Development of genetic sexing strain of the oriental fruit fly, *Bactrocera dorsalis* (Hendel)

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Abstract. A genetic sexing strain (GSS) of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) has been developed in order to release only sterile males to increase the efficiency of fruit fly control by using sterile insect technique. To compare the quality of GSS with the genetic marker strain from a mass-reared colony, it was found that eclosion, flight ability and fertility of GSS equal to 69.57, 53.14 and 47.48 % respectively, which were significantly lower than those of the genetic-marker strain. The adult eclosion, flight ability and fertility of the genetic-marker strain equal to 93.66, 87.00 and 60.48 % respectively. To study the effects of irradiation, the 2-days-before-emergence pupae of GSS were irradiated at doses of 70 and 90 Gy. It was found that sterility and competitiveness index of GSS irradiated at 70 and 90 Gy were 96.15, 97.12 % and 0.51, 0.39 respectively.

1. Introduction

Area-wide integrated pest management using the sterile insect technique (SIT) is highly efficient, specific to the targeted pest species and environmental-friendly [1]. SIT involves rearing, sterilization, and release of a large number of insects into the field, where it is expected that the sterile males will compete satisfactorily with wild males for wild females [2]. However, releasing both sexes of sterile fruit flies reduces the efficiency of controlling the population size as some sterile males will mate with sterile females [3, 4] and sterile stings of females can cause fruit damage. To avoid these problems, the release of a male-only population is being implemented for species of family Tephritidae by the development of genetic sexing strains (GSS) that allow for separation of the sexes at a specific stage of development. This separation is based on a genetic mechanism, such as the coloration of the puparium, size of the body, rate of development, or temperature sensitivity [5, 6]. By releasing only sterile males, the efficiency of SIT can be increased by several-folds [3, 7]. GSS have been developed in several Tephritid species including *Ceratitis capitata* (Wiedemann) [8], *Bactrocera cucurbitae* Coquillet [9], and *Bactrocera dorsalis* Hendel [10]. For these species, an irradiation-induced chromosomal translocation links the gene for normal brown pupal color to the male sex (Y) chromosome, resulting in male pupae having brown puparium and female pupae having the mutated white puparium color.

The fruit fly, *B. dorsalis*, is known as one of the most important pests and poses a severe threat to fruit production and trade in Thailand. SIT has been applied to control this key pest since 1987. Recently, in 2012, a GMS (genetic marker strain) of *B. dorsalis* was developed. The white-striped GMS strain, derived from hot-water treated eggs, was developed for sterile fly detection in field. In 2017, a GSS (genetic marker strain) of *B. dorsalis* was developed by an irradiation induced chromosomal...
translocation and linked gene normal brown pupae color to Y-chromosome. This strain, brown pupae is male while white pupae (mutant) is female. In this study, GSS of B. dorsalis were compared with the mass-reared bisexual GMS strain, through tests of quality and sexual competitiveness. Results of these tests would help to evaluate the potential of this new GSS strain for usage in an SIT program.

2. Methods

2.1. Fruit fly strains

2.1.1. Genetic marker strain (GMS). This strain was developed for sterile fly detection in controlling of fruit fly program [11, 12]. The strain carried a mutated white-striped phenotype on its thorax. The strain was mass-reared at the facility of Thailand Institute of Nuclear Technology (Public organization). Generation 45 was used.

2.1.2. Genetic sexing strain (GSS). This strain was developed to release only sterile male. Sterile insects [13] were used in this study, the insects were irradiated at the pupal stage 1 day before emergence at doses of 70 and 90 Gy from a cobalt-60 source installed in a gamma-ray irradiator (Gamma chamber 5000, BRIT, India) with dose rate 2.37 Gy/second.

2.2. Evaluation of pupal quality

2.2.1. Adult eclosion. At two days before emergence, 500 pupae of GMS and GSS were placed in plastic boxes, 11x11x6 cm in size. Ventilation is provided by a hole (15 cm diameter) covered with a 16-mesh screen. After emergence, dead, unemerged pupae, abnormal flies, the number of males and females were counted. Four replicates were set up, and data were analysed with t-test.

2.2.2. Flight ability. At two days before emergence, 100 pupae of GMS and GSS were placed within a ring of paper, which was centered at the bottom of a Petri dish. A black Plexiglas tube with talc was placed within the darkened Petri dish. After emerged flies flew from the tubes or died, the flies and unemerged pupae were counted. Four replicates were set up and data were analysed with t-test.

2.2.3. Sterility. Three-day-old adult flies were separated by sex. One hundred males and 100 females of GMS and GSS were placed separately into 30x30x40 cm³ Plexiglas cages, the sides and tops of which had holes covered with screens for ventilation. Flies were allowed to mate and fed with sugar mixed with yeast hydrolysate. Eggs were collected from each cage when flies were 11, 16, 21 and 26 days old and checked for hatching. Four replicates were set up, and data were analysed with t-test.

2.2.4. Evaluation of sexual competitiveness of males. 400 irradiated GSS males and 200 fertile GMS males were released into a mating cage, 30x40x30 cm. 500 virgin GSS males with 500 virgin GMS females of and 500 irradiated virgin GSS males with 500 virgin GMS females were used as the control.
Eggs of females in each cages were checked for hatchability and calculated following the Friend’s equation [14].

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C = \frac{W}{S} \times \frac{H_w - H_c}{H_c - H_s}
\]

While (W) is the number of GMS males in the competitiveness cage, (S) is the number of sterile GSS males in the competitiveness cages, (Hw) is the percentage of egg hatch from GMS females in the control cages, (Hc) is percentage of egg hatch from GMS females in the competitiveness cage and (Hs) is the percentage of egg hatch from GMS females in the sterility cage.

3. Results and Discussion

The quality of *B. dorsalis* GSS was compared with the quality of genetic marker strain (GMS) of *B. dorsalis* in Table 1. The results showed that the quality of GSS was significantly lower than that of GMS for all topics studied. Adult eclosion, flight ability and fertility were decreased by 25.72, 38.92 and 21.82 %, respectively. The situation differed from when GMS was being developed, where the quality of GMS was observably better than the wild flies [11]. While specifications for *B. dorsalis* produced for SIT programmes recommend an acceptable mean of adult emergence and flight ability not to be lower than 90 and 83 % respectively [15]. The translocations involving multiple autosomes may affect the sterility and the quality of the GSS.

| Fruit Fly Strain | % eclosion | % abnormality | % flight ability | % fertility |
|------------------|------------|---------------|------------------|------------|
| GMS              | 93.66 ±3.56a | 2.10 ±1.52a | 87.00 ±3.70a | 60.73 ±7.09a |
| GSS              | 69.57 ±6.29b | 4.89 ±2.79b | 53.14 ±6.15b | 47.48 ±1.43b |

a,b Mean values followed by the different letters in each column are significantly different (p ≤ 0.01, t-test).

Table 2. Quality comparison between the genetic sexing strain (GSS) with the genetic marker strain of *B. dorsalis*.

| Dose (Gy) | % eclosion | % abnormality | % flight ability | % sterility |
|-----------|------------|---------------|------------------|-------------|
| 0         | 73.29 ±3.33a | 4.63 ±3.50a | 53.14 ±6.14a | 53.66 ±1.77a |
| 70        | 70.74 ±4.02a | 4.49 ±2.84a | 40.71 ±16.64a | 96.15 ±5.25b |
| 90        | 71.06 ±4.15a | 4.54 ±3.76a | 39.43 ±14.94b | 97.12 ±5.57b |

a,b Mean values followed by the different letters in each column are significantly different (p ≤ 0.01, t-test).

The sexual competitiveness values of GSS, males, irradiated at 70 and 90 Gy were shown in Table 3. The results showed that GSS sterilized at 70 Gy could compete with normal GMS males better than GSS sterilized at 90 Gy.

| Mating combination | % egg hatch | Competitiveness value |
|--------------------|------------|-----------------------|
| 500 GMS males x 500 GMS females | 66.67 | |
| 500 70 Gy-GSS males x 500 GMS females | 7.48 | |
| 500 90 Gy-GSS males x 500 GMS females | 5.44 | |
| 400 70 Gy-GSS males x 200 GMS males x 200 GMS females | 36.78 | 0.51 |
| 400 90 Gy-GSS males x 200 GMS males x 200 GMS females | 39.84 | 0.39 |
4. Conclusion

The genetic sexing strain showed deceased quality of pupa due to Y- autosome translocation to link the inheritance of white pupae mutation to sex. The sterilization by irradiation at 90 Gy decreased flight ability. Radiation doses of 70 and 90 Gy could induce 96.15 and 97.12 % sterility and the index of mating competitiveness of flies irradiated at dose of 70 and 90 Gy were 0.51 and 0.39 respectively. However, efficiency of mating competition in field cages should be compared before making decision to use the GSS strain.

References
[1] Dyck VA, Hendrichs J and Robinson AS 2005 Sterile insect technique (Springer, the Netherland) p 787
[2] Knipling EF 1995 Science 130 902-4
[3] McInnis DO, Tam S, Grace C, and Miyashita D 1994 Ann.Entomol.Soc.Am. 87 231-40
[4] Orozco D, Hernandez MR, Meza JS, and Quintero JL 2012 J.Appl.Entomol (doi:10.1111/j1439-0418.2012.01748.x).
[5] Robinson A, Franz G, Fisher K 1999 Trends in Entomology 2 81-104
[6] Rendon P, McInnis D, Lance D and Stewart J 2004 J.Econ.Entomol. 97 1547-53
[7] Rendon P, McInnis D, Lance D, Stewart J 2000 Proceedings : Area-Wide Control of Fruit Flies and Other Insect Pests. Penang, Malaysia pp.517-25
[8] Robinson AS, and Van Heemert C 1982 Genetica 58 229-37
[9] McInnis DO, Tam S, Grace C, Miyashita D 2004 Annals of the Entomological Science of America 97 1026-33
[10] McCombs S, Saul S 1995 Ann. Ent. Soc. Am. 88 695-8
[11] Limohpasmanee w, Tannarin T, Khongratarpa T, Segsarnviriya S 2011 Conference on Nuclear Science and Technology. Bangkok, Thailand
[12] Boonsirichai K, Segsarnviriya s, Limohpasmanee w, Khongratarpa T, Tannarin T, Sungsinleart K 2011 Conference on Nuclear Science and Technology. Bangkok, Thailand
[13] Isasawin S, Aketarawong N, Thanaphum S 2012 Eur.J.Entomol.109 331-9
[14] Fried M 1971 J Econ Entomol 64 869-72
[15] FAO/IAEA/USDA 2014 Manual for Product Quality Control and Shipping Procedures for Sterile Mass-Reared