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Survival of sterile male Mediterranean fruit flies in large field cages after release at different ages

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Abstract

In Mediterranean fruit fly (*Ceratitis capitata*) sterile insect technique (SIT) programs worldwide, sterile flies are usually released at 2–3 days of age. However, they usually do not reach full sexual maturity until ca. 5 days of age. We tested whether holding sterile males longer at a fly emergence and release facility, until they were 5 days old, might result in significantly more flies surviving to reach sexual maturity in the field. In large field cages in Hawaii, we released sterile Mediterranean fruit fly males 2 or 5 days old under conditions where food and water were provided, or not provided. Flies were released 2 days after peak emergence in one field cage, while they were released 5 days after peak emergence in a second field cage. The numbers of flies flying out and remaining (dead, dying or non-flying) in the holding boxes were recorded on the day of fly release. At 5 and 8 days of fly age, the size of the male fly populations were estimated using trimedlure-baited traps placed into each of the two field cages for a 30-min period when the numbers of flies trapped were compared. Following six tests (three replications each with and without water and sugar provided), the differences in fly captures (i.e. survival) between 2 days vs. 5 days old releases were highly significant. With food and water provided, several times as many flies from the 5-day-old release field cage were captured at 5 and 8 days of age compared to the 2-day-old release field cage. These differences were magnified under conditions of no food and water provided. Holding Mediterranean fruit flies longer prior to release, requires more holding space and food, but will lead to significantly greater numbers of sexually mature flies in the field.

Introduction

The success of the sterile insect technique (SIT) depends critically upon released sterile males surviving in the field long enough to mate with wild females, thereby reducing the wild population. The effects of laboratory colonization, artificial mass rearing environments, sterilization procedures and other handling methods can cause mating or field survival ability to differ between laboratory-reared and wild males (McInnis et al. 1996; Briceño and Eberhard 1998; Lance et al. 2000). In addition, in most SIT programs, sterile males are not reproductively mature when released (e.g. 2–3 days old for the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (USDA 2009). The minimal age when a substantial majority of laboratory-reared males will mate has been reported to be as early as 2 days for a very old laboratory-adapted strain from Hawaii (McInnis et al. 1994), or 3 days for a mass-reared factory strain from Mexico (Papadopoulos et al. 1998; Liedo et al. 2002). However, even though some laboratory males can start mating at age 3 days when confined in mass-rearing cages with females,

observations on field-caged host trees show that a majority of such laboratory males only start joining male aggregations or leks, and actively participate in pheromone calling and courtship activities at 5–6 days (Economopoulos et al. 1988). The younger the mass-reared strain, the older will be the age of substantial mating, and for very young laboratory-reared strains, this age will approach the age at which wild flies mate substantially, i.e. 7–13 days (Liedo et al. 2002). Based on colony age, for most, if not all, currently mass-reared strains of Mediterranean fruit flies, including the Hawaii strain used in this study, this minimal age of substantial mating will be ca. 5 days. Therefore, sterile males must survive up to several days under potentially harsh conditions in the field, including desiccation, starvation and predation (Hendrichs et al. 1994) before reaching the age at which they can compete with wild males for matings with wild females.

Genetic selection for improved survival ability (McInnis et al. 2002), genetic sexing to permit more effective male-only releases (McInnis et al. 1994; Franz et al. 1996) and chemotherapeutic treatments of pre-release insects (Shelly and McInnis 2001) have all significantly improved the quality of released sterile Mediterranean fruit fly males. Other procedures, including pupal irradiation in nitrogen, adult irradiation and substitution of fluorescent dye marking with visible genetic marking of released flies, also have proven to increase sterile fly quality. In addition, there are two possible strategies to increase the proportion of released males that reach sexually maturity in the field – by maturing the released insects faster with a chemical treatment such as a juvenile hormone analogue (Faria et al. 2008; Shelly et al. 2009), or by holding the insects longer prior to release (Shelly et al. 2007). The former has, to date, shown no effect on *C. capitata*, while fly mortality for the latter has not been quantified yet under field conditions. So, it is now the focus of this study, using *C. capitata* as the test case species. This strategy could further improve the efficiency of the SIT by greatly increasing the proportion of sterile males that achieve sexual maturity and compete effectively with wild males for mates in the field.

Materials and Methods

The following describes the procedure we used to carry out the evaluation of releasing sterile Mediterranean fruit fly males into large field cages in Hawaii and comparing their survival over time for four treatments: 2 days old (with and without food/water) or 5 days old (with and without food/water).

Fly strain

The strain of Mediterranean fruit fly used was the VIENNA-7 *tsl* (temperature sensitive lethal) genetic sexing strain being mass-reared at the California Department of Food and Agriculture (CDFA) Mediterranean fruit fly mass rearing facility in Waimanalo, Oahu, Hawaii. We obtained pupae between August and December, 2008, in order to conduct the study.

Fly emergence and feeding

For each of the three replications of each of the four treatments (total of 12 large field cage tests), 600 ml of dyed [Dayglo fluorescent colour dye at 3 g/l (ca. 60 000 pupae)] VIENNA-7 pupae were irradiated at a mean dose of 145 Gy 2 days before peak emergence in a Husman Cesium-137 source (USDA-APHIS facility, Waimanalo, HI) and placed in a standard PARC box (Plastic Adult Rearing Containers – ca. 85 l capacity). Pupae were dyed in order to evaluate emerged adult flies under the same conditions as for normal fly releases in SIT programs. The tops and sides of each box were screened to allow ventilation and permit sugar-agar-water blocks to be placed on the top. In each box, the 600 ml total of pupae were divided into six paper bags each containing 100 ml, with the tops of each bag stapled closed except for the corners, to allow emerging flies to crawl out into the box and to minimize adult flies returning into the bags.

Blocks of sugar–agar–water food (10 cm × 20 cm × 5 cm thick, 17% sugar in water, w/v) were placed on the tops of each box. Adult emergence was monitored in each box in the laboratory (25–27°C, 50–70% RH) until the time of fly liberation into the field cages – ca. 80% emerged 2 days post-irradiation, and ca. 10% on each of the adjacent days (1 and 3 days post-irradiation). After 2 days, additional sugar–agar–water food was placed on top of the 5 days box to last until release into the field cage.

Fly release and field cages

Flies from one box were taken to a field cage for release on day 2 after peak fly emergence – this box comprised the 2 days treatment. A second box was taken to the other field cage for fly release on day 5 post peak fly emergence and comprised the 5 days

Fig. 1 Field cage with PARC box containing sterile male Mediterranean fruit flies ready for release (Waimanalo, Hawaii).

treatment. Two field cages (6 m × 16 m × 2.5 m tall) each containing a mixture of artificial trees (imitating *Ficus benjamina* w/ca. 700 leaves) and ca. 15 live guava trees (*Psidium guajava* L. w/ca. 1000 leaves each) were used, one field cage for each treatment (see fig. 1).

All ripe guava fruits were removed from the trees in the field cages prior to fly release. The treatments (2 or 5 days) were assigned to each field cage on a rotational basis, such that each field cage received each treatment three times out of the six total replications per treatment. On the morning (8:00–10:00) of each fly release day, the box was placed in the centre of each field cage and opened to allow flies to escape. After ca. 15 min, all dead or non-flying flies were saved to estimate (volumetrically) the number of flies that were lost during the 2- or 5-day incubation period in the boxes prior to fly release (Fig. 2). All of the pupae in the bags were saved and combined for each field cage.

Fig. 2 Collecting dead sterile male Mediterranean fruit flies or flies that did not leave the PARC box after fly release (Waimanalo, Hawaii).

Previously, a ca. 500 pupae sample had been taken from each 600 ml to calculate percentage adult emergence. The 600 ml of pupae per box was estimated to contain 36 000 pupae (60 pupae/ml). After calculating percent emergence and the number of flies that died, or could not exit from the box, the number of flies released was estimated.

Provisioning of food and water in field cages

For the six field cage tests in which food was provided to the flies in the field cages, the food (sugar–agar–water, as described for the boxes) was inserted at the time of fly release for the 2-day and 5-day field cages into three standard Jackson delta trap holders placed evenly down the middle of each field cage, each baited with a Petri dish containing ca. 100 g of a sugar–agar–water block. Each food dispenser was ant protected with birdstop, and inserted inside a delta shaped container to provide protection against wind and rain. For each of the other six field cages, no food and water was provided. However, light rain fell at least once during each test period, and flies were observed feeding on the surface of leaves and remaining unripe fruits.

Fly trapping procedure

On day 5 (i.e. 5 days after peak fly emergence), three Jackson traps with sticky insert, each loaded with 2 ml of fresh trimedlure on a 5-cm cotton wick, were spread out evenly in each of the two field cages. This was done first in the 2-day field cage, while flies were still being released from the box in the 5-day field cage, and then ca. 30 min later in the 5-day field cage after flies were distributed throughout the cage following the release. After a 30-min trapping period was run in each field cage, each trap was collected, removed from the field cage, and the flies caught were counted and recorded. On day 8, the above procedure was repeated with 3 traps in each field cage for 30 min, starting again with the 2-day field cage.

Data analysis

The trapping data for the 2-day and 5-day sterile fly release ages were compared by an analyses of variance (Proc GLM, Version 9.2, SAS Institute, 2008)

for data collected on day 5 and day 8, under field conditions of food and water provided or not provided, including means comparison tests using Tukey's HSD procedure.

Results

The data for the three replications for fly releases at 2 and 5 days of age (each with food provided or not provided) are shown in table 1.

Of the estimated 36 000 pupae (600 ml) set up in each box, between 80% and 94% emerged as adults. For the 2-day-old flies, 1280–4800 (avg. 2130) died, or could not fly out of the opened release box. For the 5-day-old flies, 6400–8900 (avg. 7173) died or were otherwise incapacitated. After correcting for adult non-emergence and mortality in the boxes, the estimated numbers of adult males flying out of the boxes were 25 620–30 780 (avg. 28 540) for the 2-day-old flies, and 21 520–25 980 (avg. 23 497) for the 5-day-old flies. The average difference between the numbers of 2-day and 5-day-old flies flying out of the boxes, 5043 flies less for the 5-day-old flies, represents a 16.0% loss of emerged flies in the boxes over the extra 3 days of adult holding time for the flies released when they were 5 days old.

The trapping data for the 2 days vs. 5 days treatments are shown in table 2. On day 5, the day on which the 5-day-old flies were released less than an hour before trapping, any surviving 2-day-old flies had already been 3 days in their field cage. On this 5th day, with no food or water provided in the field cage, the 5 days and 2 days flies averaged 1041.7 and 141.7 flies trapped, respectively (F(d.f. = 2 in all cases) = 72.88, P = 0.001). On day 8, with no food or water, the 5 days and 2 days flies averaged 104.0 and 13.3 trapped flies, respectively (F = 3.80, P = 0.123). On the other hand, on day 5 with food and water provided in the field cages, the 5 days and 2 days fly field cages averaged 1360.0 and 719.3, respectively (F = 13.88, P = 0.020). On day 8, the corresponding numbers were 469.7 and 155.3, respectively (F = 6.39, P = 0.061). So, in both cases where food and water were provided, or not provided, the numbers of flies trapped at day 5 was significantly higher for the 5-day-old released flies compared to flies released when they were 2 days old. At day 8, the differences were again strongly in favour of the 5-day-old flies, but not statistically significant (P = 0.05 level).

The ratio of surviving flies trapped on day 5 was 5.99–10.49 times greater for the 5-day-old released flies than for the 2-day-old released flies when no food or water was provided, and 1.62–2.23 times greater when food and water was provided in the field cages. For day 8, the ratio of survivors was 6.33–12.00 times greater for the 5-day-old vs. 2-day-old released flies with no food or water, and 1.92–4.75 times greater with food and water, respectively. Considering just the 2-day-old released flies, providing food and water increased fly survival 3.31–11.6 fold (avg. 6.26) at day 5 (F = 24.23, P = 0.008), and 6.29–180.0 fold (avg. 65.1x) at day 8 (F = 92.40, P = 0.001). For just the 5-day-old released flies, between day 5 (release date) and day 8, fly survival

Table 1 Estimated numbers of *Ceratitis capitata* sterile VIENNA-7 *tsl* males that emerged, died or did not leave the release box, and were released at 2 or 5 days of age into large outdoor field cages with food and water provided during half of the occasions (Waimanalo, Hawaii)

Replicate	Food ?	Fly age (days)	No. emerged	No. dead in box	No. released
1	No	2	30 420	4800	25 620
	No	5	30 420	8900	21 520
	Yes	2	29 300	1840	27 460
	Yes	5	29 300	7000	22 300
2	No	2	32 380	1600	30 780
	No	5	32 380	6400	25 980
	Yes	2	29 520	1280	28 240
	Yes	5	29 520	6800	22 720
3	No	2	31 600	1780	29 820
	No	5	31 600	6680	24 920
	Yes	2	30 800	1480	29 320
	Yes	5	30 800	7260	23 540

Table 2 Numbers of *Ceratitis capitata* sterile VIENNA-7 *tsl* males trapped at 5 or 8 days of age after fly release at 2 or 5 days of age into large outdoor field cages (Waimanalo, Hawaii)

Replicate	Food ?	Fly age (days)	No. trapped at day 5	No. trapped at day 8	Ratio: 5 days/2 days	
					Day 5	Day 8
1	No	2	198	24	5.99:1	6.33:1
	No	5	1186	152		
	Yes	2	655	151	2.23:1	4.75:1
	Yes	5	1459	717		
2	No	2	81	1	10.49:1	12.0:1
	No	5	850	12		
	Yes	2	938	180	1.62:1	1.92:1
	Yes	5	1519	346		
3	No	2	146	15	7.46:1	9.87:1
	No	5	1089	148		
	Yes	2	565	135	1.95:1	2.56:1
	Yes	5	1102	346		

increased 2.33–28.8 fold (avg. 11.95) ($F = 7.69$, $P = 0.050$), when food and water was provided.

If it is assumed that no fly mortality took place during the ca. 30 min following the release of the 5-day-old flies, then this 30 min trapping period in the 5-day field cage provided estimated percentages of the total number of flies released (from table 1) that were recaptured in the three trimedlure-baited traps. These percentages of total recapture ranged from 3.3% to 6.7% (avg. 5.2%).

Discussion

The data suggest that holding Mediterranean fruit flies ca. 3 days longer prior to release can increase the numbers surviving to reach sexual maturity (ca. 5 days old) by up to several folds. Presumably, the efficiency of the SIT would be correspondingly increased if sterile males were held accordingly longer at the fly emergence and release facility. Predation and other losses are much higher under natural conditions than inside field cages (Hendrichs et al. 1993), and this highly significant gain in surviving sterile males that reach the critical mating age when held several more days would certainly more than compensate for the ca. 16% additional loss occurring in the holding containers, and the additional food, water, and holding space required to keep them alive and well prior to release. In addition, the advantage for the 5-day-old flies does not only come from the numbers that make it to the age at which they just start mating, but also to the greater numbers that live each additional day they survive in the field, attracting and competing for wild females.

Holding flies longer prior to release invariably leads to higher mortality in the adult holding containers and also to constraints on space required to hold the fly holding containers the extra days in the fly emergence and release facility (McInnis et al. 2002; FAO/IAEA 2007; USDA 2009). Therefore, do the potential gains in relative fly survival from holding the flies longer prior to release outweigh these two limitations? The differences in fly survival based on the day 5 trapping data are strikingly in favour of the 5-day fly release treatment, especially when no food or water was provided in the field cages during the experiment. On day 8, the differences were again statistically significant for the 5-day fly release age with food provided. Also on day 8 with no food provided, the ratios of trapped flies were strongly in favour of the 5-day release age (6.33–12.0 : 1) but, due to the relatively low numbers trapped, were not statistically significant ($P = 0.05$ level).

It is assumed that the trapping data represent an accurate reflection of the relative numbers of surviving flies in the two field cages since the number of traps (3) and the trapping period (30 min) were identical; but, of course, the actual numbers of flies that survived in each field cage, and the percentage trapped, is not known exactly. The percentage of flies trapped with age does not vary significantly for lab reared flies over the range from 3 to 10 days of age (D. McInnis, unpubl. data). However, if one assumes no fly mortality during the period after fly release and before trapping on day 5 (a period of ca. 30 min), then one can obtain an estimate of the percentage trapped of the flies present in the field cage at that time. Such an estimate is not possible for any other trapping period since some unknown mortality will have occurred prior to trapping. For the 5-day release into the field cage on day 5 the percentage of released flies trapped in 30 minwas calculated to be ca. 5%.

The data show a several-fold higher fly survival when food and water was provided in the field cages for the 2-day-old flies on both day 5 and day 8, and for the 5-day-old flies on day 8. It is reasonable to assume that fly survival in the open field would be intermediate between the extreme levels of survival we tested with either food and water sources close by or where no food or water was provided at all. As expected, the data show that when food was provided, the 2-day-old released flies survived much better over the 3-day period between release (on day 2) and day 5, and for the 3 additional days until day 8. Similarly increased survival was evident for the 5-day-old released flies between day 5 and day 8 when food and water was provided in the field cage. These data suggest that providing food, perhaps cheaply in liquid form, released together with sterile flies, could significantly increase fly survival in the field. Future research could investigate this possibility further.

The efficiency of the SIT for the Mediterranean fruit fly has increased several fold with the advent of genetic sexing (Robinson and van Heemert 1982; Franz et al. 1996), aromatherapy treatments with ginger root oil (Shelly and McInnis 2001), and enriched protein diets for pre-release adults (Yuval 1998). Delayed release of sterile Mediterranean fruit flies until the insects are sexually mature would further increase, perhaps significantly so, the power of the SIT to suppress or eradicate wild Mediterranean fruit fly populations. Similar gains in SIT efficiency

might be realized, as well, in other tephritid pest species with active sterile fly release programs.

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