Methoprene and dietary yeast as pre-release supplements for Queensland fruit fly SIT

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Declaration

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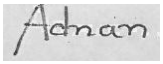
All other research described in this report is my own original work.



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Abstract

Sterile insect technique (SIT) is an environmentally benign pest management technique that relies on released sterile males mating with, and curtailing reproduction of, wild females. Juvenile hormone analogue methoprene (M+) incorporated into pre-release diet (Y+ sucrose plus yeast, Y- sucrose only) was studied as a potential enhancer of reproductive organ development, sexual maturation, and longevity of Queensland fruit fly *Bactrocera tryoni* (Froggatt), or 'Q-fly' used for SIT. Incorporation of methoprene into diets that also contain yeast was found to strongly accelerate reproductive organ development (testes and apodeme) in males, but had much weaker effects on females (ovaries). Age, dietary yeast and methoprene application all increased male mating success at early ages. Yeast and methoprene had synergistic effects on sexual maturation, since M+Y+ treated males exhibited increased mating propensity at younger ages than either M+Y- or M-Y+ flies. Flies provided yeast had increased mating success in comparison to yeast-deprived flies. In the absence of yeast, methoprene (M+Y-) treatment had only a modest effect on sexual maturation. In contrast to males, female Q-flies exhibited little response to methoprene treatment. Both when held individually in small cages and in groups in larger cages, yeast supplementation resulted in longer life spans for both males and females with and without methoprene, but methoprene did not significantly affect longevity. Longevity was higher when the flies were in larger group cages rather than in individual cages. Overall, these results show great promise and encourage direct incorporation of methoprene into yeast diet as a pre-release treatment for Q-fly SIT.

Keywords: Q-fly, Methoprene, dietary yeast, sexual maturation, sterile insect technique

1. Introduction

Tephritid fruit flies (Diptera: Tephritidae) are the world's most economically important insect pests, with dozens of species infesting a vast array of commercial and domestic fruit and vegetables (Aluja, 1994; Drew, 1989; White and Elson-Harris, 1992). Australia has ca. 80 fruit fly species (Hancock, 2000), and 16 of these have been reported to infest commercially grown fruit. In the years of 2003 - 2008, total estimated expenditure for fruit fly activities (yield losses as well as management costs) was around $128 million in Australia. National and international market access for horticultural commodities has been seriously threatened by fruit flies (Plant Health Australia, 2008). As a result, economic harm comes not only from reduced production and increased control costs, but also from loss of markets and the cost of establishing and maintaining phytosanitary measures.

The Queensland fruit fly *Bactrocera tryoni* (Froggatt) or 'Q-fly' is the most difficult and costly challenge to market access for most Australian fruit producers. It is the most devastating fruit fly in eastern Australia (Dominiak and Daniels, 2012), and infests more than 100 native and introduced hosts (Hancock, 2000; Oliver, 2007). Tropical and subtropical coastal Queensland and northern New South Wales are considered as its native distribution (Gilchrist et al., 2006), but it is now more widely distributed in south-eastern Australia and has invaded some South Pacific island nations (Drew et al., 1978).

Australian growers have relied on synthetic insecticides for almost a century to control pest Q-fly populations, and even to maintain substantial horticultural production in endemic areas. However concerns about the environmental and health consequences of insecticides used for the control of fruit fly pests has prompted a demand for more environmentally friendly methods. Amongst the available options, the Sterile Insect Technique (SIT) has attracted particular interest as an environmentally and medically benign approach that is amenable to modern area-wide management practices. In North, Central and South America, Europe, the Middle East, Asia, Africa and Australia, SIT is applied as a part of an area-wide integrated pest management (AW-IPM) approach against fruit fly pests of economic importance (Enkerlin, 2005). SIT involves the mass-rearing, sterilization and release of large numbers of insects of the target pest. The released sterile males mate with wild females, transferring nonviable sperm and accessory gland fluids to inhibit further sexual activity (Jang, 1995; Jang et al., 1999; Radhakrishnan and Taylor, 2007, 2008). As a result, reproductive potential of wild females is substantially reduced and pest populations can be effectively suppressed if the sterile males are sufficiently abundant and effective.

SIT programs rely on the survival and mating performance of released sterile males. Accordingly, SIT can be enhanced if released male flies attain earlier sexual maturity, are effective in mating, and survive longer. Detailed understanding of sexual maturation and mating behaviour of target pests is of particular value for improvement of SIT (Hendrichs et al., 2002). In order to boost sexual development and performance of sterile male fruit flies in SIT programs, there has been a substantial research focus on developing pre-release supplements. In the Q-fly, addition of yeast hydrolysate (YH) to sucrose in the pre-release diet has been associated with accelerated development (Meats and Leighton, 2004; Perez-Staples et al., 2011; Vijaysegaran et al., 2002; Weldon and Taylor, 2011), enhanced mating propensity (Perez-Staples et al., 2007), increased sperm transfer (Perez-Staples et al., 2008), increased ability of males to induce sexual inhibition in mates (Perez-Staples et al., 2008), increased fecundity (Meats et al., 2004), and increased longevity when carbohydrates are continuously available (Fanson et al., 2009; Perez-Staples et al., 2007; Prabhu et al., 2008) (for a review, see Taylor et al., 2013).

In addition to the benefits of YH, the juvenile hormone analogue methoprene has shown particular promise in some fruit flies. Juvenile hormone is responsible for regulating insect growth and development, and producing secondary sexual signals, such as pheromones (Teal et al., 2007; Teal et al., 2000). Males treated with methoprene dissolved in acetone and with access to YH rich diet have been found to have accelerated maturation and improved mating performance in melon fly *B. cucurbitae* (Coquillett) (Haq et al., 2010a) and in Q-fly (Collins et al., 2014). Males treated with methoprene and provided a diet containing YH have exhibited enhanced mating at significantly earlier ages in *Anastrepha fraterculus* (Wiedemann), *A. ludens* (Loew), *Ceratitis capitata* (Wiedemann), and *A. suspensa* (Loew) (Gomez-Simuta and Teal, 2010; Gomez et al., 2013; Pereira et al., 2009; Faria et al., 2008; Pereira et al., 2013; Segura et al., 2009; Segura et al., 2013). In contrast, effects of methoprene treatment have not been promising in *A. obliqua* (Macquart), *A. striata* (Schiner) (Aluja et al., 2009), and *Bactrocera dorsalis* (Hendel) (Shelly et al., 2009).

Although methoprene application in combination with diets containing YH is very effective in promoting early sexual maturation, there are still significant drawbacks associated with methods of incorporating methoprene into the pre-release adult diet. Methoprene is usually dissolved in acetone and applied to adults topically or pupae by dipping or bathing in acetone solution. However, significant health hazards have been associated with acetone as it is very volatile and flammable. In the context of SIT, topical application to adults and dipping of pupae are impractical due to health risks, safety and disposal issues related to the use of acetone

(Pereira et al., 2013; Segura et al., 2013). As an alternative approach, Pereira et al. (2013) and Teal et al. (2013) provided *A. ludens* and *A. suspensa* an agar-based diet containing 5–10% YH along with 0.05% methoprene, but this produced large amounts of waste, became sticky, and was not cost effective. Gomez et al. (2013) also used methoprene combined with food, but in this case as a dry mix with sugar and YH, and this was very effective for *A. ludens* in the laboratory, in field cages, and in the field. The only previous study of methoprene application in Q-fly used a standard approach of application to pupae and adults in an acetone solution, which resulted in significant mortality and diminished quality (Collins et al., 2014). Accordingly, in the present study we investigate effects of methoprene application coupled with a dry sugar or sugar+YH diet on reproductive development, sexual performance, and longevity of male and female Q-fly.

Sexual maturation and reproductive organ maturation (full development of reproductive tissues) do not necessarily follow the same trajectory; Q-flies can be sexually active and start mating before their reproductive tissues are fully developed (Perez-Staples et al., 2011). This raises the questions of whether methoprene treatment promotes the maturation of reproductive tissues or simply accelerates mating at earlier stages of reproductive development, which in turn might result in ineffective copulations. Though methoprene applied in acetone solution to pupae and flies with access to YH is known to accelerate sexual maturation in Q-fly (Collins et al., 2014), the effects on reproductive organ development have not been investigated. Incorporation of YH in adult diet is known to sustain development of reproductive tissues in both male and female Q-fly (Vijaysegaran et al., 2002; Weldon and Taylor, 2011), and it is important to investigate whether effects of methoprene provide benefits beyond those already achieved with YH.

In SIT Q-flies are currently released as a bisexual strain. The presence of sexually receptive sterile females can greatly interfere with impact of released males, and this has been recognized as a potential limit on the efficacy of SIT. As there is no genetic sexing strain available for Q-fly, both males and females would be treated with methoprene. If methoprene accelerates sexual maturation in Q-fly females, then these sterile females will mate with the sterile males, reducing the probability that the sterile males mate with wild fertile females. However, if methoprene accelerates maturation in males more than in females, then this treatment could act similarly to that of a genetic sexing strain with male-biased operational sex ratio; such effects have been reported in *A. fraterculus* (Segura et al., 2009), but were not evident in *A. ludens* (Pereira et al., 2013).

It is assumable that accelerated sexual maturity stemmed from methoprene incorporation are likely to be physiologically expensive, may result in shortened fly longevity. If methoprene treatment lowers adult lifespan to a certain point they may not be useful in SIT programs. However, methoprene treatment alone had no adverse effects on longevity in *Ceratitis capitate* (Faria et al., 2008) and *A. suspensa* (Pereira et al., 2005).

Building on our understanding of how methoprene treatment in acetone solution influences sexual performance of Q-fly (Collins et al., 2014), in the present study we focus on whether the positive effects of methoprene are maintained when this hormonal supplement is incorporated into the pre-release diet rather than applied in acetone. We have taken an important additional step beyond studies of methoprene effects in other species by specifically considering reproductive development and sexual development as related but distinct processes.

2. Methods

2.1. Insects

Fertile mass-reared Q-flies (*Bactrocera tryoni*) were obtained as pupae from the Fruit Fly Production Facility located at Macarthur Agricultural Institute, New South Wales, Australia.

2.2. Treatments

Adult males and females of Q-fly were treated with the following treatments as pre-release supplements:

- $M^{+}_{0.05}Y^{+}$: Methoprene (0.05%) + sugar and YH (3:1) as food
- $M^{+}_{0.1}Y^{+}$: Methoprene (0.1%) + sugar and YH (3:1) as food
- $M^{+}_{0.5}Y^{+}$: Methoprene (0.5%) + sugar and YH (3:1) as food
- $M^{+}_{0.05}Y^{-}$: Methoprene (0.05%) + sugar as food
- $M^{+}_{0.1}Y^{-}$: Methoprene (0.1%) + sugar as food
- $M^{+}_{0.5}Y^{-}$: Methoprene (0.5%) + sugar as food
- $M^{-}Y^{+}$: No Methoprene, sugar and YH (3:1) as food;
- $M^{-}Y^{-}$: No Methoprene, sugar as food

For the incorporation of methoprene into the adult diet, we selected NOMOZ® pellets (a trademark of Pacific Biologists, Brisbane, Queensland). These have as active ingredient PROLINK®, which contains 40 g/kg (S)-methoprene; this product was locally available, being marketed as a mosquito larvicide. NOMOZ pellets were finely powdered using mortar and pestle. Then the powdered methoprene (as required on dry weight basis) was mixed into sugar and YH diet (3:1), or only sugar, using a blender.

2.3. Effect of methoprene and YH on reproductive organ development

About 60 ml of pupae (40 flies per 1 ml of pupa) were placed in mesh cage (Megaview Bugdorm 44545, 47.5 x 47.5 x 47.5 cm) for adult emergence. First day emergence of flies was discarded to make sure that all flies were of same age (0-24hrs). After 24hrs of emergence, six different cages of flies were provided food (sugar and YH or only sugar) containing methoprene (0, 0.5% & 0.05%) for the next 48 hours *ad libitum*. After 48 hours, the treated food was replaced with sugar only in a 90 mm Petri dish and water soaked cotton wool. The flies were then sorted according to sex within 3 days after emerging by collecting and transferring individual flies in glass tubes. To assess the effect of methoprene and YH on reproductive organ

development, we measured testes & apodeme development (male) and categorised ovarian development (female). Ten males and ten females were collected from each treatment at 4, 6, 8, 10, 12, 15, 20, 25, and 30 days post emergence and preserved in 70% Ethanol solution. Each container was labelled with the age, treatment and sex before being set aside for later dissection. Flies were dissected in Phosphate-buffered saline (PBS; pH 7.4) using fine forceps on a microscope slide under a Leica MZ6 stereomicroscope.

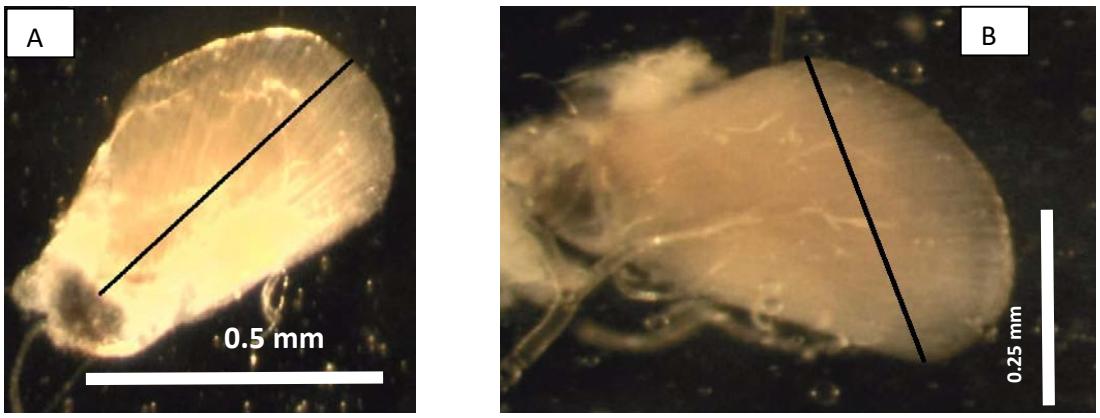

Fig. 1. Morphological traits assessed for ejaculatory apodeme Length (A) and Width (B) measurement.

For males, the ejaculatory apodeme and testes were photographed using a 1.3-megapixel Dino eye camera (AM4023X) through the phototube of the stereomicroscope. Images were calibrated and measured using Image J v.1.49. The length of the ejaculatory apodeme was measured from its lower end, where the ejaculatory sac joins the apodeme, to its upper rounded end (Fig. 1). The width was taken at its widest point, and area was measured by tracing the outline of the apodeme (Fig. 1) following Radhakrishnan and Taylor (2008). Length of the testes was measured by tracing a midline through the centre of the organ from the base to the curved tip (Fig. 2), and the area of testes was measured by tracing the outline (Radhakrishnan and Taylor, 2008).

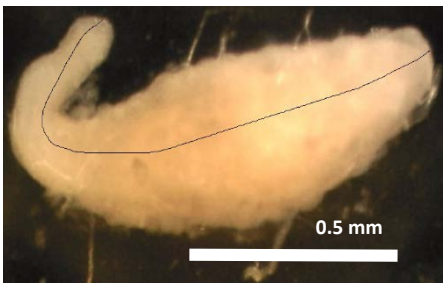

Fig. 2. Morphological traits assessed for testes length measurement of Q-fly.

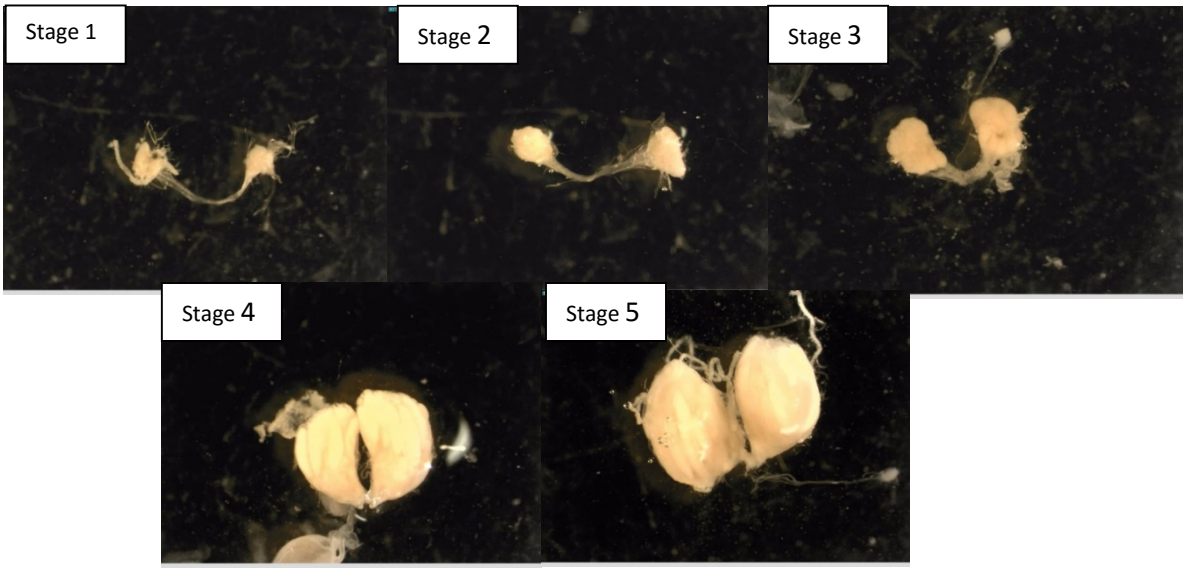

Fig. 3. Stages of ovarian development in female Q-fly. Stage 1 and 2 represent previtellogenesis development. Stage 3 indicates the initiation of vitellogenesis, and Stage 4 marks late vitellogenesis. Stage 5 ovarian follicles had mature eggs.

For females, the ovaries were photographed and ovarian development classified according to categories of Raghu et al. (2003). Raghu et al. (2003) considered Stages 1 and 2 as previtellogenic, while stages 3–4 represented the vitellogenic phase. Flies were assigned to stage 5 if the most advanced ovarian follicles had mature eggs. We did not observe stage 6, which was defined by a yellow residual follicular relic, the corpus luteum.

2.4. Effect of methoprene and YH on sexual development

2.4.1. Experimental treatments

One hundred and sixty millilitres of pupae from a single batch were placed in a mesh cage for adult emergence (Mega view Bug dorm 44545, 47.5 x 47.5 x 47.5 cm). After 24 hours of emergence, food containing four doses of methoprene (0, 0.05, 0.1, and 0.5%) was provided to the flies for the next 48 hours *ad libitum*. After 48 hours, the treated food was replaced with only sugar in a 90 mm Petri dish. The flies were then sorted according to sex within 3 days after emerging by collecting and transferring individual flies in glass tubes.

As mature flies needed to pair with treated flies, 80 ml of pupae from four different weekly batches (20 ml from each batch) were placed in mesh cages for adult emergence (Megaview Bugdorm 44545, 47.5 x 47.5 x 47.5 cm). Cages were supplied with water soaked cotton wool in 70ml sample container and with dry granular sucrose as food along with YH (3 part sugar:1 part YH) on a 90 mm Petri dish *ad libitum*; this diet is effective at supporting development of

fruit flies (Drew and Yuval, 2000; Hendrichs and Prokopy, 1994). Adult flies were sorted according to sex within 3 days after emerging by collecting and transferring individual flies in glass tubes. Approximately 200 flies were sorted into each 12-L cages, with this relatively low density to avoid effects of crowding on longevity and mating performance. All cages were of clear plastic with a mesh-covered window (ca. 80cm^2) for ventilation. No calling, courting, or mating were observed in cages prior to separating the sexes. A L12:D12 h photoperiod was maintained, with flies also experiencing a simulated dawn and dusk as the lights ramped up and down through an additional 1 h at the beginning and end of the light phase.

2.4.2. Mating trials

Mating trials were conducted at 4, 6, 8, 10, 12, 15, 20, 25 and 30 days post emergence. Observations were initiated 90 minutes prior to the onset of dusk to notice any flies that start mating early. On each mating day, at least four hours before the onset of dusk, ten males and ten females from each treatment group were placed individually in clear plastic 1.125 L containers with a mesh-covered window (ca. 28cm^2) for ventilation. Each fly was individually paired with a sexually mature (12-17 days old) fly of the opposite sex as virgin flies of this age with protein diet show a high level of sexual receptivity (Perez-Staples et al., 2007). To assess copula latency, defined as time from the start of dusk till the onset of mating, in minutes, we recorded the time of onset of copulation for each mating pair. To assess copula duration for each mating pair, we continued observation until the last pair had separated. Thus total 160 paired flies were trialled for each test day (4, 6, 8, 10, 12, 15, 20, 25 and 30 days), providing a total of 1440 test pairs. The experiment was repeated twice over a period of two months.

2.5. Effect of methoprene and YH on longevity

Application of treatments for longevity trials was identical to that of mating trials. Male and female flies were sorted within 3 days after emerging by transferring individual flies in glass tubes and placed in longevity cages (group and individual). No calling, courting, or mating were observed in cages prior to placing in the longevity trial cages. Longevity of flies was assessed in both group and individual cages.

In group cages, for each of treatment, five flies of either sex were placed in each of three 1.125 L cages having a mesh-covered window (ca. 28cm^2) for ventilation (i.e., 15 male and 15 female flies for each treatment). All cages were provided with water-soaked cotton and sugar in a 35 mm Petri dish.

In individual cages, 10 male and 10 female flies from each treatment were placed singly in 70ml containers having 8-10 2-mm holes for ventilation. Flies were provided with water-soaked cotton and sugar (as a solution dried onto 1-cm squares of porous paper). Flies were checked daily until all had died. Dead flies were removed from the cages daily. Both group and individual trials were repeated twice.

2.6. Statistical analysis

Reproductive organs of both males (testes & apodeme) and females (ovary) were analysed for each treatment using least squares regression including significance test using LSMeans differences Tukey HSD. Main effects included in the model were age (ordinal), diet treatment (binary), doses of methoprene (ordinal) and their interactions.

Variables with binary outcomes including mating probability was assessed using nominal logistic regression with significance test using likelihood ratio tests (G). Main effects included in the model were age of the flies (continuous), diet (binary) and doses of methoprene treatment (ordinal). Model parameter estimates were inspected to identify simple effects between factor levels. Mating probability followed a general trend in both replicates, therefore we pooled the data set from the two repetitions.

Copula latency and copula duration were analysed for each treatment using least squares regression including age (continuous), diet (binary) and doses of methoprene treatment (ordinal). For copula latency, values were rank transformed.

Longevity of flies were assessed for each treatment using least squares regression including diet (binary) and doses of methoprene treatment (ordinal). All the analyses were performed using JMP Statistical Software (SAS Institute, Cary, NC, USA). In all analyses, initial models considered all interaction terms. Non-significant interaction terms were removed from final models.

3. Results

3.1. Reproductive organ development

3.1.1. Ejaculatory apodeme

Ejaculatory apodeme length, width and area all varied significantly with age, diet, and application of methoprene. Apodeme length, width and area increased as the flies aged. Males with access to YH exhibited increased development of the ejaculatory apodeme compared to males provided sugar only (Fig 4). Incorporation of either methoprene dose into food significantly accelerated apodeme development (length, width and area). However, there was no difference in the effects of the two methoprene doses. Effects of age on growth in apodeme area varied with diet (significant age × diet interaction); males that had been provided with access to YH exhibited a steep increase in apodeme area followed by no further growth till 30 days whereas males provided access to sugar exhibited slower growth in apodeme area over the testing period (Fig 4).

Table 1
Analysis of variance for apodeme (length, width, and area) in relation to fly age (2, 4, 6, 8, 10, 12, 15, 20, 25 and 30 days post emergence), diet (sugar or sugar+YH) and doses of methoprene (0, 0.05 and 0.5%).

Source		d.f.	F	P
Apodeme length	Age	8	36.1033	<0.0001
	Dose	2	7.6594	0.0005
	Diet	1	862.1145	<0.0001
Apodeme width	Age	8	26.0481	<0.0001
	Dose	2	6.4900	0.0016
	Diet	1	748.3590	<.0001
Apodeme area	Age	8	55.3256	<0.0001
	Dose	2	15.6152	<0.0001
	Diet	1	96.6794	<0.0001
	Age × Diet	8	2.6348	0.0078

3.1.2 Testes

Age, diet, and methoprene significantly influenced testes length and area with significant differences between the diets in effects of age and methoprene dose (i.e., significant diet × age and diet × dose interactions). Both length and area of the testes increased with age, but the rate

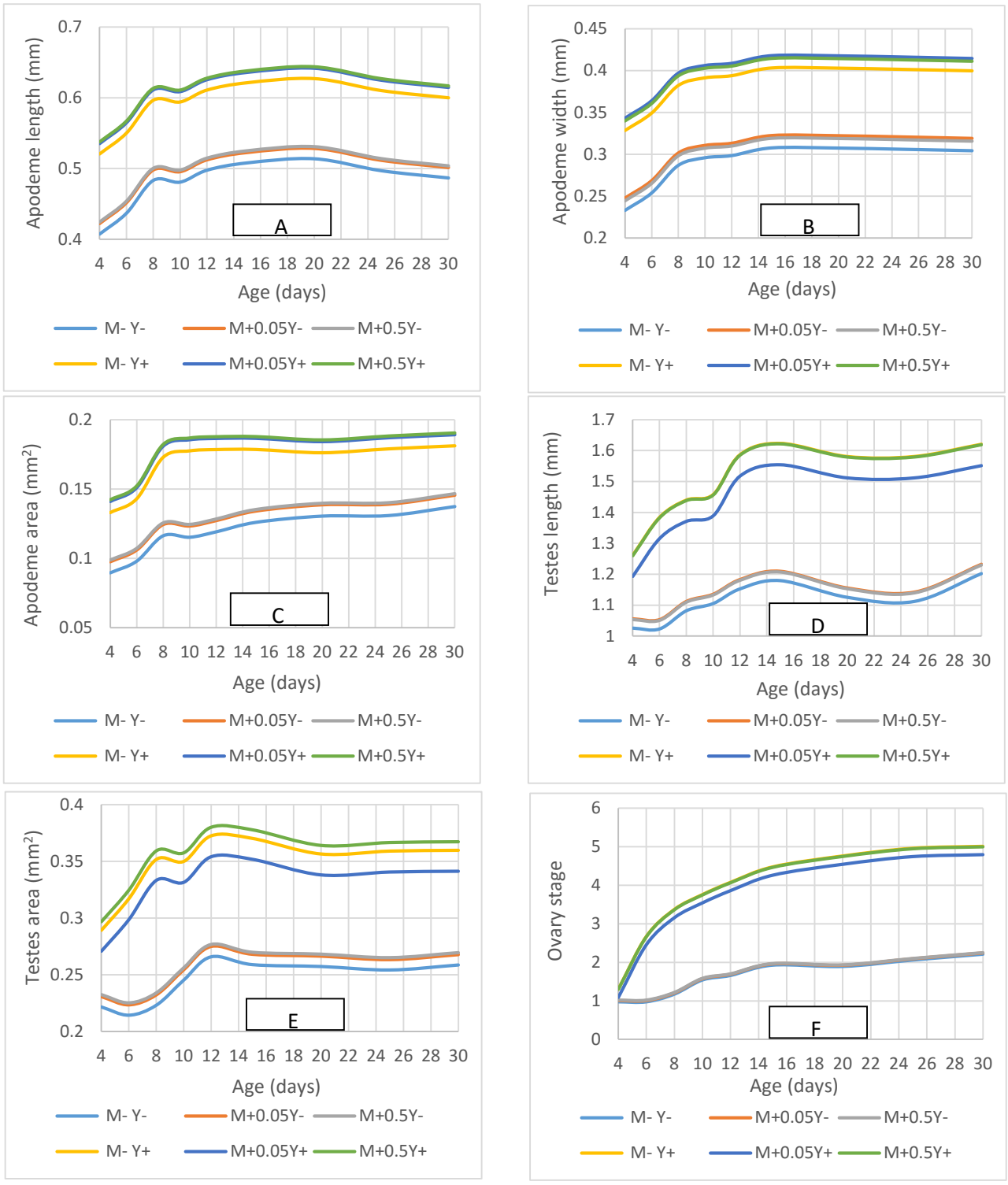

Fig. 4. Effect of different diets and methoprene treatments on reproductive organ development of Q-fly according to their age; male ejaculatory apodeme length (A), width (B), area (C), testes length (D), area (E), and female ovarian development (F). Flies were treated with methoprene at 0.05% and Yeast and Sugar ($M+_{0.05}Y+$), methoprene at 0.5% and Yeast and Sugar ($M+_{0.5}Y+$), no methoprene; Yeast and Sugar (M-Y+), methoprene at 0.05% and only sugar ($M+_{0.05}Y-$), methoprene at 0.5% and only sugar ($M+_{0.5}Y-$) and no methoprene, only sugar (M-Y-).
of growth was much steeper for flies with access to YH and plateaued at larger size (Fig 4).

For flies with access to YH, those treated with 0.05% methoprene had significantly smaller

testes length and area than those with 0 or 0.5% methoprene. In contrast, for flies with access to sugar only, those treated with 0.5% methoprene had larger testes than those treated with 0%, and those treated with 0.05% were intermediate.

Table 2

Analysis of variance for testes (length and area) in relation to fly age (2, 4, 6, 8, 10, 12, 15, 20, 25 and 30 days post emergence), diet (sugar or sugar+YH) and doses of methoprene (0, 0.05 and 0.5%).

Source		d.f.	F	P
Testes length	Age	8	47.4265	<0.0001
	Dose	2	4.7022	0.0093
	Diet	1	64.5730	<0.0001
	Age × Diet	8	7.5536	<0.0001
	Dose × Diet	2	11.3485	<0.0001
Testes area	Age	8	29.5249	<0.0001
	Dose	2	9.0657	0.0001
	Diet	1	56.1100	<0.0001
	Age × Diet	8	3.7774	0.0002
	Dose × Diet	2	10.0757	<0.0001

3.1.3. Ovaries

Female age had a significant effect on ovarian stage development. Stages of ovarian development strongly varied with diet and doses of methoprene treatment with significant differences between the diets in effects of age and methoprene dose (i.e., significant diet × age and diet × dose interactions). Ovaries of females with access to YH grew until ca. 15 days of age, at which time they were mature. In contrast, ovaries of females with access to only sugar were still pre-vitellogenesis even at 30 days of age. Females with access only to sugar showed no significant effects of methoprene on ovarian development, but females with access to YH showed significant effects that closely mirrored effects of methoprene on testes development in males; those treated with 0.05% methoprene had significantly less developed ovaries than those with 0 or 0.5% methoprene.

Table 3

Analysis of variance for ovarian stage in relation to fly age (2, 4, 6, 8, 10, 12, 15, 20, 25 and 30 days post emergence), diet (sugar or sugar+YH) and doses of methoprene (0, 0.05 and 0.5%).

Source		d.f.	F	P
Ovary stage	Age	8	502.1941	<0.0001
	Dose	2	7.4259	0.0006
	Diet	1	16.7168	<0.0001
	Age × Diet	8	118.0914	<0.0001
	Dose × Diet	2	9.4016	<0.0001

3.2. Sexual maturation

3.2.1. Mating probability

For males, mating probability varied with age, diet, and methoprene treatment, with significant diet × age interactions (Table 4). Male age had a significant influence on mating propensity for each of the diet treatments, and followed a polynomial trend that increased to a peak at ca. 20 days and then declined (Fig. 5, Table 4). The age × diet interaction is apparent when comparing diet groups in Fig. 5; flies provided YH exhibited a steep increase in mating probability, and greater mating probability overall, compared with flies provided only sugar.

Table 4

Logistic regression analysis of variation in mating probability in relation to fly age (2, 4, 6, 8, 10, 12, 15, 20, 25 and 30 days post emergence), diet (sugar or sugar+YH) and doses of methoprene (0, 0.05, 0.1 and 0.5%).

	Source	**d.f.**	**G**	**P**
Male				
	Age	1	147.465198	<0.0001
	Age × Age	1	70.4978063	<0.0001
	Dose	3	36.0834404	<0.0001
	Diet	1	219.867896	<0.0001
	Age × Diet	1	4.67508699	0.0306
Female				
	Age	2	496.175898	<0.0001
	Age × Age	2	37.3821167	<0.0001
	Dose	6	8.21398305	0.2228
	Diet	2	136.699354	<0.0001
	Age × Diet	2	9.5962173	0.0082

Although male flies fed only sugar showed a significant increase in mating probability over the tested period, the slope was shallower than that for flies fed YH. All methoprene doses increased mating propensity, and there were no differences amongst the doses in this effect (Fig. 5).

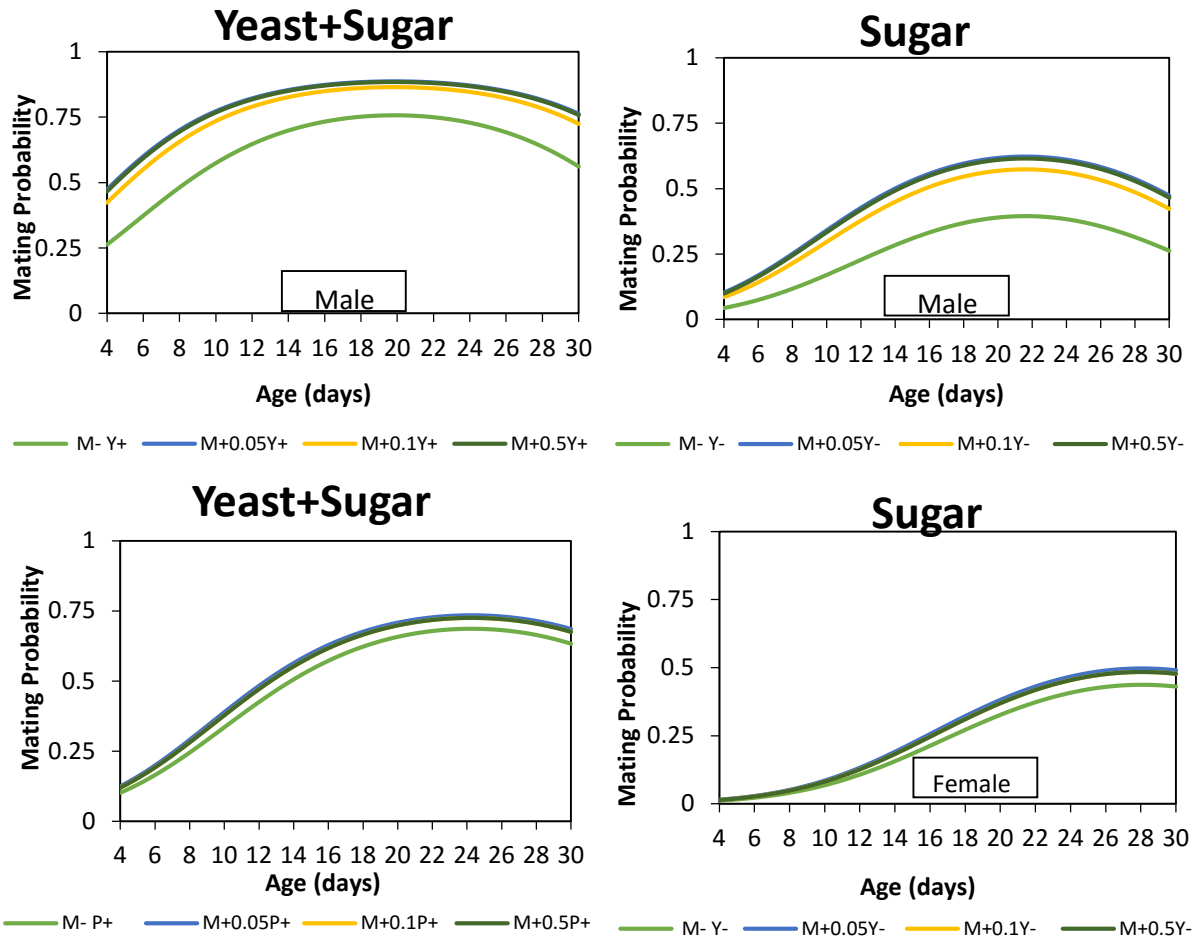

Fig. 5. Mating probability of males and females resulted from different diet-methoprene combinations according to their age. Flies were treated with methoprene at 0.05% and Yeast+Sugar ($M+_{0.05}Y+$), methoprene at 0.1% and Yeast+Sugar ($M+_{0.1}Y+$), methoprene at 0.5% and Yeast+Sugar ($M+_{0.5}Y+$), no methoprene; Yeast and Sugar (M-Y+), methoprene at 0.05% and only sugar ($M+_{0.05}Y-$), methoprene at 0.1% and only sugar ($M+_{0.1}Y-$), methoprene at 0.5% and only sugar ($M+_{0.5}Y-$) and no methoprene, only sugar (M-Y-).

For females, mating probability varied with age and diet, with significant diet × age interactions but, importantly, did not vary with methoprene treatment (Table 4, Fig. 5). Female age had a significant influence on mating propensity for each of the diet treatments, and followed a polynomial trend that increased to a peak at ca. 25 days and then declined (Fig. 5, Table 4). The age × diet interaction is apparent when comparing diet groups in Fig. 5; females fed on both diets showed a significant increase in mating probability over the tested period, but this increase was much steeper for female flies provided access to YH.

3.2.2. Copula latency

In male flies, copula latency (rank transformed) varied significantly with age and methoprene treatment, with significant differences between the diets in effects of methoprene (i.e., significant diet × dose interaction). Copula latency followed a polynomial trend, decreasing significantly with fly age till 20 days and then increasing. No effects of methoprene treatment were evident for males provided access to YH. In contrast, for males provided access to sugar only, copula latency was shortest for dose of 0.05%, longest for doses of 0.1%, and intermediate for doses of 0 and 0.5% (Fig. 6).

In female flies, effects of age on mating latency (rank transformed) differed significantly for the two diets (i.e., significant diet × age interaction). For females provided access to sugar only copula latency decreased with age whereas for females provided access to YH copula latency increased with age. Unlike males, for females there was no effect of methoprene on copula latency.

Table 5

Analysis of variance for copula latency in relation to fly age (2, 4, 6, 8, 10, 12, 15, 20, 25 and 30 days post emergence), diet (sugar or sugar+YH) and doses of methoprene (0, 0.05, 0.1 and 0.5%).

	Source	d.f.	F	P
Male				
	Age	1	7.0135	0.0083
	Age × Age	1	16.0760	<0.0001
	Dose	3	5.8392	0.0006
	Diet	1	2.1062	0.1417
	Dose × Diet	3	2.7296	0.0430
Female				
	Age	1	1.1727	0.2794
	Dose	3	0.1592	0.9238
	Diet	1	0.0799	0.7775
	Age × Diet	1	11.6040	0.0007

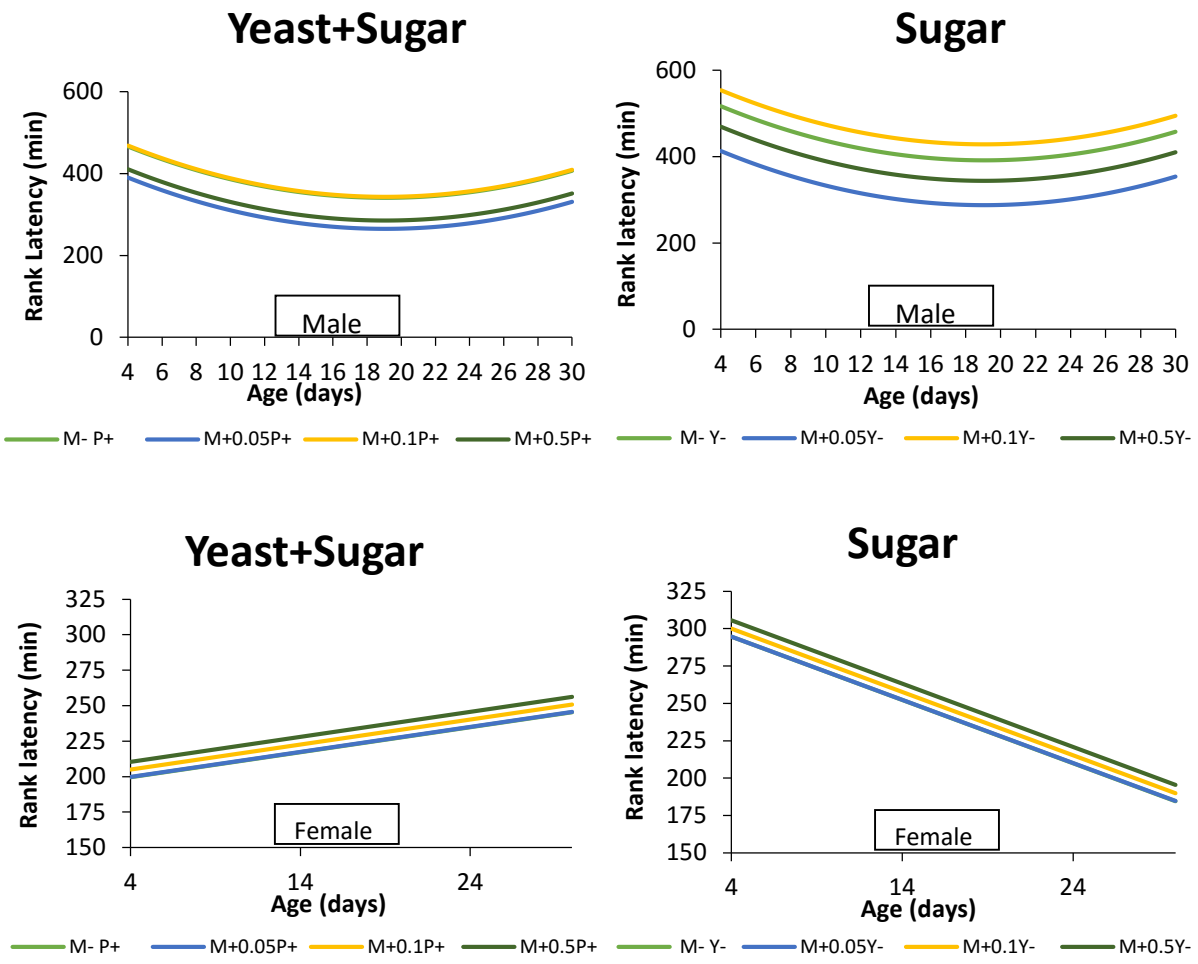

Fig. 6. Mating latency of males and females for different diet-methoprene treatments according to their age. Flies were treated with methoprene at 0.05% and Yeast+Sugar (M+0.05Y+), methoprene at 0.1% and Yeast+Sugar (M+0.1Y+), methoprene at 0.5% and Yeast+Sugar (M+0.5Y+), no methoprene; Yeast and Sugar (M-Y+), methoprene at 0.05% and only sugar (M+0.05Y-), methoprene at 0.1% and only sugar (M+0.1Y-), methoprene at 0.5% and only sugar (M+0.5Y-) and no methoprene, only sugar (M-Y-).

3.2.3. Copula duration

Male copula duration was affected significantly by age, following a polynomial trend. Males with access to YH had significantly longer copulations than males with access to only sugar. However, the difference in copula duration of males on the two diets increased with age (Fig. 7). There were also significant effects of methoprene on copula duration; males treated with 0.0 and 0.5% methoprene had longer copulations than controls, and males treated with 0.1% methoprene were intermediate.

Like males, female copula duration varied with age following a polynomial trend. Females with access to YH copulated for longer than females with access to only sugar. For each of the

diets, methoprene application resulted in longer copula duration. All doses of methoprene resulted in longer copulations than the controls.

Table 6
Analysis of variance for copula duration in relation to fly age (2, 4, 6, 8, 10, 12, 15, 20, 25 and 30 days post emergence), diet (sugar or sugar+YH) and doses of methoprene (0, 0.05, 0.1 and 0.5%).

	Source	d.f.	F	P
Male				
	Age	1	19.6541	<0.0001
	Age×Age	1	12.1965	0.0005
	Dose	3	5.1082	0.0017
	Diet	1	40.7085	<0.0001
	Age×Diet	1	4.0467	0.0446
Female				
	Age	1	1.9605	0.1621
	Age×Age	1	3.8918	0.0491
	Dose	3	2.8578	0.0367
	Diet	1	12.1884	0.0005

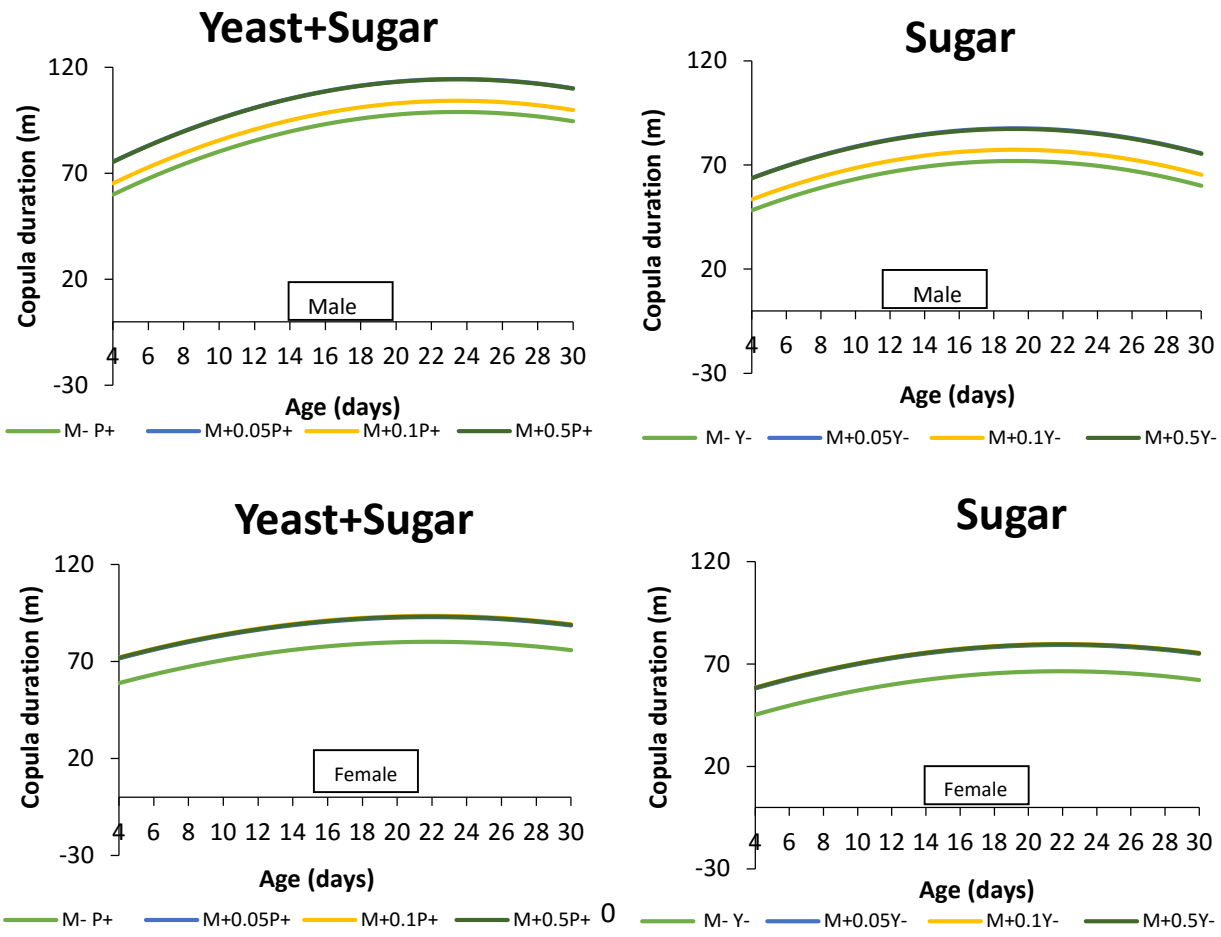

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Fig. 7. Copula duration of males and females for different diet-methoprene treatments according to their age. Flies were treated with methoprene at 0.05% and Yeast+Sugar (M+0.05Y+), methoprene at 0.1% and Yeast+Sugar (M+0.1Y+), methoprene at 0.5% and Yeast+Sugar (M+0.5Y+), no methoprene; Yeast and Sugar (M-Y+), methoprene at 0.05% and only sugar (M+0.05Y-), methoprene at 0.1% and only sugar (M+0.1Y-), methoprene at 0.5% and only sugar (M+0.5Y-) and no methoprene, only sugar (M-Y-).

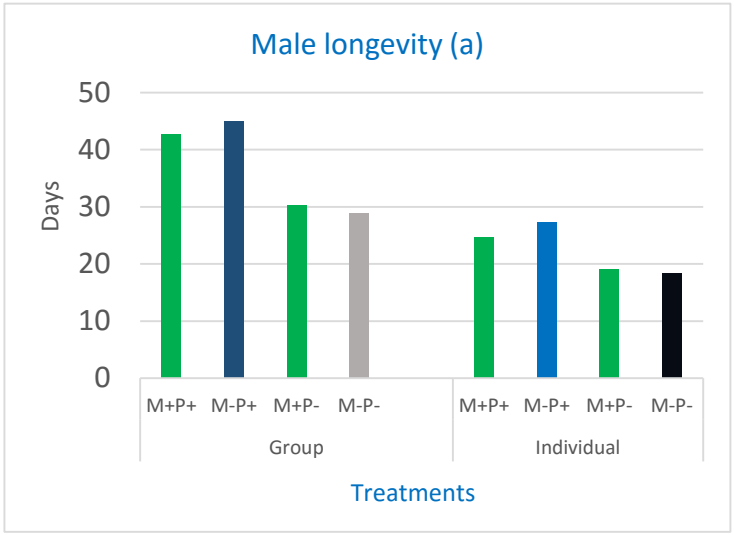
3.3. Longevity

In group cages, access to YH resulted in longer lifespan for both males and females (Table 7). In individual cages, access to YH resulted in longer lifespan for males but not females. In both group and individual cages, there was no evidence that methoprene at any dose influenced longevity.

Table 7

Analysis of variance for longevity in cages (group or individual), diet (sugar or sugar+YH) and doses of methoprene (0, 0.05, 0.1 and 0.5%).

		Source	d.f.	F	P
Group cage	Male				
		Dose	3	0.0973	0.9615
		Diet	1	53.3563	<0.0001
	Female				
		Dose	3	0.8218	0.4830
		Diet	1	34.8728	<0.0001
Individual cage	Male				
		Dose	3	0.5458	0.6519
		Diet	1	21.4511	<0.0001
	Female				
		Dose	3	1.1135	0.3467
		Diet	1	2.6569	0.1058



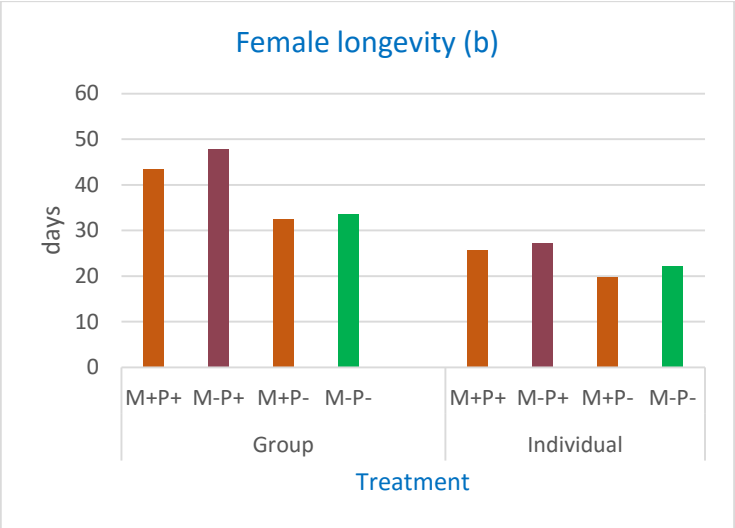


Fig. 8. Longevity of males (a) and females (b) for different diet-methoprene treatments according to their age

4. Discussion

The incorporation of methoprene into a diet containing yeast hydrolysate (YH) accelerated sexual maturity and enhanced mating success in un-irradiated male Queensland fruit fly (*Bactrocera tryoni* or 'Q-fly'), but methoprene in the diet had comparatively little effect on females. Collins et al. (2014) found that methoprene treatment promoted reproductive development in Q-flies of both sexes, with similar effects when adults were treated directly by topical application of methoprene/acetone solution and when pupae were dipped in methoprene/acetone solution prior to emergence.

4.1. Reproductive development

Access to dietary YH in the adult diet has a profound effect on male reproductive organ development. Growth of the testes and ejaculatory apodeme was significantly faster in males that received YH when compared with males that received only sugar. This finding closely resembled that of Perez-Staples et al. (2011), who reported benefits of 48 hours of access to YH in development of testes, accessory glands, ejaculatory apodeme, and ejaculatory duct of *Bactrocera tryoni*. Males fed YH had larger reproductive organs than males fed sucrose only. Similarly Weldon and Taylor, (2011) reported that inclusion of YH in the adult diet of *Bactrocera tryoni* was associated with faster growth of the ejaculatory apodeme in comparison with sugar and various natural sources (bat guano, bird droppings, citrus pollen, and wheat pollen). Application of methoprene accelerated growth of the ejaculatory apodeme regardless

of doses and diets. However, the effects of methoprene on testes growth was negligible. Compared with controls, lower levels of methoprene reduced testes area in YH-fed males. Similarly, Fry (2006) noted that in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diopsidae), control males and males receiving a medium dose of a juvenile hormone analogue had similar testes size at each age class, whereas the flies receiving a high dose had consistently smaller testes.

Like males, diet had a significant impact on ovarian development in females. Provision of YH into pre-release diet induced a substantial increase in growth of ovaries; females provided yeast were fully mature at 15 days of age whereas females provided only sugar had comparatively negligible ovarian growth. Effects of diet on ovarian maturation were in general agreement with earlier studies of Vijaysegaran et al. (2002) who found practically no ovarian development over 3 weeks for females of *Bactrocera tryoni* fed only sucrose in contrast to rapid development of ovaries in females fed yeast, with peak size at 14 days of age. Similarly, access to sugar did not support any significant ovarian maturation as all the females had previtellogenic ovaries till 30 days of age (Yap et al., 2015). Perez-Staples et al. (2011) reported that Q-fly *Bactrocera tryoni* females fed yeast hydrolysate for 48 or 24 h exhibited low levels of ovarian development, similar to females fed sucrose only. However, females provided continuous access to yeast hydrolysate showed rapid development of ovaries, reached peak size at 15 days of age.

Application of methoprene did not accelerate ovarian development at any dose. Interestingly, low doses of methoprene actually reduced ovarian development in females provided access to YH. This result contrasts with Duan et al. (1995), who found that topical application of a juvenile hormone analogue, pyriproxyfen, significantly enhanced ovarian development (number of eggs in ovaries and length of egg follicles) of female apple maggot flies, *Rhagoletis pomonella* . Juvenile hormone functions in vitellogenic oocyte development in many insects (Wyatt and Davey, 1996), and so effects on ovarian development might be anticipated. The lack of positive effects on ovarian development of Q-fly are somewhat surprising, and the negative effects of low doses even more surprising, but both of these effects are encouraging for the potential application of methoprene as a pre-release supplement for SIT.

4.2. Sexual development

Access to YH has a profound effect on sexual activity of Q-fly. YH-fed males became sexually mature earlier and achieved a steep increase in mating success compared with males provided only sugar. In the present study, for both sexes YH-fed flies showed consistently much

higher mating probability compared to sugar fed flies. Even when mating probability increased with age, sugar fed flies did not reach the same level of mating success of flies that were provided YH. Addition of YH to the pre-release diet has rendered enhanced male sexual performance in *Ceracititis capitata, Bactrocera cucurbitae, Anastrepha ludens, Anastrepha obliqua, Anastrepha fraterculus, Anastrepha suspensa, Bactrocera tryoni, Bactrocera correcta, Bactrocera dorsalis, Bactrocera philippinensis, Bactrocera zonata, Ceratitis rosa* (Faria et al., 2008; Yuval et al., 2007; Taylor and Yuval, 1999; Haq et al., 2013; Haq et al., 2010a; Haq et al., 2010b; Liedo et al., 2013; Segura et al., 2009; Pereira et al., 2010a; Pereira et al., 2009, 2010b; Pereira et al., 2013; Perez-Staples et al., 2007; Perez-Staples et al., 2009; Weldon et al., 2008; Shelly et al., 2005; Haq and Hendrichs, 2013; Quilici et al., 2013).

Besides the effects of protein source, we found marked effects of age on the mating propensity of flies in each of the diet treatments. Mating propensity increased with age for Q-flies of both sexes in both diets, followed a polynomial trend that peaked at 20-25 days in both diets. Similar polynomial effects of age on mating propensity have been reported for Q-fly by Perez-Staples et al. (2008) who, as in the present study, provided the flies to just a short period of access to dietary YH after emerging and then maintained flies with only sugar.

In addition to effects of YH, male mating propensity was increased by methoprene treatment. Similar positive effects of methoprene treatment on sexual performance have been found in *A. fraterculus, A. obliqua, A. serpentina, A. ludens, A. suspensa, B. cucurbitae* (Segura et al., 2009; Gomez et al., 2013; Pereira et al., 2009; Haq et al., 2010a; Haq et al., 2013). Methoprene treated Q-fly males fed on only sugar also showed increased mating propensity in comparison to untreated counterparts, but this trend was very weak. This may be related to the fact that protein is crucial for the development of reproductive organs of flies undergoing accelerated maturity; in the absence of YH, the flies lack the 'building blocks' for reproductive tissues (Perez-Staples et al., 2011). Although males treated with methoprene in either diet had an accelerated maturation, there was no significant variation in mating performance among the doses of methoprene. This indicates a threshold effect that is below the lowest 0.05% dose used in the present study.

Interestingly, while strong effects of YH were evident, methoprene treatment did not increase female mating propensity. These results are very similar to those reported by Segura et al. (2013) who concluded that males and females responded differently to methoprene exposure in *A. fraterculus* and this might serve as a physiological sexing system that minimizing mating between sterile males and sterile females in releases of bisexual strains.

In males, copula latency varied significantly with age, decreasing in all treatments to a trough at ca. 20 days. A similar trend of decreasing copula latency in Q-fly has been reported previously by Perez-Staples et al. (2007). Effects of diet on male copula latency were not evident in the present study, but have been reported previously by Perez-Staples et al. (2007) and Prabhu et al. (2008). However, it is important to note the differences in experimental approach - in the present study flies only had access to YH for 48 hours whereas in these other studies the flies had continuous access throughout life.

When male Q-fly had access to YH in their diet, no effects of methoprene on copula latency were evident. This contrasts findings of Haq et al. (2010a) who reported that the application of methoprene and access to a diet incorporating YH decreased male copula latency in *Bactrocera cucurbitae*. Interestingly, for sugar-fed males treatment with the lowest dose of methoprene induced shorter mating latency than was the case for males that were untreated or had been treated with higher doses.

For female Q-fly, YH-fed and sugar-fed flies had very different patterns in copula latency as they aged. YH-fed females exhibited increasing latency to mate as they aged whereas sugar fed females exhibited the opposite pattern. In female in either diet however, there were no effects of methoprene on female copula latency.

Male Q-flies with access to YH had longer copulations than those provided only sugar, and these results are consistent with previous reports for Q-fly (Perez-Staples et al., 2007) as well as for *B. cucurbitae* (Haq et al. 2010a). By contrast, in *C. capitata* and in *A. striata*, YH fed males tend to have shorter copulations than those fed sugar only (Blay and Yuval, 1997; Perez-Staples and Aluja, 2004). In *A. serpentina, A. ludens* and *A. obliqua* mating duration appears to be unaffected by adult diet (Aluja et al., 2001). In Q-fly, application of methoprene significantly increased the mating duration of both males and females compared with control for both diets. This finding is partly consistent with Haq et al. (2010a), who found that the application of methoprene significantly increased copula duration of YH-fed male *B. cucurbitae* but not sugar-fed males.

4.3. Longevity

Access to dietary protein increased longevity for both males and females. This corresponds with the previous work on Q-fly (Taylor et al., 2013) and *B. cucurbitae* (Haq and Hendrichs, 2013). Similar results have been reported in *A. ludens* and *A. obliqua* when the sugar/yeast ratio was 9:1 or 24:1, but not with the 3:1 ratio (Liedo et al., 2013). Likewise, access to YH increases

longevity in *Rhagoletis indifferens* (Yee, 2003) and *A. serpentina* (Jacome et al., 1995), whereas in *C. capitata* access to protein has an opposite effect on female and male life-span (Müller et al., 1997).

Importantly for the present study, there was no evidence that methoprene effects longevity of either male or female Q-fly, regardless of diet. Similarly, methoprene treatment alone had no adverse effects on longevity of *C. capitata* (Faria et al., 2008) and *A. suspensa* (Pereira, 2005). In *B. cucurbitae*, pre-release feeding on YH and methoprene treatment enhances male longevity (Haq and Hendrichs, 2013).

4.4. Application to SIT

Q-flies are currently released as a bisexual strain in SIT programmes, and countering benefits on males, methoprene incorporation into pre-release protein diet may also promote reproductive development and sexual performance of females. This might reduce SIT efficacy, as sterile males will predominantly mate with the mature virgin sterile females rather than with wild females, limiting their sperm transfer to wild females. However, while methoprene had strong positive effects on male sexual and reproductive development in Q-fly, methoprene did not significantly affect sexual maturation and ovarian development in females. Accordingly this hormonal supplement, along with YH diet, might to some extent induce physiological separation of the sexes in field settings; although both sexes are produced and released, males will gain vastly greater sexual performance benefits from a pre-release methoprene treatment. As there is no genetic sex strain for Q-fly our findings suggest that incorporation of methoprene into the pre-release protein diet of Q-flies could be a feasible means of releasing a bisexual strain with relatively competitive males along with sexually immature females, thereby operating somewhat like a unisexual strain.

Enhanced sexual maturation following application of methoprene and access to YH has been reported in many fruit fly species, but the problem lies in developing methods for incorporating methoprene into the pre-release adult holding system. In most of the previous studies, methoprene has been dissolved in acetone and applied to adults topically or pupae by dipping or bathing in acetone solution. In the context of SIT, millions of insects need to be released and so topical application of methoprene is impractical and hazardous (Pereira et al., 2013; Segura et al., 2013). Direct incorporation of methoprene in to pre-release diet rather than using acetone is a very promising and practical approach to accelerating sexual and reproductive development of male Q-fly, and will likely have direct application for other fruit fly species.

References

Aluja, M., 1994. Bionomics and management of *Anastrepha*. Annual Review of Entomology 39, 155-178.

Aluja, M., Jácome, I., Macías-Ordóñez, R., 2001. Effect of adult nutrition on male sexual performance in four neotropical fruit fly species of the genus *Anastrepha* (Diptera: Tephritidae). Journal of Insect Behavior 14, 759-775.

Aluja, M., Ordano, M., Teal, P.E., Sivinski, J., García-Medel, D., Anzures-Dadda, A., 2009. Larval feeding substrate and species significantly influence the effect of a juvenile hormone analog on sexual development/performance in four tropical tephritid flies. Journal of Insect Physiology 55, 231-242.

Blay, S., Yuval, B., 1997. Nutritional correlates of reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). Animal Behaviour 54, 59-66.

Collins, S.R., Reynolds, O.L., Taylor, P.W., 2014. Combined effects of dietary yeast supplementation and methoprene treatment on sexual maturation of Queensland fruit fly. Journal of Insect Physiology 61, 51-57.

Dominiak, B.C., Daniels, D., 2012. Review of the past and present distribution of Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) and Queensland fruit fly (*Bactrocera tryoni* Froggatt) in Australia. Australian Journal of Entomology 51, 104-115.

Drew, R., Hooper, G., Bateman, M., 1978. Economic fruit flies of the South Pacific Region. Economic fruit flies of the South Pacific Region.

Drew, R., Yuval, B., 2000. The evolution of fruit fly feeding behavior. Fruit Flies. CRC, Boca Raton, 731-749.

Drew, R.A., 1989. The tropical fruit flies (Diptera: Tephritidae: Dacinae) of the Australasian and Oceanian regions. Memoirs of the Queensland Museum 26.

Duan, J.J., Prokopy, R.J., Yin, C.M., Bergweiler, C., Oouchi, H., 1995. Effects of pyriproxyfen on ovarian development and fecundity of *Rhagoletis pomonella* flies. Entomologia Experimentalis et Applicata 77, 17-21.

Enkerlin, W., 2005. Impact of fruit fly control programmes using the sterile insect technique, Sterile Insect Technique. Springer, pp. 651-676.

Fanson, B.G., Weldon, C.W., Pérez-Staples, D., Simpson, S.J., Taylor, P.W., 2009. Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (*Bactrocera tryoni*). Aging Cell 8, 514-523.

Faria, M.J., Pereira, R., Dellinger, T., Teal, P.E.A., 2008. Influence of methoprene and protein on survival, maturation and sexual performance of male *Ceratitis capitata* (Diptera: Tephritidae). Journal of Applied Entomology 132, 812-819.

Fry, C.L., 2006. Juvenile hormone mediates a trade-off between primary and secondary sexual traits in stalk-eyed flies. Evolution & Development 8, 191-201.

Gilchrist, A., Dominiak, B., Gillespie, P., Sved, J., 2006. Variation in population structure across the ecological range of the Queensland fruit fly, *Bactrocera tryoni*. Australian Journal of Zoology 54, 87-95.

Gomez, Y., Teal, P.E.A., Pereira, R., 2013. Enhancing efficacy of Mexican fruit fly SIT programmes by large-scale incorporation of methoprene into pre-release diet. Journal of Applied Entomology 137, 252-259.

Gomez-Simuta, Y., Teal, P., 2010. Hormonas juveniles y su aplicación en la Técnica del Insecto Estéril. Moscas de la Fruta: Fundamentos y Procedimiemtos para su Manejo. Ed. by Pablo Montoya P, Toledo J, Hernandez E, S y G. editores, Mexico, 357-368.

Hancock, D.L., 2000. The distribution and host plants of fruit flies (Diptera: Tephritidae) in Australia. Department of Primary Industries, Queensland.

Haq, I., Caceres, C., Hendrichs, J., Teal, P., Wornoayporn, V., Stauffer, C., Robinson, A.S., 2010a. Effects of the juvenile hormone analogue methoprene and dietary protein on male melon fly *Bactrocera cucurbitae* (Diptera: Tephritidae) mating success. Journal of Insect Physiology 56, 1503-1509.

Haq, I., Caceres, C., Hendrichs, J., Teal, P.E.A., Stauffer, C., Robinson, A.S., 2010b. Methoprene modulates the effect of diet on male melon fly, *Bactrocera cucurbitae*, performance at mating aggregations. Entomologia Experimentalis et Applicata 136, 21-30.

Haq, I., Cáceres, C., Liedo, P., Soriano, D., Jessup, A., Hendrichs, J., Teal, P., Robinson, A., 2013. Effect of methoprene application, adult food and feeding duration on male melon fly starvation survival. Journal of Applied Entomology 137, 61-68.

Haq, I., Hendrichs, J., 2013. Pre-release feeding on hydrolysed yeast and methoprene treatment enhances male *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) longevity. Journal of Applied Entomology 137, 99-102.

Hendrichs, J., Prokopy, R.J., 1994. Food foraging behavior of frugivorous fruit flies.

Hendrichs, J., Robinson, A., Cayol, J., Enkerlin, W., 2002. Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: the importance of mating behavior studies. Florida Entomologist 85, 1-13.

Jacome, I., Aluja, M., Liedo, P., Nestel, D., 1995. The influence of adult diet and age on lipid reserves in the tropical fruit fly *Anastrepha serpentina* (Diptera: Tephritidae). Journal of Insect Physiology 41, 1079-1086.

Jang, E.B., 1995. Effects of mating and accessory gland injections on olfactory-mediated behavior in the female Mediterranean fruit fly, *Ceratitis capitata*. Journal of Insect Physiology 41, 705-710.

Jang, E.B., McInnis, D.O., Kurashima, R., Carvalho, L.A., 1999. Behavioural switch of female Mediterranean fruit fly, *Ceratitis capitata*: mating and oviposition activity in outdoor field cages in Hawaii. Agricultural and Forest Entomology 1, 179-184.

Liedo, P., Orozco, D., Cruz-López, L., Quintero, J., Becerra-Pérez, C., del Refugio Hernández, M., Oropeza, A., Toledo, J., 2013. Effect of post-teneral diets on the performance of sterile *Anastrepha ludens* and *Anastrepha obliqua* fruit flies. Journal of Applied Entomology 137, 49-60.

Meats, A., Holmes, H., Kelly, G., 2004. Laboratory adaptation of *Bactrocera tryoni* (Diptera: Tephritidae) decreases mating age and increases protein consumption and number of eggs produced per milligram of protein. Bulletin of Entomological Research 94, 517-524.

Meats, A., Leighton, S., 2004. Protein consumption by mated, unmated, sterile and fertile adults of the Queensland fruit fly, *Bactrocera tryoni* and its relation to egg production. Physiological Entomology 29, 176-182.

Müller, H.-G., Wang, J.-L., Capra, W.B., Liedo, P., Carey, J.R., 1997. Early mortality surge in protein-deprived females causes reversal of sex differential of life expectancy in Mediterranean fruit flies. Proceedings of the National Academy of Sciences 94, 2762-2765.

Oliver, J., 2007. Summary of national fruit fly-related activities stocktake released. Plant Protection News.

Pereira, R., Sivinski, J., Teal, P., Brockmann, J., 2010a. Enhancing male sexual success in a lekking fly (*Anastrepha suspensa* Diptera: Tephritidae) through a juvenile hormone analog has no effect on adult mortality. Journal of Insect Physiology 56, 1552-1557.

Pereira, R., Sivinski, J., Teal, P.E.A., 2009. Influence of methoprene and dietary protein on male *Anastrepha suspensa* (Diptera:Tephritidae) mating aggregations. Journal of Insect Physiology 55, 328-335.

Pereira, R., Sivinski, J., Teal, P.E.A., 2010b. Influence of a Juvenile Hormone Analog and Dietary Protein on Male *Anastrepha suspensa* (Diptera: Tephritidae) Sexual Success. Journal of Economic Entomology 103, 40-46.

Pereira, R., Teal, P., Conway, H., Worley, J., Sivinski, J., 2013. Influence of methoprene and dietary protein on maturation and sexual performance of sterile *Anastrepha ludens* (Diptera: Tephritidae). Journal of Applied Entomology 137, 191-199.

Pereira, R.M.C., 2005. Influence of a juvenile hormone analog and dietary protein on male Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae), sexual behavior. University of Florida.

Perez-Staples, D., Aluja, M., 2004. *Anastrepha striata* (Diptera: Tephritidae) females that mate with virgin males live longer. Annals of the Entomological Society of America 97, 1336-1341.

Perez-Staples, D., Harmer, A.M.T., Collins, S.R., Taylor, P.W., 2008. Potential for pre-release diet supplements to increase the sexual performance and longevity of male Queensland fruit flies. Agricultural and Forest Entomology 10, 255-262.

Perez-Staples, D., Prabhu, V., Taylor, P.W., 2007. Post-teneral protein feeding enhances sexual performance of Queensland fruit flies. Physiological Entomology 32, 225-232.

Perez-Staples, D., Weldon, C.W., Smallridge, C., Taylor, P.W., 2009. Pre-release feeding on yeast hydrolysate enhances sexual competitiveness of sterile male Queensland fruit flies in field cages. Entomologia Experimentalis et Applicata 131, 159-166.

Perez-Staples, D., Weldon, C.W., Taylor, P.W., 2011. Sex differences in developmental response to yeast hydrolysate supplements in adult Queensland fruit fly. Entomologia Experimentalis et Applicata 141, 103-113.

Plant Health Australia, 2008. Draft National Fruit Fly Strategy. March 2008, p. 63. ISBN 978-0-646-48663-5.

Prabhu, V., Perez-Staples, D., Taylor, P., 2008. Protein: carbohydrate ratios promoting sexual activity and longevity of male Queensland fruit flies. Journal of Applied Entomology 132, 575-582.

Quilici, S., Schmitt, C., Vidal, J., Franck, A., Deguine, J., 2013. Adult diet and exposure to semiochemicals influence male mating success in *Ceratitis rosa* (Diptera: Tephritidae). Journal of Applied Entomology 137, 142-153.

Radhakrishnan, P., Taylor, P.W., 2007. Seminal fluids mediate sexual inhibition and short copula duration in mated female Queensland fruit flies. Journal of Insect Physiology 53, 741-745.

Radhakrishnan, P., Taylor, P.W., 2008. Ability of male Queensland fruit flies to inhibit receptivity in multiple mates, and the associated recovery of accessory glands. Journal of Insect Physiology 54, 421-428.

Raghu, S., Halcoop, P., Drew, R.A., 2003. Apodeme and ovarian development as predictors of physiological status in *Bactrocera cacuminata* (Hering)(Diptera: Tephritidae). Australian Journal of Entomology 42, 281-286.

Segura, D.F., Caceres, C., Vera, M.T., Wornoayporn, V., Islam, A., Teal, P.E.A., Cladera, J.L., Hendrichs, J., Robinson, A.S., 2009. Enhancing mating performance after juvenile hormone treatment in *Anastrepha fraterculus*: a differential response in males and females acts as a physiological sexing system. Entomologia Experimentalis et Applicata 131, 75-84.

Segura, D.F., Utges, M.E., Liendo, M.C., Rodriguez, M.F., Devescovi, F., Vera, M.T., Teal, P.E.A., Cladera, J.L., 2013. Methoprene treatment reduces the pre-copulatory period in *Anastrepha fraterculus* (Diptera: Tephritidae) sterile males. Journal of Applied Entomology 137, 19-29.

Shelly, T.E., Edu, J., Pahio, E., 2005. Influence of diet and methyl eugenol on the mating success of males of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). Florida Entomologist 88, 307-313.

Shelly, T.E., Nishimoto, J.I., Edu, J., 2009. Influence of methoprene on the age-related mating propensity of males of the Oriental fruit fly and the Mediterranean fruit fly (Diptera: Tephritidae). Florida Entomologist 92, 193-198.

Taylor, P.W., Perez-Staples, D., Weldon, C.W., Collins, S.R., Fanson, B.G., Yap, S., Smallridge, C., 2013. Post-teneral nutrition as an influence on reproductive development, sexual performance and longevity of Queensland fruit flies. Journal of Applied Entomology 137, 113-125.

Taylor, P.W., Yuval, B., 1999. Postcopulatory sexual selection in Mediterranean fruit flies: advantages for large and protein-fed males. Animal Behaviour 58, 247-254.

Teal, P., Gomez-Simuta, Y., Dueben, B., Holler, T., Olson, S., 2007. Improving the efficacy of the sterile insect technique for fruit flies by incorporation of hormone and dietary supplements into adult holding protocols, Area-Wide Control of Insect Pests. Springer, pp. 163-173.

Teal, P., Gomez-Simuta, Y., Proveaux, A., 2000. Mating experience and juvenile hormone enhance sexual signaling and mating in male Caribbean fruit flies. Proceedings of the National Academy of Sciences 97, 3708-3712.

Teal, P.E.A., Pereira, R., Segura, D.F., Haq, I., Gomez-Simuta, Y., Robinson, A.S., Hendrichs, J., 2013. Methoprene and protein supplements accelerate reproductive development and improve mating success of male tephritid flies. Journal of Applied Entomology 137, 91-98.

Vijaysegaran, S., Walter, G., Drew, R., 2002. Influence of adult diet on the development of the reproductive system and mating ability of Queensland fruit fly *Bactrocera tryoni* (Frogratt)(Diptera: Tephritidae). Journal of Tropical Agriculture and Food Science 30, 119-136.

Weldon, C.W., Perez-Staples, D., Taylor, P.W., 2008. Feeding on yeast hydrolysate enhances attraction to cue-lure in Queensland fruit flies, *Bactrocera tryoni*. Entomologia Experimentalis et Applicata 129, 200-209.

Weldon, C.W., Taylor, P.W., 2011. Sexual development of wild and mass-reared male Queensland fruit flies in response to natural food sources. Entomologia Experimentalis et Applicata 139, 17-24.

White, I.M., Elson-Harris, M.M., 1992. Fruit flies of economic significance: their identification and bionomics. CAB International.

Wyatt, G.R., Davey, K.G., 1996. Cellular and Molecular Actions of Juvenile Hormone. ll. Roles of Juvenile Hormone in Adult Insects. Advances in Insect Physiology 26, 1.

Yap, S., Fanson, B.G., Taylor, P.W., 2015. Mating Reverses Actuarial Aging in Female Queensland Fruit Flies. PloS one 10, e0132486.

Yee, W., 2003. Effects of cherries, honeydew, and bird feces on longevity and fecundity of *Rhagoletis indifferens* (Diptera: Tephritidae). Environmental Entomology 32, 726-735.

Yuval, B., Maor, M., Levy, K., Kaspi, R., Taylor, P., Shelly, T., 2007. Breakfast of champions or kiss of death? Survival and sexual performance of protein-fed, sterile Mediterranean fruit flies (Diptera: Tephritidae). Florida Entomologist 90, 115-122.

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