Kasetsart J. (Nat. Sci.) 28:142-148 (2538)

# Miotic Karyotypes of Four Species of Fruit Flies (*Bactrocera*) in Thailand

Nattaya Hunwattanakul[1] and Visut Baimai[2]


## ABSTRACT

Giemsa and Hoechst 33258 stained mitotic chromosomes of four species of fruit flies belonging to the genus Bactrocera, namely *B. dorsalis, B. tau, B. cucurbitae* and *B. correcta*, were studied. The metaphase karyotype (2n = 12) of these species consists of one pair of heteromorphic sex chromosomes and five pairs of autosomes of different size and shape. In all the species examined, the X chromosome is metacentric while the Y is a small submetacentric which normally appears as a dot in general preparations. The autosomes of *B. dorsalis, B. tau* and *B. cucurbitae* are characterized by distinct H-bands in the centromeric regions of all the autosomes. Only in *B. cucubitae* an intercalary heterochromatin in chromosome 3 was observed. *Bactrocera correcta* differs from other by having extensive blocks of pericentric heterochromatin in all the autosomes and relatively small metacentric X chromosome.


**Key words** : mitotic chromosome, heterochromatin, fruit fly, *Bactrocera*

## INTRODUCTION

The fruit flies of the genus *Bactrocera* (Tephritidae, recorded previously as *Dacus*) are wide-spread in Asia and Australia (Hardy, 1973; Drew, 1989; White and Elson-Harris, 1992). In Thailand, these economically important insects, particularly, *B. dorsalis, B. tau, B. cucurbitae* and *B. correcta* are serious pests of fruits and vegetables such as mangoes, guavas, rose apples, pumpkins, cucumbers, *etc.* (Tigvattananont and Areekul, 1984; Tigvattananont, 1986a, 1986b; Permpoon *et al.*, 1983). These pest species have caused a reduction of yields and quality of fruits and vegetables which has consequently affected agriculturalists' income both for local and international marketing. Thus research on various aspects of the biology of fruit flies in Thailand has been carried out aiming at control measures of these insect pests (Sepsawad *et al.*, 1978; Sutantawong, 1985). However, little work has been done on the genetics and cytotaxonomy of the fruit flies in Thailand, although such basic knowledge is known in India (Bhatnagar *et al.*, 1980; Singh and Gupta, 1984).

This study is the report on mitotic chromosomes with the characterization of G- and H-bands of 4 species of fruit flies commonly occurring in Thai populations, namely *B. dorsalis, B. tau, B. cucurbitae* and *B. correcta.*

## MATERIALS AND METHODS

Samples of the four species of fruit flies, namely *B. dorsalis, B. tau, B. cucurbitae* and *B. correcta,* were kindly provided for this study by the Department of Entomology, Kasetsart University, Bangkok. The colonies of these insect pests were established from natural samples collected locally from gardens and orchards in the Bang Khen campus of Kasetsart University, except for *B. tau* which was collected from Ranong Province, southern Thailand. These colonies have been maintained in the laboratory at 25-27°C. The brain ganglia of third-instar larvae were used for mitotic chromosome preparations using the air-dried method described by Baimai (1975). Air-dried slides were set a side for a few days and then stained with 5% Giemsa staining solution for G-banding and Hoechst 33258 stain for fluorescent H-banding using a modified method as described by Latt and Wohlleb (1975). The best prophase or metaphase spreads of mitotic chromosomes with G- and H-bands were photographed with Kodak Technical Pan Film with green filter and with Kodak Tri-X Pan Film with UG-1 exciter filter,

1 Ratanakosinsompod Bang Khen, Ramintra Road, Bangkok 10230, Thailand.
2 Dept. of Biology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.

respectively, using an Olympus Photomicroscope with oil immersion (x 670).

The idiograms of the 4 species of fruit flies were constructed based on measurement of photomicrographs of 10 cells of well spread mitotic chromosomes of each species. The relative length (r.l.) was calculated as % of total chromosome length. The centromeric ratio (c.r.) was the length of the long arm divided by the length of the short arm (Levan *et al.*, 1964; Green *et al.*, 1980). The centromeric ratios were used for chromosome nomenclature as follows: 1.00-1.67 and 1.68-3.00 were taken as metacentric (m) and submetacentric (sm), respectively.

**Table 1 Karyotype analysis for relative length (r.l.), centromeric ratio (c.r.) and chromosome type (type) of 4 species of *Bactrocera*.**

| Species | Chromosome no. | | | | | | |
|---|---|---|---|---|---|---|---|
| | X | Y | 2 | 3 | 4 | 5 | 6 |
| *B. dorsalis* | | | | | | | |
| r.l. | 12.19 | 3.09 | 20.17 | 18.66 | 16.36 | 14.81 | 13.83 |
| SD | 0.81 | 0.53 | 1.26 | 1.08 | 0.98 | 0.56 | 0.95 |
| c.r. | 1.21 | 1.73 | 1.38 | 1.70 | 1.18 | 2.65 | 2.67 |
| SD | 0.18 | 0.01 | 0.10 | 0.14 | 0.10 | 0.35 | 0.68 |
| type | m | sm | m | sm | m | sm | sm |
| *B. tau* | | | | | | | |
| r.l. | 10.73 | 2.75 | 20.05 | 17.78 | 17.08 | 16.31 | 15.43 |
| SD | 0.86 | 0.54 | 1.01 | 0.63 | 0.42 | 0.53 | 0.33 |
| c.r. | 1.12 | 1.76 | 1.29 | 1.45 | 1.27 | 2.03 | 1.25 |
| SD | 0.10 | 0.04 | 0.29 | 0.29 | 0.15 | 0.35 | 0.13 |
| type | m | sm | m | m | m | sm | m |
| *B. cucurbitae* | | | | | | | |
| r.l. | 11.46 | 3.06 | 19.77 | 17.57 | 16.65 | 15.88 | 15.49 |
| SD | 1.06 | 0.44 | 1.07 | 0.88 | 0.60 | 0.56 | 0.60 |
| c.r. | 1.20 | 1.89 | 1.18 | 1.28 | 1.70 | 1.89 | 1.21 |
| SD | 0.16 | 0.03 | 0.19 | 0.12 | 0.19 | 0.24 | 0.34 |
| type | m | sm | m | m | sm | sm | m |
| *B. correcta* | | | | | | | |
| r.l. | 5.69 | 2.71 | 22.79 | 19.52 | 18.32 | 16.13 | 14.87 |
| SD | 0.73 | 0.39 | 1.38 | 1.13 | 0.95 | 1.06 | 0.73 |
| c.r. | 1.15 | 1.96 | 1.28 | 1.29 | 1.14 | 1.81 | 2.11 |
| SD | 0.12 | 0.01 | 0.19 | 0.18 | 0.12 | 0.27 | 0.47 |
| type | m | sm | m | m | m | sm | sm |

Centromeric ratios: 1.00-1.67 = metacentric (m),
1.68-3.00 = submetacentric (sm)
(based on Levan *et al.*, 1964 and Green *et al.*, 1980).

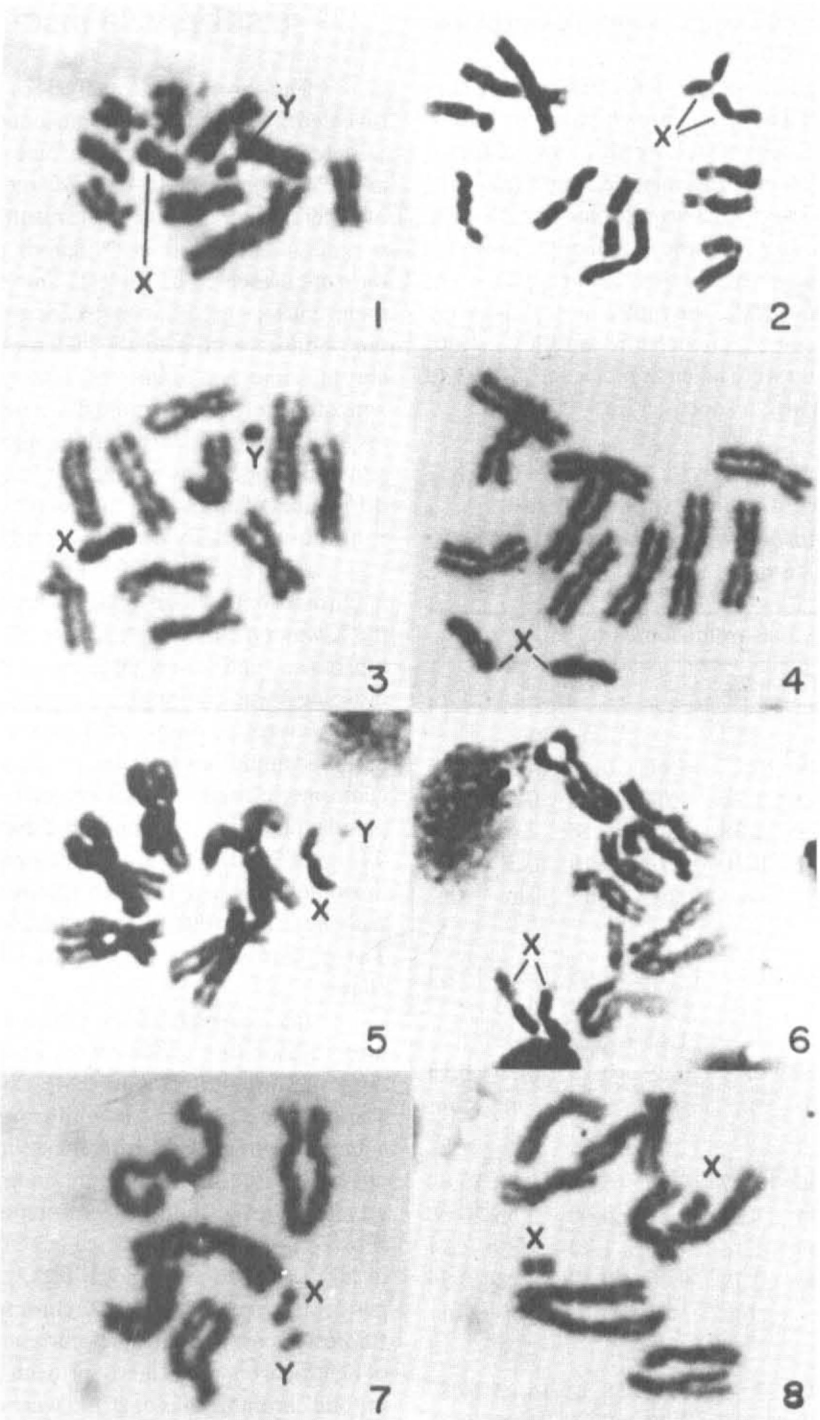
## RESULTS AND DISCUSSION

The chromosome analysis of the 4 species has revealed that the mitotic chromosomes consist of a pair of heteromorphic sex chromosomes (XX in females and XY in males) and 5 pairs of autosomes (nos. 2-6) of different size and shape. The results of chromosome measurements for relative length and centromeric ratio are presented in Table 1. The X chromosome is metacentric; one arm consists of euchromatin while the opposite arm is totally heterochromatic. The Y chromosome is a small submetacentric comprising almost entirely heterochromatic material. However, in G- and H-banding preparations, the Y chromosome generally appears as a large dot like figure. Specific differences of mitotic chromosomes of the 4 species of fruit flies are briefly described below.

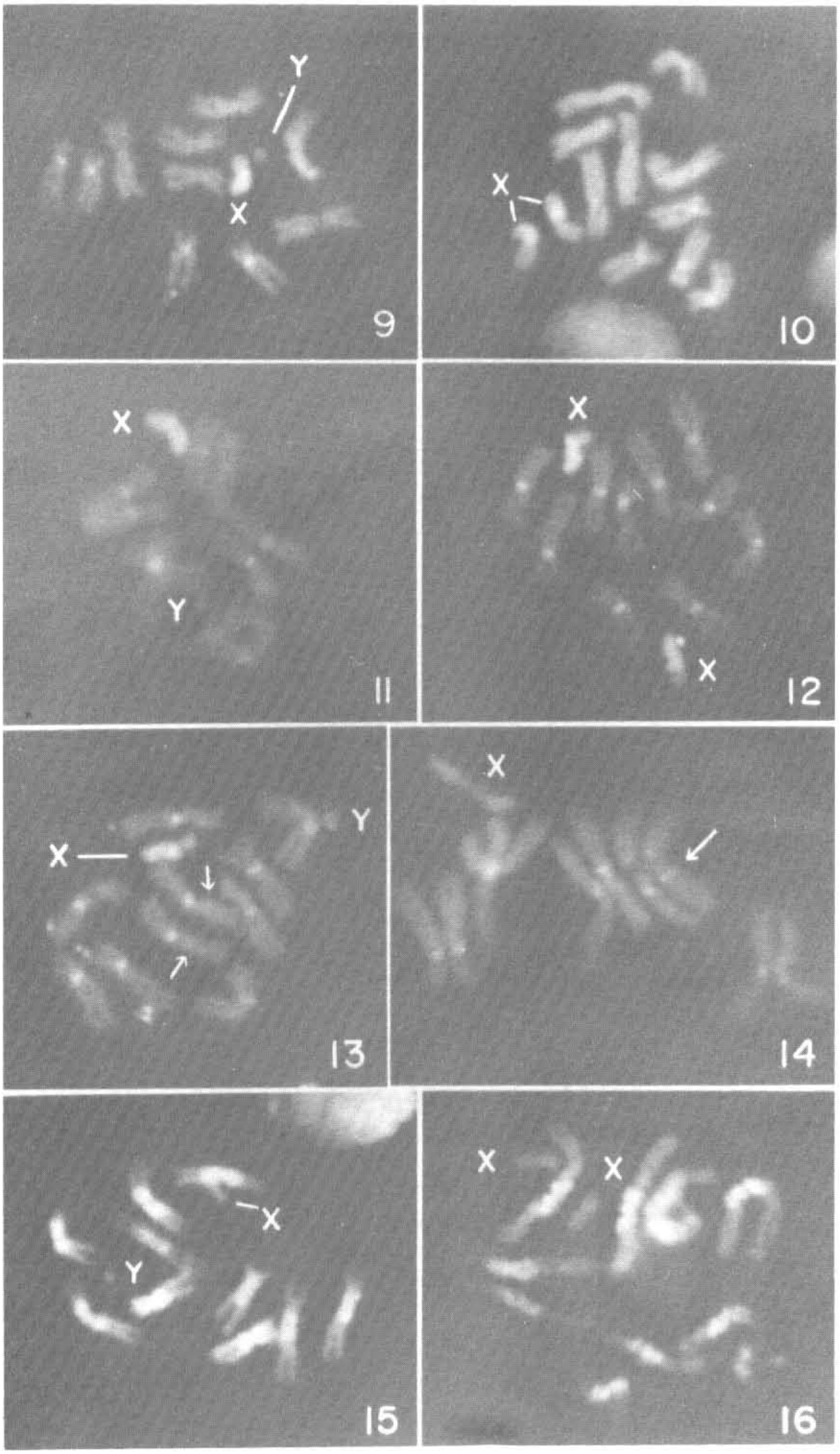
*Bactrocera dorsalis:* For Giemsa staining, the Y chromosome was a small submetacentric whereas the X was a medium metacentric (Figure 1, 2). Chromosomes 2 and 4 were large metacentric while chromosome 3 was a large submetacentric. Chromosomes 5 and 6 were medium submetacentric. The Hoechst 33258 staining has revealed brighter fluorescence in centromeric regions of chromosomes 5 and 6 compared with dull fluorescence in chromosomes 2,3 and 4 (Figure 9, 10). One arm of the metacentric X chromosome exhibited brighter fluorescence compared with the uniform dull fluorescence in the opposite arm. The Y chromosome also showed brighter fluorescence.

*Bactrocera tau*: The G-banding mitotic chromosomes of this species were generally similar to those of *B. dorsalis* (Figure 3, 4). Chromosomes 2, 3, 4 and 6 were metacentric while chromosome 5 was submetacentric. Such differences in the shape were not easily visualized from the photomicrographs. Nevertheless, the H-banding chromosomes of *B. tau* clearly showed brighter fluorescence in centromeric regions of all the autosomes (Figure 11,12). In addition, the pericentric regions of the X chromosome of *B. tau* fluorescence reflecting the occurrence of a large block of heterochromatin. Thus the Hoechst-33258 staining mitotic chromosomes of *B. tau* were obviously different from those of *B. dorsalis*.

*Bactrocera cucurbitae*: The G- and H-bandings of the mitotic chromosomes of this species (Figure 5, 6, 13, 14) were generally similar in size and shape and brighter fluorescence in the centromeric regions of the autosomes to those of *B. tau* been observed in one arm of the third chromosome where a brighter fluorescent




**Figures 1-8 Photomicrographs of the G-banding mitotic chromosomes from larval neuroblast cells of 4 species of *Bactrocera* 7-8, *B. correcta*. Left-males, right-females.**




**Figures 9-16 Photomicrographs of the H-banding of mitotic chromosomes from larval neuroblast cells of 4 species of *Bactrocera cucurbitae* (one X chromosome is missing in a female karyotype in Figure 14); 15-16, *B. correcta* right-females. Arrows indicate brighter fluorescent bands in one arm of chromosome 3.**

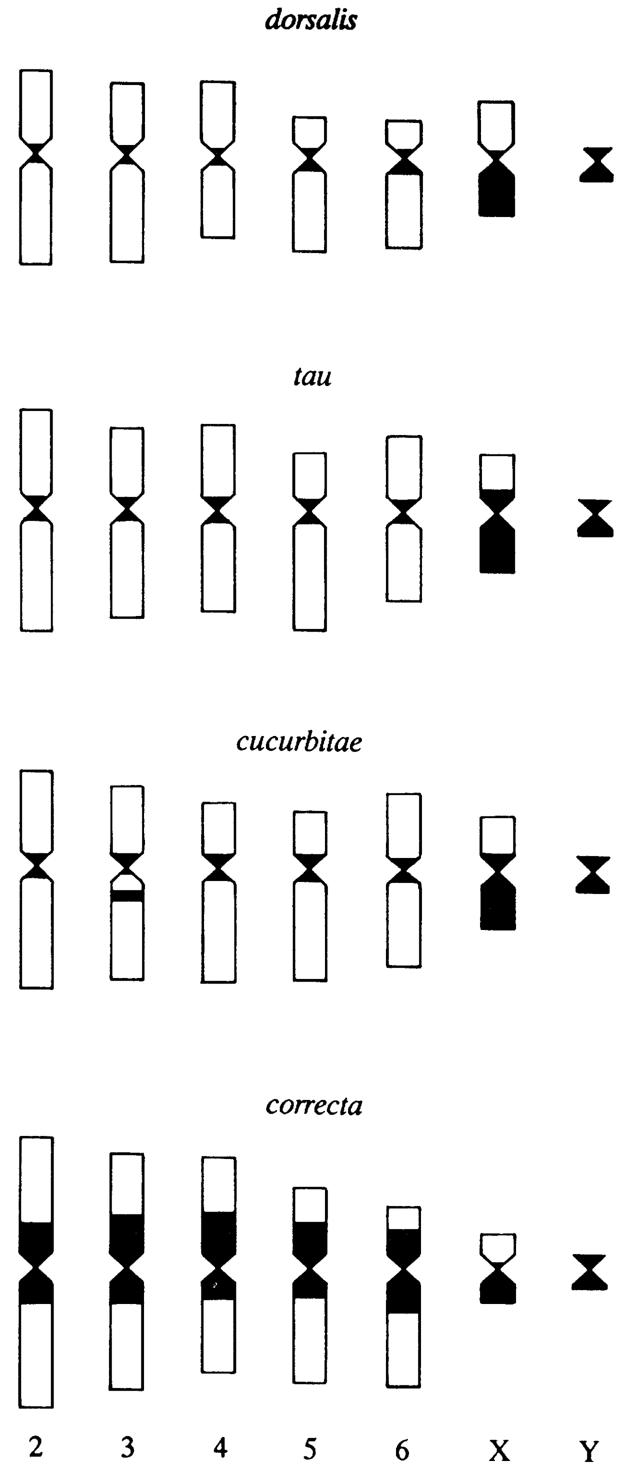



Figure 17 Diagrammatic representation and comparison of mitotic chromosomes of 4 species of fruit flies (*Bactrocera*) in Thailand. Only one set of autosomes is present. Black areas represent heterochromatic portions which show brighter fluorescence with Hoechst 33258 staining. The centromeres are indicated by constrictions of each chromosome. Chromosome lengths, arm ratios and heterochromatic portions are depicted in proportion.

banding was detected at the proximal region (Figure 13,14). This is a good diagnostic character of H-banding in separating mitotic chromosomes of *B. cucurbitae* from those of *B. tau.*

*Bactrocera correcta*: This species showed a striking difference of mitotic chromosomes from those of the other 3 species in having extensive blocks of pericentric heterochromatin in all autosomes as revealed by G-banding (Figure 7, 8) and particularly by H-banding (Figure 15, 16). This is a unique cytological characteristic of *B. correcta.* Moreover, the X chromosome was a small metacentric compared with those of the other 3 species studied here.

A diagrammatic representation of mitotic karyotype of these species are presented in Figure 17.

Previous studies of the mitotic chromosomes of *Bactrocera cucurbitae* (then reported as *Dacus cucurbitae* have been a controversial issue on metaphase karyotype of this species (Gopalan, 1972; Bhatnagar *et al.*, 1980; Singh and Gupta, 1984). Our findings on mitotic chromosomes of *B. cucurbitae* correspond well with the observations of Singh and Gupta (1984) of the Indian populations of *B. cucurbitae.*

The present chromosomal data indicate that the 4 species of fruit flies occurring in Thailand generally exhibit a similar mitotic karyotype (Figure 17). Nevertheless, remarkable differences have been found primarily in the centromeric heterochromatin and the presence or absence of a brighter fluorescent banding in the chromosome 3 and, to a lesser extent, the size of the X chromosome. Thus the mitotic karyotype of *B. correcta* is obviously distinct from that of other species in having large blocks of pericentric heterochromatin in the autosomes and the small metacentric X chromosome. On the other hand, *B. cucurbitae* presence of a clear H-band in one arm of the metacentric chromosome 3, although both species show similar brighter fluorescence in centromeric regions of the autosomes as well as similar sex chromosomes. However, *B. dorsalis* clearly differs from *B. tau* fluorescence in the centromeric regions of chromosomes 2, 3 and 4 compared with those of other species (Figure 18). The heterochromatin differentiation as revealed in mitotic chromosomes of the 4 species reported here is useful as a diagnostic character. Furthermore, this detailed comparative analysis of the mitotic karyotype may prove an excellent tool to explore the cytological interrelationships of some closely related species groups within the genus *Bactrocera* or among the genera of the family as a whole. This karyotypic approach of chromosomal evolution in the Oriental fruit flies is of interesting phenomena and deserves further investigations.

## ACKNOWLEDGEMENTS

We thank Dr. Paul Grote for useful comments on the manuscript. We also thank Prof. Sutham Areekul for providing laboratory stocks of these fruit flies. Our sincere appreciation is offered to K. Vejsanit and P. Punthusiri for preparations of illustrations.

## LITERATURE CITED

Baimai, V. 1975. Heterochromatin and multiple inversions in *Drosophila* chromosome. Can. J. Genet. Cytol. 17:15-20.

Bhatnagar, S., D. Kaul and R. Chaturvedi. 1980. Chromosomal studies in three species of the genus *Dacus* (Trypetidae: Diptera). Genetica. 54:11-15.

Drew, R.A.I. 1989. The tropical fruit flies (Diptera: Dacinae) of the Australasian and Oceanian regions. Memoirs of the Queensland Museum. 26:1-521.

Gopalan, H.N.B. 1972. The study of somatic, meiotic and salivary gland chromosomes of melon fly, *Dacus cucurbitae*. Caryologia. 25: 163-172.

Green, D.M., J.P. Bogart, E.H. Anthony and D.L. Genner. 1980. An interactive, microcomputer-based karyotypeanalysis system for phylogenetic cytotax-onomy. Comput. Biol. Med. 10:219-227.

Hardy, D.E. 1973. The fruit flies (Diptera-Tephritidae) of Thailand and borderingcountries. Pacific Insects Monograph. 31:1-353.

Latt, S.A. and J.C. Wohlleb. 1975. Optical studies of the interaction of 33258 Hoechst with DNA, chromatin and metaphase chromosomes. Chromosoma. (Berl.) 52:297-316.

Levan, A., K. Fredga and A.A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. Hereditas. 52:201-220.

Permpoon, P., M. Jirasurat, Y. Tewahasakoonthong, C. Sungthong and C. Boonyong. 1983. Destruction of guava and quantity of fruit fly attracted to methyl eugenol. Newsletterof Entomology and Zoology. 5:148-155. (*in* Thai)

Sepsawad, P., P. Senaweenin, C. Sungthong, C. Boonyong. 1978. Fruit flies and control. Kasikorn. 51:368-38. (*in* Thai).

Singh, O.P. and J.P. Gupta. 1984. Studies on mitotic and salivary chromosomes of *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). Genetica. 62: 217-221.

Sutantawong, M. 1985. The eradication of fruit flies in vegetables and fruits with gamma ray forexport. OAP Newsletter. 1:9-10. (*in* Thai)

Tigvattananont, S. and S. Areekul. 1984. Theeconomic importance of fruit fly, *Dacusc orrectus* Thailand. Kaenkaset. 18:180-185. (*in* Thai)

Tigvattananont, S. 1986a. Host plants of the fruit flies in Thailand. Kaset Prajomklao. 4:1-15. (*in* Thai)

Tigvattananont, S. 1986b. The economic importance of fruit fly, *Dacus tau* (Walker) in Thailand. Kaenkaset. 14:114-118. (*in* Thai)

White, I.M. and M.M. Elson-Harris. 1992. Fruit Flies of Economic Significance: Their Identification and Bionomics. C.A.B. International, Redwood Press, Melksham. p