Pre-release dietary supplements of methoprene and raspberry ketone increase field abundance of sterile Queensland fruit flies (Diptera: Tephritidae)

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Abstract

The sterile insect technique (SIT) is a sustainable pest management tool based on the release of millions of sterile insects that suppress reproduction in targeted populations. Success of SIT depends on survival, maturation, dispersal, and mating of released sterile insects. Laboratory and field cage studies have demonstrated that dietary supplements of methoprene and raspberry ketone (RK) promote sexual maturation of adult Queensland fruit fly, *Bactrocera tryoni* (Froggatt), and may hence shorten the delay between release and maturity in the field. We investigated the effects of methoprene and RK dietary supplements on field abundance of sexually mature sterile Q-flies relative to untreated flies fed only sugar and yeast hydrolysate before release at two days of age. Compared with untreated flies, more methoprene- and RK-treated flies were recaptured in cuelure traps to which only sexually mature males are attracted. At distances of 100 and 200 m from the release point recapture rates were higher for methoprene- and RK-treated flies than for untreated flies, but at 300 m recapture rates were low and were similar for treated and untreated flies. Rainfall, relative humidity, wind speed, and wind direction did not affect recapture rates, but temperature was positively correlated with recapture rates for all treatments. There was a strong correlation between the number of sterile and wild flies caught in traps, indicating co-location in the field. Dietary supplements of methoprene and RK can substantially increase abundance of sexually mature sterile male Q-flies in the field following release as two-day-old immature adults.

Keywords: *Bactrocera tryoni*, SIT, sexual maturation, cuelure, release-recapture

Introduction

Tephritid fruit flies attack a vast diversity of fruit crops (Enkerlin 2005, Bhattacharya et al. 2013, Qin et al. 2015) and inflict significant losses in crop yield and quality, as well as in market access (Wang et al. 2018). The Queensland fruit fly ('Q-fly'), *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), is a widespread and economically damaging pest of horticulture in the eastern states and territories of Australia and poses a significant biosecurity threat to all other states (Drew et al. 1978, White and Elson-Harris 1992, Hancock et al. 2000, Sutherst et al. 2000, Clarke et al. 2011, Dominiak and Daniels 2012). Diverse management tools have been used to manage Q-fly, including cover sprays, protein bait sprays, male annihilation technique (MAT), mass trapping, and sterile insect technique (SIT) (Jessup et al. 2007). SIT is a species-specific insect pest management approach in which millions of mass-reared sterile males are released into pest populations (Dyck et al. 2005). The large numbers of released sterile males overflood the wild population, such that the large majority of matings by wild females are with sterile males, resulting in unfertilised eggs (Knipling 1955, Krafsur 1998, Hendrichs 2002, Nagel and Peveling 2005, Gurr and Kvedaras 2010, Benelli et al. 2014a,b). Given the success of SIT in integrated pest management programs for suppressing and eradicating some of the most damaging tephritid fruit flies, including the Mexican fruit fly *Anastrepha ludens* (Loew) (Orozco-Dávila et al. 2015), the melon fly *Zeugodacus cucurbitae* (Coquillett) (Kakinohana 1994, Ito et al. 2003), the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Reyes et al. 2007), and the Oriental fruit fly *Bactrocera dorsalis* (Hendel) (Orankanok et al. 2007), there is substantial interest in developing effective SIT for control of Q-fly in Australia. SIT has been used periodically for more than 30 years to eradicate outbreaks of Q-fly in fruit fly free regions (Meats et al. 2003, Reynolds and van der Rijt 2011, Reynolds et al. 2012, Fanson et al. 2014). With increased demand for sustainable

64 and effective pest management practises, there is a need to improve the efficacy and cost-
65 effectiveness of SIT for management of Q-fly.

66 Sterile Q-flies have commonly been released as sexually immature adults at 2–3 d
67 of age (Reynolds and van der Rijt 2011, Reynolds et al. 2014). The released flies suffer
68 high mortality rates (Meats 1998, Weldon and Meats 2010), and because they may take up
69 to an additional week to mature (Pérez-Staples et al. 2011, Weldon et al. 2008) numbers
70 may be greatly reduced before the released flies become effective as control agents.
71 Successful SIT not only requires large numbers of mature and sexually active sterile
72 insects in the field (Hendrichs et al. 2002) but also spatially even distribution or co-
73 localization of sterile insects with the wild populations (Gavriel et al. 2012). Poor field
74 performance of sterile Q-fly males in terms of dispersal and survival can lead to
75 insufficient coverage of the target area, reducing the efficacy of SIT (Meats and
76 Smallridge 2007, Dominiak 2012, Rempoulakis and Nestel 2012, Navarro-Llopis et al.
77 2014, Reynolds et al. 2014).

78 Q-flies are anautogenous, requiring nutritional resources, especially protein, as
79 adults to complete sexual development (Vijaysegaran et al. 2002, Weldon and Taylor
80 2011, Fanson and Taylor 2012). In nature, nutrition from bacteria, yeasts, leaf leachates,
81 and bird faeces to sustain fruit fly development can be scarce and patchy (Hendrichs et al.
82 1993, Manrakhan and Lux 2006). Yeast hydrolysate (YH), a rich source of amino acids
83 that is commonly used as a key component of adult diet in laboratory and mass rearing
84 programs, is very effective in supporting the reproductive development of male Q-flies
85 even when provided only for the 2–3 d standard pre-release holding period and can
86 improve the overall performance of sterile Q-flies (Pérez -Staples et al. 2007, Dominiak et
87 al. 2008, Pérez-Staples et al. 2009, Weldon and Taylor 2011, Reynolds et al. 2014). Recent
88 studies have found that Q-fly development can be further accelerated by dietary

supplements of raspberry ketone (RK), a phytochemical with structural similarity to cuelure (Akter et al. 2017a, Akter and Taylor 2018), and methoprene, a juvenile hormone analogue (Collins et al. 2014, Adnan et al. 2018, 2020). Beyond enhancement of sexual maturation, RK feeding has been reported to suppress cuelure attraction in male Q-flies (Akter et al. 2017b, Khan et al. 2017) raising the possibility that MAT and SIT could be implemented simultaneously (Barclay et al. 2014).

Though recent studies indicate significant potential to increase the prevalence of sexually mature male flies in SIT programs through RK- or methoprene-mediated acceleration of sexual maturation, there remains a need to corroborate effects in an open field setting. In the present study, we assessed the effects of pre-release diet (sugar + YH, or sugar + YH + RK, or sugar + YH + methoprene) on re-capture of sterile Q-fly in cuelure traps in an open field setting. Based on the trap capture data, we also discuss the extent to which numbers of RK-treated flies in traps were affected by maturation and RK-induced reduction in responsiveness of mature male Q-flies.

Materials and Methods

Study insects

Q-flies were sourced from a laboratory colony at Macquarie University, New South Wales, Australia that originated from wild material collected in the Central Coast region of New South Wales and was maintained following Moadeli et al. (2017). The flies were kept in a controlled environment room set at 25 ± 0.5°C with 65 ± 5% RH and L13: D11 photoperiod with dawn and dusk simulated by gradually ramping the lights up and down through 0.5 h at the beginning and end of the light phase, respectively. Six releases were carried out, with each release from a different weekly production batch. Fly batches were obtained from different parental generations of laboratory rearing: G14 for the first,

second, and third field releases, G15 for the fourth release, and G16 for the fifth and sixth releases.

Experimental protocol: irradiation, marking and dietary regimes

Q-fly pupae were irradiated two days before emergence at a nominal dosage of 65 Gy (Collins et al. 2008, 2009) using a gamma irradiation source at Macquarie University. Irradiated pupae were marked according to their dietary regime and release using eight different fluorescent dyes (Comet Blue 60, Stellar Green 8, Flame Orange 4, Magenta 10, Astral Pink 1, Arc Chrome 6, Lunar Yellow 27, and Blaze 5) (Swada, 7 Stanley Street, Stalybridge, Cheshire, SK15 1SS, England) at a rate of 0.5 g of dye per 1000 pupae (Meats and Edgerton 2008, Dominiak et al. 2010, Akter et al. 2020). Approximately 50 mL of marked pupae (1 mL = ca. 40 pupae) from each diet group (treatment) (ca. 2000 pupae) were transferred to an open 90 mm diameter Petri dish and then placed in separate fine mesh cages (47.5 × 47.5 × 47.5 cm, Megaview BugDorm-4F4545). Three different dietary regimes were provided to the irradiated flies after emergence for two days before the flies were released: (1) control (YH (MP Biomedicals, Aurora, OH, USA) plus sugar (1:3)), (2) methoprene-treated (control diet + 0.5% methoprene), and (3) RK-treated (control + 1.25% RK) with a water source (soaked sponge) in a 70 mL sample container. The methoprene (NOMOZ® pellets, a trademark of Pacific Biologists, Brisbane, Queensland) and RK (≥ 98% purity, Sigma-Aldrich) supplemented diets were prepared dry by mixing the methoprene and RK with control diet using a blender, respectively. Effective doses of methoprene and RK that are known to accelerate maturation without impacting survival were selected following Adnan et al. (2018) and Akter et al. (2017a), respectively.

Field release: trapping network, release, recapture and identification

Fly releases were conducted at a ca. 200 ha site that included diverse habitats in Somersby (ca. 70 km North of Sydney), NSW, Australia. Fruit were present on citrus and kiwi fruit plants during the study period. Cages containing sterile adult flies were transported from the laboratory to the release point of the field (33°22'10.7"S 151°18'12.4"E) in an air-conditioned vehicle. Releases were carried out at two-week intervals (first release on 13 December 2016 and sixth release on 14 February 2017). All flies were released from a single point as a static ground release. The trapping network comprised 12 cuelure, specific to attract mature males traps (Biotrap V2 X containing 2 g of cuelure plus Malathion as a toxicant) (BioTrap Australia Pty Ltd) in a concentric trapping network with N-S and E-W axes to calculate the geographical coordinates of the traps. In other respects, the pattern of trap placement was random, as long as each trap was covering an estimated area of 1.5 ha and had minimal overlap with the attraction zone of other traps. The closest trap was indeed 68.2 m from the release centre and the most distant was 370 m from the release centre, based on previous studies of distance travelled from the release point by irradiated sterile flies (Meats et al. 2006, Weldon and Meats 2010, Dominiak 2012, Gilchrist and Meats 2012, Dominiak et al. 2013). The placement of each trap with its coordinates is presented in Supplementary Table 1. The traps with the lures and toxicants were placed in the field 3 d before the first release and were replaced only if damaged during the experimental period (cuelure and malathion traps are recommended for replacing every four months to maintain maximum effectiveness; Lloyd et al. 2010). Traps were inspected 3, 7, and 14 d after each release. Each sample (recaptured sterile Q-flies and wild Q-flies) was collected from the traps using forceps and transferred to 5-mL plastic vials. Flies were frozen at -20 °C upon return at Macquarie University until identification. For identification of flies, the head of each thawed fly was pressed to expose the ptilinum under a dissecting stereomicroscope (Leica S6E, Germany) with illumination

from an ultraviolet LED light source. Flies without fluorescent dye marking were assessed as wild.

Effect of fluorescent dyes on emergence and flight ability

For each batch of flies the effects of dye color on emergence and flight ability were evaluated in the laboratory using a flight ability assay (FAO/IAEA/USDA 2014). Differences between dye colors in emergence or flight ability could bias recapture data and obscure effects of treatment groups. After irradiating and marking the experimental flies, 3 replicates of 100 pupae per treatment were sampled from each pupal batch and prepared for the flight ability assay. The pupae were placed in separate 5.5-cm plastic Petri dish lids that were centered on black filter paper in a 9-cm plastic Petri dish. A black acrylic 'flight tube' (10 cm tall, with an inner diameter of 8.4 cm) was placed on the 9-cm Petri dishes containing the pupae. The inner surface of the flight tube was coated with unscented talcum powder to prevent flies from walking out. A 1 cm strip at the bottom of the flight tubes was wiped clean with a cloth to create a resting space for the emerged flies. Each flight tube containing the pupae was then transferred to a mesh cage (32.5 × 32.5 × 32.5 cm, Megaview BugDorm-43030F) positioned 5 cm beneath 20-W fluorescent tubes with a photoperiod of 14:10. Light intensity was ~1250 lx at the top and ~900 lx at the bottom of the cages. Room temperature was set at 25±0.5 °C with 65±5% RH. A second black 'fly back tube', talcum-coated but empty, was placed in each cage ~ 6 cm away from the flight tube to estimate the incidence of flies that escaped from the flight tube and then returned and died inside the flight tube. Flies that escaped the flight tube were collected every second day from the cage using 50 mL plastic vials. The remaining content of the tubes was collected one week after detecting the first adult.

Flies were classified as: (1) fully emerged (adult completely outside the puparium); (2) not emerged (adults within an unopened puparium); (3) partially emerged (adults stuck partially inside the puparium); (4) deformed (fully emerged but with morphological alterations, such as curly wings); (5) fliers (number of flies that escaped the tube and were collected from the mesh cage plus flyback); (6) flyback (number of flies inside the second tube plus the same number of morphologically normal flies inside the first tube); (7) non-fliers (morphologically normal flies that were found inside the first tube minus the number of flies inside the second tube). The following parameters were calculated:

Emergence rate: percentage of fully emerged adults calculated as (N pupae – [N not emerged + N partially emerged] / N pupae) × 100.

Percentage fliers: percentage of flies that are able to fly, calculated as (N pupae – [N not emerged + N partially emerged + N deformed + N non-fliers] / N pupae) × 100.

Statistical analysis

Except for where other packages are specified, all statistical analyses were performed using R version 3.4.0.

Emergence and flight ability

A Levene's test was first performed to assess whether variances of means were homoscedastic. A one-way ANOVA was performed on data that were found to be normally distributed. A GLM model with quasibinomial distribution was employed to test the effect of fluorescent dyes on Emergence rate and Percentage of fliers.

Field abundance

A chi-square test of independence was used to compare the relative abundance of flies from each treatment group in traps, based on the assumption that a total of ~ 24,000 sterile male flies were released for each treatment over the six releases (assuming 1:1 sex ratio, as reported previously for Q-fly reared on the diet used in the present study; Moadeli et al. 2017). A series of pairwise chi-squared tests was used for post-hoc analysis, with Holm criterion used to adjust *P* values for multiple comparison.

Proportion recaptured

We assessed the effects of pre-release diet treatment, distance, and weather on the probability of capture by fitting a linear mixed effects model, using logit-transformed estimates of capture proportions (~ 4,000 male Q-flies per release for each treatment) as the response, and trap ID (12 in total) and release date (6 in total) as random factors. Our analysis considered flies captured over the 3 recapture periods (0-3, 4-7 and 8-14-d following releases).

Candidate fixed factors included pre-release diet treatment (Control, Methoprene, RK), the straight-line distance from the release point to a particular trap (ranging from 68.2 m to 370 m), and five weather variables: average rainfall, average temperature, average relative humidity, average projected windspeed in the direction of travel from the point of release to the trap, and the absolute value of average projected windspeed in a direction of travel perpendicular to the direction of travel from the point of release to the trap. Candidate weather variables were averaged over the three recapture periods using 5 min interval data following sunrise and sunset time during the study. Average projected windspeed in the direction of travel was calculated from the average windspeed in 5 min intervals, s, and differences in bearing between the direction of travel and the wind bearing, d as mean{s*cos(d)}. Here positive values correspond to winds blowing flies

237 towards traps, and negative values correspond to winds blowing flies away from traps. The
238 absolute value of average projected windspeed in a direction of travel perpendicular to the
239 direction of travel was similarly calculated as |mean{s*sin(P)} |, where || denotes absolute
240 value. Our reasoning for using the absolute value is that cross-breezes in either direction
241 should similarly affect trapping success.
242 Influential predictors were identified using a backward stepwise procedure, starting
243 with a full model and sequentially dropping interactions and fixed-factor terms with p
244 values > 0.10, as calculated using F tests (Satterthwaite approximation for df). Both
245 random factors (release date and trap) were retained in all backward elimination steps. An
246 emmeans post-hoc test was conducted using a Tukey method for *P*-value estimation for
247 significance among treatments at estimated distances of 100 m, 200 m, and 300 m from the
248 release point, as distances was included in models as a continuous co-variate.
249
250 *Co-localization of released sterile flies with wild populations*
251 Co-localization of the sterile males with the wild males was assessed using the pooled
252 number of control, methoprene-treated and RK-treated sterile males. The 12 cuelure traps
253 were compared for the (1) ratio of number of sterile males caught by each trap to the total
254 number of sterile males caught by all traps and (2) ratio of number of wild males caught by
255 each trap to the total number of wild males caught by all traps. The proportions were then
256 log-transformed. For the analyses, GLM one-way ANOVA (SAS version 2019) was run
257 using the total number of flies captured over the 14 d of assessment. Each release was
258 considered a replication. Finally, a correlation coefficient was calculated between the
259 proportion of sterile males and the wild males captured in each cuelure trap. Figures for
260 overall recapture percentage and distances according to different pre-release treatments
261 were plotted using the R package ‘ggplot2’ (Wickham 2009).

Results

Emergence and flight ability

Fluorescent dyes had no significant effect on percentage emergence (F = 1.167; df = 7, 28; P = 0.353) or on percentage of fliers (F = 1.794; df = 7, 28; P = 0.128). As such, the numbers of flies released and the ability of those flies to disperse is expected to be independent of the dye used for each treatment in each release.

Proportion recapture

Our model selection procedure indicated that the primary determinants of the probability of recapture where recapture period (levels: 3, 7, 14 d), distance (range: 68.2 m - 370 m), and treatments (sugar + YH, or sugar + YH + RK, or sugar + YH + methoprene) (Table 1). Several weather variables were also considered as candidate predictors (average rainfall, average temperature, average relative humidity, average projected windspeed in the direction). Temperature was retained in the final model, but its direct biological importance is unclear. Temperature was correlated strongly with relative humidity (r = -0.73, P = 0.0006, n = 18) and was also correlated with rainfall (r = -0.47, P = 0.06, n = 18) and owing to such correlation temperature was no longer a significant effect in models if other climate variables were included (Supplementary Table 2). Accordingly, statistical effects of temperature likely reflect the combined effects of a set of associated weather variables.

Probability of recapture differed significantly among the pre-release diet treatments (χ^2= 105.070; df = 2; P < 0.0001, Fig. 1). The overall probability of recapture was significantly lower for control flies (95% CI: 0.0101 to 0.0128**)** than for methoprene- and RK-treated flies (both P < 0.05). Moreover, the overall probability of recapture was

significantly higher for methoprene-treated flies (95% CI: 0.0218 to 0.026**)** than for RK-treated flies (95% CI: 0.0179 to 0.0215) treatments (χ^2= 9.050; df = 1; *P* = 0.003, Fig. 1).

Recapture probability over the 14-day period declined with distance of the trap from the release point (Table 1), but significant distance by treatment and distance by period interactions indicate that the magnitude of this decline varied among the treatment groups and recapture days (Fig. 2). Post-hoc tests comparing estimated values at distances of 100 m, 200 m, and 300 m indicate that recapture rates were significantly higher for methoprene treated flies than for control flies at distances of 100 m, 200 m and 300 m (*P* < 0.001, Table 2). Recapture rates were significantly higher for RK-treated flies than control flies at 100 m and 200 m, but not at 300 m (Table 2). There were no differences in recapture rates between methoprene- and RK-treated flies at 100 m, 200 m and 300 m (Table 2).

Co-localization

There were significant differences between the traps in the proportion of wild males caught (*F* = 5.171; df = 11, 60; *P*<0.0001) and in the proportion of sterile males caught (*F* = 16.31; df = 11, 60; *P* < 0.0001). There was strong correlation (r = 0.873, *P* < 0.001) between the proportion of wild flies and proportion of sterile males caught in the traps (Fig. 3).

Discussion

We evaluated recapture rates of sterile male Q-fly in cuelure traps in the field and found that the flies that had been provided a pre-release diet supplemented with methoprene or RK were trapped at a higher rate than those that had been provided only the standard diet of sugar and YH. Because only mature males are attracted to cuelure, the number of flies

recaptured in cuelure traps indicates the abundance of sexually mature flies in the field. In Q-fly SIT programs, sterile flies have commonly been released at 2-3 d of age (Reynolds and van der Rijt 2011). Because of high mortality rates in the field, the number of sterile flies may be greatly reduced before the released flies reach sexual maturity (Weldon et al. 2008, Reynolds et al. 2012), and as an alternative extended holding of flies has been recommended in case of Mediterranean fruit fly (McInnis et al. 2013). Recapture rates of sterile Q-flies tends to be highly variable and low (Meats et al. 2003, 2006), and this may be attributed to poor rates of sexual maturation and survival resulting from inadequate foraging success. Accelerating sexual maturation through pre-release treatments can increase the prevalence of sexually mature sterile males in the field. Reynolds et al. (2014) found increased recapture rates when Q-flies were fed YH and sugar before release rather than only sugar, which had been a common alternative pre-release diet in some SIT programs. Here we find that further increases in recapture rates, and hence in abundance of sexually mature sterile males, can be achieved by use of pre-release dietary supplements of methoprene or RK. These findings provide field validation of previous studies carried out under laboratory and field cage conditions (Akter et al. 2017a, Adnan et al. 2018, 2020).

Dispersal of flies following release is important to ensure adequate coverage of SIT-treated areas, and this is especially the case for static ground releases, such as were deployed in the present study. As expected, recapture rates were highest in the traps that were closer to the release point. However, for all pre-release treatments, some sterile Q-fly flew 370 m, the maximum distance of trap placement in this study. As in Q-fly, in other tephritids the large majority (commonly ~90%) of released flies are typically found within ≈ 400 m of the release point, for example, in *Rhagoletis cerasi* L. (Leski 1969), *Ceratitis capitata* (Meats and Smallridge 2007, Navarro Lloppis et al. 2014), *Bactrocera oleae* (Rossi) (Rempoulakis and Nestel 2012), *A. ludens* and *A. obliqua* (Macquart) (Hernández

et al. 2007). Recapture rates and dispersal of released flies can also be affected by weather, host plant availability and landscapes features (Sonleitner and Bateman 1963, Bateman and Sonleitner 1966, Dominiak et al. 2003, Meats and Edgerton 2008, Raghu et al. 2000). We found that the trap catches were positively correlated with daily average temperature (19.18 °C to 27.02 °C) over the study period. However, Weldon and Meats (2010) found the total recaptures were negatively correlated with daily average temperature (16.4 °C to 23.5 °C) and attributed the decline in recapture rate to mortality caused by high temperature and adult Q-fly tendency to seek shelter when temperature is high to reduce desiccation (e.g. see Chown and Nicolson 2004). The extent to which temperature limits the movement of Q-fly in the field remains to be investigated. In laboratory studies where flies are exposed to a range of temperatures from well below to well above optimal, Q-flies have been found to settle consistently in locations that represent a narrow range of preferred conditions (Lynch et al. 2018). In field cages, Q-flies avoid direct sunlight by taking shelter under leaves in the canopy and move through the canopy during the day to avoid regions with excessive temperature (Inskeep et al. in press).

In addition, to assessing recapture rates and dispersal of sterile Q-flies, we also assessed co-localization of released sterile flies with wild flies. The positive correlation of captured wild and sterile flies likely reflects key landscape features that tend to drive the movements of both wild and sterile flies, in particular suitable plants. Regardless of whether the flies move toward attractive landscape features or encounter them by chance, they are expected to be less inclined to vacate landscape features that best suit their requirements, providing shelter and nutrition, than is the case for less hospitable landscape features (Raghu et al. 2000, Dominiak 2012, Schwarzmueller et al. 2019). While there are differences between sterile and wild flies in responses to abiotic conditions, such as temperature (Lynch et al. 2018) and humidity (Weldon and Meats 2010), and in within-

362 canopy distribution (Inskeep et al. 2021, in press), it appears that at a landscape scale the
363 broad preferences of sterile and wild flies are sufficiently similar to result in a high degree
364 of co-location. The co-location of sterile and wild flies is important for SIT, and this is a
365 particularly encouraging result.

366 Previous field cage and open field studies have reported a significant reduction in
367 number of RK-fed flies recaptured in cuelure traps compared with untreated flies (Akter et
368 al. 2017b, Khan et al. 2017; for a study of mechanism see Biswas et al. 2020). However, in
369 marked contrast to these previous studies we here recaptured significantly more RK-fed
370 flies in cuelure traps compared with control flies. In laboratory studies, these treatments
371 have very similar effects in accelerating maturation of male Q-flies, and the doses used
372 have little effect on longevity (Akter et al. 2017a, Adnan et al. 2018). The high number of
373 recaptured RK-treated flies indicates that pre-release supplementation with RK does not
374 have a consistent effect in suppressing responses to cuelure traps and casts doubt on the
375 viability of such supplements as a means of enabling simultaneous applications of SIT and
376 MAT. Given that it is likely that at least some suppression of cuelure response was
377 induced in RK-treated flies, it is hence likely that the number of RK-fed flies recaptured
378 underestimates the number of mature males of this treatment group in the field relative to
379 methoprene-fed flies, which were expected to have similar survival and maturation rates
380 without suppression of cuelure response. Further work is needed to better understand the
381 effects of pre-release RK supplements on cuelure responsiveness of Q-flies, and the factors
382 that can reduce such effects. Regardless, though, both methoprene-fed and RK-fed flies
383 were recaptured at much higher rates than untreated flies, and so both treatments offer
384 significant detectable advantages, as well as potentially some undetectable advantages in
385 RK-fed flies.

When sterile insects are released while still sexually immature, there is value in pre-release treatments that can accelerate maturation and thereby diminish the delay until released flies are able to participate as control agents and also to promote high overflooding ratios. While YH supplements can sustain sexual development, and increase prevalence of mature sterile male Q-flies in the field, results of the present study indicate that substantial additional benefits can be achieved when combining YH with methoprene or RK supplements.

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Competing interests

None to declare

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Figure Legends

Fig. 1. Percentage with 95% confidence interval (represented by whiskers) of flies recaptured from each pre-release treatment over the full study of six releases of approximately 24,000 male flies.

Fig. 2. Trap-level probability of recapture in relation to distance between the release point and trap location. Linear relationships were fitted to logit transformed capture probability data and then back-transformed to yield the prediction lines. The predictions depicted were calculated for average temperature for sterile male Q-flies collected 0-3 d after release (A), 4-7 d after release (B) and 8-14 d after release (C).

Fig. 3. Proportion of sterile flies (Control + Methoprene + Raspberry ketone) flies recaptured in each trap and proportion of wild flies captured in each trap to depict colocalization of sterile and wild flies.

688 **Table Legends**

689

690 **Table 1.** Type III Analysis of Variance for model predicting probability of recapture in
691 individual traps 0-3, 4-7- and 8-14-d following release) (Logit transformed
692 proportions) including the random effects of release date (5 levels) and trap ID (12
693 levels). Averages for temperature were calculated using data collected every 5
694 minutes following sunrise and sunset time over the sampling period. The significance
695 of fixed effects using Satterthwaite's method.

696

697 **Table 2.** Post-hoc tests at fixed distances of 100 m, 200 m, and 300 m to show the
698 significance among treatments (Kenward-Roger method for degrees of freedom,
699 Tukey’s method for P value adjustment comparing a family of 3 estimates).

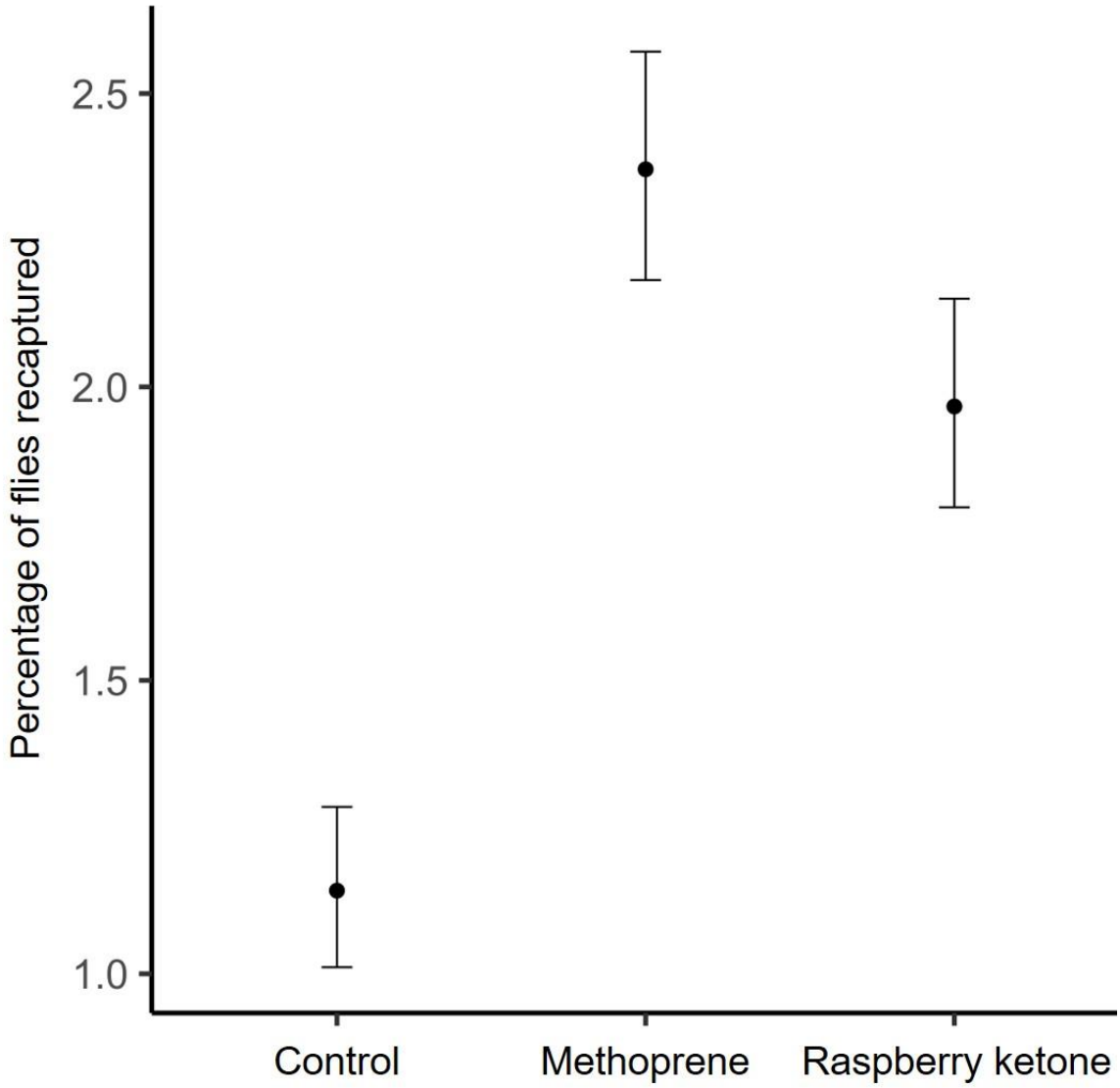
Percentage of flies recaptured
2.5
2.0
1.5
1.0
Control
Methoprene
Raspberry ketone

Fig. 1.

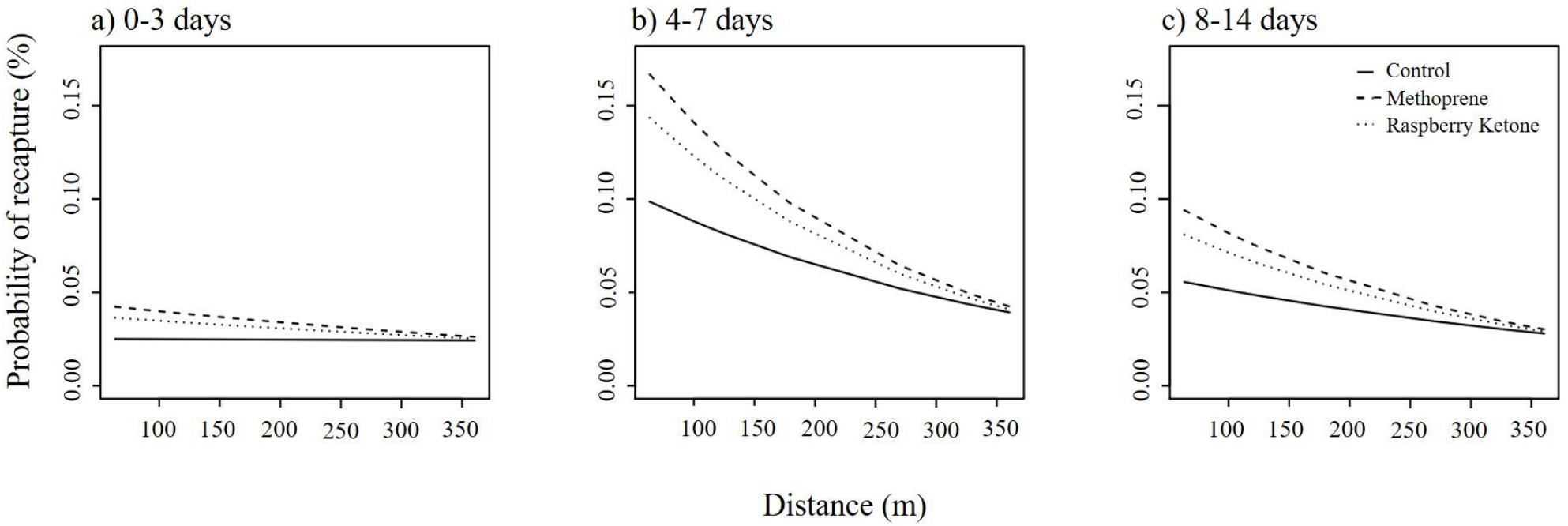
a) 0-3 days
b) 4-7 days
c) 8-14 days
Probability of recapture (%)
0.00
0.05
0.10
0.15
100
150
200
250
300
350
Distance (m)
Control
Methoprene
Raspberry Ketone

Fig. 2.

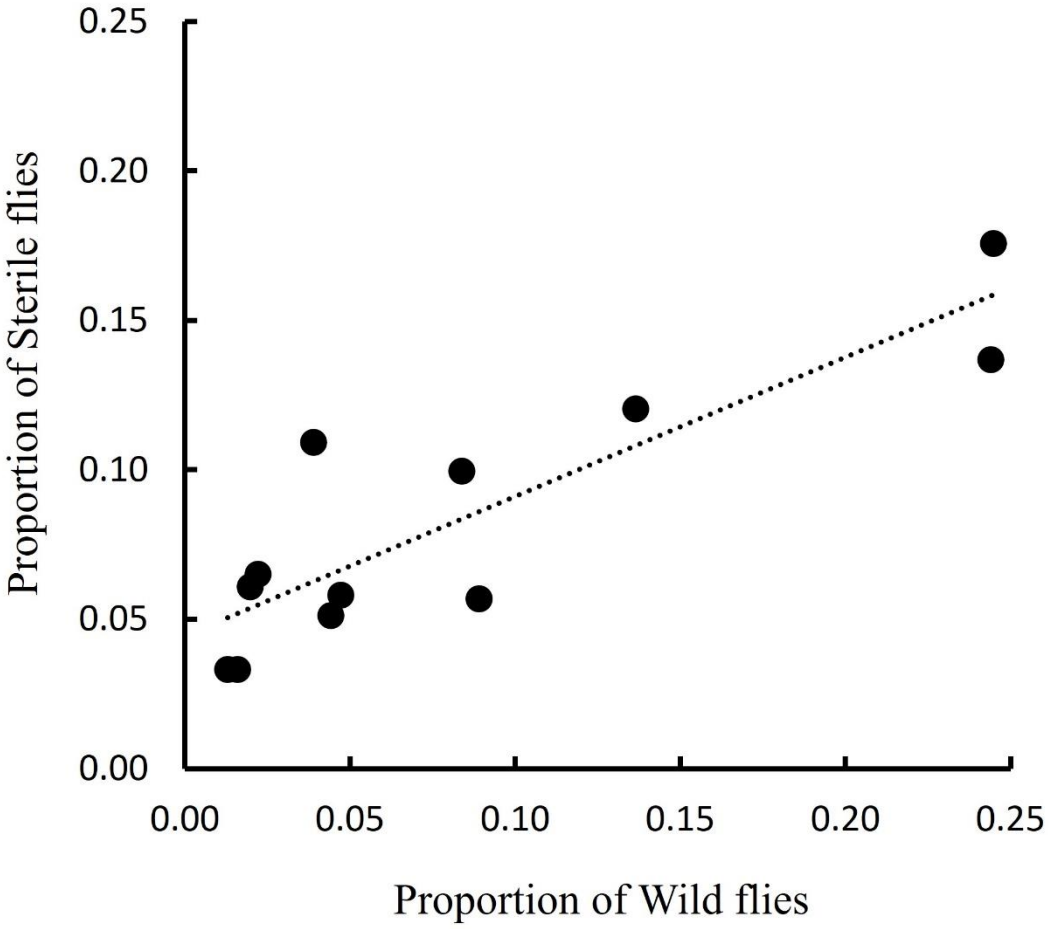
0.25
0.20
0.15
0.10
0.05
0.00
Proportion of Sterile flies
0.00
0.05
0.10
0.15
0.20
0.25
Proportion of Wild flies

Fig. 3.

Table 1.

Variables	*F*	*df*	*P*
Distance	21.996	1,10.00	< 0.001
Treatments	12.911	2, 613.00	< 0.001
Temperature	6.007	1, 14.00	0.028
Recapture period	28.362	2, 27.87	< 0.001
Distance x Treatment	4.870	2, 613.00	0.008
Distance x Recapture period	19.005	2, 613.00	< 0.001

Table 2.

Distance = 100 m					
contrast	estimate	SE	df	t.ratio	*P*
Control - Methoprene	-0.4703	0.0841	613	-5.595	<.0001
Control - RK	-0.3338	0.0841	613	-3.970	0.0002
Methoprene - RK	0.1366	0.0841	613	1.625	0.2360
Distance = 200 m					
contrast	estimate	SE	df	t.ratio	*P*
Control - Methoprene	-0.3194	0.0574	613	-5.568	<.0001
Control - RK	-0.2208	0.0574	613	-3.848	0.0004
Methoprene - RK	0.0986	0.0574	613	1.720	0.1987
Distance = 300 m					
contrast	estimate	SE	df	t.ratio	*P*
Control - Methoprene	-0.1685	0.0677	613	-2.489	0.0349
Control - RK	-0.1078	0.0677	613	-1.592	0.2498
Methoprene - RK	0.0607	0.0677	613	0.897	0.6426

Supplementary Table 1: Field trap location with distances from release point (33°22'10.7"S 151°18'12.4"E).

Trap type and number	Distances from release point (meters)	Coordinates of the traps	
		Latitude	Longitude
Cuelure 1	127.6	33°22'7.07"S	151°18'11.30"E
Cuelure 2	115.8	33°22'9.82"S	151°18'8.71"E
Cuelure 3	162.1	33°22'12.67"S	151°18'18.91"E
Cuelure 4	68.2	33°22'8.76"S	151°18'13.22"E
Cuelure 5	109.8	33°22'13.75"S	151°18'15.64"E
Cuelure 6	348.4	33°22'20.8"S	151°18'06.5"E
Cuelure 7	141	33°22'12.4"S	151°18'08.0"E
Cuelure 8	350	33°22'01.2"S	151°18'19.9"E
Cuelure 9	261	33°22'06.4"S	151°18'21.5"E
Cuelure 10	370	33°21'59.0"S	151°18'12.1"E
Cuelure 11	316	33°22'17.2"S	151°18'22.7"E
Cuelure 12	315	33°22'20.7"S	151°18'16.4"E

Supplementary Table 2: Type III Analysis of Variance table for model predicting probability of capture in individual traps over three-time periods (0-3, 4-7- and 8-14-days following release) (Logit transformed proportions) including the random effects of release date (5 levels) and trap ID (12 levels).

Variables	F	df	P
Distance	17.731	1, 10.50	0.002
Treatment	13.173	2, 602.55	< 0.001
Temperature	0.052	1, 5.73	0.828
Bearing speed (wind)	4.238	1, 353.27	0.040
Perpendicular speed (wind)	1.224	1, 369.17	0.269
Rain	0.056	1, 5.79	0.821
Relative humidity	0.575	1, 5.77	0.478
Recapture period	0.352	2, 5.73	0.718
Distance x Treatment	5.912	2, 602.55	0.003
Distance x Recapture period	15.865	2, 603.12	< 0.001
Recapture day x Temperature	0.940	2, 5.73	0.444
Recapture day x Bearing speed (wind)	5.154	2, 609.48	0.006
Recapture day x Perpendicular speed (wind)	1.046	2, 590.87	0.352
Recapture day x Rain	0.087	2, 5.79	0.918
Recapture day x Relative humidity	0.249	2, 5.76	0.787
Distance x Treatment x Recapture day	2.526	4, 602.55	0.040