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The effects of diet and semiochemical exposure on male *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) metabolic rate at a range of temperatures

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ABSTRACT

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is an invasive species that has rapidly spread across the African continent, endangering the security of agricultural industries. The sterile insect technique (SIT) is being investigated as a viable additional pest management tool to suppress *B. dorsalis* populations after its successful implementation in other parts of the world. There is evidence to suggest that pre-release nutritional and semiochemical treatments for sterilised males can enhance their competitive performance against wild type males in SIT programs. This study examined how sterilisation, a diet rich in protein (addition of yeast hydrolysate) or containing semiochemicals (methyl eugenol or eugenol) affected the resting metabolic (RMR) of male *B. dorsalis* at different temperatures (15 – 30 °C), measured using flow-through respirometry. Our results indicated that the negative effect of sterilisation on RMR decreased as temperature increased and that duration of exposure to semiochemicals for 1 to 4 days was not a significant influencing factor on male *B. dorsalis* RMR. Protein-rich diet increased average RMR, but the difference in RMR between dietary groups decreased as temperature increased. Semiochemical feeding reduced the average RMR in male *B. dorsalis*. The difference in RMR between males that consumed semiochemical and those that did not increased with as temperature increased.

1. Introduction

Energy and energetic constraints play a significant role in shaping the life history and behavior of all living organisms. This is because the pace of life of an individual is determined by the rate at which energy and materials are transformed and allocated to maintenance, growth, reproduction, and other processes, and thereby depleted (i.e., the metabolic rate) (Suarez, 2012). By studying variation in resting metabolic rate (RMR), it is possible to gain insight into insect behavior, life history and capacity to withstand stress, with improved performance in these traits sometimes associated with higher fitness (Arnold et al., 2021). Standard metabolic rate is primarily dependent on body size, temperature, and the resultant physiological status of the insect due to feeding or exposure to chemicals in their environment (Brown et al., 2004; Chown & Nicolson, 2004).

Temperature is an abiotic factor that impacts the metabolic rate of poikilothermic ectotherms, like insects (Harrison et al., 2012; Huey & Stevenson, 1979). In biological systems, reactions are generally catalyzed by enzymes, with higher temperatures leading to faster reactions due to a greater fraction of these enzymes and their substrates having the required activation energy (Harrison et al., 2012). The relationship between increase in temperature and metabolic rate remains proportional until the thermal tolerance of the insect is reached, resulting in a rapid decrease in metabolic rate and then death if the temperature continues to increase (Huey & Kingsolver, 1989). The expectation is that the metabolic rate of an ectotherm will double with every 10 °C increase in temperature (i.e., $Q_{10} = 2$) before upper thermal limits are reached (Sterratt, 2015). The Q_{10} value describes the temperature sensitivity of biochemical and physiological processes (Bělehrádek, 1926; Hegarty, 1973; James, 1953) and represents the factor by which a reaction rate increases or decreases with a 10 °C change in temperature. It can be used as a rudimentary means for assessing differences in metabolic rate between insect populations (Block et al., 1994; Nespolo et al., 2003), and different experimental groups (Berrigan & Partridge, 1997; Dingha et al., 2009).

Body mass is a fundamental determinant of metabolic rate (Chown & Nicolson, 2004; Savage et al., 2004). The standard inclusion of body mass in metabolic studies recognises that there is a scaling relationship

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between metabolic rate and body mass (Glazier, 2009) and accounts for some of the resultant variability to improve the resolution of these studies (Fielding & DeFoliart, 2008; Mispagel, 1981). By including temperature and body mass as a variable in studies of metabolic rate it is possible to more accurately determine the effects of other factors that might influence the balance of energy acquisition and expenditure.

As metabolism encompasses all the reactions in cells that supply energy to the body, diet is an important consideration in any metabolic study. Simple sugars and other carbohydrates are either used immediately upon absorption to fuel high energy activities (Gmeinbauer & Crailsheim, 1993) or synthesised into primary energy stores (Klowden, 2007). Increased concentrations of dietary sugars can lead to increases in metabolic rate and survival at high temperatures (Salvucci & Crafts-Brandner, 2000). Protein can be an important component in insect diets, aiding in sexual maturation and increasing locomotive and metabolic activity (Burger et al., 2007; Fricke et al., 2008; Neethu & Ramesh Babu, 2014). However, protein inclusive diets may cause oxidative damage that leads to a decrease in longevity in various insect species (Burger et al., 2007; Malod et al., 2022; Zanco et al., 2021).

Chemicals in the environment can also impact insect metabolism. Exposure to complex compounds in the form of hormone extracts or analogues reliably increase physiological pathways in insects (Teal et al. 2013; Adnan et al., 2020). The use of methoprene (isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate), an insect juvenile hormone analogue, triggers adult reproductive development (Adnan et al., 2020) and increases fecundity often at the cost of longevity (Wang et al., 2015). Juvenile hormone analogues also inhibit oxygen consumption in some insect species (Cotton & Anstee, 1990; VenkatRao et al., 2016). Plant secondary metabolites are another major group of compounds that have diverse effects on insects, including both stimulatory and inhibitory effects on metabolism. The plant alkaloid caffeine increases metabolism in the western honeybee, *Apis mellifera* (L.) (Strachecka et al., 2014). Exposure to caffeine slowed age-related declines in metabolism, which extended life expectancy and reduced the negative effects of Nosema disease (Strachecka et al., 2014). In contrast, nicotine imposes a significant detoxification cost, leading to reduced fitness upon exposure in *Spodoptera eridania* (Lepidoptera: Noctuidae) (Cresswell et al., 1992) and *Tenebrio molitor* (Coleoptera: Tenebrionidae) (Zafeiridou & Theophilidis, 2004). In all these cases, studying the metabolic rate of insects can lead to a better understanding of the effect that environmental factors have on their overall performance.

Respirometry is used to measure the rate of oxygen consumption or carbon dioxide production in organisms, and thereby estimate metabolic rate. Flow-through respirometry is an effective means of measuring metabolic rate in insects due to its direct correlation with energy expenditure, non-invasive nature, real-time measurement capabilities, sensitivity to metabolic variations, and suitability for comparative studies (Lighton & Halsey, 2011; Lizana et al., 2023). This technique uses a continuous flow of air passing through a chamber in which a subject is placed. The outgoing gas is then monitored with sensitive analyzing equipment to measure gases emitted and absorbed by the subject. Flow-through respirometry generates dynamic data that captures fluctuations in metabolic rate. This enables the metabolic cost associated with a specific metabolic state to be determined, as well as the straightforward identification of RMR in contrast with periods of high metabolic activity (Lighton, 2008). The sensitive recording equipment also means that flow-through respirometry works well for estimating the metabolic rate of small species (Lighton, 2008).

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is a highly invasive, quarantine pest species. To limit the economic damage caused by this species to a wide range of soft, fleshy fruit, an integrated pest management approach is needed to suppress the pest population and limit female oviposition. One of the main tactics used to suppress *B. dorsalis* populations in orchards is male annihilation technique (MAT). MAT is a male-specific variant of attract-annihilate, a behavioral control tactic that involves the attraction and targeted killing of insects (Vargas et al., 2014). Some of the most effective attractants used for MAT targeting fruit flies are semiochemicals called phenylpropanoids (Howlett, 1915; Metcalf et al., 1975). These semiochemicals attract males to the plants that produce them (Chuah et al., 1997). Phenylpropanoids attract many tephritid species in the subfamily Dacinae, including in the *Bactrocera* genus (Drew & Hooper, 1981; Fletcher, 1987). Male *B. dorsalis* are strongly attracted to methyl eugenol (4-allyl-1,2-dimethoxy-benzene) (ME), which is found across a wide range of plant orders (Raghu, 2004). It is believed that male *B. dorsalis* are attracted to ME due to mating competitiveness benefits they gain from ingesting it (Haq et al., 2014; Raghu & Clarke, 2003; Shelly et al., 2010; Tan et al., 2011; Tan & Nishida, 1996). Benefits to mating competitiveness can even be conveyed through exposure to ME volatiles (Haq et al., 2018).

Another control tactic that may be used against *B. dorsalis* in some parts of the world is the sterile insect technique (SIT). SIT programs aim to produce and release sterile males in quantities large enough to outcompete wild males for copulations with wild females (Knipling, 1979). The inability to produce sperm does very little to diminish the mating ability of sterilised fruit flies (Radhakrishnan & Taylor, 2007). Unfortunately, factors such as mass rearing or somatic damage caused by sterilization by gamma-irradiation frequently lead to the loss of natural attributes or sexual competitiveness of sterile males (Guerfali et al., 2011; Lux et al., 2003a; Lux et al., 2003b). To effectively implement the SIT, the mass-reared sterile males need to be able to compete with wild males to ensure they are transferring ejaculate to wild females. Therefore, current methods of SIT implementation rely on high overflooding ratios to overwhelm the wild males.

Pre-release dietary and chemical treatments can effectively enhance the mating competitiveness of sterilised males against wild-type males, thereby reducing overflooding ratios (Adnan et al., 2020; Pérez-Staples et al., 2009). These pre-release treatments could make SIT more successful and therefore more economically viable as a means of fruit fly control. Adult fruit flies require sources of protein and carbohydrates to become sexually mature (Raghu, 2003). In the wild, carbohydrate sources in the form of sugar come from plant exudates, while protein may be acquired through the consumption of phylloplane bacteria (Drew et al., 1983). A protein supplement in the pre-release diet of sterile males provided in the form of yeast hydrolysate also improves male sexual performance (Shelly et al., 2005), increases longevity, and accelerates the rate of sexual maturation (Perez-Staples et al., 2007). A comparison between males fed yeast hydrolysate and those deprived of it revealed that the former had larger ejaculatory ducts and accessory glands (Reyes-Hernández et al., 2019) and more successfully inhibited female remating (Blay & Yuval, 1997; Radhakrishnan et al., 2008). A dietary protein source is clearly an important component to the pre-release treatment of male fruit flies for SIT. For *B. dorsalis*, pre-release exposure to ME as either a dietary supplement (Tan & Nishida, 1996; Tan & Tan, 2013) or aromatherapy treatment (ul Haq et al., 2018) improves sexual competitiveness and leads to sterile males securing more copulations by altering their pheromone profile (Nishida et al., 1988; Raghu, 2003; Tan & Nishida, 1996). However, during semiochemical exposure studies, observations indicate that the benefits for males may extend beyond enhancement of male pheromone attraction to females. Male *B. dorsalis* supplement ed with ME displayed earlier calling behaviour than untreated groups accompanied by an increase in locomotory behaviour (Hee & Tan, 1998). These examples suggest a link between semiochemical exposure and male *B. dorsalis* energetics, which might be associated with an elevated metabolic rate.

The aim of this study was to assess the effect of a protein supplement ed diet, semiochemical exposure and sterilization on RMR of male *B. dorsalis* at a range of functional temperatures using flow-through respirometry. We expected to see a rise in average RMR with increasing temperature, a protein supplemented diet, and with exposure to semiochemicals (ME and EU). Before pursuing the main objective of the study, we evaluated the effect of gamma irradiation on RMR with the

expectation that it would be decreased by irradiation. An investigation to evaluate the appropriate semiochemical exposure time for testing was also conducted at a fixed temperature.

2. Materials and methods

2.1. Culture establishment

Male *B. dorsalis* were reared at the University of Pretoria, Department of Zoology and Entomology. The culture was initially established in 2017 from larvae in infested mangos collected from various location in Mpumalanga province, South Africa. The culture is refreshed annually (after approximately 10 generations) by mating wild-collected males with culture females to introduce wild genetic variability while retaining female acceptance of artificial oviposition substrates. Eggs were collected from culture populations using oviposition substrates constructed from a 50 ml plastic container. A tissue dampened with distilled water and a small piece of fresh guava was placed inside the container. This mimicked the smell of a host fruit and prevented eggs from drying out. The open end of the container was covered in insect screen and laboratory film (Parafilm 38 m × 10 cm, Parafilm M®, Bemis Company) with holes pierced randomly across the surface with an entomological pin. Females in the culture population were allowed to lay eggs for 6–12 h depending on egg laying rate. Once collected, eggs were transferred to a dehydrated carrot-based larval diet developed by Citrus Research International, Mbombela, South Africa, to which water was added (mixed in a ratio of 150 g dry diet in 350 ml of boiling water). The larval diet container was then placed on a layer of sand inside a larger, breathable plastic container. This container was placed in a climate room at 23.5 ± 1.8 °C for eggs to develop and larvae to hatch. The larvae fed on the diet for approximately 2–4 weeks (variability due to unstable climate room temperatures during winter) until larvae began leaving the diet to pupate in the sand. The sand was then sifted, and the retrieved pupae were placed in an adult rearing cage (32.5 × 32.5 × 32.5 cm, BugDorm-4F3030, MegaView Science Co., Taiwan) in the adult rearing room at 23.2 ± 2.7 °C. After approximately 1—2 weeks (with variability depending on season) the adults eclosed, and adult males were separated from the females using an aspirator. Adults used to maintain the colony were provided a diet of sugar and yeast hydrolysate (YH, yeast extract powder, HG00BX6.500, Merck, Wadesville, South Africa) and unlimited access to a source of water (cotton wool soaked in distilled water). *Bactrocera dorsalis* males fed sugar only are understood to perform sub-optimally when compared to sugar and yeast fed males during mating performance studies but will serve as the control group when assessing the effect of dietary protein on RMR in this study.

2.2. Range of test temperatures and times

A temperature range of 15–30 °C was used in this study. This was based on the functional temperatures for *B. dorsalis,* and the average temperatures experienced in the North-Western parts of South Africa where the species is established but subject to control (Kruger & Sekele, 2013; van der Walt & Fitchett, 2021; Van Der Walt & Fitchett, 2022). *Bactrocera dorsalis* can retain developmental and reproductive activity at acute exposure to a temperature range between 7.3 and 45.23 °C (Chen & Ye, 2007; Motswagole et al., 2019), and their optimal temperature range is between 20 and 28 °C (Cai et al., 2023), with fecundity decreasing at sustained temperatures above 28 °C (Choi et al., 2020; Michel et al., 2021).

All measurements of RMR were taken during the day between 08:00 – 16:00. This was to avoid the period of peak mating behavior when males engage in pre-copulatory mating displays involving rapid wing fanning and release of mating pheromones (Shelly & Kaneshiro, 1991), which could be associated with an elevation in metabolic rate. *Bactrocera dorsalis* engages in peak mating at dusk, a behavior strongly influenced by light intensity, with greatest activity observed at 1000–50 lx in lab trials at 26 °C (Ren et al., 2023), and a light intensity of 2000 – 35 lx at temperatures ranging between 28–31 °C in field observations (Chinvinijkul et al., 2015).

2.3. Sterilisation

To sterilise male *B. dorsalis*, pupae at the brown eye color stage, 2 days before adult eclosion (Resilva & Pereira, 2014), were exposed to a gamma radiation dose of 100 Gy from a cobalt 60 source (Gammacell 220, MDS Nordion, Ottawa, Canada) at the National Institute of Communicable Diseases, Johannesburg, South Africa. On adult eclosion, males were separated from females and the applicable dietary treatment was provided. Testing was initially conducted on sterile and fertile males fed sugar and yeast diets at 15, 25 and 30 °C to gauge whether there was any difference in metabolic rate in sterilised and fertile males. To assess the impact of male sterilization on the average RMR within a temperature range of 15, 25 and 30 °C, only males fed a sugar and yeast hydrolysate (YH) diet were used. Sample sizes of n = 10 males were used at each temperature. After this this experiment, only fertile males were used. Fertile males from the same pupal collection were considered the control group and used for assessing the effect of sterilization on RMR in *B. dorsalis*.

2.4. Diets and semiochemical application

Male *B. dorsalis* used for experiments were fed either a diet of sugar-only or a diet of sugar and yeast hydrolysate (YH) as the protein in a ratio of 3:1 (1.58 g:0.79 g). Males had unrestricted access to diet and water, replenished as needed. Consumption of the diet including the semiochemicals was likely not continuous over the period of exposure. For each male treatment group that was exposed to semiochemicals, 15 µl (1.25 % of volumetric proportion to diet) of methyl eugenol (247502; Sigma Aldrich.) or eugenol (E51791; Sigma Aldrich.) was thoroughly mixed into a portion of diet. The chosen semiochemical concentration in the diet was based on findings from another study (Pogue, Malod and Weldon, unpublished data). Eugenol (EU) was included in testing because it is the base compound for methyl eugenol (ME) synthesis in plants (Herrmann & Weaver, 1999; Sastrohamidjojo & Fariyatun, 2016) and because it performed similarly to ME during a mortality and lure attraction study (Pogue, Malod and Weldon, unpublished data)..

The effect of semiochemical exposure duration on male *B. dorsalis* metabolic rate was tested by exposing flies to a sugar-only diet containing a selected semiochemical, ME or EU, for either four, two or one day(s). Twenty 8-day old males were given a semiochemical enriched diet for 4 days, twenty 10-day old males were given the semiochemical enriched diet for 2 days and twenty 11-day old males were given the semiochemical enriched diet for one day. Respirometry measurements were conducted on these males at 14 days after eclosion with the semiochemical diet removed two days before testing to prevent cross-contamination during transport from the University of Pretoria to the University of the Witwatersrand. Based on the results from these measurements at 25 °C, a 4-day exposure was chosen for the metabolic measurements at different temperatures as this group exhibited the highest average RMR. Adults from both dietary treatment groups that did not consume semiochemicals were designated as the control groups. The effects of semiochemical consumption on RMR was assessed by comparing data from other treatment groups with that of the control group.

2.5. Measurement of metabolic rate

Resting metabolic rates (RMR) of treated and untreated males were estimated based on carbon dioxide (CO_2) emission measured with flow-through respirometry using equipment at the University of Witwatersrand. An aquarium air pump (APS300 Air Pump, Tetra®, Melle, Germany) generated air flow for the experiments. The air was scrubbed

of moisture and CO_2 by passing it through columns of calcium sulfate (10–20 mesh, indicating Drierite) and soda lime (2–5 mm pellets with indicator, Sigma Aldrich,). The flow rate was monitored and maintained at 100 SCCM using a flow meter (MC–Series: Gas Mass Flow Controllers, Alicat Scientific, Tuczon, AZ, USA). This air was then pushed into a multiplexor (Sable Systems International, North Las Vegas, NV, USA) where seven channels were used for fly respiratory measurements. The multiplexor was housed in an incubator so that the ambient temperature could be regulated. A universal interface (UI2, Sable Systems International) was used to control airflow through the different multiplexor channels. Expedata (version 1.9.27, Sable Systems International) was used to program the interface and collect data. The CO_2 levels were recorded using a CO_2/H_2O gas analyzer (model LI-6262, LI-COR, Lincoln, Nebraska, USA). Channels 1–6 in the multiplexor contained chambers (4 cm^3) into which individual males to be measured were placed. Channel 7 had an empty chamber and was used as a baseline for gas concentration measurements. This baseline provided a reference point for calibrating the respirometry system and to correct for unavoidable instrument drift. Recording cycles began with 300 s of baseline recording followed by a five-minute purge of the channel one test chamber. Following a 20-minute CO_2 level recording in Channel 1, a five-minute purge of Channel 2 preceded another 20-minute CO_2 level recording in the next channel, in this case Channel 2. This cycle was repeated until all 6 chambers containing flies had been tested. Carbon dioxide emission was determined at 25°C as well as a range of lower and higher temperatures (15, 20, 30 °C). Body mass of each tested fly was recorded before being placed in the respirometry chamber using a fine balance scale (accuracy 0.01 mg; Libror AEG-45SM, Shimadzu, Kyoto, Japan). The RMR of twenty fertile males from each diet were measured as the control group at various temperatures. Additionally, twenty males from each dietary group, exposed to both semiochemicals (ME and EU), were also measured.

2.6. Data manipulation and analyses

Expedata analysis software (Sable Systems), was used to transform the data. CO_2 output was converted from parts per million to μlh^{-1} (V_{CO2}) using the equations described by Lighton (2019). The baseline values were used to zero the data and correct for analyzer drift. The average RMR for each test was determined using the most stable section of the 20 min recording. Movement causes deviations from the specimens RMR. Therefore, each CO_2 emission trace was inspected to ensure that the respiratory pattern was constant. Metabolic data was reported as volume CO_2 (μl) per body mass (g) over a period of an hour (h) [$\mu lCO_2\ g^{-1}h^{-1}$]. Data are reported as means and standard error unless otherwise indicated.

The Q_{10} effect was used to evaluate the difference in RMR of male *B. dorsalis* based on dietary and semiochemical treatments. The Q_{10} value was calculated using:

$$Q_{10} = (R2/R1)^{(10/(T2-T1))}$$

Where Q_{10} is the temperature coefficient. T1 is the initial temperature, and T2 is the final temperature. R1 is the RMR ($\mu lCO_2\ g^{-1}h^{-1}$) at temperature T1, and R2 is the RMR ($\mu lCO_2\ g^{-1}h^{-1}$) at temperature T2.

A Shapiro-Wilk test indicated that the RMR data from male sterilization testing were not normally distributed ($W = 0.932$, $P = 0.002$). A log transformation was applied to these data before further analysis. A generalised linear model (GLM) with a Gaussian distribution was used to model the data. The model with the minimum adequate fit, determined through evaluation of residual variance and AIC value, was chosen. We then ran an analysis of variance (ANOVA) followed by Tukey's post-hoc tests to assess the effect of the explanatory variables and the differences between fertile and sterile male treatment groups.

The final resting metabolic rates from exposure testing were normally distributed based on the Shapiro-Wilk test ($W = 0.981$, $P = 0.090$). A GLM with Gamma distribution was used to model this data, before using an ANOVA to assess the effect of the explanatory variables. The model with the minimum adequate fit was determined by evaluating both residual variance and AIC value. Tukey's post-hoc tests were then used to quantify any differences between 1, 2 and 4-day exposure treatment groups and to see if there was any difference based on semiochemical.

The next stage of analysis was to assess the effects of temperature, diet and semiochemical consumption on average RMR of male *B. dorsalis*. The data were not normally distributed, based on a Shapiro-Wilk test ($W = 0.935$, $P < 0.001$) with a right-skew. To analyze the effects of diet, semiochemical consumption, temperatures, date of testing, and the significant interactions between these explanatory variables on the average resting metabolic rate, a GLM with an inverse Gaussian distribution was used to model the data. Assessing both residual variance and AIC value led to the choice of the model with the minimum adequate fit. An ANOVA was used to assess the significance of each variables contribution to the resting metabolic rate, and a post-hoc test was used to assess the difference between treatment groups.

All statistical analyses were done using R (version 4.3.2 (Eye Holes)) on R-Studio (version 2023.09.1 + 494).

3. Results

Inspection of the CO_2 output traces for *B. dorsalis* males showed continuous gaseous exchange under test conditions, with no evidence of discontinuous gaseous exchange patterns. Thus, all results were interpreted as continuous gaseous exchange recordings with peaks in output representing periods of insect activity.

3.1. Temperature

Temperature significantly affected metabolic rate across all stages of testing where more than one temperature was included in an experiment (Table 1). The Q_{10} effect was calculated from the average change in RMR between 15 °C and 20 °C, yielding a value of $Q_{10} = 2.1$, indicating that RMR doubles for every 10 °C increase in temperature. The Q_{10} trendline, extrapolated from this subset, aligns with the regression line based on the full dataset (Fig. 1). This helped to validate the use of respirometry to assess the metabolic rate of male *B. dorsalis*. When calculating Q_{10} values for the diet treatments, the protein-deprived flies had a higher Q_{10} value ($Q_{10} = 2.4$) than that calculated for flies receiving a protein-rich diet ($Q_{10} = 1.8$). The relationship between temperature and diet was confirmed in later GLM/ANOVA analyses.

3.2. Sterilisation

An analysis of the impact of sterilization, temperature, mass, and the interactions between temperature and mass, and temperature and sterilization on the average RMR showed that, sterilization ($\chi^2 = 9.016$, df = 1, $P = 0.003$), temperature ($\chi^2 = 21.338$, df = 2, $P < 0.001$) and mass ($\chi^2 = 5.772$, df = 1, $p = 0.016$) significantly influenced resting metabolic rate. The interaction between temperature and mass was also significant ($\chi^2 = 7.953$, df = 2, $P = 0.019$), while the interaction between temperature and fertility did not influence the average RMR significantly

Table 1
Mean and standard error of resting metabolic rate (RMR) for male *B. dorsalis*, fed only sugar and exposed to different environmental temperatures (15, 20, 25 and 30 °C).

Temperature (°C)	RMR ($\mu lCO_2.g^{-1}.h^{-1}$)	Mass (mg)
15	339.74 ± 87.37	15.6 ± 3.3
20	499.94 ± 113.04	15.3 ± 2.9
25	762.29 ± 170.93	15.9 ± 3.2
30	1088.91 ± 275.19	15.0 ± 3.0

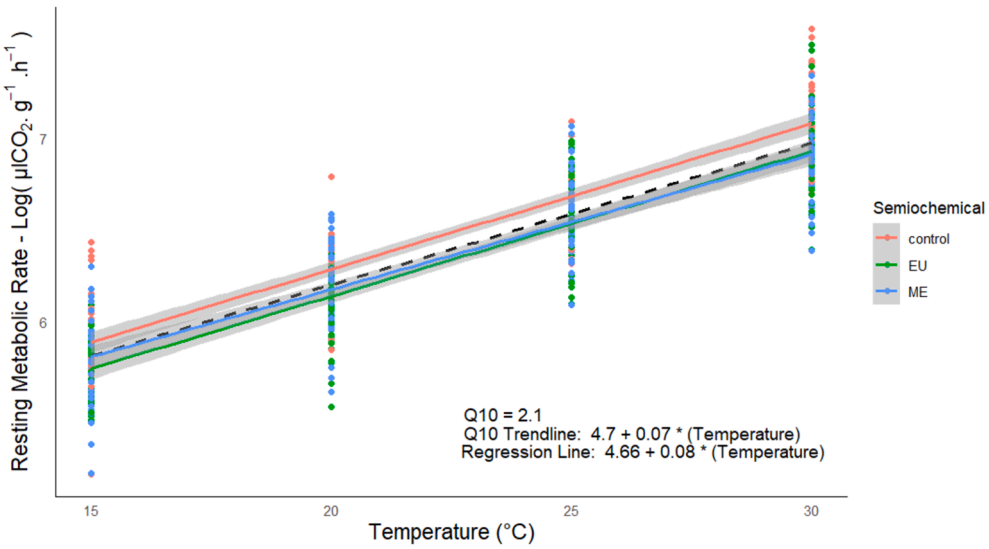

Fig. 1. Log-transformed RMR rate of male *B. dorsalis* as a function of temperature, with points coloured by semiochemical group. The black dashed line represents the Q10 trendline, and solid lines show regression trends for each semiochemical group, with shaded standard errors areas. The Q10 value and Q10 trendline equation are calculated from the change in metabolic rate between 15 °C and 20 °C, and regression equation is based the full dataset.

($\chi^2 = 4.820$, df = 2, P = 0.090). A post-hoc test revealed that, although sterility did negatively affect the RMR of male *B. dorsalis* across all temperatures (Sterile-Fertile; mean diff = -0.096, P = 0.011), there was no significant difference between average resting metabolic rates at 25 °C (Sterile:25-Fertile:25; mean diff = -0.026, P = 0.998) and 30 °C (Sterile:30-Fertile:30; mean diff = -0.045, P = 0.979) (Table 2). To determine the significance of fertility at 15 °C, we applied mass as a random variable and assessed the impact of fertility on RMR independently. The analysis showed that even when the variability of mass was considered the influence of sterility was still significant ($\chi^2 = 5.558$, df = 1, P = 0.018).

3.3. Exposure duration

The effect of duration of exposure to semiochemicals on RMR at a fixed temperature and with a sugar only diet is shown in Table 3. The semiochemical exposure duration ($\chi^2 = 33.538$, df = 2, P < 0.001), semiochemical ($\chi^2 = 6.975$, df = 1, P = 0.008), and mass ($\chi^2 = 5.359$, df = 1, P = 0.021) significantly affected the RMR measured from male *B. dorsalis*. The effect of the interaction between exposure duration and semiochemical was also significant ($\chi^2 = 10.499$, df = 1, P = 0.033). The post-hoc test showed that the results from 4 days of exposure were significantly different from exposure for 1 day (4–1; mean diff = 0.192, P < 0.001) and 2-days (4–2; mean diff = 0.270, P < 0.001). Based on these findings we chose to use 4 days of exposure for the rest of semiochemical consumption testing. The difference between ME and EU was significant when analysing the variance across all exposure periods (ME-EU; mean diff = 0.121, P < 0.001). However, the ME and EU groups only showed significantly difference in their average RMR over the same duration of exposure at 2-days exposure (ME:2-EU:2; mean diff = 129.89 129.895, P = 0.022) (Fig. 2, Table 3) .

Table 2
Mean (± SE) of resting metabolic rate (RMR) measured at 15, 25 and 30 °C for sterilised and fertile male *B. dorsalis* fed a sugar and yeast diet. A Tukey's post-hoc test was performed to comparing the RMR of sterile and fertile males at each temperature.

Temperature (°C)	Sterile metabolic rate (µl CO^2 g^{-1} h^{-1})	mass (mg)	Fertile metabolic rate (µl CO^2 g^{-1} h^{-1})	mass (mg)	P
15	339.58 ± 44.06^{a}	15.7 ± 1.7	392.80 ± 52.37^{b}	17.5 ± 3.0	0.015
25	814.74 ± 218.61^{c}	17.4 ± 2.2	839.70 ± 145.18^{c}	17.0 ± 1.3	0.998
30	1154.61 ± 93.09^{d}	17.8 ± 1.2	1288.57 ± 242.87^{d}	16.6 ± 2.4	0.979

Table 3
Mean (± SE) of resting metabolic rate (RMR) at 25 °C for male *B. dorsalis* fed a diet of sugar only and with dietary exposure to methyl eugenol or eugenol for different durations (1, 2, and 4 days). A Tukey's post-hoc test was performed to compare the RMR of ME or EU exposed males at 1, 2 and 4 days of exposure.

Exposure time (days)	Methyl eugenol metabolic rate (µl CO^2 g^{-1} h^{-1})	mass (mg)	Eugenol metabolic rate (µl CO^2 g^{-1} h^{-1})	mass (mg)	P
1	765.11 ± 167.33^{ab}	19.5 ± 2.9	663.55 ± 102.63^{bc}	18.9 ± 1.3	0.052
2	725.32 ± 122.37^{a}	18.8 ± 2.2	595.42 ± 103.95^{ab}	20.3 ± 2.3	0.005
4	871.61 ± 132.14^{cd}	14.7 ± 3.3	845.36 ± 133.02^{d}	17.5 ± 2.7	0.999

3.4. Diet and semiochemical consumption

Table 4 shows the results of the effect of temperature, diet and semiochemical consumption on RMR. The variables that significantly affected the RMR of *B. dorsalis* males were diet ($\chi^2 = 29.65$, df = 1, P < 0.001), temperature ($\chi^2 = 696.33$, df = 3, P < 0.001), and date-of-testing ($\chi^2 = 61.51$, df = 1, P < 0.001). The effect of semiochemical consumption alone was not significant in the model for RMR ($\chi^2 = 1.06$, df = 2, P = 0.589). However, the interaction between temperature and diet ($\chi^2 = 12.02$, df = 3, P = 0.007) and between temperature and semiochemical consumption ($\chi^2 = 15.19$, df = 6, P = 0.019) were both significant. The interaction between diet and semiochemical was not significant and was not included in the minimal adequate model. The model with the minimum adequate fit was determined through evaluation of residual variance and AIC value. A post-hoc test comparing treatment groups based on either diet or semiochemical consumption showed that flies fed only sugar had lower resting metabolic rates than flies fed a sugar and YH (Sugar and YH-Sugar; mean diff = 0.138, P <

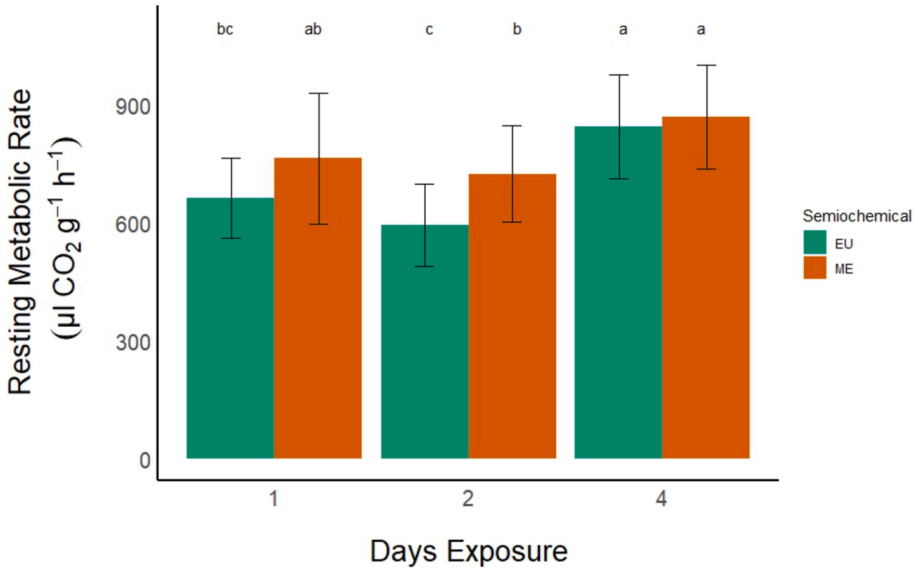

Fig. 2. RMR of male *B. dorsalis* exposed to different semiochemical for different durations. Error bars show ± 1SE. Groups labelled with the same lowercase letter are not significantly different (Tukey tests: $p > 0.05$).

Table 4
Mean (± SE) of resting metabolic rate (RMR) for male *B. dorsalis* exposure to varied environmental temperatures (15, 20, 25 and 30 °C), varied diets [sugar, sugar and yeast hydrolysate (YH)] and exposed to two semiochemicals (methyl eugenol, eugenol) for a period of 4 days.

Temperature (°C)			15	20	25	30
Control	Sugar	RMR (µl CO_2 $g^{-1}h^{-1}$)	345.60 ± 84.07	535.15 ± 101.62	767.46 ± 179.28	1249.49 ± 300.26
		Mass (mg)	13.0 ± 2.0	14.2 ± 2.4	13.6 ± 1.4	12.8 ± 2.9
	Sugar and YH	RMR (µl CO_2 $g^{-1}h^{-1}$)	409.68 ± 92.88	549.22 ± 126.44	836.36 ± 132.45	1224.65 ± 264.06
		Mass (mg)	16.3 ± 3.3	17.1 ± 2.1	17.7 ± 1.4	16.5 ± 2.7
Methyl eugenol (1.25 %)	Sugar	RMR (µl CO_2 $g^{-1}h^{-1}$)	287.86 ± 53.73	451.81 ± 113.00	671.88 ± 148.07	971.42 ± 155.50
		Mass (mg)	13.4 ± 2.5	14.7 ± 2.0	15.2 ± 1.9	14.4 ± 1.9
	Sugar and YH	RMR (µl CO_2 $g^{-1}h^{-1}$)	351.19 ± 106.74	582.07 ± 83.23	806.83 ± 166.48	1025.09 ± 254.55
		Mass (mg)	16.9 ± 3.3	14.3 ± 2.2	16.0 ± 3.9	17.2 ± 2.6
Eugenol (1.25 %)	Sugar	RMR (µl CO_2 $g^{-1}h^{-1}$)	306.01 ± 62.40	419.04 ± 86.02	667.77 ± 175.99	968.67 ± 266.16
		Mass (mg)	16.1 ± 3.5	14.1 ± 3.7	14.9 ± 4.4	12.6 ± 1.9
	Sugar and YH	RMR (µl CO_2 $g^{-1}h^{-1}$)	338.08 ± 67.68	462.36 ± 69.63	823.46 ± 161.02	1121.20 ± 272.25
		Mass (mg)	17.8 ± 2.4	17.1 ± 2.9	17.7 ± 2.9	16.4 ± 2.3

0.001), and male *B. dorsalis* not exposed to semiochemicals had a higher average RMR than those exposed to ME (ME-control; mean diff = -0.125, $P < 0.001$) or EU (EU-control; mean diff = -0.148, $P < 0.001$). The average RMR of male *B. dorsalis* exposed to EU or ME were not significantly different (ME-EU; mean diff = 0.023, P = 0.559). When assessing the interaction effect between temperature and semiochemical consumption on average RMR of treatment groups using a post-hoc analysis, results showed that at 15 °C (15: EU − 15: control; mean diff = -50.004, P = 0.973; 15: ME − 15: control; mean diff = -48.436, P = 0.979; 15: ME − 15: EU; mean diff = 1.568, P = 1) and 25 °C (25: EU − 25: control; mean diff = -56.292, P = 0.938; 25: ME − 25: control; mean diff = -62.557, P = 0.878; 25: ME − 25: EU; mean diff = -6.264, P = 1) there was no significant difference in RMR between treatment groups. This test also showed that at 30 °C there was a significant difference between semiochemical exposed males and the control group (30: EU − 30: control; mean diff = -192.134, $P < 0.001$; 30: ME − 30: control; mean diff = -252.332, $P < 0.001$), but there was no significant difference in RMR of males exposed to either semiochemical (30: ME − 30: EU; mean diff = -60.198, P = 0.903) (Fig. 3). The post-hoc test comparing treatment groups based on the interaction between diet and temperature showed that RMR was not significantly affected by diet at 30 °C (30: Sugar and YH − 30: Sugar; mean diff = 69.468, P = 0.347) (Fig. 4).

3.5. Date-of-testing

Date-of-testing was a significant explanatory variable when analyzing the effect of diet, semiochemical exposure and temperature on average resting metabolic rate. A seasonal pattern in adult mass was observed in our data as shown by the significant difference between the mass of male *B. dorsalis* tested in the months of July and October. Research has shown that there is a strong correlation between the mass fluctuations in artificially reared Tephritidae and wild type populations across seasons (Dominiak et al., 2021) (Oct-Jul; mean diff = $3.361311e^{-03}$, $P < 0.001$) (Fig. 5A, B). This fluctuation has been attributed to exposure to natural light (Deece et al., 2000) and slight temperature and humidity variations in rearing facilities (Fanson et al., 2014). Even though mass was accounted for as part of our equation to calculate metabolic rate ($\mu l CO_2\ g^{-1}h^{-1}$), the seasonal mass fluctuation had an influence on results (date-of-testing: $\chi^2 = 61.51$, df = 1, $P < 0.001$)).

4. Discussion

This study investigated the relationship between sterilization, semiochemical consumption, diet composition, and temperature on the

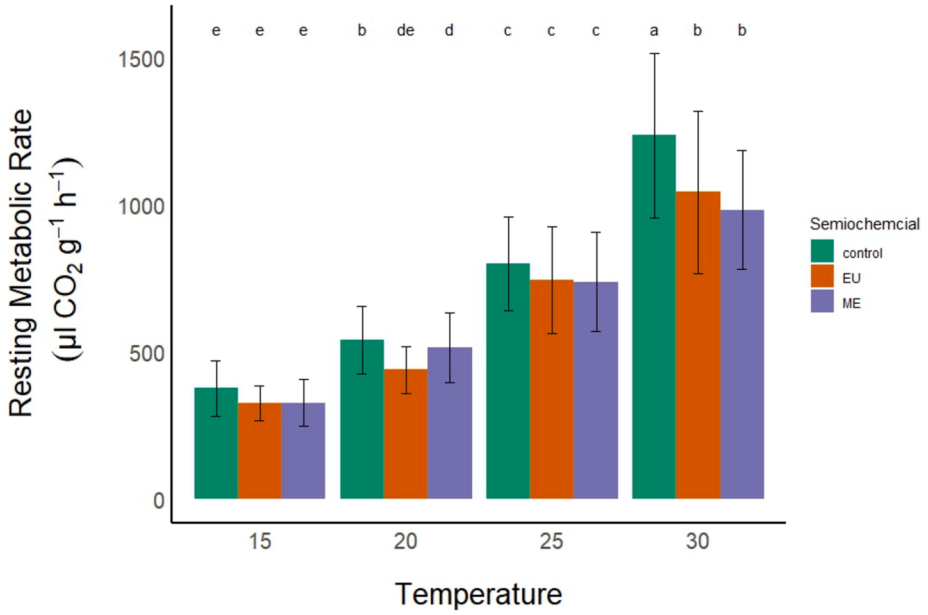

Fig. 3. RMR of male *B. dorsalis* exposed to different semiochemicals [methyl eugenol (ME), eugenol (EU)] and those exposed to no semiochemicals (control), measured at different temperatures (15, 20, 25, and 30 °C). Error bars show ± 1 SE. Groups labelled with the same lowercase letter are not significantly different (Tukey tests: $p > 0.05$).

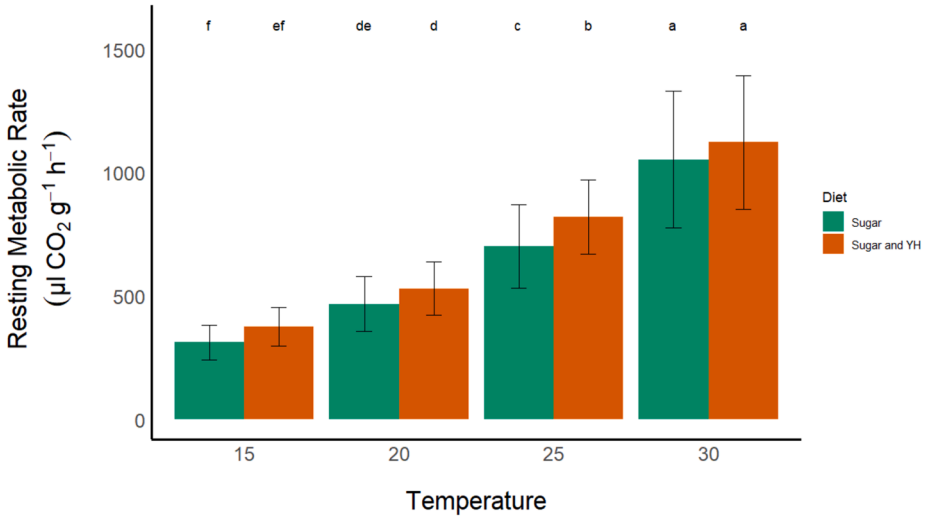

Fig. 4. RMR of male *B. dorsalis* fed different diets, sugar, and sugar and YH, measured at different temperatures (15, 20, 25, and 30 °C). Error bars show ± 1 SE. Groups labelled with the same lowercase letter are not significantly different (Tukey tests: $p > 0.05$).

RMR of male *B. dorsalis*. The average RMR recorded at 25 °C for the control group flies fed a 3:1 ratio of sugar and yeast hydrolysate (YH) (836.36 ± 132.45) was consistent with the rates recorded for male *Ceratitis capitata* (815.29 ± 671.46) fed the same diet and tested at 25 °C (Bosua et al., 2023). Although the genus *Bactrocera* and *Ceratitis* are not closely related within the family Tephritidae (Fernández et al. 2002), *B. dorsalis* does share an overlapping distribution in its invasive range (Malacrida et al. 2007; Qin et al. 2019) and host range (Pieterse et al. 2017) with *C. capitata*. This suggest that the species share similar metabolic responses to external stressors from their environment.

Our results indicated that sterilisation has no significant impact on the RMR of *B. dorsalis* at temperatures between 25 to 30 °C, but at 15 °C fertile flies have a higher metabolic rate. RMR was highly responsive to changes in temperature, $Q_{10} = 2$ at the range of 15 to 30 °C regardless of diet and semiochemical consumption. This is consistent with Q_{10} values recorded for other insect species (Chown et al., 1997; Cohen & Cohen, 1981; Cooper, 1993). At all temperatures, the RMR after 4-day exposure to ME or EU was similar, with the highest average RMR observed in males exposed to semiochemical at 4-days of exposure. The addition of yeast hydrolysate (YH) to male *B. dorsalis* diet did not significantly affect the average RMR of male *B. dorsalis* when compared at each temperature. However, the addition of yeast hydrolysate (YH) to male *B. dorsalis* diet did significantly increase the average RMR when considering the whole range of tested temperatures. Semiochemical consumption was shown to negatively affect average RMR across all temperatures, this relationship was more significant at temperatures at 25 and 30 °C.

The most important aspect of this study was to investigate the effect of pre-release treatments, specifically that of diet and semiochemical consumption on the RMR of male *B. dorsalis*. The inclusion of YH in the adult diet of tephritids can be detrimental to adult survival of acute exposure to high (28 to 52 °C), and low (14 to −10 °C) temperatures shortly after eclosion (Pullock et al., 2023). However, this diet increases

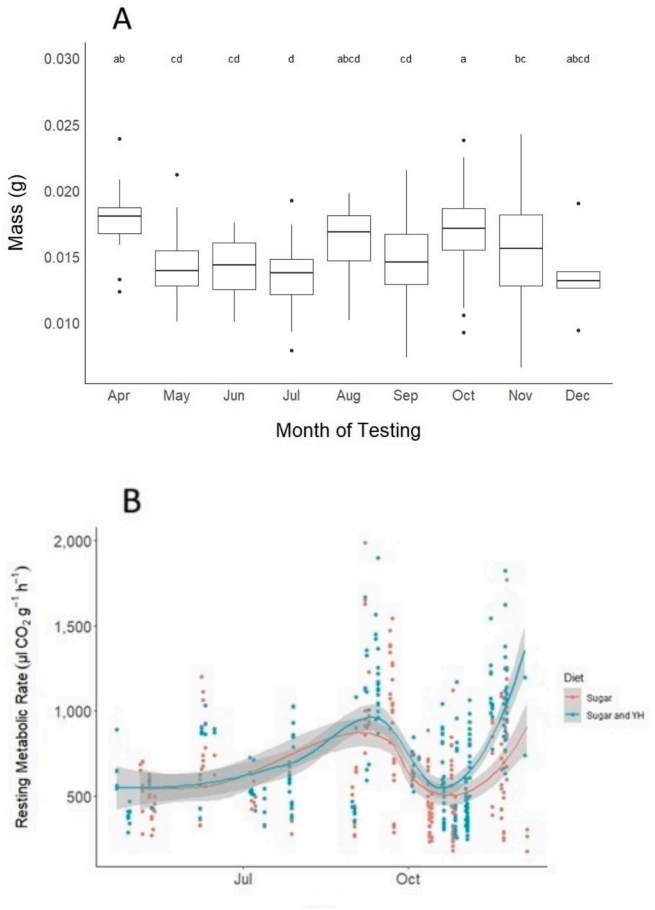

Fig. 5. Data from the semiochemical data set (Table 4) grouped by diet (Sugar, Sugar and YH). (A) Box plot depicting the difference in average mass (g) male *B. dorsalis* for each month of testing. Lowercase letters indicate that they are not significantly different (Tukey tests: $p > 0.05$). (B) Scatter plot with polynomial regression for the RMR recorded over the testing period.

the rate of sexual maturation in Tephritidae species (Pereira et al., 2013; Teal et al., 2013). The negative effects of a protein inclusive diet may be due to a prioritisation of maturation shortly after eclosion. This relationship can be seen in *Drosophila* species, where higher dietary protein increased the rate of post eclosion development (Neethu & Ramesh Babu, 2014) and capacity for energy intensive activities (Burger et al., 2007; Fricke et al., 2008; Neethu & Ramesh Babu, 2014) at the cost of accelerated senescence caused by depleting somatic tissue sterols (Lushchak et al., 2012; Zanco et al., 2021). Having tested the RMR of flies for this study at 14-days after eclosion once they had matured sexually, our results suggest that dietary protein may support optimal performance in male *B. dorsalis* by elevating their metabolic rate. Studies on other Diptera species support the idea that dietary protein plays a role in shaping the timing of male reproductive investment across different ages (Macartney et al., 2017; Papanastasiou et al., 2019). A recent study on *B. dorsalis* has also shown that dietary protein plays a pivotal role in regulated sex pheromone synthesis (Gui et al., 2023), strengthening the link between a protein enriched diet and sexual maturation. Future studies should investigate RMR at different stages of sexual maturation for *B. dorsalis* to assess whether this period was significantly reduced while still providing males with the similar average RMR observed in this study across a range of temperatures.

Semiochemical consumption for four days reduced RMR at high temperatures (25–30 °C). This was unexpected as semiochemical feeding in *B. dorsalis* is associated with increased activity (Hee & Tan, 1998), which should be associated with an elevated metabolic rate. In addition, studies investigating the effectiveness of essential oils as sustainable insecticides for the red flour beetle, (*Tribolium castaneum*) (Herbst) (Coleoptera: Tenebrionidae) show EU to have a high contact toxicity with a 50 % lethal concentration of 0.02 mg/cm^2. Consumption of potentially toxic plant secondary metabolites (e.g., alkaloids like caffeine) and insecticides on insect metabolic rate is associated with increased CO_2 emission (Muñoz et al., 2020). This is presumably associated with the energetic requirements for synthesis and action of detoxification enzymes (Rand et al., 2015). However, it may be that phenylpropanoids like ME play a role in reducing the energetic requirements of cells and tissues, and therefore whole organism metabolic rate. Citronellal, which often contains ME, strongly inhibits ATPases at a half-maximal inhibitory concentration of 9.82 mM (Saad et al., 2019). Studies on the effects of ME on ligand-gated ion channels in human cell cultures have showed that ME is a weak inhibitor of γ-aminobutyric acid type A receptors ($GABA_AR$), a primary molecular target for insecticides in the insect nervous system (Li et al., 2020). Additionally, in an experiment testing the insecticidal effectiveness of basil oil on *B. dorsalis*, males given ME along with basil oil exhibited a significant reduction in toxicity from key basil oil components (Chang et al., 2009), suggesting some unknown role of ME in reducing secondary plant metabolite toxicity. By comparing dietary ME and EU relative to a control, we also found that an extended period of exposure produced the highest resting metabolic rate. It has been shown that exposure to much higher concentrations of ME for shorter periods of exposure improve mating activity (Shelly & Dewire, 1994). However, exposure duration to ME during pre-release treatments does not significantly affect the suppress response to ME baited traps in male *B. dorsalis* (Shelly, 1994). The lack of fundamental knowledge on the physiological effects of ME shows a clear need for more detailed biochemical and gene regulation analysis on how this and other semiochemicals affect metabolic pathways in *B. dorsalis*.

Within the temperature range studied, the metabolic rate of sterilised flies significantly differed from fertile males only at low temperatures. Initially intended as a treatment group throughout the study, sterilised flies were excluded due to the temporary decommissioning of the gamma irradiator at the NICD early in the experiments. Despite this limitation, analysis of our results indicated minimal differences in resting metabolic rates between sterilised and fertile males at higher test temperatures. Gamma radiation impacts rapidly dividing cells (Hallman 2004; Bakri et al., 2005), leaving chromosomes in the somatic cells relatively intact. Nevertheless, sterilised flies are generally observed to exhibit a lower overall quality compared to wild type flies (Barry et al., 2003; Collins et al., 2008). This decline in quality may stem from damage to the midgut tissue and microbiome of the flies (Lauzon & Potter, 2012), resulting in reduced free fatty acid concentrations in irradiated male Tephritidae (Hamza et al., 2016). Furthermore, irradiation has been shown to influence the synthesis of proteins involved in the central energy generation and pheromone synthesis pathways of *B. dorsalis* (Chang et al., 2015). The physiological consequences of damage to these crucial pathways likely contributed to the observed impact on the metabolic rate of male *B. dorsalis* at the lowest test temperature of 15 °C. On the other hand, the lack of significant difference observed at high test temperature (25, 30 °C), may be due to the robust heat tolerance mechanisms of *B. dorsalis*. This species possesses DNA-stabilizing histone modification enzyme genes distributed across multiple chromosomes, which becoming more effective in response to heat with each successive generation acclimated to hot environments (Yang et al., 2023). The study strain originated from semi-arid and sub-tropical highland regions in northeastern South Africa (SAWS, 2023), suggesting acclimatization to high temperatures rather than low temperatures. This could partially explain the reduced impact of sterilization on metabolic rates at higher temperatures. Future studies should consider the inclusion of sterile flies across all testing stages for a comprehensive understanding.

Despite accounting for differences in mass when calculating resting

metabolic rate, the date-of-testing influenced the analysis of the impact of diet, semiochemical exposure, and temperature. In *B. tryoni*, there is a strong correlation between mass fluctuations in artificially reared flies and wild type populations across seasons (Dominiak et al., 2021). This fluctuation has been attributed to exposure to natural light (Deece et al., 2000) and slight temperature and humidity variations in rearing facilities (Fanson et al., 2014). Previous studies on insect flying metabolic rate (FMR) suggest that metabolites activity scales predictably with body mass ($M^{0.86}$) (Niven & Scharlemann, 2005). However, there is a distinction between large (>10 mg) and small insects (<10 mg), with smaller insects having a four-fold lower FMR on average. Our results suggest that the effect of mass on metabolic rate may not be as simplistic as assumed, at least in *B. dorsalis*.

5. Conclusion

This study aimed to enhance the efficacy of SIT implementation for control of *B. dorsalis,* by measuring the effects of temperature, sterilisation, diet and semiochemical consumption on resting metabolic rate (RMR). With RMR as an established indicator of potential performance in artificially reared male *B. dorsalis*, our results suggest no notable disparity in RMRs between fertile and sterilised males at temperatures ranging from 25 to 30 °C. Male *B. dorsalis* fed a diet containing yeast hydrolysate (YH) exhibited consistently higher RMRs across the temperature spectrum of 15–30 °C. Based on these results, we propose that releasing sterile flies at temperatures exceeding 25 °C, with a pre-release diet including YH could enhance their competitiveness against wild type flies in SIT programs.

CRediT authorship contribution statement

Dylan C. Dacre: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Frances Duncan:** Writing – review & editing, Supervision, Resources, Methodology, Formal analysis. **Christopher W. Weldon:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The experimental data that support the findings of this study are publicly available. This data can be found here: 10.25403/UPresearchdata.27314991.

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Data availability

Data will be made available on the University of Pretoria data repository on acceptance of the manuscript.

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