



Effects of semiochemical pre-feeding, physiological state, and weather on the response of *Bactrocera dorsalis* to methyl eugenol baited traps

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ABSTRACT

Plant-derived phenylpropanoids are semiochemicals that are often highly attractive to *Bactrocera* (Diptera: Tephritidae) males. One of these semiochemicals, methyl eugenol (ME), is used in the male annihilation technique (MAT) for the management of *B. dorsalis* (Hendel), a destructive horticultural pest. It is not normally viable to simultaneously implement MAT with the sterile insect technique (SIT), as released sterile males are attracted to MAT devices. However, prior semiochemical exposure can reduce the later response of *Bactrocera* males to the same or another semiochemical, which may allow the synchronous application of MAT and SIT. We determined how the interaction between semiochemical pre-feeding, weather, and fly physiology impacted the response of male *B. dorsalis* to ME baited traps. Response by a known number of males in field cages was determined in relation to temperature, relative humidity, semiochemical pre-feeding (ME, eugenol, or none), diet (protein supplemented and protein deprived) and age (4, 10 and 20 days old). Semiochemical pre-feeding of both ME and eugenol equally decreased the response of males that were 10 days old, or older, to ME baited traps. Adult diet had no effect on the response of males to ME baited traps. Response improved as temperature and relative humidity increased. These results highlight the feasibility of synchronous MAT-SIT programmes targeting *B. dorsalis* and the viability of continued protein supplementation of sterile males. We show that eugenol is an alternative to ME for suppression of male *B. dorsalis* response to MAT devices and that weather conditions significantly affect the variability and reliability of abundance estimates from trap captures.

1. Introduction

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is one of the most destructive horticultural pests in the world, increasing the cost of fruit production due to pest control, and quarantine restrictions that limit trade in lucrative export markets (Aketarawong et al., 2014). Due to its highly polyphagous nature and high capacity for dispersal and establishment in naïve areas (Clarke et al., 2019), *B. dorsalis* is classified as a quarantine pest in many parts of the world (Khamis et al., 2009; European and Mediterranean Plant Protection Organization EPPO, 2024). Management options include chemical control, quarantine efforts, and the use of biotechnical tools [i. e., male annihilation technique (MAT) and sterile insect technique (SIT)]. The integration of existing control techniques is useful for limiting the spread of and economic losses caused by this pest (Mutamiswa et al., 2021).

MAT devices, including traps and other devices or formulations applied to a target area, are baited with a male lure and an insecticide to reduce the number of male fruit flies in a population (Vargas et al., 2014). Like many *Bactrocera* species, *B. dorsalis* males are strongly attracted to a group of plant-derived phenylpropanoids that act as semiochemicals (Tan and Nishida, 2012). Semiochemical feeding, and even exposure to their volatiles (“aromatherapy”), are known to improve the mating competitiveness of males through the incorporation of semiochemical metabolites in the male sex pheromone (Haq et al., 2018; Wee and Clarke, 2020; Wee et al., 2007). One semiochemical, methyl eugenol, is a powerful male attractant used in the detection, suppression, and surveillance of *B. dorsalis* (Haq et al., 2018; Tan and Nishida, 2012) and is commonly used for MAT. Methyl eugenol has been detected in various plants (e.g. mango, banana, orchids, etc.) from over 80 different families (Tan and Nishida, 2012). The use of MAT with methyl eugenol has reduced populations of *B. dorsalis* on numerous

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occasions (Ballo et al., 2020; Fay et al., 1997; Manrakhan et al., 2011; Vargas et al., 2010). However, MAT is most effective when combined with other control techniques, such as field sanitation, protein baits and SIT, to form an area-wide integrated pest management (AW-IPM) programme because females are not targeted (Vargas et al., 2008).

SIT involves flooding wild populations with mass reared sterile insects (usually males only) (Knipling, 1964). This maximises the chance that sterile males will mate with wild females, resulting in non-viable offspring and restricting the size of the next generation. The efficacy of SIT generally improves when used on smaller populations (Shelly and McInnis, 2016). Thus, SIT is often implemented after MAT has reduced the number of wild males in the population so that there is a higher sterile:wild male overflooding ratio, which ranges between 20:1 to 50:1 for the control of *Bactrocera* species (Lance and McInnis, 2021). MAT and SIT are usually antagonistic and must be separated in either time or space to be effective. Otherwise, sterile males will be attracted to MAT devices and die, thus reducing the efficiency of SIT. However, several studies on *Bactrocera* species show that feeding on semiochemical male lures reduces subsequent attraction to these lures at a later stage (Akter et al., 2017b; Shelly, 1994, 2020). Theoretical models by Barclay et al. (2014) predict that the simultaneous use of MAT and SIT for the control of *B. dorsalis* is viable and could reduce the number of sterile males released to less than 5% of what is usually required when using SIT alone, with this improvement largely dependent on sterile males having a reduced response to methyl eugenol baited MAT traps.

The simultaneous application of MAT and SIT could also lessen the pressure on wild populations to select for lower sensitivity to methyl eugenol (Cunningham, 1989; Mandanayake et al., 2023; Mandanayake and Hee, 2023b), ensuring that MAT remains an effective control option for wild populations. Furthermore, combining MAT and SIT may improve the suppression of wild populations that are less sensitive to methyl eugenol or that have access to abundant natural sources of methyl eugenol (Shiga et al., 1989). SIT relies on the ability of sterile males to successfully compete with wild males. Exposure to semiochemicals, like methyl eugenol, increases the mating success of males through earlier sexual maturation, increased aggregation and calling rate (Shelly, 1994, 2010, 2020) and can thus improve the mating competitiveness of mass-reared sterile *B. dorsalis*. Protein supplementation also improves sexual performance and competitiveness (Perez-Staples et al., 2008; Taylor et al., 2013). It is possible that protein feeding may elevate the response of males to male lures, such as in the case of *B. tryoni* responding to cue lure (Weldon et al., 2008). However, little research has been conducted on how diet and semiochemical exposure influences the response of *B. dorsalis* to male lures.

Environmental variables are known to impact fruit fly activity, behaviour and how they respond to baits. Temperature, windspeed, rainfall and humidity have a direct influence on the population dynamics and trap capture probability of *Bactrocera* species (Chen et al., 2006; Duyck et al., 2006; Pogue et al., 2024; Shukla and Prasad, 1985). Warmer temperatures result in increased flight activity in tephritid flies (Esterhuizen et al., 2014; Makumbe et al., 2020), which enables them to respond to baits more readily. Temperature may also increase the volatilization of lures used in traps (Flores et al., 2017; Gómez-Escobar et al., 2022; Pogue et al., 2024). Environmental variables thus affect how fruit flies respond to baits by affecting their activity, mobility and the concentration of lure volatiles in the air. The role that the environment plays on the response of *B. dorsalis* is not well understood, as some studies suggest that variation in trap capture is strongly influenced by abiotic factors, whereas others only observed a minor impact (Hasyim et al., 2008; Kamala Jayanthi and Verghese, 2011; Pogue et al., 2024). Despite this, the impact of the environment on the lure response of *B. dorsalis* plays a crucial role in improving our understanding of how fruit flies respond to baited traps and MAT devices.

This study investigated the effects of semiochemical pre-feeding on the response of *B. dorsalis* to methyl eugenol baited traps under varying physiological and environmental conditions. We expected that prior

exposure to a semiochemical would reduce the response of *B. dorsalis* to methyl eugenol baited traps and that this would be more pronounced in sexually mature males (≥ 10 days old (Wong et al., 1989)). Additionally, we predicted that protein feeding would increase the response of males to methyl eugenol. Finally, it was hypothesized that the response of *B. dorsalis* to methyl eugenol would increase with temperature. Our results on the behavioural response of *B. dorsalis* to methyl eugenol after pre-exposure to semiochemicals provide empirical evidence for the simultaneous use of MAT and SIT.

2. Materials and methods

2.1. Fly husbandry

Fly cultures were maintained in a climate room at approximately 22.98 ± 3.60 °C and 40–60% relative humidity. A 14:10 light-dark photoperiod was maintained in the climate room with a 1-h dawn and dusk period simulated for the first and last hour of the light cycle. The main day lighting comprised of a combination of 20 W (G5, Eurolux, Sandton, South Africa) and 58 W (58W/840, Osram, Germany) fluorescent tubes. Whereas the dawn and dusk lighting comprised of 8 W fluorescent tubes (T4, Eurolux, Sandton, South Africa) that were placed obliquely to the fly cultures and were turned on before and after the main day lighting.

Cultures were established from wild pupae provided by Citrus Research International (Nelspruit, South Africa), from which wild females were mated with laboratory adapted males (fifth to thirteenth generation). Pupae were placed in mesh insect cages ($32.5 \times 32.5 \times 32.5$ cm, BugDorm43-030, MegaViewScience, Taichung, Taiwan, or $30 \times 30 \times 30$ cm, Small white breeding cage, Mad Hornet Entomological Supplies, South Africa) with unrestricted access to water (water-soaked cotton wool) and food [hydrolysed yeast (Yeast Extract Powder; Biolab; Merck, Germany) and sugar in a 1:3 ratio]. New generations, as well as experimental flies, were obtained by allowing females of peak reproductive age (20–40 days) to lay eggs in a 125 mL plastic container (Plastilon, South Africa). The container was covered with a layer of laboratory film (Parafilm M, Bemis, USA) that was pierced several times with a pin and contained water-soaked tissue, 3 mL of guava juice concentrate (Hall's, Tiger Consumer Brands Limited, Bryanston, South Africa) and a slice of guava to encourage oviposition. Eggs were washed out of the oviposition container with distilled water and were placed on carrot-based larval rearing medium (Citrus Research International, Nelspruit, South Africa) at a density of approximately 3 eggs/mL of medium. The container of inoculated larval medium was placed in a 2 L plastic box with a ventilated lid and a layer of sand, which was kept in the climate room. After 15 days (within the pupal phase) the sand was sifted, and pupae were placed in a Petri-dish (ϕ 65 mm) and transferred into a mesh insect cage ($32.5 \times 32.5 \times 32.5$ cm, or $30 \times 30 \times 30$ cm).

Experimental flies were produced in the same manner, except for groups with different diet treatments. Flies that were deprived of protein in their adult diet had unrestricted access to sugar and water but were not provided with hydrolysed yeast. Experimental flies were kept in mesh insect cages ($32.5 \times 32.5 \times 32.5$ cm, or $30 \times 30 \times 30$ cm) until 24 h prior to semi-field testing, wherein they were transferred to transparent plastic 1L cage with a ventilated lid and unrestricted access to water and their diet treatment specific food.

2.2. Correlates of intrinsic and extrinsic factors on lure response

Semi-field testing was conducted in hexagonal shade houses (2.3 m tall and 3 m wide at its widest point) on the University of Pretoria Innovation Africa Campus (Hatfield, South Africa). Three potted citrus trees (a combination of Midknight Valencia Orange and Star Ruby Grapefruit) were placed in each shade house as a habitat for flies to rest and feed during the day. The trees used were 1.5 m–2 m tall and were clustered in the centre of the shade houses. Flowers were removed from

all trees to prevent the trees from fruiting. A methyl eugenol lure was placed in a yellow bucket trap (Chempac, South Africa) (130 mm × 160 mm) containing a dichlorvos insecticide (Vapona, Ag-strip, Acorn Group, South Africa) block and placed in foliage in each shade house. Another unbaited trap with insecticide was also placed in each cage. A control trap is important when recapturing flies within a restricted space to account for random entry of flies into traps and possible visual bias. The exit and survival of flies that entered the trap was unlikely to occur due to the rapid action of the insecticide and plastic rings that sit inside of the trap entry holes to prevent fly escape.

Pupae were divided into three batches and dyed with three contrasting fluorescent pigment colours (T-series, Swada, UK), at a concentration of 2g L⁻¹ of pupae (Makumbe et al., 2017), to distinguish between the three age groups tested. Colours were rotated throughout age groups across the different replicates. Each cage contained pupae of the same age, which were thus dyed the same colour. Cages were furnished with sugar, or sugar and hydrolysed yeast, plus a source of water, to represent protein-deprived and protein-rich adult diets, respectively. Flies of each diet and treatment combination were divided into three to distinguish between semiochemical pre-treatment groups and placed in transparent plastic 1L cages with ventilated lids.

Semiochemical pre-treatment involved incorporating either eugenol or methyl eugenol at a concentration of 1.25% into the diet of a treatment group 48 h before semi-field testing. A third control group was also separated into smaller cages but was not given any semiochemicals. These semiochemical treatments were selected based on unpublished results showing that eugenol and methyl eugenol, both at a concentration of 1.25%, reduced response to methyl eugenol, and that this concentration and age of exposure led to little fly mortality (González-López, Malod, Pogue & Weldon, unpublished data). During the 48 h of semiochemical exposure, each semiochemical group was kept in a separate climate room (under identical conditions) to prevent potential aromatherapy effects (Haq et al., 2018) between semiochemicals. During this semiochemical exposure window, flies were given unrestricted access to water and food (with semiochemicals for experimental groups and without semiochemicals for the control group).

Fly response to methyl eugenol was determined in relation to their diet, age, and semiochemical pre-treatment. At ages four-, 10-, and 20-days after adult emergence, 20 males of each diet treatment were released at four times during the day (06:30, 11:30, 13:30, 17:00, UTC (+02:00)) and left for 90 min to move around the field cage and respond to the lure. After each 90-min period, traps were emptied, and unresponsive flies were caught with an aspirator. Flies were transferred to and stored in a freezer at -80 °C. During each 90-min period, minimum, maximum, and average temperature, and relative humidity was recorded within the shade house using a temperature logging iButton (Thermocron iButton, iButtonLink Technology, USA). Furthermore, light intensity was recorded at the base of the trap at the beginning and end of each 90-min period using a light meter (Model 407026, EXTECH Instruments, USA). The response of *B. dorsalis* to methyl eugenol was calculated for each diet, age, and semiochemical pre-treatment combination by subtracting the number of flies caught in the control trap (unbaited trap) from the number of flies in the experimental trap (baited trap). Only males were tested in their response to methyl eugenol, as females do not respond to this lure. This procedure was replicated five times.

2.3. Nutritional body composition assays

A subsample of five responsive and unresponsive flies from each experimental variable combination of the semi-field lure response tests was used to establish the role of nutrient stores on responsiveness to methyl eugenol. Flies were thawed to room temperature (~20 °C) and weighed (to 0.001 mg) to determine their wet body mass. Flies were then freeze-dried and weighed (to 0.001 mg) again to determine their dry body mass. Water content of each fly was calculated by subtracting

the dry body mass from the wet body mass.

To estimate the carbohydrate, lipid, and protein content from the same individual we used the methods described by Foray et al. (2012) that have been adapted for tephritid analysis (Weldon et al., 2019) and are detailed in Appendix A. All assays were colorimetric, with estimates of the component of interest determined by reading sample absorbances against a standard curve with a microplate reader (Eon Microplate Spectrophotometer, Biotek Instruments, Winooski, VT, USA).

2.4. Data analysis

All statistical analyses were performed using R software (v 4.1.0, The R Foundation for Statistical Computing). Data was analysed with generalized linear mixed effects models by using the “glmer” and “lmer” models from the lme4 package (Bates et al., 2015). Replicate was included as a random effect term for all models.

The response variable for intrinsic and extrinsic factor models was the number of flies caught in the baited trap minus the flies caught in the control trap. When the number of flies in the control trap exceeded that of the baited trap, trap catch was set to zero flies (this was done 22 times). Fixed effects for models testing intrinsic factors included adult diet, fly age, and semiochemical pre-treatment. Only test groups with the strongest positive response to methyl eugenol baited traps were used to assess the effect of temperature, humidity, and light intensity on fly response. This was done to ensure that the effects of weather variables were not biased by physiological variables that limited response. Linear and multivariate nonlinear regression models were tested to assess the effects of abiotic variables on the response variable. Similar to the approach used by Bunning et al. (2016), sequential model building was used to assess the linear and non-linear effects of extrinsic factors on fly response. Linear effects were tested in a regression model containing only the linear effects (temperature, relative humidity, and light intensity) and the response variable. The non-linear effects of extrinsic factors were tested in a multivariate nonlinear regression model including the linear and non-linear effects of temperature, relative humidity, and light intensity. As time of day was confounded with the measured abiotic variables it was removed as a variable in the analysis. The minimum adequate model (linear or multivariate nonlinear regression model) was selected using Akaike's information criterion. The linear fixed effects included temperature, humidity, and light intensity and the non-linear quadratic fixed effects tested were temperature × temperature, humidity × humidity, and light intensity × light intensity. The response variable for models testing the effect of body composition was binomial, differentiating between flies that were caught in the baited trap (responsive) versus flies that were not caught in the baited trap (non-responsive). Fixed effects for body composition models included: fly weight, protein content, lipid content, carbohydrate content, adult diet, and fly age. The response variable was cube root transformed for the intrinsic and extrinsic factor models and a Gaussian distribution was used for both. The response variable had a non-normal distribution. Transformation of the response variable homogenised the variance and increased model fit. A binomial distribution was used for the body composition model, with no transformation of the response variable.

Analysis of variance tables were generated using type III sums of squares to summarise the effect of factors in the minimum adequate model. If no interaction term was retained in the minimum adequate model, analysis of variance tables were generated using type II sums of squares. If a significant interaction effect was found, post hoc pairwise comparison tests were performed using estimated marginal means from the ‘emmeans’ function and the package of the same name (Russel, 2020).

3. Results

3.1. Intrinsic factors

The interaction between age and semiochemical treatment had a significant effect on response (Table 1). Furthermore, semiochemical pre-treatment had a significant effect on response of male *B. dorsalis* to methyl eugenol baited traps (Table 1).

Flies that were not exposed to semiochemicals showed an increase in response of 603.7% and 631.48% at ten days ($t = 7.113$, $p < 0.001$) and 20 days post emergence ($t = 6.447$, $p < 0.001$) respectively, when compared to four-day old flies that had no exposure to semiochemicals. However, flies exposed to eugenol or methyl eugenol showed no significant difference in response between age groups. At four days post emergence there was no significant difference in response between eugenol and methyl eugenol treatments ($t = 0.821$, $p = 0.69$), eugenol and no semiochemical treatments ($t = -0.994$, $p = 0.5814$) and methyl eugenol and no semiochemical treatments ($t = -1.295$, $p = 0.399$). At ten days post emergence, fly response significantly decreased by 65.23% when exposed to eugenol ($t = -6.89$, $p < 0.001$) and by 88.94% when exposed to methyl eugenol ($t = -7.615$, $p < 0.001$). Similarly, at twenty days post emergence fly response significantly decreased by 87.56% when exposed to eugenol ($t = -5.847$, $p < 0.001$) and by 84.42% when exposed to methyl eugenol ($t = -4.59$, $p < 0.001$).

Semiochemical exposure decreases fly response to methyl eugenol baited traps. Exposure to eugenol decreases response by 78.59% and likewise, exposure to methyl eugenol decreases response by 76.06% in comparison to flies that had no prior exposure to semiochemicals (Fig. 1).

3.2. Nutritional factors

Fresh weight of flies had a significant effect on fly response (Table 1), with overall response decreasing in heavier flies. A 50% probability of fly response to methyl eugenol (R50) occurs at a fresh weight of 14.5 mg for flies with no exposure to semiochemicals (Fig. 2a), 13.44 mg when previously exposed to methyl eugenol (Figs. 2b), and 13.73 mg for flies exposed to eugenol (Fig. 2c). There was no significant effect of protein (Fig. 3), lipid (Fig. 4), or carbohydrate (Fig. 5) content on fly response to methyl eugenol (Table 1).

3.3. Extrinsic factors

Male flies that were ten, or twenty days old and were fed a protein rich diet, without exposure to any semiochemicals were used to analyse the effects of abiotic variables due to their strong response to methyl

eugenol. The minimum adequate model included the linear effects of temperature, humidity, and light intensity (Table 2). The response of *B. dorsalis* to methyl eugenol increases with temperature (Table 2). No flies are predicted to respond to methyl eugenol baited traps at temperatures below 22.95 °C, 95% CI [21.23, 24.67], and an increase of 0.29 flies was predicted for every 1 °C increase in temperature (Fig. 6a). Similarly, response increases with relative humidity (Table 2). No flies are predicted to respond at relative humidity levels below 45.74 %RH, 95% CI [44.02, 47.46], and an increase of 0.83 flies were predicted to respond for every 10 %RH increase in relative humidity (Fig. 6b). Light intensity also affected fly response, with response decreasing with higher light intensity (Table 2). No flies are predicted to respond at light intensities above 13 158.5 lux, 95% CI [13156.78, 13160.22], and a decrease in response of 1.2 flies was predicted for every 1000 lux increase in light intensity (Fig. 6c).

4. Discussion

This study used standardised methods to determine how semiochemical pre-release feeding affected the response of *B. dorsalis* to methyl eugenol baited traps under varying physiological and environmental conditions. We found that both tested semiochemicals reduce the response of male *B. dorsalis* to methyl eugenol. Moreover, we observed that even the most responsive individuals require certain environmental conditions to respond to methyl eugenol.

As expected, the results of this study show that semiochemical feeding by sexually mature males reduces subsequent response of *B. dorsalis* to methyl eugenol baited traps. However, this effect is absent in immature flies, which are not attracted to methyl eugenol (Shelly et al., 2008). Feeding *B. dorsalis* methyl eugenol reduces later attraction to the lure (Shelly, 1994, 2020). Likewise, Wee and Rosli (2024) found that a methyl eugenol supplemented diet reduced later methyl eugenol response, and was associated with pheromone accumulation in the rectal glands. Similar suppression of response to lures have been found in other *Bactrocera* species after male feeding on raspberry ketone (Akter et al., 2017b; Khan et al., 2019). However, this study shows that feeding *B. dorsalis* either methyl eugenol or eugenol reduces subsequent response to the methyl eugenol baited traps similarly. Both eugenol and methyl eugenol provide mating benefits to *B. dorsalis* (Raghu, 2004; Tan and Nishida, 1996). Although there is little research directly comparing the mating benefits conferred by methyl eugenol and eugenol, the results of this study suggest that they both similarly satisfy the motivation for *B. dorsalis* to consume semiochemicals. As semiochemicals are bio-transformed and incorporated into the sex pheromone blend (Hee and Tan, 2004), it is likely that semiochemical exposure satiates the need for males to acquire them. This suppression of response to methyl eugenol may continue at least for as long as the metabolites are in the pheromone (Shelly, 1994, 2010).

Contrary to expectations, adult diet did not interact with semiochemical feeding to counteract the suppressed response of *B. dorsalis* males to methyl eugenol baited traps. More specifically, access to dietary protein did not elevate male responsiveness to methyl eugenol when they were sexually mature. This is in contrast with results from *B. tryoni* that showed that protein supplementation increased response to the male lure cue (Weldon et al., 2008). Semiochemical exposure accelerates the maturation of *Bactrocera* males (Akter et al., 2017b; Kumaran et al., 2013), with immature males not responding to semiochemicals (Shelly et al., 2008). Similarly, access to protein in the adult diet increases the mating competitiveness and maturation of several tephritid species, including *B. dorsalis* (Kaspi and Yuval, 2000; Pérez-Staples et al., 2011; Shelly et al., 2005). Furthermore, the addition of protein with methyl eugenol feeding has been shown to improve the mating performance of *B. dorsalis* (Orankanok et al., 2013; Shelly and Dewire, 1994; Shelly et al., 2005). Increased maturation due to protein feeding was thus proposed to increase the response of *B. dorsalis* to methyl eugenol. However, these results show that protein feeding does

Table 1

The effects of intrinsic factors and nutritional composition on the response of *B. dorsalis* to methyl eugenol.

	χ^2	df	p
Intrinsic factors			
Diet	0.125	1	0.724
Age	1.965	2	0.374
Semiochemical	37.67	2	<0.001
Diet × Age	3.356	2	0.187
Diet × Semiochemical	2.578	2	0.276
Age × Semiochemical	20.394	4	<0.001
Diet × Age × Semiochemical	4.837	4	0.304
Nutritional factors			
Wet weight	7.083	1	0.008
Protein	0.791	1	0.374
Lipid	0.572	1	0.449
Carbohydrate	0.611	1	0.434
Diet	0.228	1	0.633
Age	1.140	2	0.566

Note: Bold values indicate significant terms.

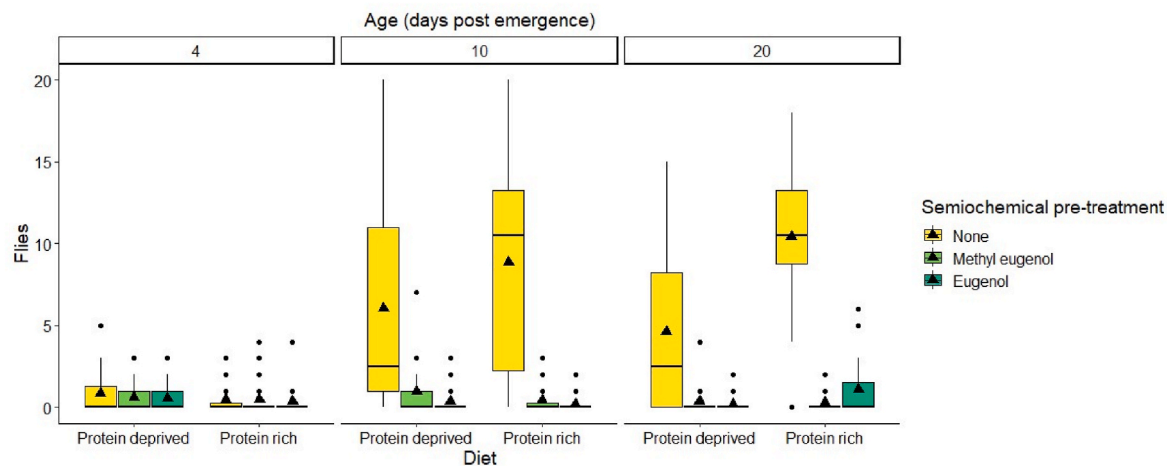


Fig. 1. Trap captures as a function of semi-chemical feeding for *B. dorsalis* of different ages, fed a protein rich or protein deprived diet. Each group represents 20 flies and was given 90 min in a semi-field cage to respond to a methyl eugenol baited yellow bucket trap. Accidental trap capture was accounted for by using an unbaited yellow bucket trap, with the number of flies caught in the control trap subtracted from those caught in the baited trap. Black triangles represent the mean trap – after accounting for accidental trap captures – catch per experimental group.

not counteract the reduction in methyl eugenol response obtained from semi-chemical feeding.

This is further substantiated in the lack of relationship found between body nutrient composition and response to methyl eugenol. Combined, these results indicate that there is no nutrient-related motivation for *B. dorsalis* to respond to methyl eugenol. However, heavier flies showed a lower response to methyl eugenol baited traps. Body mass can be used as an index of body size in *B. dorsalis*, as the two traits are positively correlated (Zhou et al., 2016). Larger *Bactrocera* males have higher fertility and are more competitive than smaller males (Ekanayake et al., 2017; Zhou et al., 2016). With larger males being more competitive, it may be that the consumption of methyl eugenol is less beneficial to their sexual performance and so they have decreased sensitivity to methyl eugenol. In contrast, smaller males may actively seek out methyl eugenol to supplement their deficit in mating competitiveness. Decreased response in heavier flies is unlikely to be a result of impaired flight ability. While the power requirements of larger insects can sometimes exceed their metabolic capabilities and impair their flight ability (Heath et al., 1971), heavier *B. dorsalis* have greater flight abilities than smaller flies (Makumbe et al., 2020). Furthermore, heavier *B. dorsalis* fly faster, disperse further and stop less often (Malod et al., 2023).

In addition to semi-chemical feeding, environmental variables played a crucial role in the response of *B. dorsalis* to methyl eugenol. In particular, there was a positive relationship between ambient temperature and male response to the lure. *Bactrocera dorsalis* can develop, reproduce, and survive at temperatures between 15 and 34 °C, but with an optimal range between 20 and 28 °C (Fiaboe et al., 2021; Manrakhan et al., 2022). Increases in ambient temperature lead to faster metabolic rates for ectothermic animals (Schulte, 2015). Thus, increases in temperature elevate activity levels in *B. dorsalis* that facilitate foraging for resources such as methyl eugenol, which can be found in over 80 plant families (see Tan and Nishida, 2012). The lower threshold for predicted trap catches occurred at 22.95 °C, with an increase in response of 0.29 flies for every 1 °C in temperature. Optimal flight temperature for *B. dorsalis* is between 20 and 32 °C (Yuan et al., 2016). Makumbe et al. (2020) found that fast, short distance flight is optimal between 12 and 36 °C, with long distance flight optimised between 20 and 24 °C. It is thus likely that the decreased probability of response below 22.95 °C occurs partly due to a drop in flight performance and mobility. At temperatures below 22.95 °C, *B. dorsalis* males have a low probability of responding to methyl eugenol, despite being in close proximity to traps baited with this powerful male attractant. Thus, management thresholds

that use trap catch without taking temperature into account to estimate population sizes will be less accurate. Only the most responsive males were used to assess the effects that environmental variables have on response. It is thus likely, that these results overestimate the response of *B. dorsalis* to methyl eugenol.

Increased response of *B. dorsalis* was found with increasing relative humidity, with a lower threshold for trap catches occurring at 45.74 % RH. Optimal flight activity (flight duration, distance, and speed) for *B. dorsalis* occurs at 60–75 %RH (Yuan et al., 2016). Increased lure response at high relative humidities is likely associated with increased flight activity. Flight is energetically costly in insects and is associated with higher rates of evaporative water loss (Nicolson and Louw, 1982; Johnson et al., 2023). However, at higher humidities the water loss rates of strenuous activities, like flight, are lessened (Sinclair et al., 2024), lowering the risks of desiccation and allowing higher activity levels. Despite less restricted flight in more humid conditions, this increased lure response will be constrained as methyl eugenol volatilization is limited at high humidities (Flores et al., 2017; Gómez-Escobar et al., 2022). In contrast, lure response decreased with increasing light intensities. The results reported in this study show that the upper threshold for response of *B. dorsalis* to methyl eugenol is 13 158.5 lux, with response being highest between the times 10:00 and 15:00. Copulatory behaviour of *B. dorsalis* increases under low light intensities, with response decreasing at light intensities above 2000 lux (Arakaki et al., 1984; Yuan et al., 2016). Whereas general activity increases during the day under brighter light intensities (Liu et al., 2018). Attraction to methyl eugenol in this study thus coincides with general resource orientated behaviours and activities such as feeding.

The suppression of response to methyl eugenol after semi-chemical feeding allows for the simultaneous application of MAT and SIT by preventing a large proportion of sterile males being killed by MAT. However, the prolonged use of methyl eugenol in fruit fly management may be problematic as it is classified as “probably carcinogenic” to humans, as well as possessing some genotoxic properties (Riboli et al., 2023; Zhang et al., 2022). Although methyl eugenol feeding reduces later response to the lure, the search for safer alternatives is key. Eugenol is a safe phenylpropanoid, stated to have antimicrobial and antioxidant effects (Zari et al., 2021). This study found that eugenol feeding reduces the response of *B. dorsalis* to methyl eugenol at a similar rate to that of methyl eugenol. Eugenol can thus be used as a safer alternative to methyl eugenol to reduce lure response. A similar reduction in response to methyl eugenol has been found in *Bactrocera zonata* previously exposed to β -caryophyllene, a safe plant compound (Haq et al., 2024).

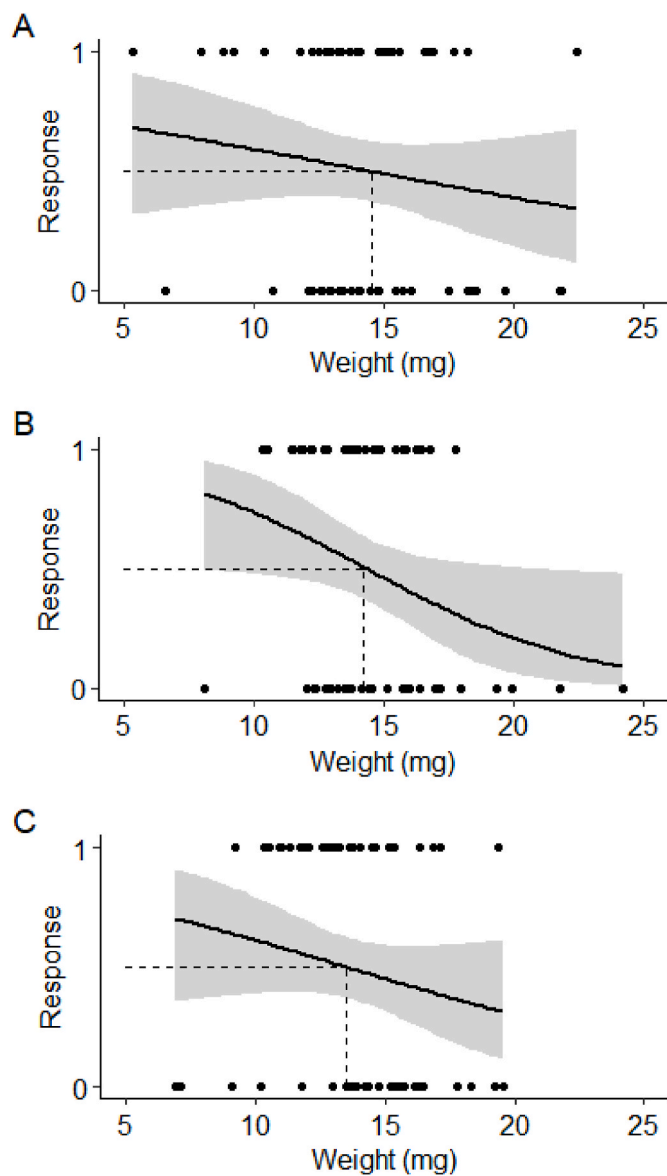


Fig. 2. Probability that *B. dorsalis* responds to methyl eugenol at varying body weights (mg) at different semiochemical feeding treatments (A) no semiochemical feeding, (B) methyl eugenol feeding, (C) eugenol feeding. Flies that responded to methyl eugenol are coded as 1 and flies that were unresponsive are coded as 0. Trendlines and the 95% confidence interval bands factors are shown. The dashed line represents the value at which there is a 50% probability of response to methyl eugenol (R50).

Although this reduction was not equal to that of prior methyl eugenol exposure, it is another feasible alternative to methyl eugenol (Haq et al., 2024). In this study we also found that protein supplementation does not counteract the reduction in methyl eugenol response caused by semiochemical feeding. Protein supplementation can thus be used to increase the mating competitiveness of sterile males (Shelly et al., 2005), without elevating male response to methyl eugenol in joint MAT-SIT programmes.

5. Conclusion

This study suggests that the simultaneous application of MAT and SIT is feasible. In accordance with our hypothesis, the pre-release feeding of *B. dorsalis* males to eugenol or methyl eugenol results in a decrease in response to methyl eugenol baited traps. Pre-exposure to these

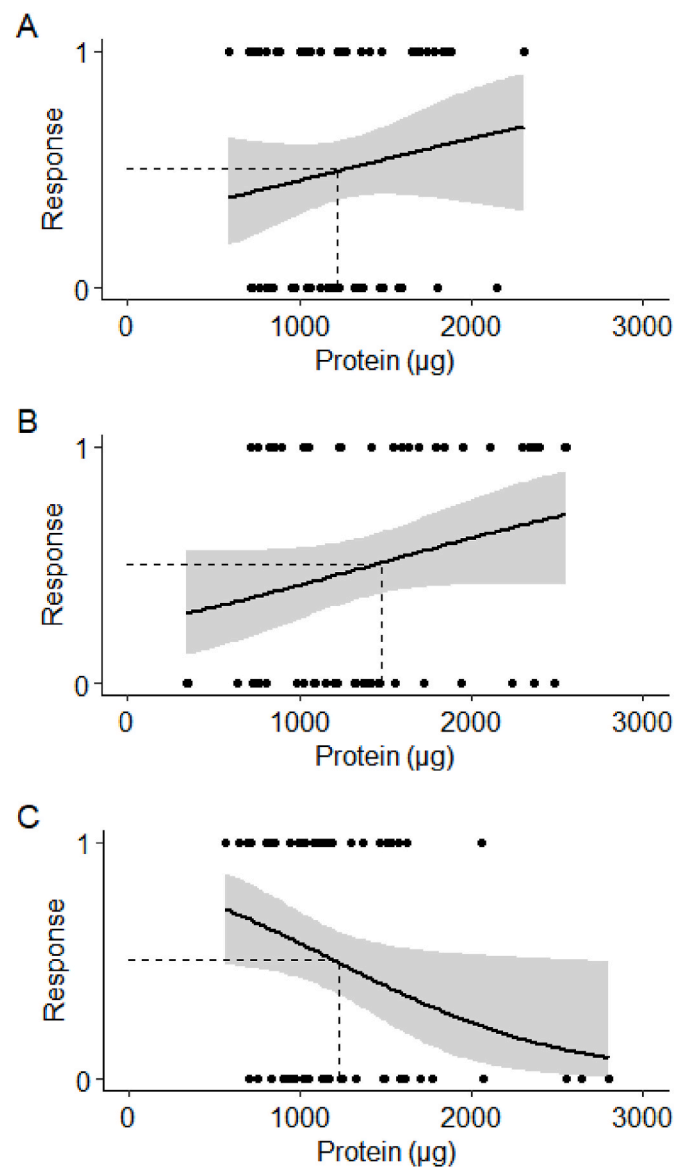


Fig. 3. Probability that *B. dorsalis* responds to methyl eugenol at varying total body protein contents (μg) at different semiochemical feeding treatments (A) no semiochemical feeding, (B) methyl eugenol feeding, (C) eugenol feeding. Flies that responded to methyl eugenol are coded as 1 and flies that were unresponsive are coded as 0. Trendlines and the 95% confidence interval bands factors are shown. The dashed line represents the value at which there is a 50% probability of response to methyl eugenol (R50).

semiochemicals is further beneficial, as studies have shown they improve the competitiveness of sterile males (Haq et al., 2018). The combined use of MAT and SIT will improve the control of *B. dorsalis*. The costs of semiochemical supplementation as well as the costs associated with implementing two control strategies at the same time may be higher than traditional forms of control. However, MAT-SIT programmes will exhibit higher levels of control of wild populations (Barclay et al., 2014). This could result in less sterile males being required to achieve an effective overflooding ratio, which in turn may lower costs. Further improvements to eradication programs can be made by reducing the application density of attract-and-kill sites, such as MAT traps (Fezza et al., 2024). Unexpectedly, protein supplementation did not increase the response of *B. dorsalis* to methyl eugenol. This suggests that protein supplementation can be used to increase male mating competitiveness (Shelly et al., 2005) in joint MAT-SIT programmes without jeopardising the reduction in methyl eugenol response. This

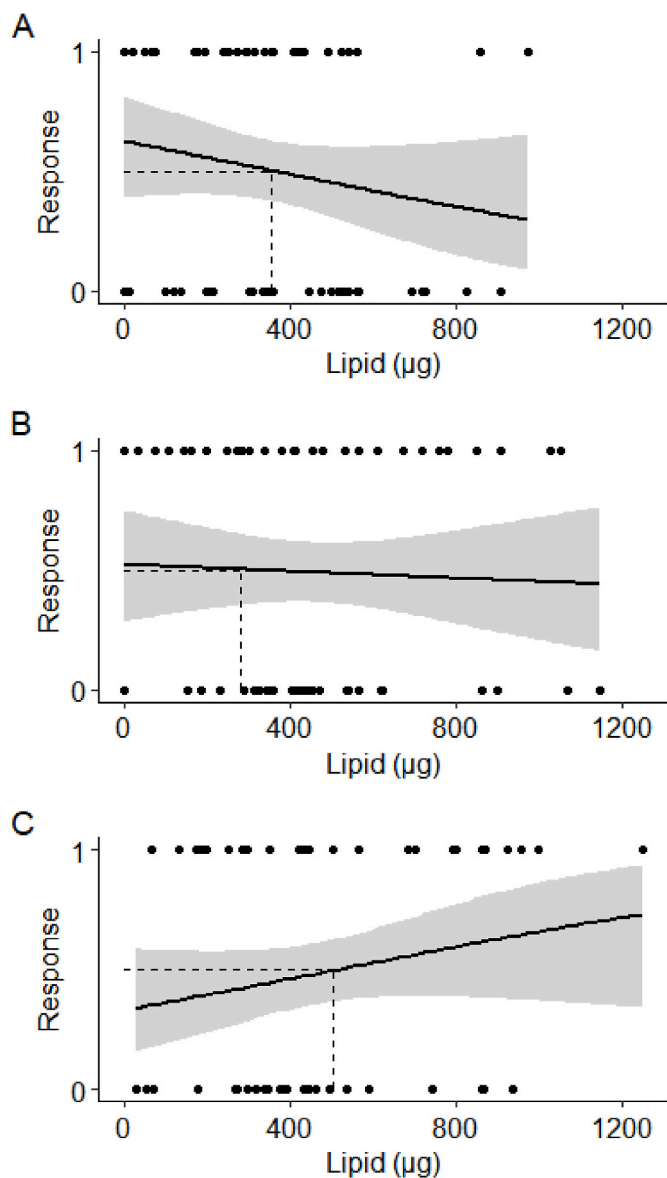


Fig. 4. Probability that *B. dorsalis* responds to methyl eugenol at varying total body lipid contents (μg) at different semiochemical feeding treatments (A) no semiochemical feeding, (B) methyl eugenol feeding, (C) eugenol feeding. Flies that responded to methyl eugenol are coded as 1 and flies that were unresponsive are coded as 0. Trendlines and the 95% confidence interval bands factors are shown. The dashed line represents the value at which there is a 50% probability of response to methyl eugenol (R50).

study also found that the response of *B. dorsalis* is largely affected by temperature, relative humidity, and light intensity. Fruit fly intervention and management thresholds should be adapted to account for this variability in trap catch that occurs due to environmental variables. Further research is needed to quantify these effects and integrate them into *B. dorsalis* management programmes.

CRedit authorship contribution statement

Tania Pogue: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Kevin Malod:** Writing – review & editing, Supervision, Investigation, Formal analysis. **Christopher W. Weldon:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

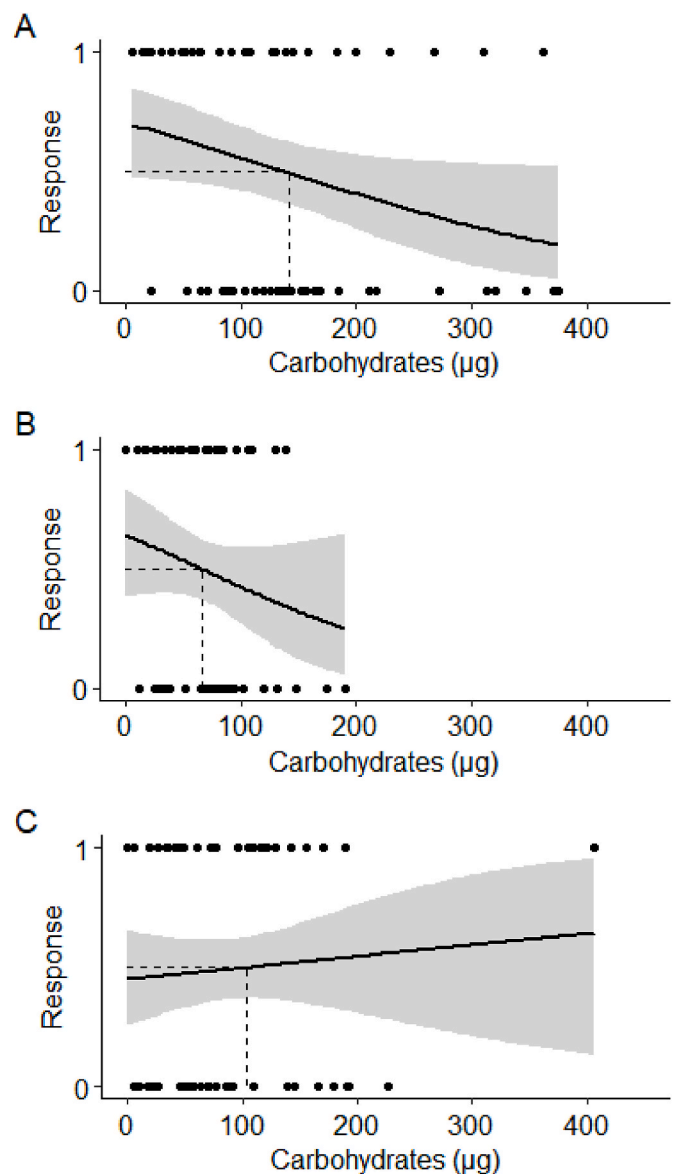


Fig. 5. Probability that *B. dorsalis* responds to methyl eugenol at varying total body carbohydrate contents (μg) at different semiochemical feeding treatments (A) no semiochemical feeding, (B) methyl eugenol feeding, (C) eugenol feeding. Flies that responded to methyl eugenol are coded as 1 and flies that were unresponsive are coded as 0. Trendlines and the 95% confidence interval bands factors are shown. The dashed line represents the value at which there is a 50% probability of response to methyl eugenol (R50).

Table 2

The effects of extrinsic factors on the response of *B. dorsalis* to methyl eugenol.

	Coefficient \pm SE	χ^2	df	p
Linear effects				
Temperature	0.123 \pm 0.020	37.208	1	< 0.001
Humidity	0.020 \pm 0.006	10.264	1	0.001
Light	-0.000 \pm 0.000	10.486	1	0.001

Note: Bold values indicate significant terms.

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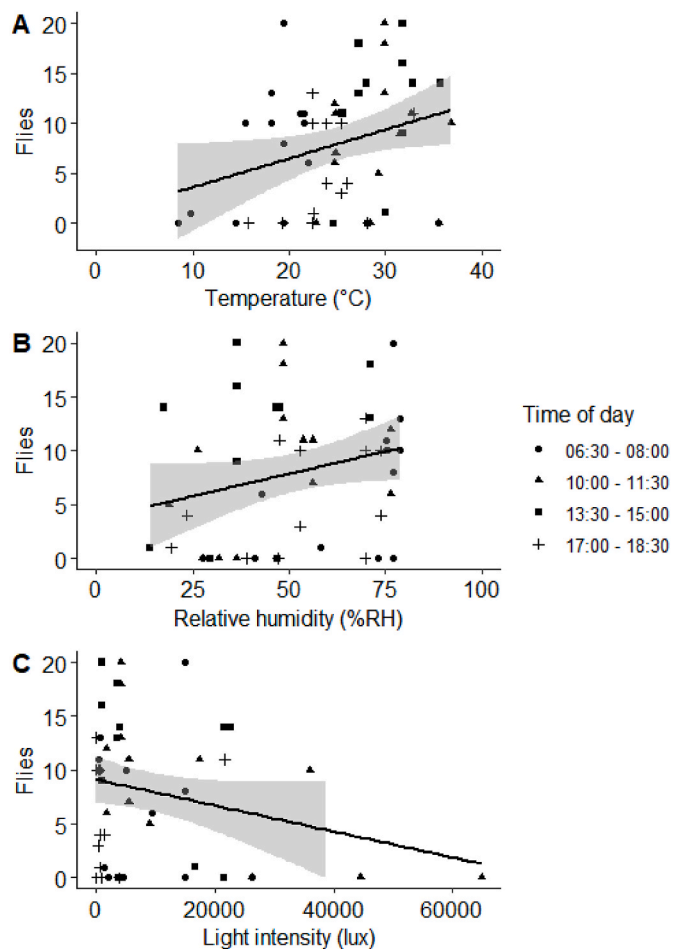


Fig. 6. Trap captures of the most responsive experimental groups of *B. dorsalis* to methyl eugenol (10- or 20-day old males fed a protein rich diet) at different (A) temperatures ($^{\circ}\text{C}$), (B) relative humidities (%RH), and (C) light intensities (lux). For each group, 20 flies were given 90 min in a semi-field cage to respond to a methyl eugenol baited yellow bucket trap. Lure response was tested at four times of the day and accidental trap capture was accounted for by using an unbaited yellow bucket trap, with the number of flies caught in the control trap subtracted from those caught in the baited trap. Trendlines and 95% confidence interval bands are shown.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Christopher W. Weldon reports financial support was provided by the International Atomic Energy Agency. Tania Pogue reports financial support was provided by Citrus Academy. The other author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cropro.2024.107015>.

Data availability

Data for this study are openly available in figshare at [10.25403/UPresearchdata.25880326.v1](https://doi.org/10.25403/UPresearchdata.25880326.v1), reference number 25880326.v1.

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