Journal of Insect Physiology 61 (2014) 51–57

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Combined effects of dietary yeast supplementation and methoprene treatment on sexual maturation of Queensland fruit fly

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ARTICLE INFO

Article history:
Received 14 June 2013
Received in revised form 28 December 2013
Accepted 2 January 2014
Available online 11 January 2014

Keywords:
Tephritidae
Sterile insect technique
Diet
Methoprene
Maturation

ABSTRACT

Yeast hydrolysate supplements promote maturation of many tephritid flies targeted for control using the sterile insect technique (SIT), including Queensland fruit fly (*Bactrocera tryoni*; 'Q-fly'). Recently, application of the juvenile hormone analogue methoprene has been demonstrated to further promote maturation in some species. We here investigate the separate and combined effects of yeast hydrolysate and methoprene treatment on sexual maturation of sterile male and female Q-flies. Two methods of applying methoprene solution were used; topical application to adults and dipping of pupae. Consistent with previous studies, access to yeast hydrolysate greatly increased maturation of both male and female Q-flies. Maturation was further promoted by methoprene treatment, with similar effects evident for males and females and for both application methods. For flies provided access to yeast hydrolysate supplements, methoprene treatment advanced maturation by approximately 2 days. No effects of diet or methoprene treatment were found on timing of copulation or copula duration. Countering the positive effects on sexual maturation, dipping of pupae in methoprene/acetone solution did diminish emergence rates and flight ability indices, and increased rates of wing deformity. Promising results of the present study encourage further investigation of treatment methods that maximise maturation while minimising detrimental effects on other aspects of fly quality.

1. Introduction

Nutrition plays an integral role in the ecology and developmental biology of tephritid fruit flies (Diptera: Tephritidae). In addition to nutritional requirements of somatic maintenance, many tephritids are an autogenic, relying on adult nutrition to sustain the development of reproductive tissues (Drew and Yuval, 2000). In particular, a source of protein is needed by many species to complete reproductive development and for sexual performance. In nature nutrition may come from bacteria, yeasts, plant leachates, fruit juices, pollen, and faeces (Hendrichs et al., 1993; Drew and Yuval, 2000), which can vary both in quality and abundance (Courtice and Drew, 1984; Manrakhan and Lux, 2006).

Just as investigation of nutritional requirements is important for understanding the biology of tephritids in nature, it is also important for understanding how best to maintain domesticated tephritids for management programs. Many tephritid flies are devastating pests of horticultural crops, and some are managed using the sterile insect technique (SIT) in which flies are mass-reared, sterilized, and released in very large numbers to disrupt reproduction of wild populations (Knipling, 1955; Krafsur, 1998). Most tephritid breeding colonies in laboratories and mass-rearing programs supply adult flies with yeast hydrolysate (YH) as a rich source of amino acids (i.e., protein) and micronutrients (see Fanson and Taylor, 2012), with positive effects on reproductive development and mating propensity reported in numerous species, including *Anastrepha ludens* (Periera et al., 2013), *Anastrepha obliqua* (Liedo et al., 2013), *Anastrepha suspensa* (Pereira et al., 2009), *Anastrepha serpentina* (Aluja et al., 2001), *Bactrocera cucurbitae* (Haq et al., 2010a,b), *Bactrocera philippinensis* (Obra and Resilva, 2013), *Ceratitis capitata* (Yuval et al., 2002), and *Rhagoletis pomonella* (Webster and Stoffolano, 1978). In the Queensland fruit fly, *Bactrocera tryoni* (Q-fly'), addition of YH to sucrose in the diet has been associated with accelerated development (Vijaysegaran et al., 2002; Meats and Leighton, 2004; Pérez-Staples et al., 2011; Weldon and Taylor, 2011), increased mating propensity (Pérez-Staples et al., 2007), increased sperm transfer (Pérez-Staples et al., 2008), increased ability of males to induce sexual inhibition in mates (Pérez-Staples et al., 2008), increased fecundity (Meats et al., 2004), and increased longevity when carbohydrates are continuously available (Pérez-Staples et al., 2007; Prabhu et al., 2008; Fanson et al., 2009) (for a review, see Taylor et al., 2013b). Given the importance of nutrition for reproduction and survivorship,

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0022-1910/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jinsphys.2014.01.002

and the potential for nutritional constraints in the field, there has been substantial interest in the potential of YH as a pre-release supplement for tephritid flies used in SIT, including Q-fly (Reynolds and van der Rijt, 2011). SIT requires that released males reach sexual maturity and compete effectively for mates in the field. Daily mortality rates of flies released in SIT can be very high (Meats, 1998; Meats et al., 2003; Dominiak et al., 2000), and so pre-release treatments that accelerate maturation, promoting sexual activity at younger ages when survivorship is higher, are of particular value.

Paralleling interest in YH as a pre-release supplement, there has been interest in additional treatments that might accelerate maturation and enhance sexual performance of released sterile flies. The juvenile hormone analogue methoprene has shown particular promise in many tephritids. Juvenile hormone regulates insect growth and development, and production of secondary sexual signals, such as pheromones (Teal et al., 2000, 2007). When dissolved in acetone and applied to adults or pupae, methoprene has been found to enhance maturation or sexual performance in *Anastrepha fraterculus* (Segura et al., 2009, 2013), *A. ludens* (Aluja et al., 2009; Periera et al., 2013), *A. suspensa* (Pereira et al., 2009), *B. cucurbitae* (Haq et al., 2010a,b), and *C. capitata* (Faria et al., 2008), although these effects do vary across contexts or studies in *A. ludens* (Aluja et al., 2009) and *C. capitata* (Shelly et al., 2009; Faria et al., 2008). In contrast, effects of methoprene treatment have not been detected in studies of *A. obliqua*, *A. striata* (Aluja et al., 2009), and *Bactrocera dorsalis* (Shelly et al., 2009).

Because positive effects of YH supplementation on maturation were known previously, several studies investigating effects of methoprene have considered the combined effects of YH and methoprene (e.g., Faria et al., 2008; Haq et al., 2010a,b; Liendo et al., 2013; Pereira et al., 2009, Periera et al., 2013; for a review, see Teal et al., 2013). In particular, it is important to know whether effects of methoprene provide benefits beyond those already achieved with YH. Building on our detailed understanding of how YH influences development and sexual performance of Q-flies, in the present study we investigated the effects of methoprene treatment on sexual maturation in combination with and apart from effects of YH. The effects of YH and methoprene were assessed using mating trials (Pérez-Staples et al., 2007), and effects of methoprene treatment on fly quality was assessed using standard measures of flight ability and longevity under stress (IAEA/FAO/USDA, 2003; Collins et al., 2009).

2. Materials and methods

2.1. General methods and diet treatments

Q-fly pupae were obtained from the Fruit Fly Production Facility at Elizabeth Macarthur Agricultural Institute at Camden, New South Wales, Australia (for production details, see Dominiak et al., 2008). Pupae were sealed in plastic 'zip-lock' bags at ~2000 pupae per bag and left overnight to achieve a hypoxic environment. Bags were then exposed to a 70–75 Gy dose of gamma radiation from a Cobalt 60 Gammacell source (dose rate 9 Gy min^{-1}) located at Macquarie University, Sydney, one day before emergence (following Collins et al., 2009). This is the standard dose used in Q-fly SIT and induces > 99.5% sterility (Collins et al., 2009). Only flies that emerged within 24 h of irradiation were used for diet and methoprene treatment experiments. All flies emerged in a controlled environment laboratory at Macquarie University in Sydney and were housed in 5 l cages each containing approximately 110 flies. Flies were sorted by sex on the day of emergence, with half of the test flies provided access to YH for 48 h post emergence and then maintained on a sugar only diet (YH+), while the remainder were maintained solely on a sugar diet (YH−). This time frame is consistent with the standard pre-release holding period of Q-fly SIT (Reynolds and van der Rijt, 2011). Both males and females were assessed for the effects of diet and methoprene treatment on maturation.

Un-irradiated flies were set up in a similar manner but with constant access to a full diet (sugar and YH in separate dishes), a week prior to sterile fly irradiation to obtain mating partners at peak sexual maturity, 10–14 days old, to be paired with diet and methoprene treated flies in mating experiments.

2.2. Methoprene treatment

Flies were treated with methoprene (M+) using two methods: topical and dipping. For topical treatment, 1 μl of methoprene dissolved in acetone (5 μg/μl) was applied onto and females at one day post emergence. Flies were gently aspirated into a cloth mesh bag and gently held in place by pulling the mesh tight. A micropipette (Eppendorf AG, Hamburg Germany) was used to apply the methoprene solution by gently placing the pipette tip through a hole in the mesh and dripping the required volume onto the dorsal thorax of the fly. Treated flies were then released into a standard 5 l holding container and provided with one of the diet treatments. For the dipping treatment, irradiated pupae were immersed in a 1:100 dilution of the previously mixed 5 μg/μl methoprene solution (following Segura et al., 2013). Immediately after irradiation treatment one hundred pupae were poured into a glass Petri dish so that each pupa rested flat on the floor of the dish. Pupae were submerged for 5 min in methoprene/acetone solution so that each pupa was covered completely. Pupae were removed and poured gently onto a paper towel and left to dry for 10 min. Pupae were then placed in 5 l cages to emerge. Dipped males and females were sorted by sex on the day of emergence into separate 5 l cages with approximately 110 flies per cage. All flies were provided a diet of sugar from the day of emergence, but YH+ flies were provided YH in a separate dish for the initial 48 h post-emergence.

2.3. Mating

Mating trials were conducted at 2, 4, 6, 8, 10 and 12 days post emergence of male and female flies. All flies were maintained in the lab on a 1 dusk:12 light:1 dawn; 10 dark cycle in which the lights turned on in stages over the period of an hour during dawn and turned off in stages over the period of an hour at dusk. Q-fly mating takes place only at dusk (Tychsen and Fletcher, 1971). Observations began 90 min prior to the onset of dusk to observe any flies that initiated copulation early. No copulations commenced after all lights were off at the end of the dusk period.

At least four hours before the onset of dusk, twenty males and twenty females from each treatment group (topical M+YH+, topical M+YH−, dipped M+YH+, dipped M+YH−, M−YH+, M−YH−) were placed individually in clear plastic 1.25 L containers. Each fly was individually paired with an un-irradiated sexually mature (10–14 days old) fly of the opposite sex. The time of onset of copulation was recorded for each mating pair to assess copula latency, defined as time from the start of dusk till the onset of mating, in minutes. To assess copula duration for each mating pair, observations continued until all copulations had terminated. On each test day new flies from each batch were paired with new mature partners. This resulted in 240 paired flies trialled for each test day post emergence, providing a total of 1440 test pairs for each replicate batch of flies. A total of three replicate batches of flies were assessed over a period of three months.