Mating competitiveness of the adult oriental fruit fly reared as larvae in liquid vs. those raised on standard wheat-based diets

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Abstract

Three strains (standard laboratory, *dorsalis* translocation white pupae (DTWP) pupal colour sexing strain and wild strain) of adult oriental fruit flies, which were reared as larvae on liquid diet (LD), mill-feed diet (MF) (Tanaka's diet) or natural host fruit diet, were evaluated for mating competitiveness in both indoor and outdoor Boller's mating cages and outdoor field cages. The Relative Sterility Index (RSI) was used as an indicator of mating capability. The RSI between standard strain adults that grew as larvae in LD and those in Tanaka's MF indicated no statistically significant difference in Boller's mating cage results after irradiation and dye marking. These results show that irradiation and dye marking had no effect on laboratory mating ability. Adults of the DTWP genetic sexing strain based on pupal colour that were reared as larvae in a LD (DTWP-LD) or MF (DTWP-MF) had a lower mating ability than the standard laboratory strain in Boller's cages, and against the wild strain in both Boller's and field cages. However, there was no statistically significant difference between irradiated DTWP-LD strains in competition with non-irradiated standard laboratory strain or DTWP-MF strain in Boller's cages. These findings indicate that (i) the quality of the DTWP sexing strain may be less capable in mating than the standard laboratory and wild strains and (ii) LD reared larvae can produce adults of equal mating ability compared with adults reared on a conventional diet. LD reared fruit flies should therefore be ready for mass scale rearing for Sterile Insect Technique programmes following the completion of other field quality control tests, including those measuring dispersal abilities.

Introduction

Bactrocera dorsalis, the oriental fruit fly, (Hendel), is one of the most destructive fruit and vegetable pests in Hawaii and worldwide. Its existence has caused significant losses in crops as well as marketing opportunities in our global market economy. A group of scientists in Hawaii initiated an area wide pest management programme in 2000. The technologies developed by this programme have been transferred to other parts of world that share the same need to suppress fruit fly populations in support of Sterile Insect Technique (SIT) programmes. Fruit flies are sterilized using radiation then released into the field to compete with wild flies, leading to suppression of the population. Therefore, strong and healthy laboratory sterilized fruit flies are needed to be able to compete successfully with wild flies in the field.

One of the most important components of an Area Wide Pest Management programme is to have well-established and cost-effective methodologies in place to rear high-quality fruit flies and their parasitoids. United States Department of Agriculture (USDA)-Agricultural Research Service (ARS) recently developed a liquid diet (LD) method for larval rearing of fruit flies (Chang et al. 2004, 2006, 2007). The core

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of this diet is to replace the currently used wheat product as bulking agent with a sponge cloth that is highly water absorbent, reusable, recyclable, biodegradable and light weight while maintaining a similar standard quality for fruit flies reared as larvae in this diet. A starter kit of this LD has been distributed to interested groups that, so far, has involved 35 countries and 26 species of fruit flies worldwide (Chang, unpublished data). Mass rearing for field release will be implemented following the success of the starter kit evaluation.

Fly, pupal production, pupal weight, developmental time, adult emergence, flight ability, mating, egg production and egg hatch, from larvae reared on a LD before irradiation and colour marking, have been evaluated and reported as satisfactory compared with those of the standard laboratory strain (Chang et al. 2006, 2007). However, whether adults reared from larvae on a LD would have the same mating competitiveness in the field after irradiation, colour marking and release (Shelly et al. 2000; McInnis et al. 2004; Dyck et al. 2005) remains unknown. The objective of this study was to compare the mating competitiveness of oriental fruit fly males from two different strains reared as larvae reared on a LD, or on a millfeed diet (MF) (standard laboratory diet), under both laboratory and outdoor field cage conditions.

Materials and Methods

Insects

The two strains used were the standard laboratory strain [*bactrocera dorsalis* (BD)] and a pupal colour sexing strain [*dorsalis* translocation white pupae (DTWP)] (McCombs and Saul 1995). The diets tested were the standard MF (Vargas et al. 1984, 1996) and the recently developed LD (Chang et al. 2004, 2006). According to the strain and diet used, flies were named as follows: BD-MF, BD-LD, DTWP-MF and DTWP-LD. A wildish strain of larvae collected from host papaya plants in the field and reared on papaya in the laboratory for the first five generations, was also used in this study as the target/control strain (i.e. representing the wild population to be controlled in the field). A stack of five trays of each of the tested strains were reared side by side in the laboratory under conditions of 25°C, 65–80% relative humidity (RH) and 12 : 12 h [dark : light (D : L)] at the ARS Tropical Plant Pest Research Laboratory in Honolulu, Hawaii. The eggs used in each diet were collected from the same cohort of adult insects.

Insect preparation before mating tests

Two days before adult emergence, some of pupae were dyed with pink fluorescent dye (Dayglo®) (4 g dye/litre pupae) and irradiated with 100 Gy dose using a Gammacell 220 (Nordion Co., Ontario, Canada) irradiator located at the USDA Manoa laboratory. These irradiated pupae were abbreviated IRR to be differentiated from non-irradiated pupae (NIRR). The pink dye is used to mark emerging flies, so they can be identified after field release and recapture.

Upon emergence, adults were chilled at 5°C for 1 h for easy sexing. Designated numbers of males and females (110 each of BD-MF strain, BD-LD strain and W strain) for each treatment were separated into separate metal screened cages (24 × 24 × 24 cm) based on their sex, and supplied with sugar : protein hydrolysate (3 : 1) and water. Sexes were kept in separate rooms at 25°C, 80% RH and 12 : 12 h (D : L) for later use. One day before the laboratory mating test, 100 10-day-old non-irradiated (NIRR) BD-MF strain males or 100 35-day-old W strain males, and 100 10-day-old males of a competing irradiated (IRR) strain including IRR-BD-MF or IRR-DTWP-LD or IRR-DTWP-MF were placed into acrylic Boller's cages (39.5 cm × 30 cm × 30 cm) for each test (http://www.iaea.org/programmes/nafa/d4/index.html). Additionally, 100 NIRR BD-MF strain or wildish females each were placed into 8 oz plastic cups with sugar protein hydrolysate and water, and stored in a separate room for later use. In the afternoon of the mating day, the 11-day-old females were introduced into the male-filled Boller's cages, or both females and males were released simultaneously into the field cages (2.5 m × 2.5 m × 3 m) with 2–3 artificial 2-m-tall *Ficus benjamina* trees for a field cage test. Each test was repeated at least three times (table 1) except for the case of field cages with the wildish target strain (two replicates).

Experimental design

The mating competitiveness of adults which were reared from larvae on a LD was compared with the MF (conventional diet) from either the DTWP sexing strain, the standard laboratory strain (BD) or the wildish strain (W). The released females corresponded to the same strain of the competing male.

Because of the limitation of number of field cage and manpower, we designed that the best six mating tests were performed, combining the different strains and diets (table 1): (1) NIRR-BD-MF strain vs. IRR-BD-MF strain; (2) NIRR-BD-MF strain vs.

Table 1 Information on all the tests, including test types, no. of flies per type, sex of insects first introduced to laboratory or field test cages

Tests	Target strains	Competing strains	No. of replicates		No. of flies		Sex first introduced to test cages	
			Boller	Field	Females	Males	Boller	Field
NIRR-BD-MF × IRR-BD-MF	NIRR-MF	IRR-MF	3	3	100	100	Females	Females
NIRR-BD-MF × IRR-BD-LD	NIRR-MF	IRR-LD	3	3	100	100	Females	Females
NIRR-BD-MF × IRR DTWP-MF	NIRR-MF	IRR DTWP-MF	6	0	100	100	Females	NA
NIRR-BD-MF × IRR DTWP-LD	NIRR-MF	IRR DTWP-LD	6	0	100	100	Females	NA
NIRR wildish × IRR DTWP-MF	NIRR wildish	IRR DTWP-MF	4	2	100	100	Females	Females
NIRR wildish × IRR DTWP-LD	NIRR wildish	IRR DTWP-LD	4	2	100	100	Females	Females

BD, standard laboratory strain; DTWP, pupal colour sexing strain; MF, mill-feed diet; LD, liquid diet; IRR, irradiated; NIRR, non-irradiated.

IRR-BD-LD; (3) NIRR-BD-MF strain vs. IRR-DTWP-MF strain; (4) NIRR-BD-MF vs. IRR DTWP-LD strain, to compare DTWP-LD with DTWP-MF (results from test 3 above); (5) NIRR wildish strain vs. IRR-DTWP-MF strain and (6) NIRR Wildish strain vs. IRR DTWP-LD strain.

The NIRR-BD-MF females were the target insects and the IRR-BD-MF and IRR-BD-LD males were the competing insects for (1) and (2). The NIRR-BD-MF females were the target insects and IRR-DTWP-MF and IRR-DTWP-LD males were the competing insects for (3) and (4). The NIRR wildish strain females were the target strain and IRR-DTWP-MF and IRR-DTWP-LD males were the competing insects for (5) and (6).

Mating tests in the laboratory (Boller's cages)

One hundred males for each of a target and a competing strain were released into a cage containing 100 target females at ca. 4:30 PM. The flies usually mated within an hour after release, and pairs copulated for more than several hours (including overnight) without separation. Mating pairs were collected into plastic or glass vials and were later identificated by using a UV lamp or under a dissecting scope, to detect the presence of dye in the ptilinum (area between the eyes). Mating tests were completed at about 8:00 PM.

Mating tests in field cages

One hundred males for each of the target and competing strains were released into each cage at ca. 4:30 PM, then 100 target females were introduced ca. 15 min later. About an hour later, collection of the mating pairs commenced and continued for ca. 2 h. Mating pairs were collected in plastic or glass vials. The male type in each pair was determined later, using the fluorescent dye as the indicator of the sterile strain. In a few cases, where <20 mating pairs were collected in a cage, the data was considered to be inadequate and was not included. Therefore, because of the limited number of wild strain insects, we only had enough flies to set up two field cage tests.

Data analysis

The mating tests were repeated to obtain at least three different dates/batches per treatment except the wild strain. Number of replications for each treatment performed are listed in table 1. The performance of males from each test/treatment were compared using an analysis of variance with SAS software (SAS Institute Inc., Cary, NC, USA), using PROC ANOVA, based on the Relative Sterility Index (RSI) of each cage (McInnis et al. 1996), as well as the mating proportions on the combined results of the respective replicates of each treatment. (SAS system 2002). Comparisons between tests (1) and (2) revealed differences between the two diets for the BD strain. Differences between the two diets in the DTWP strain would be detected by comparing test (3) and (4) and between tests (5) and (6) (indicated as [c, d, e, f] in table 2) in both Boller's cage and field tests. In the later case, the use of a wildish strain would confer more reliable results as wildish females may be more selective and hence more capable of detecting subtle differences in male competitiveness. In addition, test (1) can be used to assess the effect of irradiation on the standard laboratory strain, and comparisons of tests (1) and (3) can be used to detect differences between strains reared on a MF.

Results and Discussion

Laboratory Boller's cage test

The major focus of this study was to test for the mating competitiveness of flies reared on a LD as compared to flies reared on a MF, considering both the

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Table 2 Summary of the competitive mating test results

Target strains	Competing strains	RSI	Mean mating proportions*		PROC ANOVA (α = 0.05)
			Target	Competing	
Boller's cage tests					
(1) NIRR-BD-MF	IRR-BD-MF	0.62 ± 0.09	0.38 ± 0.09a	0.62 ± 0.09a	F = 3.56; d.f. = 1, 5; P = 0.1322
(2) NIRR-BD-MF	IRR-BD-LD	0.70 ± 0.05	0.30 ± 0.07b	0.70 ± 0.07a	F = 15.95; d.f. = 1, 5; P = 0.0162
IRR-BD-MF[c]	IRR-BD-LD	0.70 ± 0.07	0.62 ± 0.09a	0.70 ± 0.07a	F = 0.49; d.f. = 1, 5; P = 0.5216
(3) NIRR-BD-MF	IRR DTWP-MF	0.35 ± 0.04	0.65 ± 0.04a	0.35 ± 0.04b	F = 30.89; d.f. = 1, 11; P = 0.0002
(4) NIRR-BD-MF	IRR DTWP-LD	0.43 ± 0.005	0.56 ± 0.005a	0.43 ± 0.005b	F = 364.41; d.f. = 1, 11; P < 0.0001
IRR DTWP-MF[d]	IRR DTWP-LD	0.43 ± 0.005	0.35 ± 0.04a	0.43 ± 0.005a	F = 4.66; d.f. = 1, 11; P = 0.0563
(5) NIRR Wildish	IRR DTWP-MF	0.38 ± 0.05	0.62 ± 0.05a	0.38 ± 0.05b	F = 10.41; d.f. = 1, 7; P = 0.0180
(6) NIRR Wildish	IRR DTWP-LD	0.38 ± 0.06	0.62 ± 0.06a	0.38 ± 0.06b	F = 7.33; d.f. = 1, 7; P = 0.0353
IRR DTWP-MF[e]	IRR DTWP-LD	0.38 ± 0.06	0.38 ± 0.05a	0.38 ± 0.06a	F = 0.01; d.f. = 1, 7; P = 0.9419
Field cage tests					
(5) NIRR Wildish	IRR DTWP-MF	0.38 ± 0.07	0.62 ± 0.07a	0.38 ± 0.07a	F = 5.74; d.f. = 1, 3; P = 0.1388
(6) NIRR Wildish	IRR DTWP-LD	0.40 ± 0.09	0.60 ± 0.09a	0.40 ± 0.09a	F = 2.48; d.f. = 1, 3; P = 0.2560
IRR DTWP-MF[f]	IRR DTWP-LD	0.40 ± 0.09	0.38 ± 0.07a	0.40 ± 0.09a	F = 0.01; d.f. = 1, 3; P = 0.9188

[c, d, e]Resulted from Boller's cage tests (1) and (2); (3) and (4); (5) and (6) respectively.
[f]From field cage tests (5) and (6).
BD, standard laboratory strain; DTWP, pupal colour sexing strain; MF, mill-feed diet; LD, liquid diet; IRR, irradiated; NIRR, non-irradiated.
*Within a row, mean values with the same letter are not significantly different. PROC ANOVA, Tukey test.

standard laboratory (BD) and a sexing strains (DTWP). Standard laboratory (BD-MF) and wildish (W) (reared on papaya) strains were used only as target strains.

Standard laboratory strain (BD)

The NIRR-BD-MF and IRR-BD-MF strain males competed for NIRR-BD-MF strain females. The NIRR-BD-MF strain females showed no significant preference toward either irradiated (0.62) (IRR-BD-MF) or non-irradiated standard laboratory strains (0.38) (NIRR-BD-MF) males (P = 0.1322) although the mating proportion was higher in the irradiated strain. However, when both irradiation and diet were involved in the evaluation [test (2) with NIRR-BD-MF males and IRR-BD-LD males], NIRR-BD-MF females preferred IRR-BD-LD strain males (0.7) more than NIRR-BD-MF strain (0.3) males (P = 0.0162). In addition, there was no statistically significant difference between the RSIs for the IRR-BD-MF strain and the IRR-BD-LD strain (P = 0.5216) (table 2).

These results indicate that there was no significant effect of irradiation on mating capability. Results also showed that LD reared fruit flies, dyed and irradiated, displayed equal or similar laboratory mating ability to flies reared on a standard mill-feed diet.

DTWP strain

Both the RSIs of the irradiated DTWP-MF strain (0.35) and the irradiated DTWP-LD strain (0.43) in the Boller's cage test were found to be statistically lower than that of the BD-MF strain (0.65 and 0.56, respectively; P = 0.0002 and <0.0001). Therefore, the RSIs for the IRR-DTWP-MF strain (0.35) and IRR-DTWP-LD (0.43) were not statistically different from each other (P = 0.0563) (table 2).

These results indicate that the irradiated DTWP-MF sexing strain did not perform as well as the irradiated BD-MF strain, based on the test conditions stated above. Additionally, these results again reveal that a different strain (in this case, the DTWP sexing strain) consisting of adults reared on a LD can produce sterile male adults of equal mating ability to those reared on a conventional standard mill-feed diet.

Wildish strain

Both the RSIs of the irradiated DTWP-MF strain (0.38) and DTWP-LD strain (0.38) were significantly lower than that of the wildish strain (0.62 and 0.62; P = 0.018 and 0.0353 respectively), while the RSIs for the DTWP-MF strain and DTWP-LD strain were the same (P = 0.9419) (table 2) in Boller's cage tests. These results show that the wildish strain males were more attractive to the wildish strain females than the DTWP males reared either on the liquid or mill-feed diets. Again, results showed that sterile males reared on the LD displayed equal mating ability in comparison to mill-feed reared sterile males when competing against wildish males for

wildish females in standard laboratory (Boller's) cages.

Field cage test

The RSIs from the irradiated DTWP-MF strain and the irradiated DTWP-LD strain males were not found to be statistically different (P = 0.9188), while the RSIs of these two strains (0.40 and 0.38) were not significantly less competitive than those of the wildish strain (0.60 and 0.62) (P = 0.12560 and 0.1388) in the field cage setting although the RSIs are lower for the irradiated males. More tests should be done in the future to confirm the competitiveness between DTWP and wild strains. However, whether DTWP-MF or DTWP-LD are significantly different from the wildish strain or not, they both were not significantly different from each other (table 2). Again, the mating competitiveness of the DTWP strain reared on MF or LD is equal.

For the standard strain, data showed that there was no significant difference between NIRR-BD-MF and IRR-BD-MF although the mating proportion of irradiated strains are larger than those of target non-irradiated strains in both Boller's cage tests and field cage tests. That is, irradiation did not adversely affect mating performance. Additional replications would better confirm these results. However, in the Boller's cage test, it favoured IRR-BD-LD strain in mating proportion while it favoured IRR-BD-MF strain in the field cage test. Therefore, the results from these (1) and (2) tests revealed that no difference exists between the irradiated standard strain from rearing in MF (IRR-BD-MF) or from LD (IRR-BD-LD), while a significant difference existed between these two strain in the field cage test.

For the genetic sexing strain, DTWP, to compare the DTWP and wildish strains, tests were made in both Boller's cage and in field cages. In Boller's cage test, NIRR-wildish was more competitive than those of IRR-DTWP-MF or IRR-DTWP-LD. But there was no significant difference between IRR-DTWP-MF and IRR-DTWP-LD. In field cage tests, there was no significant difference among NIRR-Wildish, IRR-DTWP-LD and IRR-DTWP-MF. Therefore, there is no significant difference in mating between IRR-BD-MF and IRR-BD-LD. Again, data from these two tests did not show a significant difference although the mating proportions were higher in irradiated strains compared with the target, non-irradiated strains. Again, insects from MF reared and LD reared were the same in terms of mating competitiveness under the stated laboratory or field cage conditions.

We conclude that the mating ability of the LD reared sterile males is equal to that of males reared on a standard mill-feed diet for the two laboratory strains (the standard laboratory strain and the DTWP genetic sexing strain). This was confirmed under laboratory and field cage conditions and when using females from the standard or a wildish strains. These results indicate that sterile flies produced from a LD (Chang et al. 2006) can satisfy mating quality control standards required for *Bactrocera dorsalis* SIT programmes (FAO/IAEA/USDA 2003).

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