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ORIGINAL PAPER

Reproductive performance of the European grapevine moth *Lobesia botrana* (Tortricidae) is adversely affected by warming scenario

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Abstract

The European grapevine moth, *Lobesia botrana* (Denis and Schiffermüller), is an important grape pest worldwide. To forecast how this species might respond to climate change has emerged as one major challenge in recent viticultural research. Predictions about *L. botrana* population dynamics under global warming scenario are usually derived from physiologically based demographic models (PBDMs). Despite their ecological realism, PBDMs are based on physiological equations derived from constant, unrealistic thermal regimes, and they exclude the effects of temperature on male's reproductive traits, which importantly mediates population dynamics in this species. Focused on Burgundy (a viticultural region of France), we compared the adult performance of *L. botrana* between the Current Fluctuating Regime (CFR) based on in situ meteorological data and the Future Fluctuating Regime (FFR) based on the outputs of climate models. Under controlled conditions complying to the scenario of local warming termed FFR, *L. botrana* individuals incurred reduced adult lifespan, while their body amounts of four major energetic components (proteins, lipids, glycogen, soluble carbohydrates) remained unchanged compared to individuals exposed to the CFR. Furthermore, each sex endured reproductive costs associated with local warming scenario: females incurred reduced mating success, and males bore decreased fertility (lower number of eupyrene sperms within spermatophore). Our results indicate that global warming should adversely impact the reproductive success of *L. botrana* and the local abundance of this pest. In terms of pest management, our findings might contribute to the increase in the ecological realism of PBDMs and improve the reliability of their predictions about the population dynamics of *L. botrana*.

Keywords Fluctuating thermal regimes · Global warming · Grapevine pest · *Lobesia botrana* · Population dynamics · Sperm quality

Key Message

- Using realistic fluctuating thermal regimes, we explored the consequences of global warming in Eastern France (Burgundian winegrowing region) for the adult performance of the grapevine pest *Lobesia botrana*.
- Under thermal conditions complying to a scenario of local warming, individuals of both sexes incurred reduced lifespan and decreased reproductive performance: females experienced reduced mating success, and males endured decreased sperm fertility.
- These findings suggest that warming should negatively impact the reproductive success and local abundance of *L. botrana*.

Introduction

Viticulture is facing new threats and challenges worldwide, owing chiefly to anthropogenic disturbances (Fraga et al. 2012). In the current climatic context, rising temperatures are predicted to impact grapevine cultivation by modulating the risks of pest outbreaks and emergence of insect-borne diseases (Fraga et al. 2012; Reineke and Thiéry 2016).

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Indeed, grapevine is targeted by numerous insect pests exerting detrimental effects on grape production through feeding on plant tissues and/or by acting as vectors of plant diseases (Reineke and Thiéry 2016; Thiéry et al. 2018). As ectothermic animals, phytophagous insects are very sensitive to environmental temperature, which regulates their physiology, individual performance and population dynamics in space (distribution range) and time (life cycle duration, phenology) (Bale et al. 2002; Reineke and Thiéry 2016). In view of the many threats global warming poses to the long-term viability of vineyards, to predict how the projected increase in global mean temperature might affect the population dynamics of major grapevine pests and anticipate the possible repercussions on grapevine cultivation has emerged as one major challenge in recent viticultural research (Reineke and Thiéry 2016; Castex et al. 2018; Thiéry et al. 2018).

The European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae), is one of the most harmful viticultural pests in the Palearctic region (Thiéry et al. 2018). Quantitative and qualitative damages are triggered by caterpillars while attacking vine fructiferous organs (floral buds, ripening and mature berries). Larval feeding on berries induces severe yield losses because of direct consumption of agricultural production. Moreover, it allows the intrusion of several fungal agents responsible for bunch rot development (*Botrytis cinerea*, *Aspergillus carbonarius*, *A. niger*) and increases the plant susceptibility to other grape berry boring pests like fruit flies (Cozzi et al. 2006; Reineke and Thiéry 2016). The economic impacts of *L. botrana* have been rated as ‘high’, provided that one caterpillar might infest between 2 and 10 berries throughout its development and that density might reach up to 20–30 larvae per bunch in heavily infested vineyards (Delbac and Thiéry 2016). High levels of economic injury due to *L. botrana* activity are also suggested by considerable yield losses like those recorded in Greece, amounting from 13.3 to 27% of the total grape production over a 4-year period (Moschos 2006).

Predictions about *L. botrana* population dynamics under global warming scenario are usually derived from physiologically based demographic models (PBDMs) (Gutierrez et al. 2012, 2017). These mechanistic models rely on equations describing the effects of temperature on physiological processes enabling to infer population growth, above all developmental rates, survival and *per capita* fecundity (total number of eggs laid by one female) (Gutierrez et al. 2012, 2017; Gilioli et al. 2016). PBDMs can be coupled with in situ weather data and climate change scenarios to project the current and future geographical distribution of *L. botrana* across a lattice of spatial cells. This way, PBDMs allow to anticipate the local dynamics of *L. botrana* populations in terms of abundance and phenology (Gutierrez et al. 2012, 2017). These models predict that, concurrently with grape yield, the abundance of *L. botrana* populations should increase northward and in higher elevations of the Euro-Mediterranean region, while the opposite trend should be observed for southern areas where summer conditions will adversely impact the moth vital functions and reduce grape yields (Gutierrez et al. 2017).

Notwithstanding their ecological realism (i.e. the possibility to include the bottom-up effects of grapevine phenological stage on *L. botrana* physiology), PBDMs suffer from shortcomings that could limit their ability to accurately forecast the future of *L. botrana* populations in a rapidly changing climatic context. First, their equations are based on data obtained under constant temperatures (Gutierrez et al. 2012). For that reason, their predictions might differ from biological conclusions drawn by comparing the performance of *L. botrana* under daily fluctuating thermal regimes, which reflect more realistically the day/night variations of temperatures experienced by insects in their natural environment (Colinet et al. 2015). Second, by expressing fecundity as a function of temperature and female’s age, PBDMs implicitly rule out the importance of male’s reproductive performance on female’s egg production. Nonetheless, in *L. botrana* as in many other lepidopteran species, the spermatophore delivered during copulation does not only include sperm but also accessory gland secretions containing nutrients absorbed by female to boost her egg production (South and Lewis 2011). Since *L. botrana* is a capital breeding species (i.e. with a non-feeding adult stage), the quality of the nutritive ejaculate delivered by male during copulation importantly mediates the resources allocated to egg production and the reproductive output of the pair (number of eggs laid, hatching success) (Muller et al. 2015, 2016a). Provided that ejaculate quality is usually temperature-dependent in arthropod species (Zeh et al. 2012; Vasudeva et al. 2014), addressing the effects of warming on male’s ejaculatory traits is required for a better understanding of the consequences of climate change for *L. botrana* reproduction and population dynamics.

In view of the concerns raised above, we performed laboratory experiments to investigate the consequences of local warming scenario for the performance of *L. botrana* adult females and males. More specifically, our study aimed at investigating the consequences of temperatures experienced by *L. botrana* individuals during their whole life on the expression of reproductive traits. For this purpose, we compared the performance of each sex separately between two daily fluctuating thermal regimes designed to reflect current or simulate future thermal conditions in our study area (Burgundy, Eastern France). We recorded several surrogates for overall performance in both sexes, including body amounts of four major energetic components available upon moth emergence (proteins, lipids, glycogen, soluble carbohydrates), and adult lifespan. Additionally, we assessed the effects of temperature on the reproductive traits of females (mating success, number of eggs laid, hatching success, oviposition dynamics) and males (mating success, spermatophore volume, sperm quality).

Previous works focused on the consequences of rising temperatures for *L. botrana* larval performance reported that caterpillars of this species respond to warmer conditions by speeding up their larval development, which implies a reduced time allowed for feeding (Iltis et al. 2018, 2019). Importantly, such findings have been observed when comparing the two fluctuating regimes used in this study (Iltis et al. 2019). We therefore expect that this plastic reduction in the duration of the feeding stage should negatively adult performance, resulting in lower stocks of energetic components available upon moth emergence, shortened adult lifespan and decreased reproductive performance of both sexual partners, which crucially hinges on resources acquired through the alimentation of larval stages (Moreau et al. 2006; Muller et al. 2015).

Materials and Methods

Thermal Regimes

This study involved two daily fluctuating thermal regimes designed to reflect current and expected future thermal conditions in Burgundy, Eastern France (Longvic-Dijon weather station, 47.27°N; 5.09°E; altitude = 219 m) (www.meteofrance.com). Both these two regimes aimed at emulating an average day during summer period (15th July–15th August), because *L. botrana* exhibits high levels of activity (i.e. one peak of adult emergence) over this date range (Barnay et al. 2001; Martín-Vertedor et al. 2010). The regime used to simulate summer conditions currently found in Burgundy, called the Current Fluctuating Regime (CFR), was built based on a 20-year recording of in situ hourly temperatures over a recent period (1995–2014). The CFR was composed of six segments of temperatures, each lasting 4 h and calculated as the mean segment over the 30 days (15th July–15th August) and the 20 years studied.

Alongside the CFR, we designed a scenario termed Future Fluctuating Regime (FFR), aimed at simulating one possible trajectory for local climate in Burgundy, and based on summer thermal conditions likely to occur in this region for a period covering the years 2081 to 2100. The FFR was generated according to the projections provided by six different climate simulations (spatial resolution: 8 km), each involving a combination of one General Circulation Model and one Regional Climate Model nested within (Taylor et al. 2012; Jacob et al. 2014). All these simulations were run with the most pessimistic of the Representative Concentration Pathway (RCP) scenarios, termed RCP 8.5, which predicts the highest positive radiative forcing (i.e. high absorbance of solar and infrared radiation) as a consequence of massive and growing future emissions of greenhouse gas (Moss et al. 2010). The FFR included six segments of temperatures, each lasting 4 h and corresponding to the mean segment over the six simulations performed. All meteorological data used to design the thermal regimes are freely available on the Drias portal (www.drias-climat.fr). The comparison of CFR and FFR indicates that global warming is likely to increase both the mean temperature (+ 5.3 °C) and the daily thermal range (+ 0.6 °C) in our focal area (Table 1).

Moth Rearing and Larvae Collection

An inbred strain of *L. botrana* provided the individuals used in this study (French National Institute for Agricultural Research, Villenave-d'Ornon, France). Insect stock arose from large numbers of caged adults (several thousand each week), kept without diapause under standard laboratory conditions (20 ± 0.5 °C, 60 ± 5% relative humidity, photoperiod of L17/D6 with 1 h of dusk). During photophase, luminosity varied between 600 lx (daytime) and 100 lx (dusk). Bands of waxed paper (5 × 10 cm) were hung inside rearing cages for eggs laying. Once these oviposition supports received a sufficient number of eggs (after 2–3 days), they were renewed and placed in plastic boxes covered with a daily humidified piece of paper towel to protect eggs from desiccation until hatching.

Adults were obtained by initially collecting 2043 newly hatched larvae (age < 24 h) from the rearing stock with a fine paintbrush. To avoid any experimental bias attributable to larval competition and subsequent food deprivation (Thiéry et al. 2014), larvae were individually reared in Eppendorf tubes filled with 1.5 ml of artificial culture medium (composition for 1000 ml: 1000 ml water, 15 g agar, 86.6 g maize flour, 41.3 g wheat germ, 45.5 g beer yeast, 6 g ascorbic acid, 3.4 g mineral salt, 128 mg pyrimethanil,

Table 1 Thermal conditions (°C) for the two daily fluctuating thermal regimes involved in this study, Current Fluctuating Regime (CFR) and Future Fluctuating Regime (FFR), that were divided into 6 periods of 4 h

Hour	CFR	FFR
0–4 h	16.4	21.4
4–8 h	17.8	22.9
8–12 h	22.3	27.8
12–16 h	24.9	30.5
16–20 h	22.9	28.3
20–0 h	19.0	24.2
Mean temperature (°C)	20.5	25.8
Daily thermal range (°C)	8.5	9.1

Daily thermal range corresponds to the difference between daily maximum and minimum temperatures

2.7 g benzoic acid, 2.8 g methyl 4-hydroxybenzoate and 5 ml 95% ethanol). Tube lids were pierced for air circulation and covered with a piece of fine mesh fabric to prevent larvae from escaping. Freshly hatched larvae were then randomly allocated to one thermal regime (CFR or FFR) programmed with climate chambers (ST 2/2 BASIC, Pol-Eko Aparatura, Wodzisław Śląski, Poland). Abiotic conditions inside these incubators were controlled (temperature ± 0.1 °C, 50 ± 10% relative humidity, L18/D6, 650 lx). According to a previous study, daily monitoring of pupation started only 2 weeks (if caterpillars were reared in the FFR) or 4 weeks (if they were reared in the CFR) after initial collection to minimise disturbance (Iltis et al. 2019).

Immediately following pupation, chrysalises were delicately extracted from their cocoon and weighted to the nearest 0.1 mg with a balance (Pioneer PA214C, OHAUS, Greifensee, Switzerland). They were then individually transferred into glass tubes (70 × 9 mm diameter) closed with cotton and were returned to their rearing thermal regime to end their development. Tubes were inspected once a day to monitor adult emergence. Freshly emerged moths were sexed and supplied with water ad libitum through daily soaked cotton plugs. They were then randomly distributed among three groups all along the experiments, because the array of measurements included in the study could not have been performed on the same individuals. In Group 1, adults were frozen to subsequently quantify their body amounts of four major energetic components upon adult emergence. In Group 2, adults were held in their rearing thermal regime until death to record adult lifespan. In Group 3, adults were mated with standard individuals (originating from the laboratory insect stock) to assess the reproductive performance of each sex separately.

Group 1: Body Amounts of Four Major Energetic Components Upon Emergence

Energetic assessments were run on 75 females ($n = 37$ for CFR, $n = 38$ for FFR) and 80 males ($n = 36$ for CFR, $n = 44$ for FFR). Within the 12 h following their emergence, moths were individually placed in Eppendorf tubes and immediately frozen in liquid nitrogen. They were kept at − 80 °C for later quantification of their energetic states. Wings were cut before moths were crushed in lysis buffer, as they are covered with scales which might disturb the biochemical assays based on absorbance measurements. Four major energetic components involved in energy production in insects were quantified through spectrophotometry methods: proteins, lipids, glycogen and soluble carbohydrates. The total amounts of soluble proteins available were quantified using a DC Protein Assay kit involving Bradford reagent (Bio-Rad, Hercules, USA). The assessments of other energetic components (lipids, glycogen, soluble carbohydrates) were performed with the method described by Foray et al. (2012). The repeatability of the measurements was assessed by calculating the coefficient of variation over two replicates for protein and lipid body amounts, and excluding the individuals for which this coefficient exceeded 30% (see Iltis et al. 2019). Repeatability was relatively high, as indicated by the low percentages of individuals removed from the protein dataset (3.2%) and the lipid dataset (0.6%). More specifically, this selection procedure led to the exclusion of 3 females ($n = 2$ for CFR, $n = 1$ for FFR) and 2 males ($n = 2$ for FFR) from the protein dataset, and 1 female ($n = 1$ for FFR) from the lipid dataset.

Group 2: Adult Lifespan

This group involved 76 females ($n = 28$ for CFR, $n = 48$ for FFR) and 73 males ($n = 24$ for CFR, $n = 49$ for FFR). Immediately following their emergence, freshly emerged moths were individually kept in clean glass tubes (100 × 15 mm diameter) within their rearing thermal regime. They were supplied daily with water ad libitum through soaked cotton plug, and monitored once a day (during water provisioning) until death. We then recorded the adult lifespan (in days), expressed as the time elapsed between imaginal emergence and death.

Group 3: Reproductive Performance of Females and Males

A total of 99 females ($n = 42$ for CFR, $n = 57$ for FFR) and 105 males ($n = 53$ for CFR, $n = 52$ for FFR) were allowed to mate in order to assess their reproductive performance. All these individuals were virgin and 24-h-old. At dusk, one randomly selected 24-h-old virgin individual (female or male) reared in either the CFR or FFR was confined in a glass mating tube (100 × 15 mm diameter) with one 24-h-old virgin standard individual of the opposite sex. These standardised individuals were obtained from the inbred stock population and were weighted as pupae (precision: ± 0.1 mg) with a balance (Pioneer PA214C, OHAUS, Greifensee, Switzerland). They were considered equivalent in terms of quality as sexual partners given the relatively low genetic variation between them and the same standard environmental conditions (see above) they met during development (Moreau et al. 2006). Using standardised individuals as sexual partners for individuals assigned to the two thermal treatments enabled to specifically assess the effects of rearing temperature on the reproductive performance of each sex separately (see Moreau et al. 2006; Muller et al. 2015). Mating trials lasted 3 h under controlled conditions (23.1 ± 0.5 °C, 60 ± 5% relative humidity, 100 lx). This duration was selected because the latency to mate in this species does not exceed 3 h in cases of no-choice mating trials (Muller et al. 2016b). Temperature was set at 23.1 °C (intermediate temperature between the mean temperature of the CFR and the FFR) during mating experiments, irrespectively of the thermal regime from which tested individuals originated. Luminosity during mating trials was set at 100 lx to reflect dusk conditions, as the reproduction of this species occurs at dusk in natural settings. Mating tubes were examined every 15 min to check if genital coupling was initiated. In this species, the complete transfer of the spermatophore during copulation requires approximately 1 h (Muller et al. 2015, 2016a, b). Hence, a time lapse of 15 min between two successive inspections is short enough to ensure that every effective mating could have been detected. Individuals for which no copulation was observed within 3 h were considered unmated and were discarded from the rest of the experiments.

In cases where a female reared in either the CFR or FFR was mated with a standard male, the male was immediately removed after the pair separation and no longer used in the experiments. The female was left in her mating tube and her rearing thermal regime for oviposition. Water was daily provided ad libitum through soaked cotton plug. Each tube was checked every morning (during water supply) through visual inspection to count the number of eggs deposited by female until death. Ten days after the end of laying period (which extended over approximately 6 days within the CFR and 4 days within the FFR), egg tubes were examined with a stereomicroscope at 8x magnification to calculate the proportion of the eggs that successfully hatched (fertile eggs). An incubation period of at least 10 days ensured that all viable larvae had hatched from the fertile eggs (Torres-Vila et al. 1999; Moreau et al. 2006; Muller et al. 2015). Mating was considered successful when the tested female laid at least one fertile egg, thus indicating an effective delivering of the spermatophore during copulation. Based on these observations, we recorded four metrics indicative of female's reproductive performance: (1) mating success (proportion of the females that laid at least one fertile egg), (2) fecundity (total number of eggs deposited by each mated female), (3) fertility (proportion of the eggs that successfully hatched) and (4) laying rate (in eggs day^{-1}, expressed as the ratio between the total number of eggs deposited by each mated female and the number of days elapsed between the appearance of the first and the last eggs laid) (Moreau et al. 2006).

In cases where a male reared in either the CFR or FFR was mated with a standard female, the female was removed at the end of copulation and frozen at − 80 °C, while the male was no longer used in the experiments. The standard female was further dissected on a glass side to extract the bursa copulatrix containing male's spermatophore. The dissection first enabled to check for the presence of male's spermatophore inside female's reproductive tract (thus indicating successful mating), provided that spermatophore transfer might sometimes fail despite genital coupling. Once the spermatophore was collected, we extrapolated its size (volume), which provides information about the quantity of sperm and energetic substances transferred to female during copulation (Torres-Vila et al. 1999; Muller et al. 2015). For this purpose, we measured spermatophore dimensions—including length l, width w and thickness t—using a stereomicroscope (Stemi 508, Zeiss, Göttingen, Germany) at 50× magnification (precision ± 1 μm). Considering the spermatophore as an ellipsoid balloon, we then assessed its volume V using the mathematical expression $V = \pi/6\ (l \times w \times t)$. Analyses of these photographic data were performed with the ZEN imaging software (version 2.3, Zeiss, Göttingen, Germany). Alongside spermatophore volume, we also evaluated sperm quality following the standard procedure developed by Muller et al. (2015). This involved rupturing the spermatophore in a drop of distilled water, delicately stirring the sperm mass to distribute the sperm evenly throughout the water and to isolate encysted eupyrene bundles. Each bundle contains 256 nucleated sperms involved in egg fertilisation. For this reason, the number of eupyrene bundles in the spermatophore can be interpreted as a surrogate for sperm quality, with demonstrated consequences for female's reproductive output (Muller et al. 2015, 2016a). In summary, we included three metrics related to male's reproductive performance: (1) mating success (expressed as the proportion of males that successfully delivered a spermatophore during copulation), (2) spermatophore volume and (3) number of eupyrene bundles (sperm quality).

Statistical Analyses

For the body amounts of four energetic components, statistical models incorporated thermal regime, sex, their interaction and pupal mass as a single covariate to control for allometric effect on these energetic stocks. The data for body amounts of proteins, lipids and glycogen satisfied both normality and homoscedasticity assumptions. Hence, the variation in the amounts of these energetic components was decomposed by means of analyses of covariance (ANCOVAs). The distribution of soluble carbohydrates data was skewed towards zero and thus required a Generalised Linear Model (GLM)-negative binomial error to be analysed. Adult lifespan was treated as a function of thermal regime, sex and their interaction within a Cox proportional hazards regression model. For all reproductive traits measured in females (mating success, fecundity, fertility, laying rate) and males (mating success, spermatophore volume, number of eupyrene bundles), statistical models included thermal regime as an explanatory variable and the pupal mass of tested individuals along with the one of their standard partners as two covariates. Mating success of both females and males was compared among thermal regimes with GLM-binomial error. Data for fecundity and spermatophore volume met the criteria of normality and homoscedasticity, while for laying rate a square root transformation was needed to reach normality. Consequently, all these data were studied with ANCOVAs. Fertility was expressed in proportions and therefore tested using a GLM-quasi binomial error with a logit link function. As discrete count data, the number of eupyrene bundles was analysed with a GLM-negative binomial error. Normality and homoscedasticity were evaluated with a Shapiro–Wilk and a Levene test, respectively. All statistical analyses were carried out using R 3.6.2 software.

Results

Group 1: Body Amounts of Four Major Energetic Components Upon Emergence

The four energetic components were impacted by sex in the same way, but did not respond to thermal regime or to the interaction between thermal regime and sex (Table 2). Females stocked higher amounts of proteins, lipids, glycogen and soluble carbohydrates than males (Table 2). Furthermore, the amounts of the four energetic components were positively influenced by pupal mass: heaviest individuals possessed higher amounts than lightest ones (proteins: $F_{1,145} = 81{,}2$, $P < 0.001$, slope = 80.1; lipids: $F_{1,149} = 11.1$, $P < 0.001$, slope = 45.1; glycogen: $F_{1,150} = 60.7$, $P < 0.001$, slope = 13.0; soluble carbohydrates: $\chi^2_1 = 12.7$, $P < 0.001$, slope = 0.21).

Group 2: Adult Lifespan

Adult lifespan was impacted by thermal regime (Cox proportional hazards regression model, $\chi^2_1 = 55.3$, $P < 0.001$), but not by sex ($\chi^2_1 = 3.06$, $P = 0.08$) or by the interaction between these two factors ($\chi^2_1 = 1.19$, $P = 0.28$). Individuals of both sexes reared and kept in warm conditions died faster than those reared and held in cool conditions (Fig. 1).

Group 3: Reproductive Performance of Females and Males

Females reared in warm conditions had a lower mating success compared with females reared in cool conditions (GLM-binomial error: $\chi^2_1 = 7.28$, $P = 0.01$) (Fig. 2a). By contrast with mating success, fecundity (ANCOVA: $F_{1,59} = 0.22$, $P = 0.64$), fertility (GLM-quasi binomial error: $\chi^2_1 = 0.88$, $P = 0.06$) and laying rate (ANCOVA: $F_{1,59} = 2.70$, $P = 0.10$) did not respond to temperature (Fig. 2b–d). Among female's reproductive traits, only fecundity was affected (positively) by female's pupal mass ($F_{1,59} = 4.71$, $P = 0.03$, slope = 10.5) and the pupal mass of the standard male ($F_{1,59} = 4.06$, $P = 0.04$, slope = 11.1): the heavier the female and the standard male mated with, the higher the number of eggs produced.

Among male's reproductive traits, mating success (GLM-binomial error: $\chi^2_1 = 0.77$, $P = 0.38$) and spermatophore volume (ANCOVA: $F_{1,77} = 0.14$, $P = 0.71$) remained unaffected by thermal regime (Fig. 3a, b). However, spermatophores from males reared in warm conditions contained fewer eupyrene bundles than those produced by males reared in cold conditions (GLM-negative binomial error: $\chi^2_1 = 18.3$, $P < 0.001$) (Fig. 3c). None of the male's reproductive traits

Table 2 Mean values and their 95% confidence intervals of the body amounts of four energetic components for the two thermal regimes and sexes

	Proteins (μg)	Lipids (μg)	Glycogen (μg)	Soluble carbohydrates (μg)
Thermal regime				
CFR	664 [586; 742]^a	474 [417; 530]^a	57.5 [47.6; 67.5]^a	36.1 [28.5; 43.5]^a
FFR	649 [575; 724]^a	454 [404; 504]^a	53.4 [44.3; 62.6]^a	37.4 [30.9; 43.9]^a
Test value	$F_{1,145} = 0.66$	$F_{1,149} = 0.49$	$F_{1,150} = 1.41$	$\chi^2_1 = 0.09$
Sex				
Females	967 [924; 1011]^a	617 [568; 666]^a	90.1 [82.5; 97.7]^a	55.8 [48.6; 62.9]^a
Males	369 [354; 384]^b	321 [288; 354]^b	22.8 [19.7; 25.8]^b	19.0 [15.3; 22.6]^b
Test value	$F_{1,145} = 1093$	$F_{1,149} = 108$	$F_{1,150} = 387$	$\chi^2_1 = 71.3$

For each energetic component, significant differences ($P < 0.05$) between regimes or sexes are shown by superscript letters

Data for proteins, lipids and glycogen were compared among thermal regimes and sexes with Analyses of Covariance while for soluble carbohydrates, a Generalised Linear Model-negative binomial error was performed. All these statistical models accounted for pupal mass as a single covariate

There was no significant interaction between thermal regime and sex for any of the four energetic components (proteins: $F_{1,145} = 0.88$, $P = 0.35$; lipids: $F_{1,149} = 0.16$, $P = 0.68$; glycogen: $F_{1,150} = 0.93$, $P = 0.34$; soluble carbohydrates: $\chi^2_1 = 0.24$, $P = 0.63$)

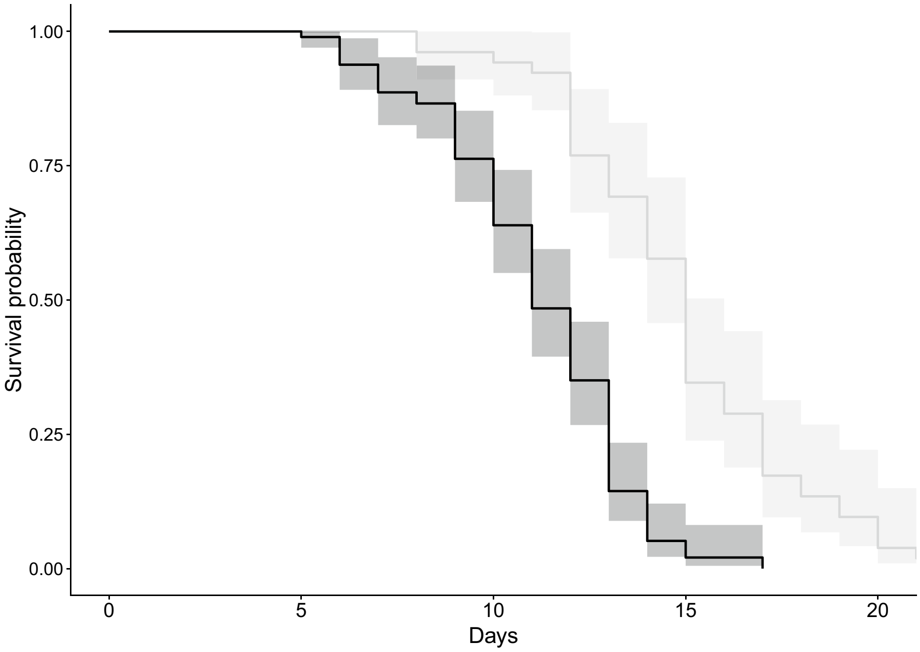

Fig. 1 Effect of thermal regime (grey: Current Fluctuating Regime, black: Future Fluctuating Regime) on adult lifespan (±95% confidence interval), expressed as a survival probability function of individual age. Data for males and females were pooled together as non-statistically different

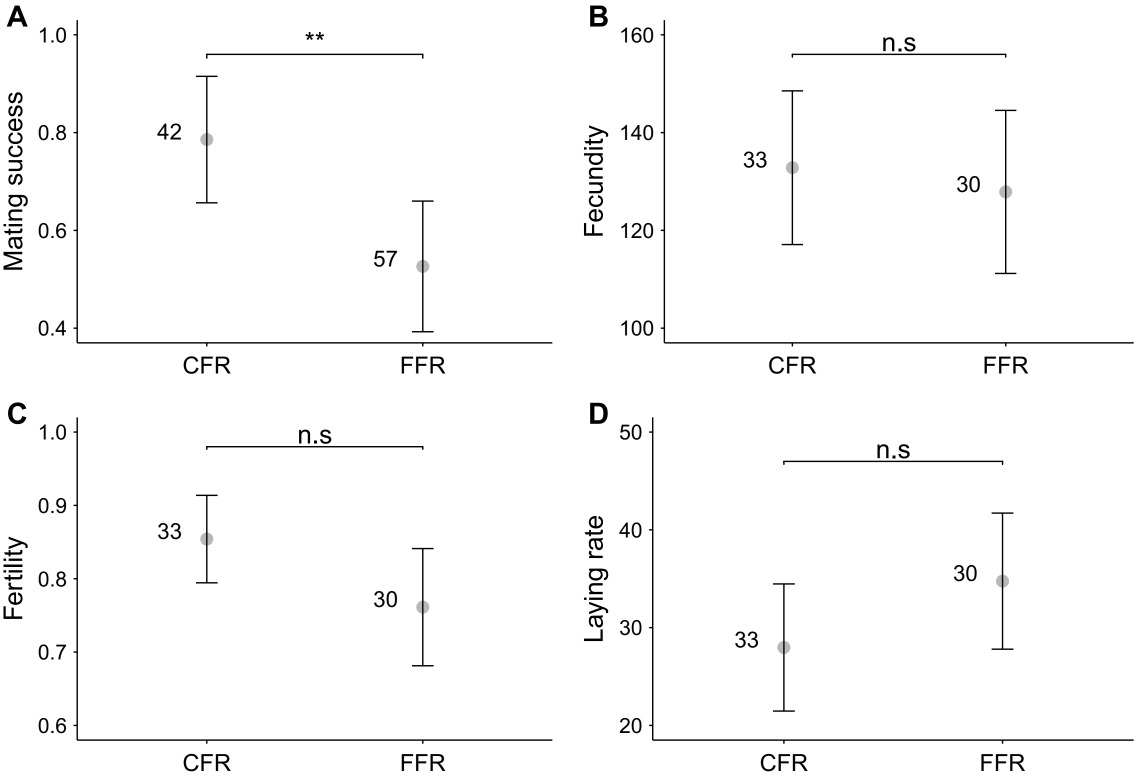

Fig. 2 Effect of thermal regime (CFR = Current Fluctuating Regime, FFR = Future Fluctuating Regime) on the mean (±95% confidence interval) of four reproductive traits measured in females: **a** mating success, **b** fecundity (total number of eggs laid by one female), **c** fertility (proportion of the eggs that successfully hatched), and **d** laying rate (fecundity/oviposition duration). Asterisks indicate significant differences (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$), n.s means non-significant, and numbers refer to sample sizes

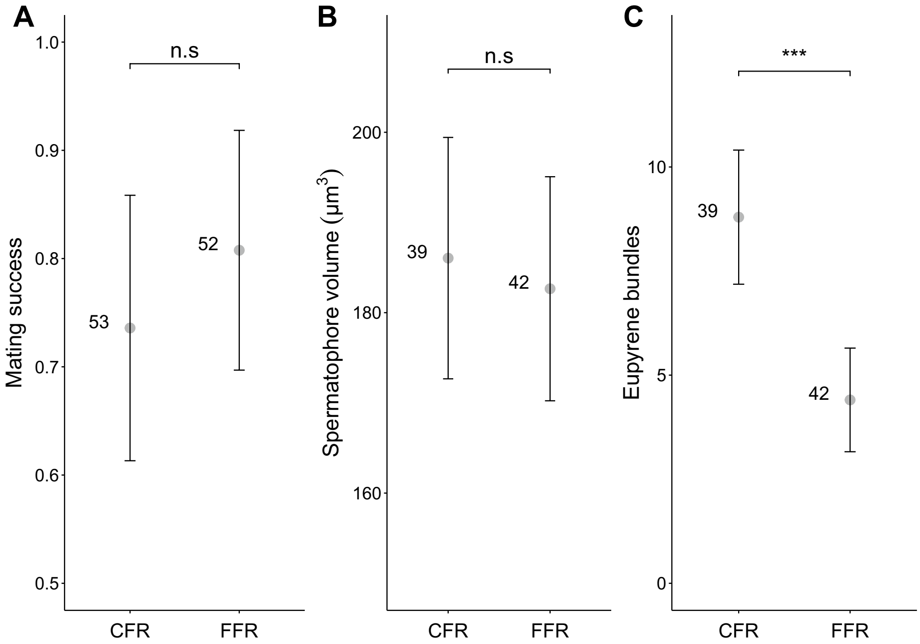

Fig. 3 Effect of thermal regime (CFR = Current Fluctuating Regime, FFR = Future Fluctuating Regime) on the mean (±95% confidence interval) of three reproductive traits measured in males: **a** mating success, **b** spermatophore volume, and **c** number of eupyrene bundles contained in sperm. Asterisks indicate significant differences (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$), n.s means non-significant, and numbers refer to sample sizes

was impacted by either male's pupal mass or the pupal mass of the standard female mated with.

Discussion

Using realistic fluctuating regimes, this study sought to investigate the impacts of global warming on the reproduction of a major grapevine pest, the European grapevine moth (*L. botrana*). We found clear evidence revealing that moth physiology and reproduction are influenced by temperatures experienced throughout ontogeny. When reared in warmer conditions complying to a scenario of local warming (FFR), individuals of both sexes incurred a reduced adult lifespan, but their body amounts of four major energetic components remained unchanged compared with individuals reared in thermal conditions similar to those currently found in Burgundy (CFR). Focusing on reproductive traits, females that developed in warm conditions underwent reduced mating success (−26%), while males reared in warm conditions suffered decreased sperm quality, that is, a lower number of eupyrene bundles contained in their spermatophore (−50%).

The absence of temperature-related differences in the body amounts of four energetic components suggests that caterpillars are able to compensate for a shortened time allowed for feeding (accelerated larval development) in warm conditions. These findings match the results of a previous study conducted on *L. botrana*, which outlined an increase in daily gain of body mass (i.e. growth rate) with mean temperature (Iltis et al. 2018). In agreement with these conclusions, several phytophagous species—with examples found in the Lepidoptera order—are known to elevate their food intake rate as temperature increases, probably to fuel higher basal metabolic rates induced by warming (Lee and Roh 2010; Bauerfeind and Fischer 2013; Pandey et al. 2015). We also reported here a reduced adult lifespan for individuals reared and held in warm conditions, presumably because, as capital breeders, *L. botrana* adults cannot feed to compensate for the higher energetic losses and reserves consumption occurring with warming (for similar results on capital breeding species, see Laughton et al. 2017). Such a reduction in adult lifespan should have important repercussions for the reproduction of *L. botrana*. In males, it should imply a shorter time allowed for mate sampling in natural and complex habitats, thereby possibly impairing the encounter between sexual partners. In females, it can entail a reduced time available for oviposition and egg dispersion in space, potentially leading to complex consequences for larval density, levels of intraspecific competition and offspring survival on infested bunches (Thiéry et al. 2014).

In females, the 26% reduction in mating success observed when comparing the two thermal regimes can be explained by the effects of temperature on the precopulatory phase of reproduction, that is, the pheromonal communication between females willing to mate and males. In *L. botrana*, females signal their sexual receptivity by emitting sex pheromones through calling behaviour (Muller et al. 2015, 2016a). In our study, rearing temperatures experienced by females before mating tests are likely to have influenced calling behaviour by altering pheromone production in quantitative (titres, duration of calling behaviour) and/or qualitative ways (blends of chemical compounds constituting the olfactory signal for mating) (Ono 1994; Konopka and McNeil 2017). This thermal modulation of pheromone emission should further affect the outcomes of the behavioural interaction between sexual partners (e.g. specific recognition, mating success). With respect to this hypothesis, we expect the negative effects of warming on female's mating success to be exacerbated in natural, highly heterogeneous environments, where pheromonal signal plays a key role in facilitating the encounter between sexual partners. Despite its negative effects on mating success, warming did not influence fecundity and fertility, in accordance with the absence of differences regarding female's body amounts of energetic components observed between thermal treatments.

In males, thermal conditions did not influence mating success, suggesting no differences between males reared in the CFR and the FFR in terms of access to reproduction and ability to transfer the sperm material during copulation. Temperature did not either modify spermatophore volume as a surrogate for the quantity of sperm and accessory gland secretions delivered to female during mating. As spermatophore was included in our energetic assays, these results are congruent with the absence of differences in body amounts of energetic components found when comparing males reared in the two thermal regimes. However, our study showed that male's sperm quality—approximated by the number of eupyrene bundles contained in the ejaculate—was severely reduced (– 50%) in warm conditions. The negative impacts of warming on male's fertility traits are becoming more and more evident in arthropod species, including sperm number (Zeh et al. 2012; Sales et al. 2018), viability (Stürup et al. 2013; Sales et al. 2018), size (Vasudeva et al. 2014; Iossa et al. 2019) and ability to migrate through the female's genital apparatus (Sales et al. 2018). Our study brings new evidence showing that eupyrene spermatogenesis in butterfly species is very sensitive to prevailing environmental conditions, particularly temperature (Hiroyoshi and Mitsuhashi 1998; Iossa et al. 2019). Considering that the number of eupyrene bundles included in male's spermatophore primarily mediates female's egg production in *L. botrana* (Muller et al. 2015, 2016a), further experimental investigations are needed to determine to what extent the decline in male's fertility observed for individuals reared in warm conditions will lower the reproductive success of the pair. In this regard, it is reasonable to think that the 50% reduction in sperm quality we highlighted will perceptibly reduce female's egg production after mating. For example, a less important decline in the amount of eupyrene sperm (approximately – 33%) due to larval alimentation had been shown to negatively impact both female's fecundity (– 30%) and fertility (– 10%) in a previous study (Muller et al. 2015).

In terms of pest management, our study has two main implications for PBDMs design, which could contribute to improve the ecological realism of these models and the accuracy of their local and global predictions about the future of *L. botrana* populations. First, our results show that, using realistic fluctuating thermal regimes, climate change would not impact female's fecundity. By contrast with this absence of difference in female's fecundity observed when comparing fluctuating regimes, equations constituting PBDMs—based on constant temperatures—predict that *per capita* egg production will likely increase over the range of temperatures here examined (considering an increase in mean temperature from 20.5 to 25.8 °C) (Gutierrez et al. 2012, 2017). We argue that differences between constant and fluctuating regimes probably arise from the negative effects of thermal fluctuations on egg production, which would likely expose the organism to stressful thermal conditions during the hottest hours of the cycle. Such a phenomenon is likely to become more and more frequent if mean temperature and the range of daily fluctuations increase concomitantly as two facets of global warming, as predicted in Colinet et al. (2015). Second, by demonstrating that male's fertility was halved with respect to warming scenario, our study highlights the need for PBDMs to consider (1) how male's reproductive performance is expected to vary according to temperature and (2) how this variation in male's traits modulates the offspring production of the pair. In light of our findings, we are firmly convinced that the implementation of realistic patterns of fluctuations together with male's reproductive traits as an integral component of *L. botrana* reproductive success within PBDMs should substantially improve their reliability as tools to develop adequate pest management practices and policies.

In conclusion, we expect climate change to reduce the reproductive success of *L. botrana* and the abundance of this pest over generations in Eastern France due to the negative effects of the local warming scenario observed for adult lifespan and the reproductive performance of both sexual partners. At this point, two considerations should be examined before inferring future pest population dynamics based on the results of this study. First, this work explored the plastic response of one single *L. botrana* generation that experienced an abrupt shift in mean temperature between rearing conditions—whose mean temperature basically corresponded to the one of the CFR—and FFR. Hence, our findings do not account for the insect ability to adapt to a gradual warming experienced over several successive generations, as expected in natural conditions (Bale et al. 2002; Hoffmann 2017). It could therefore be hypothesised that *L. botrana* populations will be able to cope with an incremental increase in mean temperature through evolutionary response, such that the negative effects of warming on the reproduction of this pest species should be less important within natural populations than suggested in this study. Second, the abundance of *L. botrana* populations is not solely be determined by the reproductive success of this pest, but also by the mortality of immature stages (mostly eggs and larvae) induced by parasitoids in agroecosystems, which will likely be affected by changing thermal conditions (for reviews, see Jeffs and Lewis 2013; Thierry et al. 2019). The physiological response of these natural enemies to rising temperatures in terms of development, fecundity and adult longevity (which all influence effective parasitism rates among pest populations) is thus pivotal to develop a more thorough understanding of the consequences of climate change for *L. botrana* populations (Reineke and Thiéry 2016; Castex et al. 2018). Sound knowledge about the influence of temperature on parasitoid vital rates could be incorporated to PBDMs in order to provide predictions about future *L. botrana* abundances relevant in the context of tritrophic interactions (for an example with another pest species, see Gutierrez et al. 2011).

Author Contribution Statement

All authors conceived the experiments. CI and KP run out the experiments. CI and PL analysed the data. CI, PL, JM and DT led the writing of the manuscript. All authors critically revised the intellectual content of the draft and gave their approbation for the final version to be published.

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Compliance with Ethical Standards

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

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

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