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Synergistic effects of elevated temperature with pesticides on reproduction, development and survival of dung beetles

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Abstract

In times of global change, high temperatures can increase the negative effects of pesticides and other stressors. The goal of this study was to evaluate, under controlled laboratory conditions, the effect of a moderate increase in temperature in combination with ivermectin (an antiparasitic medication used in cattle that is excreted in dung), an herbicide, and parasitic pressure, on the reproductive success, development time and adult survival of dung beetles *Euoniticellus intermedius*. Whereas high temperature increased the number and proportion of emerged offspring, it had synergistic negative effects in combination with the ivermectin, herbicide and parasite treatments. Moreover, high temperature in combination with ivermectin and with parasitism caused a synergistic increase of adult offspring mortality and, in combination with the herbicide, it synergistically accelerated development. These results indicate that high temperatures can enhance the negative effects of other stressors and act synergistically with them, harming dung beetles, a group with high ecological and economic value in natural and productive ecosystems. Although adult sex ratio was not affected by experimental treatments, contrasting responses were found between males and females, supporting the idea that both sexes use different physiological mechanisms to cope with the same environmental challenges. The effects that combined stressors have on insects deepen our understanding of why we are losing beneficial species and their functions in times of drastic environmental changes.

Keywords Independent action model · Invasive species · Ivermectin · Parasitism · Fitness

Introduction

Current global warming acts in combination with other environmental factors and influences the reproductive success and abundance of insects (Noyes et al. 2009; Gunderson et al. 2016; Tran et al. 2018; Iltis et al. 2020; Turschwell et al. 2022). Factors linked to changes in land cover and biodiversity loss, such as the presence of chemical contaminants, higher rates of parasitism, or the decrease in the availability of resources, can have negative consequences on the survival and reproduction of insects (Folt et al. 1999; Siviter et al. 2021; Verheyen and Stoks 2023). When two or more stressors occur simultaneously, their combined effects on organisms can be additive (the sum of their individual effects), antagonistic (smaller effect together than alone), or synergistic (greater effect in combination of stressors than the sum of their individual effects). The latter are of particular concern, due to the great threat they represent to living organisms (Folt et al. 1999; Verheyen et al. 2019; Siviter et al. 2021; Verheyen and Stoks 2023).

Insects are particularly vulnerable to temperature changes since they depend on environmental temperature to control their internal temperature (Atkinson 1994; González-Tokman et al. 2020a; Mastore et al. 2023). Therefore, insects have evolved physiological and behavioral strategies to avoid the noxious effects of warming (González-Tokman et al. 2020a; Bodlah et al. 2023), but such mechanisms can be highly costly and constrain insect adaptation to warm temperatures (García-Robledo et al. 2016). Consequently, insects are highly threatened by increasing temperatures and strongly rely on phenotypic plasticity to be able to face these thermal challenges

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(DeWitt et al. 1998; Agrawal 2001; Davidson et al. 2011; Balzani et al. 2021).

High temperatures can increase the toxicity of polluting compounds found free in the environment (Noyes et al. 2009). For example, temperature increase combined with a moderate dose of the insecticide chlorpyrifos (1 μg/L, causing 45% mortality) increases mortality and decreases growth rate and thermal tolerance in larvae of the damselfly *Ischnura elegans* (Vander Linden, Odonata: Coenagrionidae; Verheyen et al. 2019). Similarly, *Culex pipiens* (Linnaeus, Diptera: Culicidae) mosquitoes exposed to high temperature combined with a low dose of chlorpyrifos (0.23 μg/L, causing 9.4% mortality) had lower survival, shorter developmental time, and smaller size at emergence, indicating that some pesticides are more toxic at higher temperature (Tran et al. 2018).

Temperature also plays an important role in the activation of the immune system, since a thermal shock can be decisive in the survival of parasitized insects or their offspring (Wojda 2017). For example, *Gryllus texensis* (Cadde and Otte, Orthoptera; Gryllidae) and *Tenebrio molitor* (Linnaeus, Coleoptera: Tenebrionidae) can adaptively modify their behavior when they are parasitized, to face the immune challenge. For example, illness induced anorexia or behavioral fever can help them cope with the allocation of energy to enhance survival upon parasite exposure (Adamo et al. 2010; Catalán et al. 2012). Therefore, small changes in environmental temperature can induce physiological, morphological and behavioral changes, in addition to enhancing the effect of other biological and chemical stress factors (Sunday et al. 2014; Wojda 2017).

Cattle pastures are habitats where different stressors occur, including residues of herbicides (Martínez et al. 2001) and veterinary medications (Lumaret et al. 2012) that are excreted in dung, in addition to naturally occurring pathogens and parasites (Adamo et al. 2010; Servín-Pastor et al. 2021). These stressors threaten coprophagous fauna, mainly flies and beetles. Worryingly, these harmful effects could be enhanced by the increasing environmental temperature, resulting in negative effects on insect fitness (Verdú et al. 2020; Ambrožová et al. 2021). For example, in dung flies *Scatophaga stercoraria* (Linnaeus, Diptera: Scathophagidae), exposure to high temperature in combination with residues of the veterinary medication ivermectin (6.57 mg/kg, a concentration causing low mortality in juveniles and adults), synergistically decreases the survival of developing larvae (González-Tokman et al. 2022).

Dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae, Aphodiinae and Geotrupidae: Geotrupinae) play a fundamental role in the functioning of cattle pastures (Doube 1990; Nichols et al. 2008; Cruz et al. 2012; Doube 2018; Mamantov and Sheldon 2023). When they bury cattle dung to feed and reproduce, they fertilize the soil, reducing cattle exposure to feces, and reducing the emission of methane from dung into the atmosphere (Cruz et al. 2012; Miranda-Flores et al. 2020; Verdú et al. 2020). However, the presence of different stressors at high temperatures can reduce the survival and reproductive success of these organisms, affecting their function in the ecosystem (González-Tokman et al. 2017a; Ishikawa and Iwasa 2019; Servín-Pastor et al. 2021). For example, an increase in temperature modifies the production of brood balls (each containing dung and an egg; Mamantov and Sheldon 2020) and parasitic pressure reduces the size of such balls (Servín-Pastor et al. 2021).

Even though some experiments have evaluated the effect of isolated stressors on some aspects of dung beetle life history, it is still unknown whether an increase in temperature enhances the negative effects of other stressors that are applied to grasslands, such as veterinary medications and agrochemicals, or natural factors such as parasites. Pesticides are the second major threat for dung beetles in cattle pastures from some regions of the world, only after habitat loss (Alvarado et al. 2017), but their effect in combination with high temperature has not been evaluated. The same is true for the effect of parasitism, which has not been evaluated in dung beetles but could also become more dangerous at high temperatures. Furthermore, since males and females may differ in their sensitivity to different environmental stressors, stressful conditions may cause changes in sex ratio, with implications for reproductive behavior and mate availability, and ultimately with consequences on population sizes and dynamics (Kappeler et al. 2023).

This study evaluated the hypothesis that a high temperature enhances the negative effects of each of three stressors: an antiparasitic medication (ivermectin), an herbicide mixture, and parasitism, on reproductive success, larval development time, sex ratio and survival of adult dung beetles *Euoniticellus intermedius* (Reiche, Coleoptera: Scarabaeidae), an important ecosystem engineer. Synergistic effects were expected between heat and ivermectin, which is known to be highly toxic for these insects.

Materials and methods

Study species

Euoniticellus intermedius (Coleoptera: Scarabaeinae) is an African species that was introduced into America more than 30 years ago to bury cattle dung in pastures (Montes de Oca and Halffter 1998; González-Tokman et al. 2024). It is a diurnal, multivoltine species that depends on bovine dung for its reproduction. In this species, reproductive females use dung to build brood balls (14–19 mm long), where a single egg is laid; the egg will develop in a chamber, buried at 7–14 cm of depth in the soil; larval development lasts 28 days (Blume 1984; Martínez et al. 2017; Pokhrel et al. 2020). Each female can lay up to 100 eggs in her lifetime and adult individuals live up to 5 months in captivity (González-Tokman et al. 2022). Its reproductive strategies allow this species to establish in non-native sites (Bornemissza 1976; Doube et al. 1991; Pokhrel et al. 2020), and can disperse despite the excessive use of contaminants registered in some regions (Montes de Oca and Halffter 1998; González- Gomez et al. 2023). Similar to other dung beetles, this coprophagous species provides important ecosystem services, including soil fertilization and aeration, reduction of greenhouse gases emission from dung and fly pest control, among others (Nichols et al. 2008; Pokhrel et al. 2020). This species is well known for its response to different sources of environmental stress, and it is known that its reproductive success, physiological condition, and stress tolerance are traits with plasticity in response to stressors such as contaminants in the dung and parasitism (Cruz et al. 2012; González-Tokman et al. 2017a, b; Servín-Pastor et al. 2021).

Study site and beetle breeding

Two hundred adult *E. intermedius* were collected directly from the dung at a 17 ha cattle pasture, San Román Ranch, Medellín de Bravo, Veracruz, Mexico (18° 58′19.37″ N, 96° 04′5143″ W). In the region, cattle pastures are sites where the natural habitat has been deforested to plant a specific grass for cattle grazing (Bonilla-Moheno and Mitchell 2020). Local soil was collected and transported to the laboratory. The soil was sifted, bagged in plastic bags and sterilized in a centrifuge at a pressure of 20 kg/cm2 during 80 min at 100 °C to be used in the experiment. Cow dung was collected from Coacoatzintla Ranch (19° 43′ 10.3656″ N, 19° 43′ 10.3656″ W), a ranch free of medications and pesticides, transported to the laboratory, homogenized with an industrial mixer and frozen at −20 °C for 72 h to reduce parasite populations (Schurer et al. 2014). Dung was thawed before use. After homogenizing the dung and combining it according to the chemical treatment (control, medication or herbicide, see below), the dung was refrigerated at 4 °C until the end of the experiment. *Euoniticellus intermedius* individuals were transported to the laboratory which was maintained at 28 ± 1 °C, 70% humidity and 13:11 light:dark. They were separated into 30 pairs consisting of a male and a female, and were placed into terraria (plastic container size: 57 × 30 × 37 cm) with a 15 cm layer of moist sandy soil with a mesh covering. Beetles were fed 500 g of homogenized cattle dung three times a week. Adults were allowed to reproduce in terraria for 15 days, after which they were removed to allow the emergence of the next generation. This procedure was repeated for another generation before starting the experiment to avoid environmental effects on experimental beetles. Newly emerged adult beetles were separated by terrarium and sex to avoid reproduction between relatives. After two generations in the laboratory, emerged adult beetles of the third generation were used to carry-out the experiment.

Experimental setup

An experiment under controlled laboratory conditions was carried out to determine the effect of different temperatures in combination with other stressors. Two temperatures, 29 °C and 33 °C (73% and 70% humidity, respectively), were used in the experiment. Whereas 29 °C is a standard temperature for breeding this species in the laboratory (Esquivel-Román et al. 2023), 33 °C simulates a moderate temperature increase estimated to be caused by climate change for year 2100 (IPCC (2007)) and because previous evidence in insects has shown important effects of this moderate temperature increase on insect life-history traits (Dinh Van et al. (2014); Verheyen et al. 2019). Temperature was controlled in climatic chambers, where reproductive pairs were exposed to the respective stressor treatments applied in dung (see below, Fig. 1). The pairs of adult beetles (a male and a female) were placed in 1 L plastic, transparent containers, filled 75% with sterile sieved soil and covered with a mesh, using the same photoperiod as above (Villada-Bedoya et al. 2020). The soil was moistened with a sprayer three times a week to maintain similar moisture conditions across the experimental containers. At each experimental temperature, three stressors (medication, herbicide and parasitism), plus a control treatment, were used, leaving a total of 8 experimental treatments. Fifteen replicates per treatment were used, for a total of 120 replicates (Fig. 1).

The used medication (ivermectin, Sigma-Aldrich, CAS-Number: 70288-86-7,10 μg per kg of fresh dung) was chosen because it has been recognized as a main threat to dung beetles from cattle pastures (Alvarado et al. 2017) and the used concentration simulates the excretion of a cow treated a month before with the recommended dose (Wohde et al. 2016). This dose is relatively low but evidence in *E. intermedius* shows that it has effects on the metabolic rate, muscle mass and triggers a physiological stress response (González-Tokman et al. 2017b; Villada-Bedoya et al. 2020). The used herbicide (0.4 mg per kg of dung) was the combination of picloram +2-4-dichlorophenoxyacetic acid, which is frequently used in the study area (Martínez et al. 2001; González-Gómez et al. 2018; Villada-Bedoya et al. 2019) and is one of the most persistent herbicides in the environment (Volodymyr et al. 2018). The used concentration was based on the estimation of how much herbicide a 1 kg dung pad receives after herbicide application in the field at the recommended dose (González-Tokman et al. 2017a). Although it has been found that this herbicide does not have an obvious negative effect on *E. intermedius* in natural or laboratory conditions (González-Tokman et al. 2017a; Villada-Bedoya et al. 2019), its effect could be enhanced in combination with high temperature. In

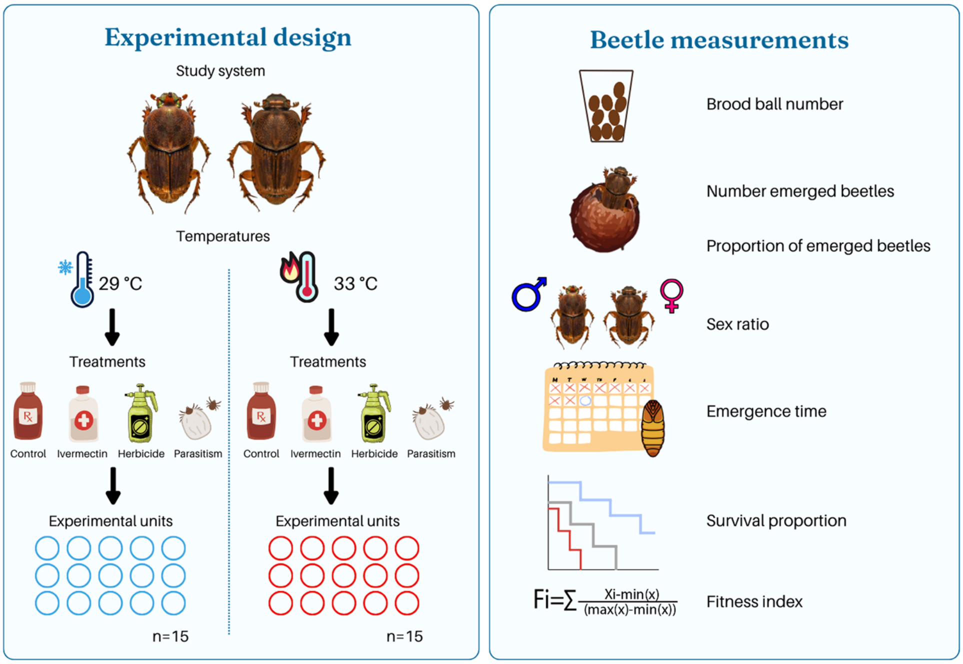

Fig. 1 Experiment designed to evaluate the effect of the combination of heat with other stressors on the survival and reproductive success of *E. intermedius*, under controlled laboratory conditions. Co Control, Me Medication, He Herbicide, Pa Parasitism, N number of replicates per treatment

addition to beetles being in direct contact with herbicides at the time of spraying, these contaminants also have the ability to easily move to the roots, which affects insect soil fauna (Martínez et al. 2001; Teixeira et al. 2007; Burns and Swaen 2012; Song, 2013; Villada-Bedoya et al. 2019). Medication and herbicide treatments were diluted in acetone (10 ml of acetone per kg of fresh dung) and applied in homogenized dung. Acetone was used as a control (see similar procedures in González-Tokman et al. 2017a, 2017b). In addition, the effect of parasitism was evaluated, since it can cause changes in the reproduction and thermal tolerance of the study species (Servín-Pastor et al. 2021; González-Tokman et al. (2021)), indicating a link between the response to heat and the immune system (Wojda 2017). Parasitism was simulated with the implantation of a 2 mm long and 0.8 mm thick nylon monofilament between the pronotum and the elytra to both parents, a procedure that activates the immune system (Pomfret and Knell 2006; Rantala et al. 2000; Reaney and Knell 2010; Catalán et al. 2012), and may have effects on the reproductive investment of the study species (Servín-Pastor et al. 2021).

Each pair was exposed to the treatment for 15 days, during which they could reproduce and form brood balls. After this period, both parents were removed from the experimental container. Brood balls were removed from terraria, counted and returned to the bottom of the same terrarium (12 cm depth) and left there until the emergence of the adults. At this moment, a trap (a 5 ml plastic bottle filled with soil and dung) was set in each terrarium and replaced three times a week, so that the newly emerged beetles, which needed to feed, were captured and the emergence date recorded. Three recently emerged adult beetles of each sex (5–7 days old) were randomly selected per terrarium and were isolated in 15 ml glass bottles (covered with moistened cotton, at 28 °C and at a photoperiod of 12:12 h of light and darkness, deprived of food) to record adult survival. Mortality was registered every 6 h until the last individual died. A fitness index (values between 0 and 1; Garrido et al. 2012) was calculated with the product of the sum of each standardized response variables plus 0.01. Higher index fitness values indicate higher number of brood balls, emerged beetles, and proportion emerged, fast emergence time, and high survival of the offspring.

Statistical analysis

Analyses were performed in R (version 4.0.2, R Core Team 2020), using RStudio, following Crawley (2005) and Zuur (2009). The analyses evaluated the effects of temperature (a factor with two levels; 29 and 33 °C) stressor treatment (with four levels; control, medication, pesticide, parasitism) and the interaction temperature × stressor treatment on the number of nest masses, number and proportion of emerged beetles, emergence time, sex ratio of emerged beetles, survival and fitness index. The interaction between temperature and treatment was eliminated from the models when it was non-significant (Engqvist 2005).

To evaluate the significance of the explanatory variables in all the models, analyses of variance with type 2 sum of squares were performed, using the “car” package (Fox and Weisberg 2019). When stressor treatment had a significant effect, either alone or in combination with temperature, a priori contrasts were performed with a false discovery rate correction given the elevated number of evaluated pairwise comparisons (Benjamini and Hochberg 1995) (Supplementary Information).

To analyze the number of brood balls and the number of emerged beetles, generalized linear models (GLM) were used, with negative binomial error distribution because the Poisson models showed overdispersion (Resid. Dev./Resid. D. f. >2). To analyze the proportion of emerged adults, a quasibinomial GLM with logit function was implemented. The quasibinomial model was preferred over a binomial model due to high overdispersion. To analyze the sex ratio, a binomial GLM with logit function was performed. To analyze differences in the emergence time, a Gaussian GLM was carried out. To analyze survival, a mixed-effects Cox model was performed with the Coxme package (Therneau 2022), in which no case was censored, since all organisms died at the end of the experiment. Survival curves were made for each treatment with the survminer package (Kassambara et al. 2021). In this survival analysis, the effect of the interaction temperature×treatment×sex, with the terrarium (family), as a random factor, was also tested given that more than one sibling was used per family. As the triple interaction was significant, Cox mixed effects models were performed separately by sex. To analyze the fitness index, a linear mixed model (LMM) was modeled with terrarium as random factor with the nlme package (Pinheiro and Bates 2023). The results are graphically represented as means and 95% confidence intervals, except for survival, where survival curves by treatment are shown.

When temperature, stressor treatment or the interaction of temperature × stressor treatment were significant in the statistical analyses, independent action (IA) models were calculated to know the effect (i.e., additive, antagonistic, or synergistic) of the combination of heat and stressor treatments. IA models were carried out following Coors and De Meester (2008) and Verheyen et al. (2019):

$$Ei = \frac{e_i - e_{control}}{e_{\max} - e_{control}}$$

Where ei is the effect of the combination of heat with each stressor, $e_{control}$ is the effect of the control, e_{max} is the difference between the maximum value minus the minimum. To convert the effect into relative units, the following formula was used:

$$Ejoint = 1 - Prod(1 - E_1) * (1 - E_2)$$

Where E1 and E2 are the effects of each stressor divided by e_{max}. Finally, the value in absolute units was obtained:

$$e_{joint} = E_{joint} * (e_{\max} - e_{control}) + e_{control}$$

When the calculated value (e_{joint}) falls within the calculated 95% confidence intervals, the effects are additive. When the calculated value falls above the 95% confidence intervals, the effects are synergistic. When calculated value falls below the 95% confidence intervals, the effects are antagonistic.

Results

The total number of brood balls laid by *E. intermedius* females did not differ with temperature ($\chi^2 = 563$, $p = 0.453$) or stressor treatments ($\chi^2 = 0.993$, $p = 0.803$; Table 1; Table A2). However, the number and proportion of emerged beetles tended to be higher at 33 °C than at 29 °C

Table 1 Model outputs analyzing the effects of different stressors on dung beetles *E. intermedius*, breeding at different temperatures

Response variables/Explanatory variables	Temperature			Treatment			Temperature × Treatment		
	χ^2	*df*	*p*	χ^2	*df*	*p*	R.D.	*df*	*p*
Number of brood balls	0.563	1	0.453	0.993	3	0.803	NS	NS	NS
glm negative binomial									
Number of emerged beetles	3.780	1	0.051	10.895	3	**0.012**	NS	NS	NS
glm negative binomial									
Proportion of emerged beetles	3.874	1	**0.049**	20.843	3	**0.0001**	7.864	3	0.049
glm quasibinomial									
Sex ratio of emerged beetles	0.001	1	0.980	3.023	3	0.388	NS	NS	NS
glm binomial									
Fitness index	0.006	1	0.940	3.773	3	0.287	5.158	3	0.161
Linear mixed model									
Development time	*F*	*df*	*p*	*F*	*df*	*p*	*F*	*df*	*p*
Linear model	70.52	1	**<0.001**	0.614	3	0.608	4.427	3	**0.005**

The contrasts between treatments are described in the supplementary material. Significant values with false discovery rate (FDR) p correction method are shown in bold

df degrees of freedom, *NS* non-significant interaction

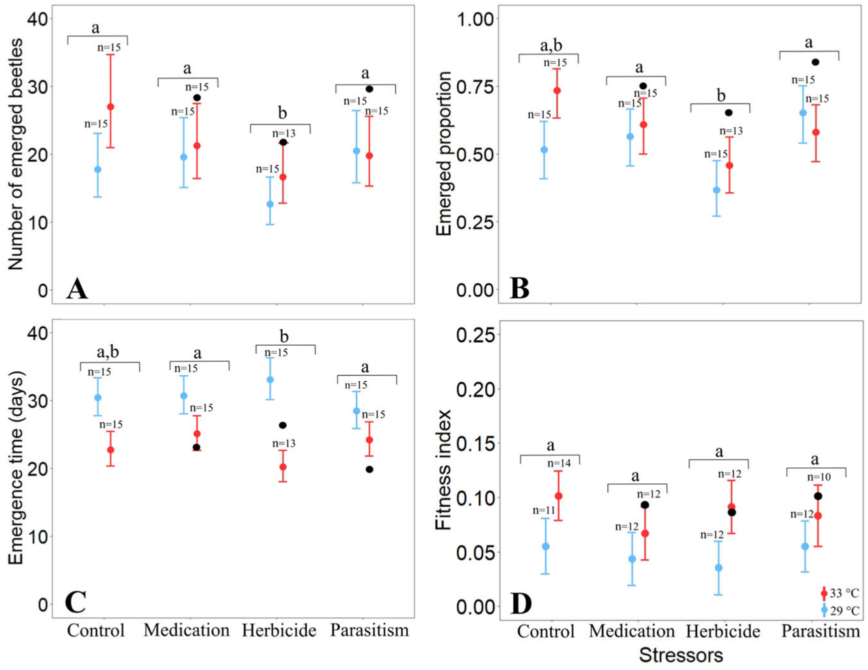

Fig. 2 Effect of different stressors on the number of emerged beetles (**A**), the proportion of masses with emerged adults (**B**), developmental time (days) (**C**) and fitness index (**D**), at different temperatures of beetles *E. intermedius*. N = number of replicates in each treatment. The points represent the means and the lines the 95% confidence intervals. Letters indicate significant differences between treatments. Black dots indicate additive effect calculated from independent action models

Table 2 Effects of different stressors on the survival of emerged *E. intermedius* beetles under controlled laboratory conditions

	Survival Coxme regression			Female survival Coxme regression			Male survival Coxme regression		
	χ^2	*df*	*p*	χ^2	*df*	*p*	χ^2	*df*	*p*
Temp	1.86	1	0.172	2.055	1	0.152	0.483	1	0.487
Treat	14.34	3	**0.003**	6.657	3	0.084	19.723	1	**0.0001**
Sex	0.75	1	0.388	NA	NA	NA	NA	NA	NA
Temp × Treat	2.86	3	0.413	7.518	3	0.057	NS	NS	NS
Temp × Sex	0.76	1	0.384	NA	NA	NA	NA	NA	NA
Treat × Sex	9.82	3	**0.020**	NA	NA	NA	NA	NA	NA
Temp × Treat × Sex	9.73	3	**0.021**	NA	NA	NA	NA	NA	NA

Contrasts between treatments are described in the supplementary material. Significant values after false discovery rate correction are shown in bold

df degrees of freedom, *NS* non-significant interaction, *NA* factor not tested

(temperature effect: $\chi^2 = 3.78$, p = 0.051 for the number of emerged beetles and $\chi^2 = 3.87$, p = 0.049 for the proportion of emerged beetles; Tables 1, A1, A2; Fig. 2A, B), and decreased with exposure to stressors (stressor treatment effect: $\chi^2 = 10.90$, p = 0.012 for the number of emerged beetles; $\chi^2 = 20.843$, $p < 0.0001$ for the proportion of emerged beetles; Tables 1, A1, A2, A5, A6; Fig. 2A, B), particularly with exposure to herbicide treatment (contrast herbicide vs. control treatment: $z = 3.116$, p = 0.001 for the number and $t = 4.773$, p < 0.001 for the proportion emerged; Table A6). According to the IA model, the combination of heat and the other stressors had synergistic negative effects on the number and proportion of emerged beetles (Fig. 2A, B).

The sex ratio of the emerged adults was not affected by temperature ($\chi^2_1 = 0.001$, $p = 0.980$) or stressor treatment ($\chi^2 = 3.023$, p = 0.388; Tables 1, A2). However, emergence time of *E. intermedius* decreased at 33 °C compared to 29 °C (temperature effect: F = 70.52, p < 0.001), but the temperature effect depended on the specific stressor (temperature × stressor treatment: F = 4.42, p = 0.005; Tables 1, A7; Fig. 2C), with such reduction being particularly notable in the herbicide treatment (contrast herbicide vs. control treatment: $t = 2.005$, p = 0.047; Table A7; Fig. 2). According to the IA model on emergence time, the heat had an additive effect when combined with medication, a synergistic effect when combined with herbicide and an antagonistic effect when combined with parasitism (Fig. 2C).

Regarding the survival of emerged adults under starvation conditions, a significant effect of the interaction temperature × stressor treatment × sex was found ($\chi^2 = 9.73$, p = 0.021, Tables 2, A3; Fig. 3), so both sexes were

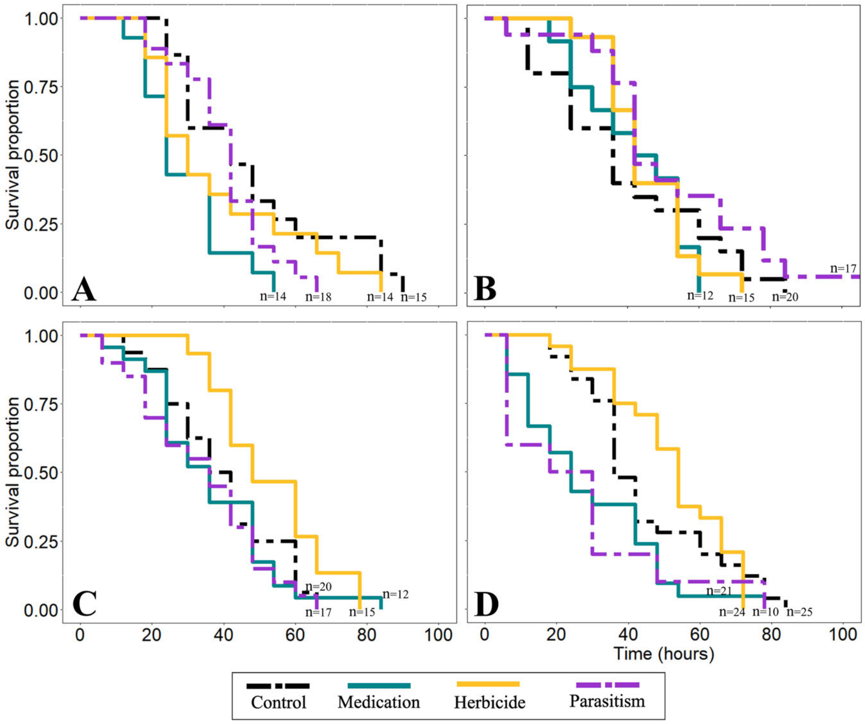

Fig. 3 Effect of different stressors on the survival of emerged beetles of the species *E. intermedius* under starvation conditions in captivity. Top panels: females at 29 °C (**A**) and 33 °C (**B**); bottom panels: males at 29 °C (**C**) and 33 °C (**D**). N = number of beetles exposed to starvation in each treatment. The results of the independent action model are shown in Table A2

analyzed separately. Female survival was not affected by temperature ($\chi^2 = 2.055$, $p = 0.152$), stressor treatment ($\chi^2 = 6.657$, $p = 0.084$), or the temperature × stressor treatment interaction ($\chi^2 = 7.518$, $p = 0.057$; Tables 2, A3; Fig. 3A,B). Conversely, male survival was not affected by temperature ($\chi^2 = 0.483$, $p = 0.487$), but it was affected by stressor treatment ($\chi^2 = 19.723$, $p < 0.0001$; Tables 2, A3; Fig. 3C,D). Compared to the control group, both medication ($z = 2.17$, $p = 0.030$) and parasitism ($z = 2.35$, $p = 0.019$) decreased male survival (from 42 to 32 and 31 h, respectively; Tables A1 and A8). According to the IA model on female survival, high temperature combined with other stressors caused antagonistic effects (Table A4). For male survival, the IA model showed that high temperature combined with medication and parasitism had synergistic effects, while the effect of the combination with herbicide was additive (Table A3).

The fitness index was higher at 33 °C than at 29 °C ($\chi^2 = 19.234$, $p < 0.001$) but did not change with stressor treatment ($\chi^2 = 3.911$, $p = 0.271$; Tables 1, A2; Fig. 2D). According to the IA model on the fitness index, high temperature had only additive effects with the stressor treatments (Fig. 2D).

Discussion

In a changing and warming world, organisms are increasingly being subjected to multiple, intense, and novel stressors (Gunderson et al. 2016; Verheyen et al. 2019: Siviter et al. 2021). How multiple stressors interact, and affect biologically and/or economically important insects, remains largely unknown. Importantly, the negative effects of stressors can be potentiated synergistically, meaning that multiple stressors can affect organisms more strongly together than expected by the simple addition of their effects (Noyes et al. 2009; Verheyen et al. 2019; Verdú et al. 2020). Here, high temperature (33 °C) increased the proportion and (marginally) the number of offspring produced by *E. intermedius* dung beetles. However, high temperature also potentiated the negative effect of ivermectin (medication treatment), herbicide, and parasitism, synergistically reducing the proportion of emerged offspring and increasing mortality of adult males. Furthermore, heat combined with an herbicide synergistically accelerated larval development. Finally, heat increased a global measure of fitness. These results confirm that a four °C increase in temperature can enhance the effect of other stressors, affecting these insects with important functions in natural and productive ecosystems.

In this study, heat synergistically increased the negative effect of ivermectin and parasitism on the survival of adult offspring. A similar effect was observed in the dung fly, *Scathophaga stercoraria* exposed to ivermectin and high temperature, which suffered a synergistic reduction in survival (González-Tokman et al. 2022). Synergistic effects in survival caused by ivermectin and parasitism might result from trade-offs in the use of the energy between body maintenance and reproduction (Rantala et al. 2000; Villada-Bedoya et al. 2020; González-Tokman et al. 2022). Here, only male survival was affected, while even antagonistic effects between high temperature and parasitism were observed in females. These contrasting responses indicate that males and females use different strategies to deal with environmental stress, with males typically favoring reproduction and females prioritizing self-maintenance (Villada-Bedoya et al. 2020; Córdoba-Aguilar and Munguía-Steyer 2013). Transcriptomic analyses of stressed male and female insects could shed light on the physiological mechanisms explaining sex differences in response to multiple stressors.

As predicted (Atkinson 1994), heat accelerated larval development, an effect that was enhanced synergistically by the presence of the herbicide. Faster emergence may increase the possibilities of having more reproductive events per year, which can be beneficial in some cases, such as more variable and less predictable environments (i.e., a bet-hedging strategy). Nevertheless, earlier emergence might bring fitness costs caused by mismatches between animals and their resources (Trakimas et al. 2019; Buckley et al. 2017). Future experimental studies in natural dung beetle populations from cattle pastures could evaluate the costs and benefits of emerging earlier, and whether these costs and benefits co-vary with specific ecological traits such as dung quality, which is highly dependent on cattle diet (Heddle et al. 2024). Besides changes in emergence time, behavioral responses could contribute to dealing with high temperature and other stressors. For example, modifying the depth of burial of nest masses (Mamantov and Sheldon 2020), or moving to cooler places (Sunday et al. 2014; González-Tokman et al. 2020a) are potential behavioral responses contributing to dung beetle adaptation to novel conditions that could accompany physiological adaptations and should be experimentally evaluated in the future.

The results of this study show contrasting responses in survival between adult emerged females (antagonism) and males (synergies) exposed to different combinations of stressors. This is likely caused by genetic differences between the sexes (Xirocostas et al. 2020), which translate into sex-specific morphological and physiological responses in the face of environmental challenges. Specifically, in *E. intermedius*, ivermectin activates sex-specific damage repair systems under heat, with females activating heat shock proteins, while males using their antioxidant capacity (Villada-Bedoya et al. 2020). Likewise, an immune challenge causes a differential use of energy reserves between male and female *E. intermedius*, where females are the most affected (Servín-Pastor et al. 2021). Activating the stress defense system can have consequences on fertility (Rantala et al. 2000; Reaney and Knell, 2010; González-Tokman et al. 2013; Clint et al. 2018; González-Tokman et al. 2020a), and could bring costs in terms of survival (Sokolova (2013)). The observed differential responses between females and males can also be explained by differences in the concentrations of juvenile hormone (Edwards et al. 1995; Yu et al. 2007; Villanueva et al. 2013; Nijhout and Laub 2018), that is a main developmental and reproduction controller in insects that causes sex-specific effects, such as changes in body size (Stilwell et al. 2010; Goodman and Cusson 2012; Villanueva et al. 2013), or 20-hydroxyecdysone (Bhardwaj et al. 2017), which have different functions in adult insects of both sexes. Future studies in males and females exposed to different stressors could clarify the physiological mechanisms responsible for the differential responses between sexes. More broadly, different responses between the sexes to similar ecological and climatic pressures can modify population dynamics and life history traits, such as operational sex ratios, sexual selection, and parental care evolution.

For other fitness-related variables in *E. intermedius*, no effects of heat were found in combination with other stressors. Females of this species produced the same number of brood balls in all treatments, even when stressors were combined with heat, reflecting a similar reproductive investment by females in this first reproductive event. Whether this reproductive effort under stressful conditions has costs on future reproductive events remains to be tested. Maintaining the same investment in reproductive aspects, even in scenarios where multiple stressors are acting, can result from small amounts of stress stimulating some biological functions, an effect known as hormesis (Guedes and Cutler 2013; Rix et al. 2022). This effect has been observed in dung beetles, that have higher survival rates when exposed to low concentrations of ivermectin (Manning and Cutler 2020). Future studies could analyze the physiological aspects responsible for hormesis processes in insects facing multiple stressors.

In this study, there were no differences between the sex ratio of emerged beetles exposed to any of the combinations of stressors, reflecting that the eggs and larvae of both sexes have similar sensitivity to the combination of stressors. Although previous studies have shown that the sex ratio for *E. intermedius* does not change because of ivermectin (Cruz et al. 2011; Baena-Díaz et al. 2018), another study found that this substance at certain concentrations causes a decrease in the emergence of females, which would increase competition between males for reproductive partners (González-Tokman et al. 2017b). Future studies could evaluate combined effects of multiple stressors on sex ratios and measure aspects of competition between males or female choice, which may further increase the evidence of the ubiquity of the equal proportion of males and females (i.e., Fisher's principle; Fisher 1930).

Conclusions

Experimental evidence presented here indicates that high temperature increased the negative effects of ivermectin, an herbicide, and parasitism, on life-history and fitness of dung beetles *Euoniticellus intermedius*. These results from laboratory conditions present a pessimistic scenario for dung beetles in cattle pastures, where many factors are acting at the same time while environmental temperatures are rapidly increasing. Evidence from natural conditions in different dung beetle species is needed to identify the conditions associated with cattle farming that are threatening these valuable insects.

Data availability

Data is provided within the supplementary information files.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s10646-024-02825-0.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by AER. The first draft of the manuscript was written by AER and DGT, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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