculture uses large quantities of synthetic pesticides. The numerous non-target effects of these pesticides on human health, environment, and beneficial organisms (Baldi et al. 2013; Bonner and Alavanja 2017; Brühl and Zaller 2019; Rajput et al. 2019; Rehman et al. 2019; Moreau et al. 2022) have led to a reduction in the use of these products. In this context, Integrated Pest Management (IPM) is increasingly valued, as it uses current and comprehensive information on the life cycle of pests in their agroecosystems to optimize the combination of agricultural practices, biological and chemical controls to manage pest populations (Stern et al. 1959; Peshin and Dhawan 2009), while maintaining high yields (Stenberg 2017). Although this global pest management method aims at reducing the use of pesticides, it does not forbid a well-reasoned use of such products as a last resort. However, the use of multiple methods of pest

---

Communicated by Chris Cutler.

✉ William Nusillard
William.Nusillard@u-bourgogne.fr

[1] AgroParisTech, 91120 Palaiseau, France

[2] Biogéosciences, UMR 6282, CNRS, Université Bourgogne, 6 Boulevard Gabriel, 21000 Dijon, France

[3] Centre d'Études Biologiques de Chizé, CNRS and La Rochelle Université, UMR 7372, 79360 Villiers-en-Bois, France

[4] UMR 1065, INRA (French National Institute for Agricultural Research), BSA, Centre de Recherches INRAe Nouvelle-A quitaine-Bordeaux, 33882 SaveVillenave d'Ornon Cedex, France

[5] UMR Institut Sophia Agrobiotech, INRAE, UCA, CNRS, Sophia Antipolis, France

[6] Recherche Et Développement, Bioline Agrosciences, 26250 Livron-Sur-Drôme, France

control can result in synergistic or antagonistic effects, making it hard to predict the overall success of IPM (Barzman et al. 2015). For example, the use of pesticides can lead to several negative effects on the natural enemies used in IPM programs. Predators and parasitoids used as biocontrol agents are generally more sensitive to pesticides than their prey or hosts, and can be greatly affected by short and long-term exposure to pesticides (Aveling 1981; Martinou et al. 2014; Di et al. 2016). Due to their low specificity (Aktar et al. 2009; Gill and Garg 2014; Rani et al. 2021), pesticides sometimes consequently lead to secondary pest outbreaks by reducing their natural enemies populations (Gill and Garg 2014; Guedes et al. 2016). Pesticides used in IPM programs may thus reduce the performance of biological control programs when non-target effects on biocontrol agents are underestimated (Desneux et al. 2007; Stark et al. 2007).

Viticulture requires high amounts of pesticides in order to control various pests and pathogens (Agreste 2021; Fouillet et al. 2022). Therefore, it represents an ideal agricultural system to explore the interplay between pesticides and biological control agents used in IPM programs. For instance, the most devastating diseases of grapevines, downy mildew and powdery mildew, are caused by fungal pathogens (*Plasmopara viticola* and *Erysiphe necator*). These cryptogamic diseases are responsible for severe yield and economic losses in vineyards (Šrobárová and Kakalíková 2007; Pertot et al. 2017). To control these pathogens, fungicides are widely used and represent up to 96% of the pesticides applied in vineyards (El-Wakeil et al. 2013; Agreste 2021). Among fungicides, copper-based fungicides such as Bordeaux Mixture are the most widely used, even in organic agriculture for which their active substances are authorized (European Commission 2018). In France for example, copper-based fungicides are used on 85% of vineyards in conventional agriculture and 98% in organic agriculture (ANSES 2022). Copper-based fungicides currently remain necessary as no efficient enough alternatives exist (Dagostin et al. 2011; Andrivon et al. 2019). This extensive use of copper-based fungicides has led to copper accumulation in vineyard soils (Brun et al. 1998; SOGREAH 2007; Andrivon et al. 2019), which may have indirect effects on phytophagous insects like moth pests (Lepidoptera: Tortricidae), *e.g.,* the European grapevine moth (*Lobesia botrana*) (Denis and Schiffermüller, 1775) or the grape berry moth (*Eupoecilia ambiguella*) (Hübner, 1796). These insect pests cause damage throughout the grape fructification period and facilitate the development of fungal infections (Cozzi et al. 2006; Delbac and Thiéry 2016). The application of copper-based fungicides may increase the damage caused by insect pests as it has been shown to reduce predation and parasitism of pest eggs and pupae by their natural enemies (Pennington et al. 2018; Vogelweith and Thiéry 2018; Reiff et al. 2021). As such, the efficacy of commonly used insect parasitoids to control moth pests, *e.g.,* oophagous parasitoids of the genus *Trichogramma* (El-Wakeil et al. 2009; Sentenac 2011; Walton et al. 2012; Thiéry et al. 2018; Di Giovanni et al. 2022), could be reduced by the use of copper-based fungicides in vineyards. *Trichogramma* species are sensitive to environmental conditions, such as temperature (Chen et al. 2021), or the use of several pesticides (insecticides and fungicides), which have been shown to reduce the survivorship, longevity and fertility of *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae) (Fontes et al. 2018). *Trichogramma* species are also sensitive to the developmental conditions of their hosts (*e.g.,* temperature, diet), which have been shown to affect the parasitism rate, the emergence rate of parasitoids and increase the size of emerging parasitoids (Moreau et al. 2009; Thiéry and Desneux 2018; Iltis et al. 2020). Based on previous studies, exposure to copper fungicides, however, seems to have minor effects on *Trichogramma* species, when exposed on the surface of host eggs or after parasitoid emergence (Vieira et al. 2001; Garcia et al. 2009). On the other hand, exposure to copper through bioaccumulation in their host's tissue could lead to sublethal effects, which has not been studied. Studying these pernicious effects is crucial to quantify the long-term effects of copper on biological control methods.

We assessed the effects of an extensively used copper-based fungicide (Bordeaux Mixture) on *Trichogramma cordubensis* performance, by offering wasps host eggs from European grapevine moths that ingested different concentrations of copper. *T. cordubensis* has recently been identified as a species with a high parasitism rate *in natura* on the European grapevine moth (Lucchi et al. 2016), and thus represents a promising biological control agent. Larval diet is known to influence host egg quality and the parasitism and life history traits of *Trichogramma* species (Thiéry and Desneux 2018; Moghaddassi et al. 2019). We thus expected the exposure of *L. botrana* larvae to copper to have indirect effects on *T. cordubensis*. Copper is indeed a key oligo-element and a heavy metal, involved in the mechanisms of cellular respiration (Collins 2016) and oxygen transport in connection with hemocyanins (Coates and Costa-Paiva 2020). We expected copper to impact the development and reproductive success of *T. cordubensis*, as it has been shown on other parasitoids before (Ye et al. 2009). We also expected copper to have indirect effects on the survival and development of the parasitoids in their host eggs, through alterations of their host metabolism, as copper can affect *L. botrana* survival, development and immune parameters (Iltis et al. 2022).

Materials and Methods

Parasitoids production

All parasitoids originated from an industry-reared thelytokous population (only constituted by females) of *T. cordubensis* used as biological control agents. The strain was maintained in climatic chambers (ST 2/2 BASIC, Pol-Eko-Aparatura, Wodzisław Śląski, Poland) under standardized conditions (temperature: 22 ± 0.5 °C, relative humidity: 60 ± 5%, photoperiod: L18:6D, and luminosity: 650 lx) for the duration of the experiments. Rearing took place on adhesive strips coated over a width of 1 cm with alternative host eggs of *Ephestia kuehniella* (allowing mass rearing of *Trichogramma*) in glass tubes (100 × 15 mm) containing female *T. cordubensis* for three days. These strips covered with parasitized host eggs were then isolated in new glass tubes of the same size so that emerging parasitoids could be used in experiments after twelve days. Each day, the parasitoid rearing tubes were emptied of previously emerged parasitoids to obtain adult females that were no more than 24 h old for the experiments. For *Trichogramma* species, the oviposition rate, developmental time, and emergence rate (of the offspring) depend strongly on the age the females (Pizzol et al. 2012; Iltis et al. 2020); therefore, parasitoid age was restricted to less than 24 h. These females had not parasitized any host eggs prior to contact with *L. botrana* eggs.

Host eggs production for Trichogramma exposition

All *L. botrana* individuals originated from a laboratory-reared population (INRAe, Villenave d'Ornon, Fance) maintained under standardized conditions (temperature: 22 ± 0.5 °C, relative humidity: 60 ± 5%, photoperiod: L18:6D, and luminosity: 650 lx). Pupae were introduced into a cylindrical cage (28 × 25 cm in diameter). A few days after the emergence of the adults, oviposition papers carrying *L. botrana* eggs near hatching were isolated every day in climatic chambers (ST 2/2 BASIC, Pol-Eko-Aparatura, Wodzisław Śląski, Poland) under the same standardized conditions as the rearing. Larvae less than 24 h old ($n = 1168$) were gently isolated with a brush and placed single in 2 ml pierced-lid Eppendorf tubes containing 1.5 ml of nutrient medium considered ad libitum (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 et al. 2016; Iltis et al. 2020).

In order to be representative of real field conditions, Bordeaux Mixture (20% copper content in the form of copper sulfate $CuSO_{4,}$ RSR Disperss NC, UPL) was used as the copper source, as it is one of the most widely used fungicides in vineyards. Different copper concentrations were chosen to encompass a wide range of possible copper concentrations usually found in vineyards, from a control treatment with distilled water, to an increasing concentration of copper reflecting low (25 mg kg^{-1}) (Angelova et al. 1999; Miotto et al. 2014), high (100 mg kg^{-1}) (Lai et al. 2010; Miotto et al. 2014), and extreme copper concentrations in highly treated vineyards (225 mg kg^{-1}) (Pietrzak and McPhail 2004; Mirlean et al. 2007; Mackie et al. 2012; Ballabio et al. 2018; Hummes et al. 2019).

Lobesia botrana larvae were separated into four groups exposed to increasing copper concentrations contained in the medium, which was prepared several times for each concentration ($n = 296$ larvae for 0 mg kg^{-1}, $n = 298$ for 25 mg kg^{-1}, $n = 295$ for 100 mg kg^{-1}, $n = 279$ for 225 mg kg^{-1} of copper). Larvae were individually deposited over four days in 2 ml Eppendorf tubes filled with 1.5 ml of nutrient medium either containing 0 mg kg^{-1}, 25 mg kg^{-1}, 100 mg kg^{-1} or 225 mg kg^{-1} of copper. Each larva remained in the same tube until pupation, which was monitored daily. The three-day-old pupae (strong enough to be handled) were gently extracted from their cocoons with fine flexible forceps and weighed (± 0.1 mg, Pioneer PA214C, OHAUS, Greifensee, Switzerland). Pupae were isolated in glass tubes (100 × 15 mm in diameter), sealed with moistened cotton, then stored and monitored every day until the emergence. The 48-h-old females were transferred into new glass tubes (100 × 15 mm), sealed with moistened cotton, and then placed in contact with 24–96 h-old males for four hours in the dark. Each mating was performed between partners previously developed with the same copper treatment. After the couple's separation, mated females were then isolated in their tubes for 24–96 h to lay their eggs on the glass wall. The number of eggs laid on the inner surface of each glass tube was monitored every morning. Only the tubes containing at least 30 eggs laid in less than 24 h were used for the experiments as the classical procedure using both *L. botrana* and *Trichogramma* species (Moreau et al. 2009; Pizzol et al. 2012; Iltis et al. 2020). Oviposition tubes were observed through a 5 × amplification stereomicroscope (Stemi 508, Zeiss, France), and five random photographs of eggs per tube were taken in order to estimate the average egg diameter as an indicator of egg size (Becker et al. 2022).

Host eggs exposition to Trichogramma females

On the day of the experiments, a single 24 h-old *T. cordubensis* laboratory-reared female was gently introduced into each host egg tube using a lighted flat surface to avoid their flight due to phototropism. Only actively moving female parasitoids were selected for the experiments, to avoid using unresponsive parasitoids and those potentially harmed by manipulations. Host eggs were exposed to *T. cordubensis* females for six hours. This exposure time was chosen in conjunction with studies conducted on the number of *L. botrana* eggs parasitized by *Trichogramma cacoeciae* females (Moreno et al. 2009; Iltis et al. 2020), so that the number of host eggs (> 30 per tube) would not be limiting. After six hours of exposure, *T. cordubensis* females were isolated in 1 ml Eppendorf tubes and frozen. A total number of 149 parasitized host egg tubes were analyzed ($n = 57$ for

0 mg kg^{-1}, $n=37$ for 25 mg kg^{-1}, $n=37$ for 100 mg kg^{-1}, $n=18$ for 225 mg kg^{-1} of copper).

Parasitism measures and data acquisition

All *T. cordubensis* females used to parasitize host eggs were frozen. The hind tibia length of each female was measured using a 40 × amplification stereomicroscope (Stemi 508, Zeiss, France) and measurement software (± 0.01 µm, Motic Image Plus, MoticEurope, Spain) (Olson and Andow 1998; Farahani et al. 2016; Iltis et al. 2020). Oviposition tubes containing parasitized host eggs were left unmanipulated in climatic chambers for 20 days. This duration was sufficient to allow the complete development of either *L. botrana* non-parasitized eggs until hatching, or of parasitoid larvae until their emergence (Moreau et al. 2009; Moreno et al. 2009). After 20 days of incubation, oviposition tubes were observed with a stereomicroscope (Stemi 508, Zeiss, Göttingen, Germany) in order to determine: (1) the total number of eggs (> 30 eggs) laid by *L. botrana* females in less than 24 h, (2) the number of unfertilized and unparasitized eggs, with an undifferentiated yellow vitellus, (3) the number of fertilized and unparasitized eggs from which a *L. botrana* larva emerged, (4) the number of unparasitized eggs in which there was a dead *L. botrana* larva, (5) the number of parasitized eggs with a fully developed parasitoid, appearing blackened with a hole, and (6) the number of parasitized eggs with an unsuccessfully developed parasitoid, appearing blackened (Hutchison et al. 1990).

The total number of parasitized eggs (with or without parasitoid development) was used to assess the success of parasitism (Iltis et al. 2020). Parasitoid emergence rate was evaluated as the ratio of the number of eggs from which a parasitoid emerged to the total number of parasitized eggs. The emerging parasitoids were gently collected with a brush and then observed under a 40 × amplification stereomicroscope (Stemi 508, Zeiss, France) to measure their hind tibia length in the same way as for their mother parasitoids. For each tube of parasitized host eggs, the size of all emerging parasitoids was measured if they were less than five; otherwise, five parasitoids randomly selected. As super-parasitism (two parasitoid larvae in the same host egg) was observed sporadically (4.4% of the parasitized eggs), the number of host eggs exhibiting super-parasitism was included in the total number of parasitized eggs and each emergence was taken into account in the total number of emerging parasitoids per host egg tube and then factored into the parasitoid emergence rate.

Statistical analyses

Statistical analyses were divided into two steps and were performed using R software version 4.1.2 (R Core Team 2021). The first step aimed at quantifying the effects of copper treatments on measured traits of *L. botrana* and *T. cordudensis*. The effects of copper on the pupal mass and the development time of *L. botrana* females and males were analyzed using Generalized Linear Mixed Models (GLMM, package 'lme4') (Bates et al. 2015) according to Poisson distributions with a log link function. The effects of copper treatments on success, the emergence rate, and emergent size (estimated by their tibia) were evaluated using statistical models factoring in the four copper treatments, female and male *L. botrana* pupal mass, *L. botrana* egg size, and the size of the parasitoid mothers (estimated by their tibia). As development time was negatively correlated with *L. botrana* male and female pupal mass ($\chi^2_1 = 17.87$, $p < 0.001$, slope $= -0.25$ for females; $\chi^2_1 = 9.77$, $p = 0.002$, slope $= -0.16$ for males), we evaluated the variance inflation factor (function vif, package 'car') (Fox and Wiesberg 2019) for models encompassing every variable showing a multicollinearity for the development times of both males and females (VIF > 3.8 for each model). To avoid effects of multicollinearity in the analyses, only the pupal masses were used in the models to analyze the potential effects of host egg parental generation. Since no transformation achieved normality, GLMMs were used to analyze parasitism performances, incorporating tube ID as a random effect. The number of parasitized eggs and hind tibia length of emerging parasitoids were analyzed using GLMMs according to Poisson distributions with a log link function. Parasitoid emergence rate was analyzed using a GLMM based on a binomial distribution with a logit link function. For each mixed model, overdispersion (presence of more variability in the data set than expected based on the model) was checked and no model modifications were required. Tests and *p* values were obtained after type II ANOVAs applied on the different models.

The second step aimed at revealing the nature of the effects of copper on *T. cordubensis*. We considered here that parasitoids may be exposed to copper transferred into the host eggs by *L. botrana* parents, which may be responsible for direct toxic effects on *T. cordubensis*. We also considered that copper may indirectly affect parasitoids through any transgenerational alteration of *L. botrana* host eggs, resulting from exposure of their parental generation to copper. In order to disentangle the relative influence of these two modes of action, (*i.e.,* direct and indirect) of copper treatments on parasitism, the emergence rate and the size of the emerging parasitoids, path analyses were used (package 'lavaan') (Rosseel 2012). Path analysis is a statistical method based on the analysis of a theoretical model which enables one to evaluate linear relationships among variables in a system and the comparative strength of direct and indirect relationships among these variables (Wright 1934). The model used for the path analyses was conceptualized according to information gathered in the literature leading to hypotheses linking the variables between them. In our experiment, parasitoid mothers were not exposed to copper during their development, so that direct and indirect effects of copper were supposed to act on all measured variables except for the size of the parasitoid mothers. Since copper was introduced into the diet that the parental generation of *L. botrana* fed on, one of the first hypotheses was that copper may alter the larval development time, known to be linked with pupal mass and the size of the eggs laid by the adults (Iltis et al. 2021). Parasitism response variables were then assumed to be dependent on the size of the host eggs, which has previously been shown as an indicator of egg quality (Moreau et al. 2016). Finally, parasitism response variables were assumed to be dependent on the size of the parasitoid mothers used for the experiments (Iltis et al. 2020). The conceptual model was divided into five levels: (1) the copper treatments, (2) the parental generation of the *L. botrana* hosts represented by the development time and the pupal masses of the males and females, (3) the host generation of *L. botrana* represented by the size of the host eggs, (4) the parasitism performances represented by the number of parasitized eggs, the emergence rate and the hind tibia length of the emerging parasitoids, and (5) the parental generation of *T. cordubensis* parasitoids represented by the hind tibia length of the mothers. Once the conceptual model was established, the analyses assessed the valence and strength of the relationships between the variables in the conceptual model. Direct effects were evaluated by observing the paths from one variable to another that depends directly on it. Indirect effects were evaluated by multiplying the coefficients of the successive paths between them (either within or between levels). The total effects were evaluated by adding the direct and indirect effects.

Results

Host development

Copper had a significant effect on developmental time (day) of both female and male *L. botrana* larvae ($\chi^2_3 = 16.87$, $p < 0.001$ for the female larvae; $\chi^2_3 = 17.01$, $p < 0.001$ for the male larvae). High copper concentrations (100 mg kg^{-1} and 225 mg kg^{-1}) increased the development time (by 17%) (Fig. 1a). Copper concentrations had a negative effect on male and female pupal mass (mg), with 100 mg kg^{-1}and 225 mg kg^{-1} of copper showing lower mass (Fig. 1b). The pupal mass of *L. botrana* females had a positive effect on the host egg size (μm) ($\chi^2_1 = 16.95$, $p < 0.001$, slope = 5.3). The age and pupal mass of males had no effects on the host egg size ($\chi^2_1 = 0.73$, $p = 0.39$ for the age; $\chi^2_1 = 0.65$, $p = 0.41$ for the mass).

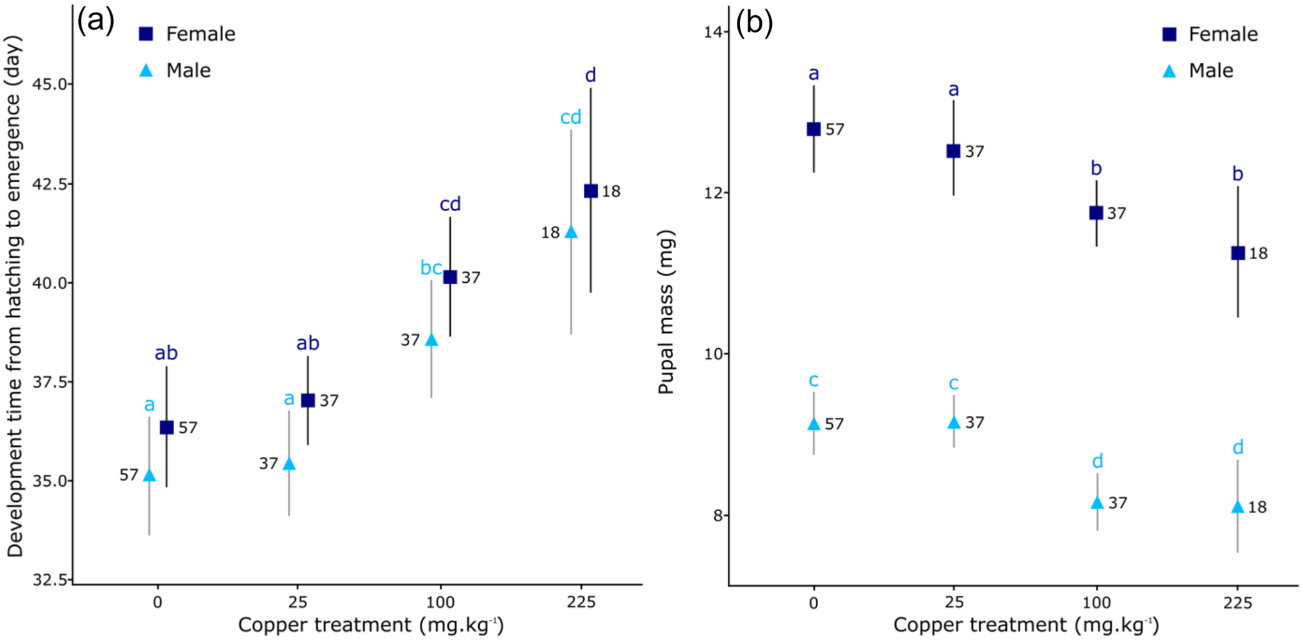

Fig. 1 Effects of copper treatments on the development time of *L. botrana* females and males from hatching to emergence (**a**) and on the pupal mass of female and male *L. botrana* (**b**). The represented values correspond to the means ± 95% confidence interval. the values written next to the dots indicate the sample sizes. Each letter **a**, **b**, **c** or **d** denotes a significantly different result according to the Newman–Keuls post hoc test

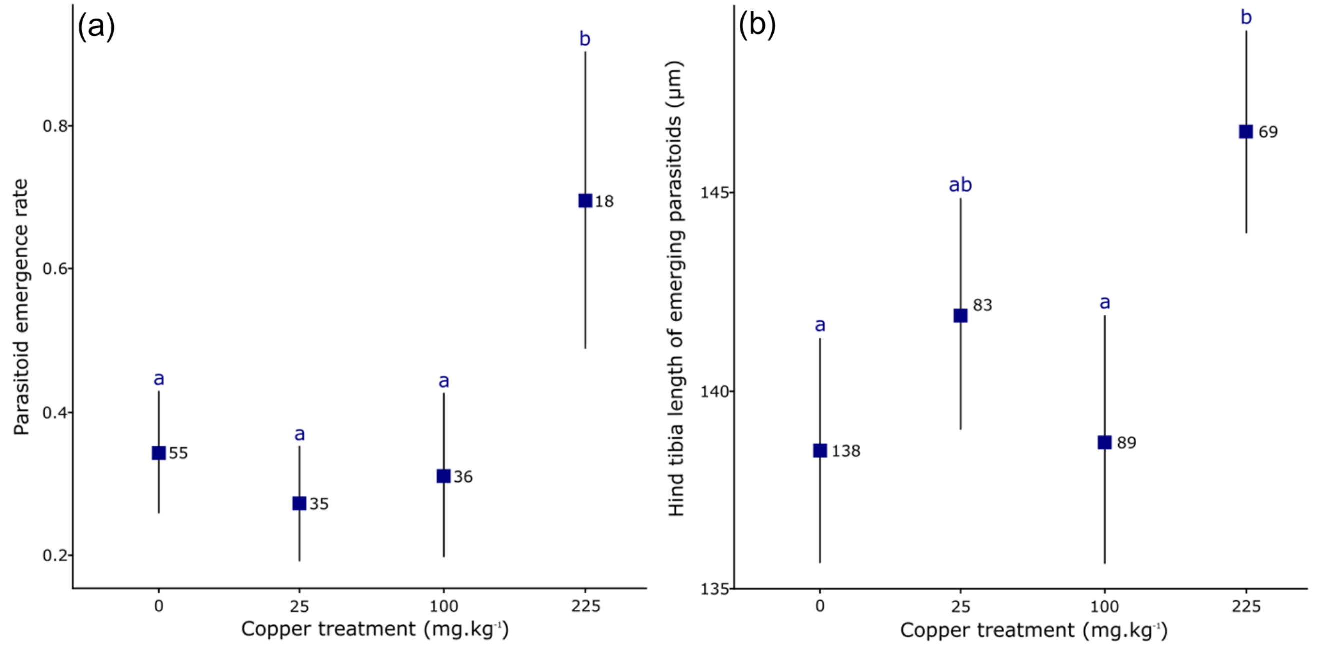

Fig. 2 Effects of copper treatments on the parasitoid emergence rate (**a**) and the size of emerging parasitoids (**b**). The represented values correspond to the means ± 95% confidence interval. The values written next to the dots indicate the sample sizes. Each letter **a** or **b** denotes a significantly different result according to the Newman-Keuls post hoc test

Parasitism performances

Copper had no effect on the number of parasitized eggs (mean 12.81 $CI_{95\%} = [8; 18]$ parasitized host eggs for all treatments taken together) (Table 1). However, the parasitoid emergence rate and the size of emerging parasitoids (µm) were affected by copper treatments (Table 1). These effects were observable at the highest copper concentration (225 mg kg^{-1}), which doubled the average parasitoid emergence rate (Fig. 2a) and increased the size of emerging parasitoids, as denoted by hind tibia length (Fig. 2b). None of the three response variables used to assess parasitism performances were significantly affected by pupal mass or host egg size (Table 1). The number of parasitized eggs and the size of emerging parasitoids were significantly affected by the size of the female parasitoids (Table 1). Larger female parasitoids parasitized significantly more eggs than smaller females ($y = 0.19x - 15.16$, $r = 0.37$) and had larger progeny ($y = 0.23x + 106.68$, $r = 0.25$).

Path analysis

The path analysis showed that copper had two direct positive effects, one on the developmental time of host eggs of the parental generation and one on the parasitoid emergence rate (Fig. 3). The pupal masses of the parental generation were not directly affected by copper, but indirectly through their development time (respective correlation coefficients of $0.37 \times (-0.67) = -0.25$ for the females and $0.36 \times (-0.56) = -0.20$ for the males). Host egg size was indirectly influenced by copper through parental generation developmental time and pupal mass of females (coefficient of -0.084). According to the path analysis, copper had no significant influence (direct or indirect) on the number of parasitized eggs or the size of emerging parasitoids (Fig. 3). The size of emerging parasitoids was indirectly influenced by the size of their mothers through the number of parasitized eggs (coefficient of 0.098).

Discussion

The aim of this study was to evaluate the non-target effects of a copper-based fungicide commonly used in vineyards on the parasitism performance of *T. cordubensis* when consumed by grapevine moth larvae. Our results showed that the highest copper concentration used in this study (225 mg kg^{-1}) had positive effects on the emergence rate and the size of emerging parasitoids, which could affect their performance as biological control agents. Effects of copper on *T. cordubensis* could have at least two origins, a direct one and an indirect one. The first hypothesis is that *T. cordubensis* could have been exposed to copper in their host eggs (direct effect), this hypothesis is supported by the direct effect of copper on the emergence rate shown by the path analysis.

The second hypothesis is that *T. cordubensis* was indirectly affected by copper, via stress responses of their hosts to copper (at the parental generation or at the host egg stage), including traits which were not considered in this study (*e.g.,* host egg immunity). In addition, copper negatively impacted larval developmental time and the pupal mass of the parental generation. Taken together, these results indicate that the stress caused by high copper concentrations could reduce pest pressure on grapevines, both by altering pest development and by favoring their control by parasitoids. The effects of copper pesticides on *T. cordubensis* have only been observed at the highest copper concentration, which should be the rarest as well in viticulture. We can thus assume that the use of biological control methods facing exposures to copper pesticides through trophic accumulation remains effective in most of the vineyards.

Our study has shown that host exposure to the highest concentration of copper had positive effects on the emergence rate and the size of emerging parasitoids. According to the path analysis, copper had a direct positive effect only on the emergence rate of parasitoids. Copper may have been transferred to the host eggs and made available to the parasitoids at a favorable concentration (Calabrese 2014; Cutler and Guedes 2017) as a nutrient, promoting the development of the parasitoids at the larval stage. Copper is involved in mitochondrial respiration processes via cytochrome c oxidases and in the transport of oxygen in the hemolymph by hemocyanins (Coates and Costa-Paiva 2020), which can directly affect insect development (Collins 2016). The positive effects of copper on the parasitoid emergence rate suggests that parasitoids encountered copper concentrations below their hypothetical sensitivity or resistance thresholds during their development. The hypothesis that copper has been transferred from insect parents to their eggs seems consistent as heavy metals such as nickel and cadmium are known to accumulate in reproductive organs of insects (Schmidt and Ibrahim 1994; Su et al. 2014; Sun et al. 2016), and a heavy metal such as zinc is known to be transferred from exposed parents to their eggs (Shu et al. 2009; Kim et al. 2012). Although there is little evidence in the literature that copper is transferred maternally from terrestrial insect parents to their eggs, this phenomenon was described in aquatic insects (Cid et al. 2010). It is thus plausible that copper accumulated in the reproductive organs of *L. botrana* parents has been transferred into their eggs, which could support the hypothesis that parasitoids have been exposed to copper during their development in the host eggs.

We showed overall that high copper exposure of *L. botrana* as host for *T. cordubensis* can positively impact the performance of parasitoids. Under the hypothesis that copper has been transferred into the host eggs, both species may have not been exposed to similar copper concentrations. Following the example of zinc accumulated in aquatic insects, the concentration of a heavy metal transferred maternally to the eggs does not seem proportional to the concentration in the contaminated environment (Shu et al. 2009), but may be impacted by detoxification mechanisms of the parental generation. Copper had a positive effect on the development time of *L. botrana* parental generation but a negative effect on the pupal mass, which suggests that costs for detoxification are paid at high concentrations. Further studies would be necessary to determine the dose–response to copper on the interaction between *L. botrana* or *Trichogramma* species, as dose–response to copper has been shown to strongly vary between aquatic insects (Leland et al. 1989; Van Der Geest et al. 2000; Mebane et al. 2020). There could have been competition between growth and detoxification processes as a stress response of *L. botrana* larvae, including copper inactivation by proteins such as glutathione and metallothioneins (Tibbett et al. 2021), copper storage in mineral-rich granules in the lysosomal systems (Hopkin 1990; Barka 2007), copper excretion through the exoskeleton or the digestive system (Pigino et al. 2005). The implementation of these detoxification mechanisms is however very energetically costly, which could explain the increase of the development time and the reduction of the pupal mass of *L. botrana* with copper exposure. Although these mechanisms may have reduced copper toxicity and concentration in the parental generation and hence maybe in the host eggs, their high energetic costs in addition to copper toxicity could have led to the negative effects on the development of *L. botrana*. However, parasitoids may have faced reduced and benefic copper

Table 1 Results of the generalized linear mixed models (GLMM) on 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

	Number of parasitized eggs[a]		Parasitoid emergence rate[b]		Emerging parasitoids' size[a]	
	Test value	p	Test value	p	Test value	p
Copper treatments	$\chi_3^2 = 2.77$	0.43	**$\chi_3^2 = 21.17$**	**< 0.001**	**$\chi_3^2 = 11.67$**	**0.009**
Female pupal mass	$\chi_1^2 = 1.84$	0.17	$\chi_1^2 = 3.34$	0.07	$\chi_1^2 = 0.41$	0.52
Male pupal mass	$\chi_1^2 = 0.90$	0.34	$\chi_1^2 = 1.24$	0.26	$\chi_1^2 = 1.69$	0.19
Host egg size	$\chi_1^2 = 0.02$	0.88	$\chi_1^2 = 1.12$	0.29	$\chi_1^2 = 0.50$	0.48
Parasitoid mothers' size	**$\chi_1^2 = 6.08$**	**0.01**	$\chi_1^2 = 0.28$	0.59	**$\chi_1^2 = 6.67$**	**0.01**

[a] GLMM with Poisson distribution; [b] GLMM with binomial distribution

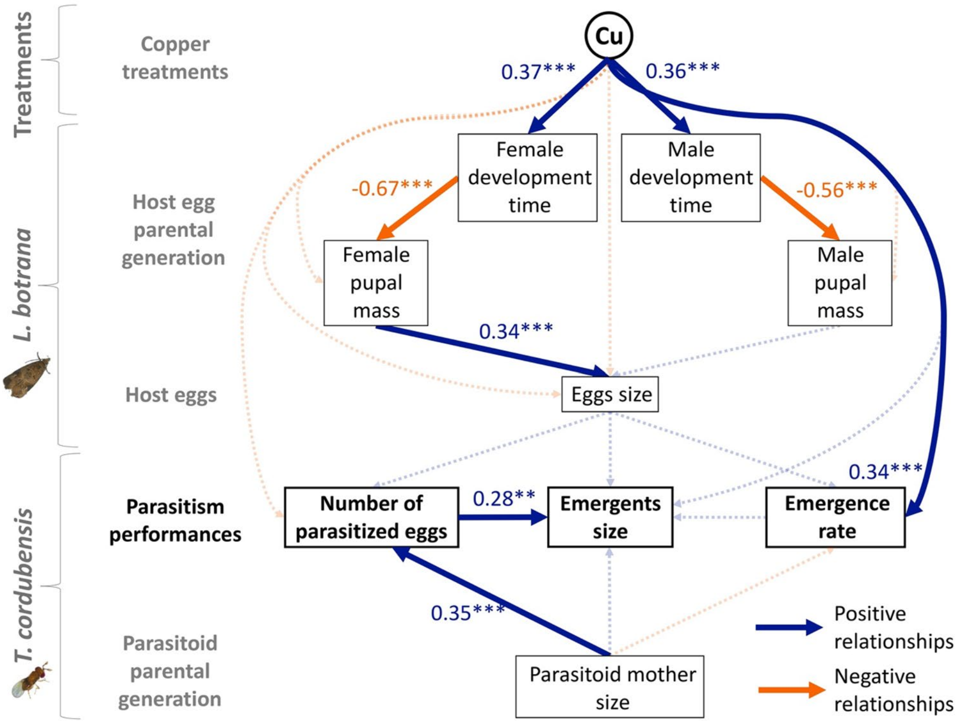

Fig. 3 Evaluation of the conceptual model of direct and indirect effects of copper through a path analysis. The model was built by reflecting on the dependence or independence of the variables between them. Arrows indicate dependence relationships between variables which have been included in the model. Blue arrows indicate positive relationships. Red arrows indicate negative relationships. Relationship significance is given by asterisks: (**) $p<0.01$, (***) $p<0.001$. Non-significant relationships are represented by transparent dotted arrows. Path coefficients indicate the strength of direct relationships between variables. The strength of indirect relationships is obtained by multiplying the coefficients along the path

concentrations due to detoxification mechanisms used by *L. botrana* facing the stress of a heavy metal contaminant in its diet.

The path analysis showed no effects of copper on the size of emerging parasitoids as opposed to the GLMMs. This apparent statistical inconsistency could be because copper may have had an indirect effect on the size of emerging parasitoids through an alteration of traits in the host that have not been considered in the present study, such as immune parameters. Previous studies carried out on several insects such as *Epirrita autumnata* (Lepidoptera: Geometridae) (van Ooik et al. 2007, 2008), or on *Formica aquilonia* (Hymenoptera: Formicidae) (Sorvari et al. 2007), as well as on *L. botrana* (Iltis et al. 2022), have shown that copper reduces immune activity of larvae at high concentrations due to its role as regulator of these mechanisms (Cerenius and Söderhäll 2004; González-Santoyo and Córdoba-Aguilar 2012). Other studies carried out on insects have suggested that immune and dietary stresses experienced by the parental generation are exacerbated in the offspring, including the egg stage (Sadd et al. 2005; Sadd and Schmid-Hempel 2007; Pölkki et al. 2012; Triggs and Knell 2012; Grueber et al. 2018). In line with this, we hypothesize that the host eggs exposed to the highest parental copper concentration may have been less able to mount an immune response against the parasitoids, improving their development and emergence.

The treatments tested in the present study encompass a wide range of copper concentrations in grapes and three parasitism variables which can be used to evaluate the performance of biological control agents used in IPM programs. Our results showed that copper had no effects on the number of parasitized eggs and positive effects on the emergence rate and the size of emerging parasitoids. These results indicate that the use of copper fungicides can have positive effects on the performance of *T. cordubensis* as biological control agents. Our study also showed that copper exposure of host parents had positive effects on the fertility (emergence rate) of parasitoids but no effects on fecundity (number of parasitized eggs). In line with previous studies showing effects of copper fungicides on the fecundity and fertility of other beneficial insects (Michaud and Grant 2003; Ye et al. 2009; El-Sheikh et al. 2010), we propose that the next generation of biological control agents could be indirectly affected by copper. The emerging parasitoids, although more numerous at the highest copper concentration, could then show altered abilities to locate and reach their hosts. Thus, studying a wider range of exposures and effects of copper fungicides would be useful to evaluate the overall performance of biological control agents used in IPM programs.

Conclusion

This study provides new insights into the non-targeted effects of copper pesticides on oophagous parasitoids used as biological control agents. Exposure to a copper fungicide through trophic accumulation generates sublethal effects on the size and emergence of *T. cordubensis* after exposure of the parental generation of *L. botrana* host eggs, itself affected by copper. The results showed positive effects of copper at a very high copper concentration on the parasitism performance of the first generation of *T. cordubensis*. Similarly, only high copper concentrations appear to affect pest development by increasing its development time and reducing its mass. Thus, exposure of *T. cordubensis* to copper fungicides through their hosts does not seem to reduce their effectiveness as biological control agents against the grape berry moth *L. botrana*, at the most commonly observed copper concentrations in vineyards. At a very high concentration, copper even had positive effects on the performance of *T. cordubensis* as a biological control agent. The effects of copper observed at the highest concentrations seem to be related to alterations in the metabolism of the pests and potentially in their immune system, favoring the development and survival of *T. cordubensis*, depending on the differences in sensitivity or resistance of these organisms to copper. However, this study does not take into account the full range of exposures and environmental conditions that *T. cordubensis* may face under field conditions, including for example, leaching after rainfall, bioaccumulation or exposure through the multiple treatments during pest development. Further studies will therefore be necessary to evaluate the overall efficacy of *T. cordubensis* as a biological control agent in vineyards.

Author contribution

WN and PL conceived and designed the research; JM and DT were associated to this reflection. WN and TG maintained the insect stock and conducted the experiments. WN, YL and PL analyzed the data. WN, PL, JM, DT, JF, GG led the writing of the manuscript. All authors contributed to the intellectual content and the writing of the manuscript and gave their final approval for submission.

Acknowledgements We are grateful to the company Bioline Agro-Sciences which provided the parasitoids for the experiments. We are also grateful to the « *UMR 1065 Santé et Agroécologie du Vignoble* » and Lionel Delbac who provided the pest insects.

Funding This work was supported by the « *Conseil Régional de Bourgogne Franche-Comté*» through the « *Projet E-SITE BFC Pesticlim–Louâpre*», and the « *ANR plan de relance TrichoVigne*». Acknowledgements are expressed to the French Ministry of Agriculture and Food Sovereignty which funded the research activities.

Declarations

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

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

References

Agreste (2021) Enquête pratiques culturales en viticulture en 2019. Chiffres and Données 19:13

Aktar W, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol 2:1–12. https://doi.org/10.2478/v10102-009-0001-7

Andrivon D, Savini I (2019) Peut-On Se Passer Du Cuivre En Protection Des Cultures Biologiques ? éditions Quae. https://doi.org/10.35690/978-2-7592-2998-7

Angelova VR, Ivanov AS, Braikov DM (1999) Heavy metals (Pb, Cu, Zn and Cd) in the system soil–grapevine–grape. J Sci Food Agric 79:713–721. https://doi.org/10.1002/(SICI)1097-0010(199904)79:5%3c713::AID-JSFA229%3e3.0.CO;2-F

ANSES (2022) Cartographie des utilisations des produits phytopharmaceutiques à base de cuivre en France en considérant leur application en agriculture biologique et conventionnelle. (saisine 2021- AUTO-0060). Maisons-Alfort: Anses, 133 p

Aveling C (1981) The role of *Anthocoris* species (Hemiptera: Anthocoridae) in the integrated control of the damson-hop aphid (*Phorodon humuli*). Ann Appl Biol 97:143–153. https://doi.org/10.1111/j.1744-7348.1981.tb03007.x

Baldi I, Cordier S, Coumoul X, Elbaz A, Gamet-Payrastre L (2013) Pesticides: Effets sur la santé. Institut national de la santé et de la recherche médicale (INSERM). EDP Sciences (ISSN: 1264-1782), 1014 p, Paris

Ballabio C, Panagos P, Lugato E et al (2018) Copper distribution in European topsoils : an assessment based on LUCAS soil survey. Sci Total Environ 636:282–298. https://doi.org/10.1016/j.scitotenv.2018.04.268

Barka S (2007) Insoluble detoxification of trace metals in a marine copepod *Tigriopus brevicornis* (Müller) exposed to copper, zinc, nickel, cadmium, silver and mercury. Ecotoxicology 16:491–502. https://doi.org/10.1007/s10646-007-0155-z

Barzman M, Bàrberi P, Birch ANE et al (2015) Eight principles of integrated pest management. Agron Sustain Dev 35:1199–1215. https://doi.org/10.1007/s13593-015-0327-9

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Statist Softw. https://doi.org/10.18637/jss.v067.i01

Becker C, Herrmann K, Reineke A (2022) Biological control in a changing climate: plant-mediated impact of elevated CO_2 concentration on *Lobesia botrana* eggs and egg parasitism by *Trichogramma cacoeciae*. J Pest Sci. https://doi.org/10.1007/s10340-022-01545-w

Bonner MR, Alavanja MCR (2017) Pesticides, human health, and food security. Food Energy Secur 6:89–93. https://doi.org/10.1002/fes3.112

Brühl CA, Zaller JG (2019) Biodiversity decline as a consequence of an inappropriate environmental risk assessment of pesticides. Front Environ Sci. https://doi.org/10.3389/fenvs.2019.00177

Brun LA, Maillet J, Richarte J et al (1998) Relationships between extractable copper, soil properties and copper uptake by wild plants in vineyard soils. Environ Pollut 102:151–161. https://doi.org/10.1016/S0269-7491(98)00120-1

Calabrese E (2014) Hormesis: a fundamental concept in biology. Microbial Cell 1(5):145–149. https://doi.org/10.15698/mic2014.05.145

Cerenius L, Söderhäll K (2004) The prophenoloxidase-activating system in invertebrates. Immunol Rev 198:116–126. https://doi.org/10.1111/j.0105-2896.2004.00116.x

Chen L, Sørensen JG, Enkegaard A (2021) Acclimation for optimisation: effects of temperature on development, reproduction and size of *Trichogramma achaeae*. Biocontrol Sci Technol 32:60–73. https://doi.org/10.1080/09583157.2021.1963679

Cid N, Ibáñez C, Palanques A, Prat N (2010) Patterns of metal bioaccumulation in two filter-feeding macroinvertebrates: exposure distribution, inter-species differences and variability across developmental stages. Sci Total Environ 408:2795–2806. https://doi.org/10.1016/j.scitotenv.2010.03.030

Coates CJ, Costa-Paiva ME (2020) Multifunctional roles of hemocyanins. In: Hoeger U, Harris J (eds) Vertebrate and Invertebrate Respiratory Proteins, Lipoproteins and other Body Fluid Proteins. Springer, Subcellula, pp 233–250

Collins JF (2016) Copper: basic physiological and nutritional aspects. In: Collins JF (ed) Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals. Academic Press, pp 69–83

Cozzi G, Pascale M, Perrone G et al (2006) Effect of *Lobesia botrana* damages on black aspergilli rot and ochratoxin a content in grapes. Int J Food Microbiol 111:88–92. https://doi.org/10.1016/j.ijfoodmicro.2006.03.012

Cutler GC, Guedes RNC (2017) Occurrence and significance of insecticide-induced hormesis in insects. ACS Symp Ser 1249:101–119. https://doi.org/10.1021/bk-2017-1249.ch008

Dagostin S, Schärer HJ, Pertot I, Tamm L (2011) Are there alternatives to copper for controlling grapevine downy mildew in organic viticulture? Crop Prot 30:776–788. https://doi.org/10.1016/j.cropro.2011.02.031

Delbac L, Thiéry D (2016) Damage to grape flowers and berries by *Lobesia botrana* larvae (Denis and Schiffernüller) (*Lepidoptera: Tortricidae*), and relation to larval age. Aust J Grape Wine Res 22:256–261. https://doi.org/10.1111/ajgw.12204

Desneux N, Decourtye A, Delpuech JM (2007) The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol 52:81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Di Giovanni F, Ricciardi R, Loni A et al (2022) Back to the wild: the *parasitoid* community of *Lobesia botrana* (*Lepidoptera: Tortricidae*) in a grapevine-free natural environment. InSects 13:627. https://doi.org/10.3390/insects13070627

Di N, Hladun KR, Zhang K et al (2016) Laboratory bioassays on the impact of cadmium, copper and lead on the development and survival of honeybee (*Apis mellifera* L.) larvae and foragers. Chemosphere 152:530–538. https://doi.org/10.1016/j.chemosphere.2016.03.033

El-Sheikh TMY, Fouda MA, Hassan MI et al (2010) Toxicological effects of some heavy metal ions on *Culex pipiens* L. (*Diptera: Culicidae*). Egypt Acad J Biol Sci F Toxicol Pest Control 2:63–76. https://doi.org/10.21608/eajbsf.2010.17465

El-Wakeil NE, Farghaly HT, Ragab ZA (2009) Efficacy of *Trichogramma evanescens* in controlling the grape berry moth *Lobesia botrana* in grape farms in Egypt. Arch Phytopathol Plant Prot 42:705–714. https://doi.org/10.1080/03235400701390422

El-Wakeil N, Gaafar N, Sallam A, Volkmar C (2013) Side effects of insecticides on natural enemies and possibility of their integration in plant protection strategies. In: Trdan S (ed) Insecticides-Development of Safer and More Effective Technologies. InTech. https://doi.org/10.5772/54199

European Commission (2018) Commission implementing regulation (EU) 2018/1981. Off J Eur Union 317:16–20

Farahani HK, Ashouri A, Zibaee A et al (2016) The effect of host nutritional quality on multiple components of *Trichogramma brassicae* fitness. Bull Entomol Res 106:633–641. https://doi.org/10.1017/S000748531600033X

Fontes J, Roja IS, Tavares J, Oliveira L (2018) Lethal and sublethal effects of various pesticides on *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae). J Econ Entomol 111:1219–1226. https://doi.org/10.1093/jee/toy064

Fouillet E, Delière L, Chartier N, Munier-Jolain N, Cortel S, Rapidel B, Merot A (2022) Reducing pesticide use in vineyards. evidence from the analysis of the French DEPHY network. Eur J Agron 136:126503. https://doi.org/10.1016/j.eja.2022.126503

Fox J, Wiesberg S (2019) An R companion to applied regression, Third Edit. Sage, 608p, Thousand Oaks CA

Garcia PV, Pereira N, Oliveira LM (2009) Side-effects of organic and synthetic pesticides on cold-stored diapausing prepupae of *Trichogramma cordubensis*. Biocontrol 54:451–458. https://doi.org/10.1007/s10526-008-9186-5

Gill HK, Garg H (2014) Pesticides: environmental impacts and management strategies. Pestic Toxic Asp. https://doi.org/10.5772/57399

González-Santoyo I, Córdoba-Aguilar A (2012) Phenoloxidase: a key component of the insect immune system. Entomol Exp Appl 142:1–16. https://doi.org/10.1111/j.1570-7458.2011.01187.x

Grueber CE, Gray LJ, Morris KM et al (2018) Intergenerational effects of nutrition on immunity: a systematic review and meta-analysis. Biol Rev 93:1108–1124. https://doi.org/10.1111/brv.12387

Guedes RNC, Smagghe G, Stark JD, Desneux N (2016) Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. Annu Rev Entomol 61:43–62. https://doi.org/10.1146/annurev-ento-010715-023646

Hopkin SP (1990) Critical concentrations, pathways of detoxification and cellular ecotoxicology of metals in terrestrial arthropods. Funct Ecol 4:321–327. https://doi.org/10.2307/2389593

Hummes AP, Bortoluzzi EC, Tonini V et al (2019) Transfer of copper and zinc from soil to grapevine-derived products in young and centenarian vineyards. Water Air Soil Pollut 230:150. https://doi.org/10.1007/s11270-019-4198-6

Hutchison WD, Moratorio M, Martin JM (1990) Morphology and biology of *Trichogrammatoidea bactrae* (Hymenoptera: Trichogrammatidae), imported from Australia as a parasitoid of pink bollworm (*Lepidoptera: Gelechiidae*) eggs. Ann Entomol Soc Am 83:46–54. https://doi.org/10.1093/aesa/83.1.46

Iltis C, Moreau J, Manière C et al (2020) Where you come from matters: temperature influences host–parasitoid interaction through parental effects. Oecologia 192:853–863. https://doi.org/10.1007/s00442-020-04613-z

Iltis C, Louâpre P, Vogelweith F et al (2021) How to stand the heat? Post-stress nutrition and developmental stage determine insect response to a heat wave. J Insect Physiol 131:104214. https://doi.org/10.1016/j.jinsphys.2021.104214

Iltis C, Moreau J, Hübner P et al (2022) Warming increases tolerance of an insect pest to fungicide exposure through temperature-mediated hormesis. J Pest Sci 95:827–839. https://doi.org/10.1007/s10340-021-01398-9

Kim KS, Funk DH, Buchwalter DB (2012) Dietary (periphyton) and aqueous Zn bioaccumulation dynamics in the mayfly *Centroptilum triangulifer*. Ecotoxicology 21:2288–2296. https://doi.org/10.1007/s10646-012-0985-1

Lai H, Juang K, Chen B (2010) Copper concentrations in grapevines and vineyard soils in central Taiwan. Soil Sci Plant Nutr 56:601–606. https://doi.org/10.1111/j.1747-0765.2010.00494.x

Leland HV, Fend SV, Dudley TL, Carter JL (1989) Effects of copper on species composition of benthic insects in a Sierra Nevada, California, stream. Freshw Biol 21:163–179. https://doi.org/10.1111/j.1365-2427.1989.tb01356.x

Lucchi A, Scaramozzino PL, Michl G et al (2016) The first record in Italy of *Trichogramma cordubense* Vargas and Cabello 1985 (Hymenoptera: Trichogrammatidae) emerging from the eggs of *Lobesia botrana* (Denis and Schiffermüller, 1775)

(*Lepidoptera: Tortricidae*). Vitis J Grapevine Res 55:161–164. https://doi.org/10.5073/vitis.2016.55.161-164

Mackie KA, Müller T, Kandeler E (2012) Remediation of copper in vineyards—a mini review. Environ Pollut 167:16–26. https://doi.org/10.1016/j.envpol.2012.03.023

Martinou AF, Seraphides N, Stavrinides MC (2014) Lethal and behavioral effects of pesticides on the insect predator *Macrolophus pygmaeus*. Chemosphere 96:167–173. https://doi.org/10.1016/j.chemosphere.2013.10.024

Mebane CA, Schmidt TS, Miller JL, Balistrieri LS (2020) Bioaccumulation and toxicity of cadmium, copper, nickel, and zinc and their mixtures to aquatic insect communities. Environ Toxicol Chem 39:812–833. https://doi.org/10.1002/etc.4663

Michaud JP, Grant AK (2003) Sub-lethal effects of a copper sulfate fungicide on development and reproduction in three *coccinellid* species. J Insect Sci 3:16. https://doi.org/10.1093/jis/3.1.16

Miotto A, Ceretta CA, Brunetto G et al (2014) Copper uptake, accumulation and physiological changes in adult grapevines in response to excess copper in soil. Plant Soil 374:593–610. https://doi.org/10.1007/s11104-013-1886-7

Mirlean N, Roisenberg A, Chies JO (2007) Metal contamination of vineyard soils in wet subtropics (Southern Brazil). Environ Pollut 149:10–17. https://doi.org/10.1016/j.envpol.2006.12.024

Moghaddassi Y, Ashouri A, Bandani AR et al (2019) Effect of *Ephestia kuehniella* (*Lepidoptera: Pyralidae*) larval diet on egg quality and parasitism by *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae). J Insect Sci 19:1–7. https://doi.org/10.1093/jisesa/iez076

Moreau J, Richard A, Benrey B, Thiéry D (2009) Host plant cultivar of the grapevine moth *Lobesia botrana* affects the life history traits of an egg parasitoid. Biol Control 50:117–122. https://doi.org/10.1016/j.biocontrol.2009.03.017

Moreau J, Monceau K, Thiéry D (2016) Larval food influences temporal oviposition and egg quality traits in females of *Lobesia botrana*. J Pest Sci 89:439–448. https://doi.org/10.1007/s10340-015-0695-6

Moreau J, Monceau K, Gonnet G et al (2022) Organic farming positively affects the vitality of passerine birds in agricultural landscapes. Agric Ecosyst Environ 336:108034. https://doi.org/10.1016/j.agee.2022.108034

Moreno F, Pérez-Moreno I, Marco V (2009) Effects of *Lobesia botrana* (*Lepidoptera: Tortricidae*) egg age, density, and UV treatment on parasitism and development of *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). Environ Entomol 38:1513–1520. https://doi.org/10.1603/022.038.0520

Muller K, Arenas L, Thiéry D, Moreau J (2016) Direct benefits from choosing a virgin male in the European grapevine moth, *Lobesia botrana*. Anim Behav 114:165–172. https://doi.org/10.1016/j.anbehav.2016.02.005

Olson DM, Andow DA (1998) Larval crowding and adult nutrition effects on longevity and fecundity of female *Trichogramma nubilale Ertle* and *Davis* (Hymenoptera: Trichogrammatidae). Environ Entomol 27:508–514. https://doi.org/10.1093/ee/27.2.508

Pennington T, Reiff JM, Theiss K et al (2018) Reduced fungicide applications improve insect pest control in grapevine. Biocontrol 63:687–695. https://doi.org/10.1007/s10526-018-9896-2

Pertot I, Caffi T, Rossi V et al (2017) A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. Crop Prot 97:70–84. https://doi.org/10.1016/j.cropro.2016.11.025

Peshin R, Dhawan AK (2009) Integrated Pest Management: Innovation-Development Process. Springer, Netherlands. https://doi.org/10.1007/978-1-4020-8992-3

Pietrzak U, McPhail DC (2004) Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. Geoderma 122:151–166. https://doi.org/10.1016/j.geoderma.2004.01.005

Pigino G, Migliorini M, Paccagnini E et al (2005) Fine structure of the midgut and Malpighian papillae in *Campodea* (*Monocampa*) *quilisi* Silvestri, 1932 (*Hexapoda, Diplura*) with special reference to the metal composition and physiological significance of midgut intracellular electron-d. Tissue Cell 37:223–232. https://doi.org/10.1016/j.tice.2005.02.001

Pizzol J, Desneux N, Wajnberg E, Thiéry D (2004) Parasitoid and host egg ages have independent impact on various biological traits in a *Trichogramma* species. J Pest Sci 85:489–496. https://doi.org/10.1007/s10340-012-0434-1

Pölkki M, Kangassalo K, Rantala MJ (2012) Transgenerational effects of heavy metal pollution on immune defense of the blow fly *Protophormia terraenovae*. PLoS ONE 7:e38832. https://doi.org/10.1371/journal.pone.0038832

R Core Team (2021) R: a language and environment for statistical computing. Vienna, Austria. URL https://www.R-project.org

Rajput V, Minkina T, Sushkova S et al (2019) ZnO and CuO nanoparticles: a threat to soil organisms, plants, and human health. Environ Geochem Health 42:147–158. https://doi.org/10.1007/s10653-019-00317-3

Rani L, Thapa K, Kanojia N et al (2021) An extensive review on the consequences of chemical pesticides on human health and environment. J Clean Prod 283:124657. https://doi.org/10.1016/j.jclepro.2020.124657

Rehman M, Liu L, Wang Q et al (2019) Copper environmental toxicology, recent advances, and future outlook: a review. Environ Sci Pollut Res 26:18003–18016. https://doi.org/10.1007/s11356-019-05073-6

Reiff JM, Kolb S, Entling MH et al (2021) Organic farming and cover-crop management reduce pest predation in austrian vineyards. InSects 12:220. https://doi.org/10.3390/insects12030220

Rosseel Y (2012) lavaan: an R package for structural equation modeling. Of Statist Softw. https://doi.org/10.18637/jss.v048.i02

Sadd BM, Schmid-Hempel P (2007) Facultative but persistent transgenerational immunity via the mother's eggs in bumblebees. Curr Biol 17:1046–1047. https://doi.org/10.1016/j.cub.2007.11.007

Sadd BM, Kleinlogel Y, Schmid-Hempel R, Schmid-Hempel P (2005) Trans-generational immune priming in a social insect. Biol Lett 1:386–388. https://doi.org/10.1098/rsbl.2005.0369

Schmidt GH, Ibrahim NMM (1994) Heavy metal content (Hg2+, Cd2+, Pb2+) in various body parts: its impact on cholinesterase activity and binding glycoproteins in the grasshopper *Aiolopus thalassinus* adults. Ecotoxicol Environ Saf 29:148–164. https://doi.org/10.1016/0147-6513(94)90016-7

Sentenac G (2011) La faune auxiliaire des vignobles de France. France Agricole, 442p

Shu Y, Gao Y, Sun H et al (2009) Effects of zinc exposure on the reproduction of *Spodoptera litura* Fabricius (*Lepidoptera: Noctuidae*). Ecotoxicol Environ Saf 72:2130–2136. https://doi.org/10.1016/j.ecoenv.2009.06.004

Sogreah (2007) Bilan des flux de contaminants entrant sur les sols agricoles de France métropolitaine. Angers, France: ADEME

Sorvari J, Rantala LM, Rantala MJ et al (2007) Heavy metal pollution disturbs immune response in wild ant populations. Environ Pollut 145:324–328. https://doi.org/10.1016/j.envpol.2006.03.004

Šrobárová A, Kakalíková L (2007) Fungal disease of grapevines. Eur J Plant Sci Biotechnol 1:84–90

Stark JD, Vargas R, Banks JE (2007) Incorporating ecologically relevant measures of pesticide effect for estimating the compatibility of pesticides and biocontrol agents. J Econ Entomol 100:1027–1032. https://doi.org/10.1603/0022-0493(2007)100[1027:IERMOP]2.0.CO;2

Stenberg JA (2017) A conceptual framework for integrated pest management. Trends Plant Sci 22:759–769. https://doi.org/10.1016/j.tplants.2017.06.010

Stern M, Smith RF, van den Bosch R, Hagen KS (1959) The integration of chemical and biological control of the spotted alfalfa aphid. Hilgardia 29:81–101. https://doi.org/10.3733/hilg.v29n02p081

Su HH, Hu MM, Harvey-Samuel T, Yang YZ (2014) Accumulation and excretion of cadmium in three successive generations of *Spodoptera exigua* (*Lepidoptera: Noctuidae*) and impact on the population increase. J Econ Entomol 107:223–229. https://doi.org/10.1603/EC13436

Sun H, Wu W, Guo J et al (2016) Effects of nickel exposure on testicular function, oxidative stress, and male reproductive dysfunction in *Spodoptera litura* Fabricius. Chemosphere 148:178–187. https://doi.org/10.1016/j.chemosphere.2015.10.068

Thiéry D, Desneux N (2018) Host plants of the polyphagous grapevine moth *Lobesia botrana* during larval stage modulate moth egg quality and subsequent parasitism by the parasitoid *Trichogramma cacoeciae*. Entomol Gen 38:47–59. https://doi.org/10.1127/entomologia/2018/0675

Thiéry D, Moreau J (2005) Relative performance of European grapevine moth (*Lobesia botrana*) on grapes and other hosts. Oecologia 143:548–557. https://doi.org/10.1007/s00442-005-0022-7

Thiéry D, Louâpre P, Muneret L et al (2018) Biological protection against grape berry moths. Rev Agron Sustain Dev. https://doi.org/10.1007/s13593-018-0493-7

Tibbett M, Green I, Rate A et al (2021) The transfer of trace metals in the soil-plant-arthropod system. Sci Total Environ 779:146260. https://doi.org/10.1016/j.scitotenv.2021.146260

Triggs AM, Knell RJ (2012) Parental diet has strong transgenerational effects on offspring immunity. Funct Ecol 26:1409–1417. https://doi.org/10.1111/j.1365-2435.2012.02051.x

Van Der Geest HG, Greve GD, Kroon A et al (2000) Sensitivity of characteristic riverine insects, the caddisfly *Cyrnus trimaculatus* and the mayfly *Ephoron virgo*, to copper and diazinon. Environ Pollut 109:177–182. https://doi.org/10.1016/S0269-7491(99)00269-9

van Ooik T, Rantala MJ, Saloniemi I (2007) Diet-mediated effects of heavy metal pollution on growth and immune response in the geometrid moth *Epirrita autumnata*. Environ Pollut 145:348–354. https://doi.org/10.1016/j.envpol.2006.03.008

van Ooik T, Pausio S, Rantala MJ (2008) Direct effects of heavy metal pollution on the immune function of a geometrid moth, *Epirrita autumnata*. Chemosphere 71:1840–1844. https://doi.org/10.1016/j.chemosphere.2008.02.014

Vieira A, Oliveira L, Garcia P (2001) Effects of conventional pesticides on the preimaginal developmental stages and on adults of *Trichogramma cordubensis* (Hymenoptera: Trichogrammatidae). Biocontrol Sci Technol 11:527–534. https://doi.org/10.1080/09583150120067553

Vogelweith F, Thiéry D (2018) An assessment of the non-target effects of copper on the leaf arthropod community in a vineyard. Biol Control 127:94–100. https://doi.org/10.1016/j.biocontrol.2018.08.011

Walton VM, Daane KM, Addison P (2012) Biological control of arthropods and its application in vineyards. In: Bostanian NJ, Vincent C, Isaacs R (eds) Arthropod Management in Vineyards:: Pests, Approaches, and Future Directions. Springer Netherlands, Dordrecht, pp 91–117. https://doi.org/10.1007/978-94-007-4032-7_5

Wright S (1934) The method of path coefficients. Ann Math Stat 5:161–215. https://doi.org/10.1214/aoms/1177732676

Ye GY, Dong SZ, Dong H et al (2009) Effects of host (*Boettcherisca peregrina*) copper exposure on development, reproduction and vitellogenesis of the ectoparasitic wasp, *Nasonia vitripennis*. Insect Sci 16:43–50. https://doi.org/10.1111/j.1744-7917.2009.00252.x