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THESIS

BIONOMICS OF GUAVA FRUIT FLY, *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae) AND EFFECT OF GAMMA RADIATION

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A Thesis Submitted in Partial Fulfillment of
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Kessuda Puanmanee 2010: Bionomics of Guava Fruit Fly, *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae) and Effect of Gamma Radiation. Master of Science (Entomology), Major Field: Entomology, Department of Entomology. Thesis Advisor: Professor Praparat Hormchan, Ph.D. 72 pages.

Life cycle study of *Bactrocera correcta* (Bezzi) fed on papaya under laboratory conditions of 25-27 °C and 70-80 %RH was conducted. It revealed mating at the average female age of 11.85±1.18 days. The egg was either laid singly or in mass, 4-5 eggs/mass, with 92.25 % hatching. The averages of egg, larval (3rd instar), pupal, female and male adults stages were 38.30 h, 6.77, 9.62, 82.30 and 70.12 days respectively. The analysis of partial life table of *B. correcta* using 100 eggs for the start showed the net reproductive rate (R_o) to be 197.2200, the capacity for increase (r_c) 0.0910, the cohort generation time (T_c) 58.1235 days and the finite rate of increase (λ) 1.0952. Partial life table in 3 diets namely mango, papaya and artificial diet of wheat-yeast formula were also studied. It was found that for the mortality and survival in the mango, the 3rd and 2nd instars larvae had the highest percentages of 48.98 and 97.45 respectively; in papaya, the pupal stage and 1st instar larva obtained the highest percentages of 31.36 and 97.17 respectively whereas in the artificial diet, those of 9.36 and 92.00 were found in the 2nd and 3rd larval instars respectively.

The investigation of gamma irradiation on the fruit fly pupae from ^{137}Cs source at the doses of 0, 5, 10, 15 and 30 Gy indicated percentages of adult emergence, adult abnormality and male age not to be significantly different from one another and the sterilities were 23.85, 21.78, 59.10, 72.57 and 98.34 respectively. The percent sterilities at 5 Gy was not significantly different from the control (0 Gy). Mating competitiveness of the males when irradiated at 30 Gy was almost equally competitive to the untreated males. The total competitiveness values of treated males were estimated to range from 1.45 to 2.09 at three different ratios from 1:1:1 to 3:1:1. The observation on melanization of the treated larvae after killing by freezing resulted in the larval color appearing from black to creamy white. The degree of melanization decreased with the increasing dose. The total haemocyte counts (THCs) of the irradiated 1st instar larvae and observed in the 3rd instar larvae were also found to be the averages of 3150, 2200, 700, 550 and 350 h/mm^3 respectively. THCs at every dose were noticed to be significantly different from that of the control.

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Kessuda Puanmanee

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LIST OF ABBREVIATIONS

Cs-137	Cesium 137
cm	centimeter
DHCs	differential haemocyte counts
EDTA	Ethylene diamine tetra-acetic acid
Gy	gray
h/mm^3	haemocytes per cubic millimetre
kGy	kilogray
mg	milligram
mm	millimetre
mm^3	cubic millimetre
min	minute
ml	millilitre
h	hour
RH	relative humidity
THC	total haemocyte count
µl	microlitre

BIONOMICS OF GUAVA FRUIT FLY, *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae) AND EFFECT OF GAMMA RADIATION

INTRODUCTION

The guava fruit fly, *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae), is one of the most destructive pests in the genus *Bactrocera* (Wang, 1996). The fly was first recorded in 1916 at Bihar, India (Bezzi, 1916) and is now found throughout most countries in Southeast Asia, including Pakistan, India, Nepal, Burma, Sri Lanka, Vietnam China and Thailand (Wang, 1996; Drew and Raghu, 2002). It is polyphagous with a wide host range, infesting tropical and subtropical fruits that covers more than 30 plant families (Allwood *et al*., 1999; Maynard *et al*., 2004). Normally found in guava, jujube, mango, rose apple, carambola and tropical almond (*Terminalia catappa*) (White and Elson-Harris 1992), it is widely distributed in North and Central parts of Thailand (Department of Agriculture, 2001). The fly causes a great loss in fruit and vegetable production (Drew and Raghu, 2002) and is listed as a quarantine pest by most countries worldwide (White and Elson-Harris, 1992).

Tradition control method for this fruit fly is based on the extensive use of chemical insecticides. Awareness in public health and environmental concerns, the use of biological and genetic methods is now being encouraged. One of them is the sterile insect technique (SIT) also referred as 'sterile male technique' or 'sterile insect release method' or 'autocidal control'. It is based on the release of large amount of sterilized insects in order to reduce/suppress natural pest population (Knipling 1959, 1968). This method is made possible to control and/or eradicate insect populations.

Sterilization with chemicals and radiation for flies is getting popular. The induction of sterility, particularly by ionizing radiation, does not cause any problem. Management of insect pests using ionizing radiation can be achieved by the application of lethal or sterilizing dose. Radiation can render the pest species sterile immediately or it can sub-sterilize the species, causing reduced reproduction in subsequent generations (Alam *et al*., 2001).

The practicability of sterile insect tecgnique (SIT) to control insect pests has been demonstrated for a number of species in various parts of the world e.g., *Cochliomyia hominivorax* (Coquerel) in Florida (Knipling, 1960), *Dacus cucurbitae* (Coquillett) in the Pacific (Steiner *et al*., 1965); *Ceratitis capitata* (Wiedemann) in Nicaragua (Rhode *et al*., 1971); *Dacus tryoni* (Froggatt) in Australia (Andrewartha *et al*., 1967); *Pectinophora gossypiella* (Henneberry, 1994) and *Cydia pomonella* (L.) (Calkin *et al*., 2000). Other tephrititids, such as *C. capitata*, the irradiation process may reduce the mating performance of the sterilized males (Alcagno *et al*., 2002); Lux *et al*., 2002). Calkins *et al*. (1988) reported that the lower irradiation doses (30 Gy) applied 24-48 h before emergence of genus *Anastrepha* induced high levels of sterility in adult. Rhode *et al*. (1961) demonstrated *Anastrepha ludens* (Loew) pupae irradiated 96 h before emergence with 40 Gy to show 100% male sterility and dose (70 Gy) did not affect survival of the laboratory- reared flies (Cendra *et al*., 2004). Rull *et al*. (2007) found that a low dose of 40 Gy was sufficient to completely suppress egg production in females. Similarly, a mild carryover of genetic damage might have been transferred to the F1 progeny of males irradiated at 40 Gy crossed with fertile wild females. Allinghi *et al*. (2007) showed that the pupal age at the time of irradiation did not affect the sterility induced by gamma radiation in males. Nahar *et al*. (2006) reported *B. cucurbitae* pupae irradiated before emergence with 30 Gy. The mating of unirradiated females with those of irradiated males did not affect the production of eggs but egg viability was reduced to 0.93 %. The males irradiated at a dose of 40 Gy produced 100 % sterilization. The exposure of females to gamma irradiation sharply reduced the production of eggs by the latter. Irradiation of females had an effect on both fecundity and fertility.

Irradiation is technically effective in quarantine treatment for pests. At low doses, irradiation does not cause immediate death of pests (Ignatowicz and Brzostek, 1990). Particularly, in products irradiated for quarantine purpose, it must be ascertained that living insects will not be able to survive or proliferate in a new location. Therefore, some methods to detect previous exposure of insect pests to ionizing radiation are needed (ICGFI, 1986). Nation *et al*. (1995a) suggested that an indicator which could be used easily for identifying irradiated insects might lie in the

irradiation causing inhibition of the darkening or melanization that usually followed by death or injury in a living insect. The haemocyte number was found to change with the level of irradiation. Elbadry (1964) demonstrated the effects of gamma irradiation on total haemocyte counts (THCs) and differential haemocyte counts (DHCs) of potato tuberworm. Nation *et al*. (1995a) suggested that ionizing radiation inhibited the production of one or more enzymes involving in melanization.

Demographic population analysis has diverse applications: predicting life history traits, an analyzing population stability and structure, estimating extinction probabilities, predicting outbreaks in pest species, and examining the dynamics of colonizing or invading species (McPeek and Kalisz, 1993). Life tables are tables of data on survivorship and fecundity of individuals within a population. Life tables constructed this way are called cohort life tables. They can then be used to determine age or stage-specific fecundity and mortality rates, survivorship, and basic reproductive rates, which in turn can be compared from cohort to cohort enabling an analysis of their annual variation.

OBJECTIVES

1. To study the biological development of the guava fruit fly, *B. correcta* on papaya fruit under laboratory conditions.

2. To investigate the effect of substerilizing doses of gamma radiation on the guava fruit fly reproduction.

3. To study the effect of substerilizing doses of gamma radiation on mating competitiveness.

4. To differentiate the gamma irradiated and unirradiated guava fruit fly larvae with substerilizing doses by haemocyte counts and their melanization.

LITERATURE REVIEW

Biology of *Bactrocera correcta* (Bezzi)

The guava fruit fly, *B. correcta* is in family Tephritidae belonging to Order Diptera *B. correcta* is an important pest for fruit production in Thailand. The distribution of this species covers most Southeast and Southwest Asian countries including Thailand.

The account on morphology and life history of *B. correcta* was given by Department of Agriculture, (2001) as follows: Eggs small, elongate-ovoid (banana fruit or cresent-shaped), creamy or milky and polished skin, 1.13 mm long and 0.27 mm wide, hatching in 36-48 h, frequently laid in groups in a hole under fruit skin.

Larva jointed legs absent, head-capsule reduced, mouthpart with a pair characteristic mouth-hooks articulated to a distinctive internal cephalo-pharyngeal skeleton, head black, body white, colour changed by food, the ability to jump and size about 8.17 mm, three larval stages differentiated by the relative length of paired cephalopharyn and spiracle, larval stadium five to six days according to the environment, in each stage, larvae found in the fruits eating the flesh or other parts of plants, full-grown larvae white and opaque. Before pupation, larvae leave the fruits by jumping action then drop on the soil to pupate.

Pupa ovoid or barrel shaped, coarctate type, tough, newly pupa white pale and brown at last, 4.0 mm long and 2.0 mm wide. During this stadium, pupae exist in two to five cm soil depth. Adult stadium is variated according to the environment and food. Adults usually emerge through forcing off a circular cap by expanding a sac (ptilinum) everted from the head.

Brightly colored adult, predominately black with lateral yellow stripes, approximately 5.4 mm in length. Hardy (1973) stated that *B. correcta* was differentiated from the other species known from Thailand and surrounding regions by having the face with the black transverse band at the lower third and by having the costal end of the wing interrupted in cell R_3, beyond the tip of vein R_{2+3}. Wings almost entirely hyaline with the subcostal cell yellow, a very faint tinge of yellow along the costal margin in apex of cell R_1, and a narrow brown spot at lower apex of cell R_3 and upper apex of cell R_5.

Management and control

In the development of integrated pest management (IPM) strategies for fruit flies control tactics which have been evaluated against fruit flies include cultural manipulation of the crop and its environment, biological control and genetic methods. Sterile insect technique (SIT) also referred to as the 'sterile male technique', the 'sterile insect release method' or 'autocidal control' and based on the release of mass-reared and sterilized insects in order to reduce pest populations (Knipling 1959, 1968) is a possible method for controlling or eradicating insect populations.

Life table

The basic information needed to study density changes and rates of increase or decrease is contained in a life table. A life table contains such vital statistics as the probability of an individual of a certain age dying or, conversely, the average number of offspring produced by a female of a given age. The most reliable method of determining these statistics is to begin with life of the cohort, noting the death of individuals and the birth of offspring until the demise of the last individual. It is convenient in the calculations to use cohorts of 100 or 1,000 individuals (Poole, 1974).

Life table construction termed demography by Stilling (1992) contains such vital statistics as the probability of an individual of a certain age dying, or conversely,

the average number of offspring produced by female of a given age (Poole, 1974). The demographic parameters like the intrinsic rate, mean generation time, and population doubling time are useful indices of population growth of an insect under a given set of growing conditions (Tsai, 1998).

Radiation and radioisotopes in agriculture

The use of radiation in agriculture started around 1940s when gamma radiation was applied to control livestock insect pests by inducing reproductive sterility. The sterile insect technique (SIT) is the most well-known for its use to control the screwworms of cattles in the United States (Anonymous, 1992). The radiation biology publications show that sterility in insect is caused by exposure to ionizing radiation. Development of new approach along with its demonstrated usefulness has led to related research on other insects. A better understanding of the technique including its potential and limitation and more data on insect population dynamics, movement, migration and management problems has evolved from these studies (Anonymous, 1992).

Post-harvest quarantine treatments of fresh agricultural commodities are devised to prevent migration of potentially damaging organisms to the new areas. Traditional treatments which most commonly involved chemical fumigants, such as ethylene dibromide and methyl bromide, and both hot (43-48 °C) and cold (0-3 °C) temperatures, work by killing essentially 100% of all stages of quarantined pests that might be present in the commodity.

Effects of radiation on living organisms

Radiation may damage biologically important molecules in two ways. Direct action, as the name implies, is the alteration of a biological molecule through deposition of energy in it as the result of a primary interaction with the radiation. Indirect action takes place when the primary action of radiation is on water. The biological molecules are then attacked by the highly reactive products of that

radiolysis. A biological system suffers mostly from indirect action since it is mostly water. However, action on water of hydration of a molecule is regarded as being direct rather than indirect (Thornburn, 1972).

Radiation can interfere directly with mitosis in cell, and as with whole insects, the stage development influences the response and can cause mutation in insect, a common tool in studies with *Drosophila*, for example, where important information on population is being obtained. The environments in which insects are irradiated interacts with the effects of irradiation (Desrosier and Rosenstock, 1960).

Radiation effect on insects

Insect, in general, are sensitive to radiation. As with other organisms, the effects of radiation on insects are closely related to the effects on constituent cells. For insect cells, radiation sensitivity is directly proportional to the reproductive activity of the cells and inversely proportional to their degree of differentiation. During the larval period of insect, very little cell differentiation occurs. Cell division and tissue differentiation occur during the embryonic development in eggs and also during brief period before moulting and later stages of pupation. The dividing cells of insect are quite radiation sensitive, whereas the static adult stage is more resistant to radiation. Hence, low radiation doses cause insect sterilization or genetically deformed gametes, while higher doses are required to induce insect death (Molin, 2001).

Radiation effects manifested in an insect's life cycle are lethality (i.e. immediate kill), reduce longevity, delay moulting, cause infecundity and aspermia, reduce egg hatch, delay development, reduce feeding and locomotion and inhibit respiration. At low radiation doses, increased longevity, higher feeding, higher egg laying and higher egg hatching may also occur. The effects of radiation vary in developmental stages. Eggs are most sensitive to radiation. Sublethal radiation doses in eggs give rise to malformed or sterile adults. If a moderate dose of radiation is applied to larvae, a prolonged larval stage occurs. Diapausing larvae of insects are

more resistant to radiation. However, these larvae normally die after pupation. Female pupae are more radiosensitive than are their male counterparts. As eggs and larvae, mortality varies with the ages of pupae. Adult insects emerging from irradiated pupae are preferentially males (Molin, 2001).

Physical factors also have influence on the effects of radiation on insects. Raising of temperature during treatment positively influences the radiation sensitivity of insects. Modified atmosphere (i.e., absence of oxygen) decreases the radiosensitivity of insects. It has been generally observed that the more active insect, the more sensitive radiation.

The radiation sensitivity of insects varies from order to order. The family of Bruchidae, order Coleoptera (beetles), is mostly sensitive to radiation, whereas moths (Lepidoptera) are the most resistant group. The moths have diffused centromeres in their chromosomes. Low doses of radiation cause few breaks in chromosomes, allowing them to participate in cell division. Higher radiation doses applied to moths cause multiple breaks in the chromosome, thus causing death or sterility. Normally, sterilizing doses vary from 50 Gy in beetles (Bruchidae) to around 1000 Gy in moths. Doses over 1 kGy may be needed to achieve 100% sterility in some moths. However, for all practical purposes, 500 Gy should be considered adequate as it produces over 95% sterility in most of the species and partial sterility to the rest. As a result, the commodity disinfestation objective is achieved. In contrast, a dose of 3-5 kGy would be required to achieve immediate insect kill. As mentioned earlier, this is unnecessary because lower doses can be used to cause death in developmental stages of insect, accomplishing the objective of the treatment (Molin, 2001).

Radiation has effects on insect such as disinfestation, changes in physiology and morphology. In the study of substerilizing doses of gamma radiation, Pransopon (2000) reported that *Helicoverpa armigera* emergence was found not to be significantly different at different radiation doses whereas moth deformation following irradiation of mature pupae varied significantly with the irradiation doses and positive correlation was also noticed to exist between moth deformation and

radiation doses. The numbers of moth deformation were not significantly different when their pupae irradiated at 50, 100 and 150 Gy while those were comparatively higher when the pupae irradiated at 200 Gy. In addition, Burditt *et al*. (1989) found a significant decrease in adult emergence when mature cocoons and larvae of nondiapausing codling moth, *Cydia pomonella* (L.), were irradiated as the dose rate increased from 1 to 200 Gy per min using a cobalt 60 source.

Depending on dose, irradiation could result in mortality by preventing egg hatch, larval development, pupation or adult emergence of codling moth. Similarly, depending on dose, any adults developing from irradiated eggs or larvae could not possibly produce fertile offspring due to sterility or other abnormalities (Ouye and Gilmore, 1985).

Azelmat *et al*. (2005) reported that the Indian meal moth *Podia interpunctella* (Hübner) larvae were exposed to different gamma irradiation doses ranging from 300 to 900 Gy. Feeding, pupation, adult emergence and survival were very sensitive to ionizing irradiation. At a dose of 300 Gy and higher, no adults emerged. Irradiation at 450 Gy caused 100% mortality. The dose at which 100% sterility was achieved in treated females mated to treated males was 300 Gy for the parental generation of irradiated pupae. Fertility of the parental males from irradiated pupae was 48.17% at 300 Gy in treated males crossed with untreated females, while male progeny of irradiated male parents had a residual fertility of 11.06% at the same dose. (Ayvaz *et al*., 2007)

Boshra and Mikhaiel (2006) found that mature pupae of *Ephestia calidella* (Guenée) irradiated at dose of 1000 Gy prevented the emergence of both sexes. Females and males were sterilized with doses of 350 and 400 Gy respectively. Adult longevity and mating ability varied with regard to the dose. Males irradiated with sub-sterile doses (100, 150, and 200 Gy) were more sexually competitive with normal males than those irradiated with the sterilizing dose (400 Gy). Ozyardimci *et al*. (2006) reported that the rate of egg hatch was 7.6% at 300 Gy and completely inhibited at 450 Gy with almond moth, *E. cautella*.

Radiation effect on fruit fly

The findings suggested that the sterile insect technique (SIT) might be applied successfully against Tephritidae at least at regional scale, such as *C. capitata*. The irradiation process may reduce the mating performance of the sterilized males (Alcagno *et al*., 2002 and Lux *et al*., 2002). Calkins *et al*. (1988) reported that lower irradiation dose (30 Gy) applied 24-48 h before emergence of genus *Anastrepha* induced high levels of sterility in adult. Rhode *et al*. (1961) also stated that *A. ludens* pupae irradiated 96 h before emergence with 40 Gy showed 100% male sterility and dose (70 Gy) did not affect survival of the laboratory-reared flies (Cendra *et al*., 2004). Rull *et al*. (2007) found that a low dose of 40 Gy was sufficient to completely suppress egg production in females. Similarly, a mild carryover of genetic damage might have been transferred to the F1 progeny of males irradiated at 40 Gy crossed with fertile wild females.

Haemocyte classifications

Haemocyte classification, both in insect and other arthropods, has been variously based on morphology, function and staining or histochemical reactions of haemocytes. Thus, it is not unusual to find the same haemocyte type or its various forms referred to by different names in various arthropods by different authors. Consequently, it becomes very difficult to compare haemocytes of one species with those of the others. The insect haemocyte classification generally used has evolved over more than half a century.

In order to adopt a uniform haemocyte classification for discussing haemocytes and their physiological significance in various insects, it is necessary to homologize terminologies used by different authors on the bases of description, observed functions, line drawings and micrograph of haemocytes studied by several authors. Gupta (1979) demonstrated that two to seven types were reported in Lepidoptera, Coleoptera and Diptera as well as five types in Hymenoptera, Neuroptera and Megaloptera but only three types in Trichoptera. There is

disagreement among insect haemotologists about the numbers of haemocyte type. From one or a few to as many as nine or more types have been described, particularly by light microscopy. Ultrastructurally, however, only seven types have so far been identified: prohaemocyte (PR), plasmatocyte (PL), granulocyte (GR), spherulocyte (SP), adipohaemocyte (AD), oenocytiod (OE) and coagulocyte (CO). Most insects do not have all seven classes, if indeed may have all seven. Based on the classification scheme of Gillespie *et al*. (1997) that established application of monoclonal antibodies (Mabs) to the identification and classification of haemocytes, it has become a useful tool.

Haemocyte number

Suspended in the blood plasma are blood cells or haemocytes. The number of haemocytes in the circulation of insects is quite variable from species and even within the same individual at different times depending on its physiological state. Stage of development, sex, age and activity are known to influence the observed number of cells in some insects. Measurements of haemocyte count per microlitre (counts/ul) of haemolymph over time can be influenced by fluctuation in blood volume. The total number of haemocytes present in the circulating blood of various adult species varies from about 1,000 haemocytes per cubic millimeter (h/mm^3). While many insects have total haemocyte count (THCs) below 10,000 h/mm^3, many other insects have from 20,000 to 100,000 h/mm^3 (Chapman, 1998).

Females tend to have higher counts than males and Endopterygote larvae have relatively more cells than the corresponding adults, though the reverse is true of Exopterygote nymphs (Richard and Davies, 1994). THCs usually determined on blood collected from a severed appendage or some other wounds representing the number of haemocytes in a particular known volume of haemolymph. THCs undertaken in this way have been used as indirect evidence for multiplication of haemocytes and changes in haemocyte populations in general under various experimental conditions. In some cases, differential haemocyte counts (DHCs) were also made to show the changes in proportion of haemocyte types in relation to THCs.

Radiation effects on haemocytes

Abnormal and pathological changes in insect haemocytes have been reviewed by Wittig (1962) for many types of injury and disease but nothing has been mentioned on the changes that may be induced by gamma irradiation. Such changes may lead to cytological, haematological or physiological studies. Elbadry (1964) demonstrated the effects of gamma irradiation with 30 and 90 Gy on THCs and DHCs of the potato tuberworm, *Gnorimoschema operculella* (Zeller). The THC and DHC decrease in treated larvae and small numbers of the haemocyte of the irradiated insects showed some morphological changes.

Hoffmann (1972) studied the haemograms of nymph and adult *L. migratoria* after selective X-irradiations of the haemocytopoietic tissue in 24 h. It was found that the total haemocyte number fell by approximately 50 percent of its initial figure. The dramatic decrease in the number of circulating haemocyte after irradiation of the highly radiosensitive haemocytopoietic tissue occurred. Eppensteiner and Karp (1989) showed the effect of gamma irradiation in the American cockroach, *Peliplaneta americana* exposed to 50 Gy to deplete circulating haemocytes to one third of normal. After irradiation by X-rays, the characterized alteration of the adult insects were observed in the haemocytes (*L. migratoria*; 100 Gy). The process decreased the number of haemocytes in the haemograms and decreased in the size (Gregioire, 1974).

Radiation effects on melanization

The presence of these enzymes on the hemocytes, offers option in the control of metabolic pathways leading to melanization and tanning process (Gupta, 1979). Melanization is defined as a process by which the cuticle becomes dark and sometime can be induced in normally nonmelanized cuticle (or blood) by mechanical injury which somehow induces tyrosinase activity (Richard, 1978). Phenoloxidase (PO) can be quantified easily and exists in all insects. The enzyme plays an important role in the melanization of insect cuticle. The cuticle would be required by generating the

necessary pigments through the conversion of aromatic quinones and its products into melanin (Anderson, 1985; Salt, 1970).

Melanins are common pigments of insect, and produce their red to brown and black colors. Melanin formation is a complex process involving numerous enzymatically-controlled steps, catalyzed by several kinds of phenoloxidase. It occurs in many insects following wounding (wound healing) (Lai-Fook, 1996), during encapsulation of foreign objects (Gotz and Boman, 1985; Gupta, 1985), tanning, pigmentation, and after death. The first three functions are generally considered to be protection of melanin formation; melanization after death probably results from loss of control over chemical reactions leading to melanin formation (Nicholas, 1968).

With some biochemical changes, the level of amino acid in the plasma may be able to act as biochemical indicator of radiation damage (Thornburn, 1972). All enzymes can be inactivated when irradiated in solution, although the radiation doses necessary to inactivate different enzymes vary greatly (Casarett, 1968). The frozen larva could be removed after 10-15 min from the freezer and observed at room temperature for degree of darkening. If it was not darkened, the result would indicate that it had been irradiated (Nation *et al*., 1995b).

Nation *et al*. (1995a) reported the gamma radiation to induce changes in melanization and phenoloxidase in Caribbean fruit fly larvae. When irradiated at >20 Gy, larvae failed to show typical melanization. Irradiated larvae showed greatly decreased enzyme activity at >20 Gy and substantial reduction at lower doses. Irradiation reduces or eliminates melanization after death and reduces PO activity in third instars irradiated as first instars. Mansour and Franz (1996) reported the results of research conducted on the effects of gamma irradiation on PO activity in the mediterranean fruit fly, *C. capitata* larvae and on the melanization process. The results indicated that measuring the activity of PO in larvae was a good, sensitive and reliable indicator of irradiation treatment. The melanization in larvae exposed as eggs to doses 10-160 Gy and examined as old larvae showed that the degree of melanization in treated larvae decreased with the increasing dose.

Supawan (2005) reported the degree of melanization in non-irradiated azuki bean weevil larvae to be significantly different from the irradiated larvae. Colors of larval body appeared black, light gray and creamy white color. In treated larvae at 100, 300 and 500 Gy, the degree of melanization decreased with the increasing dose. Ignatowicz and Banasik-Solgala (1997) reported that after the irradiation treatment with doses ranging from 0.1 to 0.5 kGy, the melanization process was significantly inhibited in young larvae of khapra beetle cold-killed after irradiation and old larvae melanized so slowly that after their death some visible in the body color were noted as late as after 24 h. However, the changes in melanization of the khapra beetle larvae can not be used for indicating previous exposure of the insects to irradiation because of the great variability in response of the melanization process to the irradiation treatment.

Ionizing radiation might inhibit the production of one or more enzymes rather than alter the enzymatic reactions involving in the melanization. Melanization in some pest larvae may be strongly inhibited by irradiation doses applied at a sufficient level (Nation *et al*., 1995b).

MATERIALS AND METHODS

Fruit fly mass rearing in laboratory

Rearing room: The colonies of the fruit flies were held in temperature and humidity controlled room of 25 – 27 ^{o} C, 70-80 % RH and light : dark cycle of 12 : 12. The photophase was from 6:00 am to 6:00 pm. Lighting system of the rearing room was provided by fluorescent lamps.

Adult fly: Approximately 10,000 adult flies were housed in 16 mesh-wire screening cage. The dimension of the cage was 30 x 30 x 50 cm. Adult flies were fed with artificial diet consisting of 3 parts of sugar and 1 part of yeast hydrolysate by weight. The diet was put in a shallow plastic dish in the cage. Water was supplied in a plastic container (16 cm diameter x 7.5 cm high) with 3 holes (1mm) on the lid. It was placed upside down on the filter paper on the top of the cage.

Adult flies were held in the cage for 6 weeks. After that, the remaining flies were destroyed and the cage was cleaned in preparation for new emerging flies.

Egg collecting: The perforated polyethylene container was used as an egg receptacle. This container was 17 cm long and taper from 7 cm to 5.5 cm in diameter. Eggs were deposited through 0.4 mm holes punched through the side of the container. A small amount of guava juice provided as the ovipositional stimulus and to prevent the eggs from desiccating was placed inside the egg receptacle.

Eggs were collected 15 days after the adult emergence and once a week from 10:00 to 12:00 am. Egg collecting was made with egg receptacles. The eggs were then washed under running water into a fine-mesh cloth from which they were transferred into the beaker. Eggs were kept in the water and later transferred onto the larval diet. Periodic check on egg hatching rate was made with the amount of hatched eggs recorded.

Larval diet: Larval diet based on wheat-yeast formula was used for laboratory rearing of the fruit flies (Sutantawong *et al*., 1985). The formulation was as follows:

Methyl-p-hydroxybenzoate	1	g
Sodium benzoate	1	g
Sugar	120	g
Dried yeast	36	g
Wheat bran	260	g
HCl (conc.)	2	ml
Distilled water	580	ml

The prepared diet was put in the shallow plastic tray (23 x 32 x 5 cm). Each tray was filled up to a depth of 2 cm (1,000 g of diet). The eggs were put on top of 2 tissue paper strips (5.5 x 11 cm) placed across the surface of larval diet. Eggs were smeared on the larval diet using a fine camel's hair-brush. The diet tray was covered by the addition of second inverted tray to maintain high humidity necessary for larval hatching. Subsequently, the diet was held in rearing room until the larvae were fully grown.

Collection of mature larvae: The fruit fly larvae became mature 6 days after eggs were transferred, then they began to leave the artificial diet. At the end of larval period, the cover was removed from diet tray. The diet trays were placed in pupal collecting boxes (43 x 74 x 23 cm) containing moistened saw dust (20 mesh) to prevent crawling of larvae and to encourage pupation. The larvae crawled and popped off the sides of the stacked larval diet trays and pupated in collecting boxes.

Pupal handling: Pupae were held in saw dust. Two days before the expected emergence, the pupae were collected from the saw dust by sieving through a 20-mesh screen sieve. They were then put in plastic tray (23 x 32 x 5 cm) and placed inside new cage until eclosion. Pupal weight, emerging rate and sex ratio were periodically recorded.

Life history study

Newly laid eggs were transferred onto fresh thin papaya slices for the study of egg and larval development. Observations were made at 1 hour intervals from the egg stage. Mature larvae were allowed to pupate in saw dust. Two days before expected emergence, pupae were separated from the pupation medium and held in plastic cups until eclosion. Twenty pairs of newly emerged adults were placed in separate containers to assess fecundity, longevity, mating and characteristics of male and female. The life history and biological data of *B. correcta* were recorded throughout the span of developmental period.

Life table study

Partial life table study was carried out using 100 newly laid eggs. When eggs hatched, larvae were placed on papaya. As larvae matured, they crawled out of the diet onto the saw dust under the trays where they pupated. The time required for larval development and the numbers of pupae obtained were recorded. Pupae were placed in a cage for adult emergence. Water and adult diet were provided. Life table started with 100 eggs was investigated by checking every 2 days of egg laying and hatching, larval and pupal ages and mortality of the fly until the last adults died. The number of adult survival and egg laying activity data was used for the construction of the biological life table using techniques given by Laughlin (1965), Southwood (1968), Napompeth (1973), Harcourt (1969) and Varley and Gradwell (1970).

The net reproductive rate of increase (R_o) is calculated from equation:

$$R_o = \sum_{x=0}^{\alpha} l_x m_x \qquad \ldots(1)$$

Where, 0 to α = life span

l_x = proportion at birth of females being alive at age X

m_x = number of female births during age X

$l_x m_x$ = egg curve

The cohort generation time (T_c) is calculated from the equation

$$T_c = \sum_{x=o}^{\alpha} l_X m_X .X / \sum_{x=o}^{\alpha} l_X m_X \quad \ldots(2)$$

The capacity for increase (r_c) of Laughlin (1965) is as approximation of the innate capacity for increase (r_m), the calculation of which is complicated. The r_c could be calculated from equation:

$$r_c = \frac{\log_e R_o}{T_c} \quad \ldots(3)$$

The finite rate of increase (λ) is calculated from equation:

$$\lambda = \text{antilog}_e r_c \quad \ldots(4)$$

The egg curve is obtained by plotting $l_x m_x$ against X. This curve represents the egg schedule of births and deaths in terms of the age-schedule fecundity and probability at births of females being alive at each age group and egg productivity within age group through the life history.

The partial life table study was carried out using 400 newly laid eggs. The larvae reared in 3 diets namely mango, papaya and artificial diet of wheat-yeast formula. Daily observation was made and the number of individuals survived in each developmental stage were recorded to construct the partial life table using technique given by Napompeth (1973).

Radiation effects on mature pupae study

Mature pupae (2 days before adult eclosion) from the laboratory culture were irradiated with 5, 10, 15 and 30 Gy at dose rate of 72.38 Gy / min in a ^{137}Cs gamma irradiator (Mark I). The similar group of pupae was held as the control. Treated and untreated pupae were held in the same conditions previously described. Data on adult fly emergence, deformation, longevity and sterility were recorded.

The sterility of *B. correcta* was recorded from the following crosses for each radiation dose:

UTF x UTM (control)

UTF x TM

Where UTF = untreated female
UTM = untreated male
TM = treated male

Each treatment was replicated three time using oviposition cages with ten pairs of flies per cage per replication. The data were analysed by the analysis of variance (ANOVA) and the means compared by Duncan's new multiple range test (DNMRT) at p=0.05.

Mating competitiveness

Mating competitiveness of irradiated males was studied. The irradiated male with 30 Gy as mature pupae, normal male and normal female were caged in oviposition cages. Data from four treatments showed the ratio of irradiated male : normal male : normal female to be 0:1:1, 1:0:1, 1:1:1 and 3:1:1. Each cage was provided with adult diet and water. The eggs were collected daily and the number of hatched eggs in each population was recorded. The competitiveness value and expected egg hatch rates were computed as described by Fried (1971).

$$\% \text{ Expected egg hatch} = \frac{N(Ha) + S(Hs)}{S + N}$$

$$\text{Competitiveness value (CV)} = \frac{\% \text{ Expected egg hatch}}{\% \text{ Observed egg hatch}}$$

Where Ha = % egg hatch of UTM x UTF
Hs = % egg hatch of TM x UTF
N = Number of normal males
S = Number of irradiated males

Assessment of melanization

After the irradiation treatment, larvae were reared under laboratory conditions and selected for evaluation of cuticle melanization. When the larvae reached the late third instar, they were then placed in a freezer (-4 °C) for 24 h. After that, the larvae were removed from the freezer and placed on a sheet of white paper at room temperature for observation. Within the next hour, melanization was evaluated visually. The observation on melanization process was made using stereomicroscope (10x). The color of melanized body portion (black colour) was shown by photo.

The haemocyte count (THC)

The larvae of each irradiated dose were heat-fixed (60 °C for 1 min) and bled from the abdominal segment cut with fine scissors. Haemolymph was allowed to flow onto a clean glass slide and drawn into a Thoma white blood cell pipette, diluted to 1:100 by physiological versene (EDTA: Ethylene diamine tetra-acetic acid, 1 g: 100 ml distilled water). After vigoroustly shaken and discarded the first three drops, the haemocytes were counted using a haemocytometer (counting chamber). Haemocytes from 1-mm squares (the four corner and center square) were counted (El-Mandarawy *et al*., 2000). The experiment was carried out in triplicate. Data obtained for THC were statistically analyzed at 0.05 level of probability and means separated by Duncan's new multiple range test (DNMRT).

Place and durations

The studies were conducted in the laboratory of the Department of Entomology, Faculty of Agriculture and Gamma Irradiation Service and Nuclear Technology Research Center, Kasetsart University, Bangkhen, Bangkok during October 2007 – November 2009.

RESULTS AND DISCUSSION

Life history study of *B. correcta*

Life cycle study was conducted under laboratory conditions of 25-27 °C, 70-80 % relative humidity (RH) and 12:12 (light : dark) photoperiod.

Egg (Figure 1A): *B. correcta* laid eggs beneath the rind of fruit. Egg was elongate-ovoid (banana fruit or crescent shape) with an average egg size of 1.08 ± 0.05 mm in length and 0.27 ± 0.02 mm in width. Hatching rate was 92.95 %. Egg stadium was 35-50 h averaging 38.30 ± 1.56 h.

Larva (Figure 1B): Newly hatched larva was translucent and white in color varied by the food color. Larvae had three developmental stages distinguished by the relative length of paired cephalopharyn and spiracle. Mean body size of the first second and third instar larvae were 0.91 ± 0.12, 2.31 ± 0.31 and 7.92 ± 0.39 mm in length, and 0.20 ± 0.04, 0.43 ± 0.06 and 1.77 ± 0.12 mm in width respectively. Measurement was made in the early day of each instar. Larval stadium was 5–9 days with the average of 6.77 ± 1.33 days.

Pupa (Figure 1C): The pupa was coarctate type with an average size of 4.42 ± 0.14 mm in length and 2.04 ± 0.10 mm in width. Pupa stadium was 8-10 days with an average of 9.62 ± 1.24 days. The body sizes of egg, larva and pupa are shown in Table 1.

Adult (Figure 1D-E): The adult was bright in color. The female body size was 6.06 ± 0.27 mm in length and 5.01 ± 1.10 mm in wing length. The male body size was 5.62 ± 0.23 mm in length and 4.89 ± 0.09 mm in wing length (Table 1). The first reproduction stage was 10-14 days after emerging with the average of 11.85 ± 1.18 days. The egg was either laid singly or in mass, 4-5 eggs/mass. The longevity of adult male and female were 70.10 ± 28.79 days ranging from 10 to 112 days and 82.30 ± 21.46 days ranging from 15 to 120 days respectively. The total period from egg to adult was 93.10 ± 30.58 days ranging from 31 to 136 days (Table 2).

Similar results were reported by Srikachar *et al*. (2005) who studied the biology of *B. correcta* on guava fruit in laboratory condition. They found the egg to be an average of 1.13 mm in length and 0.27 mm in width. Hatching rate was 91.80 %. Egg stadium was 36-48 h. The average body size of first instar larva was 1.28 mm in length and 0.30 mm in width. The mean size of mature larva was 8.17 mm in length and 1.95 mm in width. Larval stadium was 7-10 days. The average pupal size was 5.16 mm in length and 2.26 mm in width. Pupa stadium was 7-10 days. Duangsupa (2002) also reported that the mean egg size of *B. correcta* from natural host plant in Chaingmai province was 1.29 mm in length and the mature larvae 10.55 mm in length and 1.48 mm in width. The average pupal size was 4.6 mm in length and 2.16 mm in width.

Table 1 Body and wing sizes of the guava fruit fly, *Bactrocera correcta* (Bezzi) fed on papaya at each developmental stage

Stage	Sample no. (N)	Body size (mm) [1/]	
		Width	**Length**
Egg	20	0.27 ± 0.02	1.08 ± 0.05
1st instar larvae	20	0.20 ± 0.04	0.91 ± 0.12
2nd instar larvae	20	0.43 ± 0.06	2.31 ± 0.31
3rd instar larvae	20	1.77 ± 0.12	7.92 ± 0.39
Pupae	20	2.04 ± 0.10	4.42 ± 0.14
Adult		Wing length	Body length
Male	20	4.89 ± 0.09	5.62 ± 0.23
Female	20	5.01 ± 1.10	6.06 ± 0.27

[1/] Average sizes ± standard deviation

Table 2 Life cycle of the guava fruit fly, *Bactrocera correcta* (Bezzi) fed on papaya under laboratory conditions

Stage	Sample no. (N)	Range (day)	Average age [1/] (day)
Egg	30	35-50 h	38.30 ± 1.56 h
1st instar larvae	30	2-3	2.17 ± 0.38
2nd instar larvae	30	3-4	3.2 ± 0.41
3rd instar larvae	30	5-9	6.77 ± 1.33
Pupae	30	8-10	9.62 ± 1.24
Adult			
Female	50	15-120	82.30 ± 21.46
Male	50	10-112	70.10 ± 28.79
Total life cycle		31-136	93.10 ± 30.58

[1/] Average sizes ± standard deviation

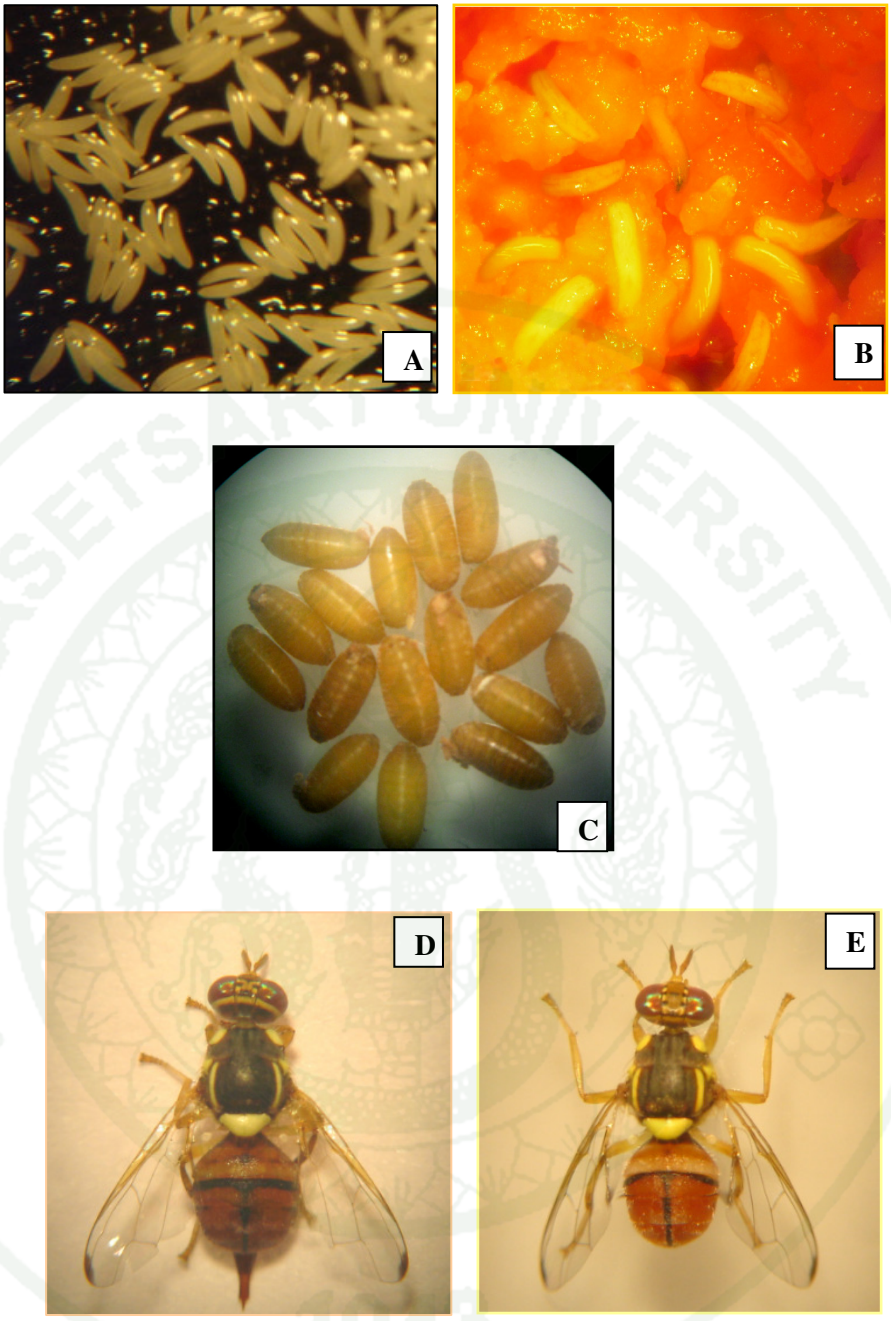

Figure 1 Developmental stages of the guava fruit fly, *Bactrocera correcta* (Bezzi)

A. Eggs (10x)

B. Larvae (10x)

C. Pupae (10x)

D. Adult female (10x)

E. Adult male (10x)

Partial life tables of *Bactrocera correcta* (Bezzi)

The construction of the partial life table of *B. correcta* is shown in Table 3. Using the techniques given by Laughlin (1965), the population statistics were calculated. Various population parameters calculated from this table were the gross reproductive rate (GRR) = 348.8218, net reproductive rate (R_o) = 197.2200, the capacity for increase (r_c) = 0.0910, the finite rate of increase (λ) = 1.0952 and the cohort generation time (T_c) = 58.1235 days. From these parameters, it was found that the population of *B. correcta* could be multiplied 197.2200 times in each generation or 1.0952 times in two days. The mean length of generation time was 58.1235 days.

The egg curve (l_xm_x), designated by Laughin (1965), was obtained by plotting against x (Figure 2). It was obvious from the curve that maximum number of egg was laid during the first ten days after the adults started to lay their eggs. The egg deposition gradually decreased thereafter.

The results for partial life table found in this study were similar to those recorded for other species of tephritids. In *B. cucurbitae* and *B. dorsalis* gross reproductive rates were estimated to range from 236 to 1,243 eggs at temperatures within the range tested here (Vargas *et al*., 2000) and *B. invadens* of 1,057 eggs reared on artificial diet in the laboratory (Ekesi *et al*., 2006). Carey (1984) reported a net reproductive rate of over 1,000 egg/female for *C. capitata*. Vargas *et al*. (2000) obtained the net reproductive rate, intrinsic rate, doubling time and mean generation for *B. dorsalis* and *B. cucurbitae* of 169.9, 11.0, 0.065 and 78.2 respectively and 42.1, 0.053, 14.0 and 72.7 respectively. Ekesi *et al*. (2006) reported the net reproductive rate, finite rate of increase, mean generation time, intrinsic rate of increase and population doubling time for *B. invadens* to be 273, 1.120, 30.7, 0.113 and 6.16 respectively. El-Aw *et al*. (2006) found that the net reproductive rate, the finite rate of increase and the population doubling time of *B. zonata* were 93.4, 1.56 and 1.93 respectively, on banana fruits. Foote and Carey (1987) reported a ten-fold increase in the fecundity of lab strains over wild strains *B. dorsalis*. Perez (1987) found a net reproductive rate of 4.13 for wild *A. ludens* compared with 10.24 for laboratory flies.

On the other hand, developmental rates did not differ between wild and laboratory strains. Furthermore, the high increased rates of population and shorter mean generation time were reported for laboratory-adapted compared to wild populations of tephritids (Vargas and Carey, 1989). In most fruit fly species, reproductive and life history trait of population parameters are known to vary among different geographic areas (Vargas and Carey, 1989).

The life table of *B. correcta* was far from completion and still required additional investigation. However, very useful data were obtained as a basic for further development, precision and utilization.

Table 3 Partial life table, age-specific fecundity rates and net reproductive rate of *Bactrocera correcta* (Bezzi) under laboratory conditions

Age (days) (X)	Proportion at birth[1/] of female being alive at age x (l_x)	Age-specific[2/] fecundity (m_x)	Egg curve[3/] (l_xm_x)
0 Egg	1.0000	-	-
2 Larva	0.8700	-	-
4	0.8400	-	-
6	0.8000	-	-
8 Pupa	0.7400	- Immature	-
10	0.7400	- stages	-
12	0.7400	-	-
14	0.7400	-	-
16	0.7400	-	-
18 Adult	0.6900		-
20	0.6900	-	-
22	0.6900	- Preoviposition	-
24	0.6800	- period	-
26	0.6800		-
28	0.6700	0.2390	0.1600
30	0.6500	2.9080	1.8900
32	0.6500	3.9850	2.5900
34	0.6500	17.7080	11.5100
36	0.6500	11.8920	7.7300
38	0.6500	14.5690	9.4700
40	0.6500	20.9080	13.5900
42	0.6200	9.7260	6.0300
44	0.6200	9.6610	5.9900
46	0.6100	11.9840	7.3100
48	0.6100	22.1310	13.5000
50	0.6100	6.2950	3.8400
52	0.6000	18.8670	11.3200
54	0.6000	15.2500	9.1500
56	0.6000	16.1330	9.6800
58	0.6000	12.8000	7.6800
60	0.6000	12.3170	7.3900
62	0.6000	8.2170	4.9300
64	0.5800	9.2930	5.3900
66	0.5700	13.1930	7.5200

Table 3 (Continued)

Age (days) (X)	Proportion at birth[1/] of female being alive at age x (l_x)	Age-specific[2/] fecundity (m_x)	Egg curve[3/] (l_xm_x)
68	0.5700	7.0350	4.0100
70	0.5600	7.9460	4.4500
72	0.5600	6.4290	3.6000
74	0.5600	3.4110	1.9100
76	0.5600	4.6070	2.5800
78	0.5600	4.4290	2.4800
80	0.5400	2.2780	1.2300
82	0.5400	3.3700	1.8200
84	0.5200	4.2880	2.2300
86	0.5200	5.0190	2.6100
88	0.5000	5.7000	2.8500
90	0.4600	7.6520	3.5200
92	0.4600	4.4130	2.0300
94	0.4300	4.5580	1.9600
96	0.4200	6.9050	2.9000
98	0.4200	4.5710	1.9200
100	0.4200	2.8330	1.1900
102	0.4200	2.6900	1.1300
104	0.3900	2.4360	0.9500
106	0.3800	1.0260	0.3900
108	0.3700	1.0270	0.3800
110	0.3700	2.2430	0.8300
112	0.3400	1.0000	0.3400
114	0.3300	1.1520	0.3800
116	0.2400	2.3330	0.5600
118	0.2400	0.1670	0.0400
120	0.2300	1.8260	0.4200
122	0.2200	1.2730	0.2800
124	0.2200	0.9550	0.2100
126	0.2200	0.5910	0.1300
128	0.2200	0.6820	0.1500
130	0.2200	0.4550	0.1000
132	0.2200	0.1820	0.0400
134	0.2100	1.0000	0.2100
136	0.2000	0.4000	0.0800

Table 3 (Continued)

Age (days) (X)	Proportion at birth[1/] of female being alive at age x (l_x)	Age-specific[2/] fecundity (m_x)	Egg curve[3/] (l_xm_x)
138	0.1800	0.6110	0.1100
140	0.1800	0.3890	0.0700
142	0.1800	0.5560	0.1000
144	0.1800	0.2220	0.0400
146	0.1600	0.6880	0.1100
148	0.1500	1.0670	0.1600
150	0.1500	0.3330	0.0500
152	0.1100	0.0000	0.0000
154	0.1100	0.0000	0.0000
156	0.1100	0.0000	0.0000
158	0.1100	0.0000	0.0000
160	0.1100	0.0000	0.0000
162	0.0800	0.0000	0.0000
164	0.0500	0.0000	0.0000
166	0.0400	0.0000	0.0000
168	0.0400	0.0000	0.0000
170	0.0400	0.0000	0.0000
172	0.0300	0.0000	0.0000
174	0.0100	0.0000	0.0000
178	0.0100	0.0000	0.0000
180	0.0100	0.0000	0.0000
182	0.0000	0.0000	0.0000
		Ro =	**197.2200**
		GRR =	**348.8218**

[1/] l_x = The probability of individual being alive at the beginning of the age-interval

[2/] m_x = The number of female eggs of offspring for each age-interval

[3/] l_xm_x = Egg curve

Table 4 Parameters calculated for biological attributes of *Bactrocera correcta* (Bezzi) under laboratory conditions (25-27 ^{o}C, 70-80 % RH)

Biological attributes	Notation	Calculated value
Net reproductive rate of increase	R_o	197.2200
Capacity for increase	r_c	0.0910
Finite rate of increase	λ	1.0952
Cohort generation time	T_c	58.1235

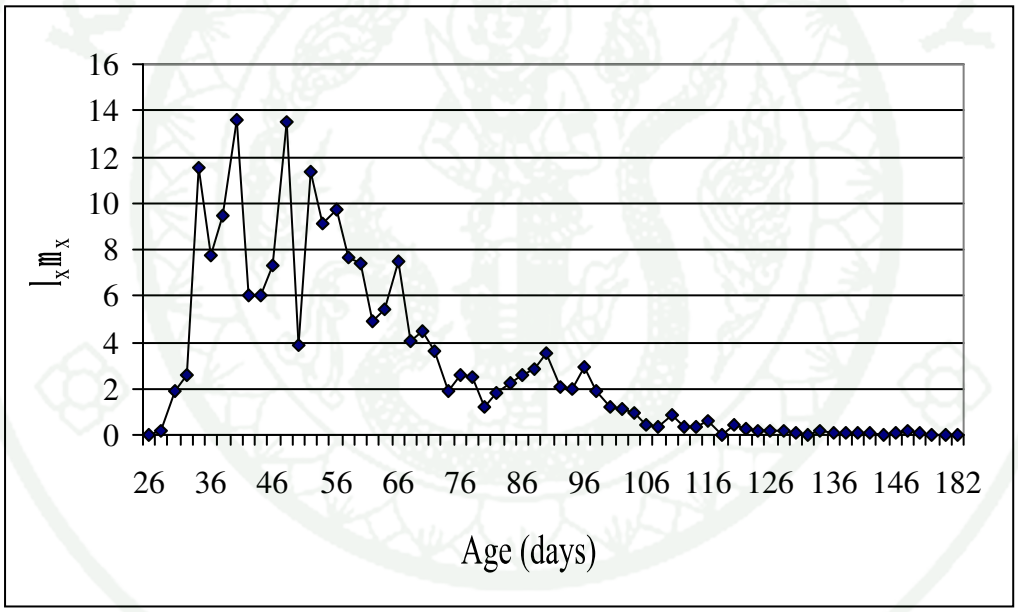

Figure 2 Egg curve of *Bactrocera correcta* (Bezzi) under laboratory conditions (25-27 ^{o}C, 70-80 % RH)

The partial life table of *B. correcta* reared from 3 kinds of diet namely mango, papaya and artificial diet of wheat-yeast formula were as follows:

Mango: The highest mortality of 48.98 % was found in the 3rd instar larvae. Other mortalities were 22.28 % in pupal stage, 7.25 % in egg stage, 5.12 % in 1st larval instar and 2.58 % in the 2nd larval instar. Sex ratio was 1 male : 2 female.

The highest mortality occurred in the 3rd instar larvae in mango due to rotten mango fruits and the rind kept the ooze inside the fruit thus the larvae were drowned in the liquid. This was the key factor to cause the larval death. While the percentages of highest survival rate of the 2nd and 1st instar larva were 97.45 and 94.88 % respectively, the pupal stage had the low survival of 67.28 % (Table 5).

Papaya: The highest mortality of 31.36 % was found in the pupal stage. Other stages mortalities were 8.13 % in the 2nd instar larva, 5.01 % in the 3rd instar larva, 5.0 % in egg stage and 2.89 % in the 1st instar larva (Table 6). Sex ratio was 1 male : 2 female.

The highest mortality was found in pupal stage in papaya due to the thin layer of saw dust in tray, hence, the larvae could not pupate. According to Srikachar *et al*. (2005) reporting pupa of *B. correcta* to live in 2-5 cm depth, it was possible that the thin layer of saw dust affected the pupation.

The survival rates of *B. correcta* were 97.17 % for the 1st larval stage. This was the highest survival rate, similar to the egg stage and the 3rd larval instar with 95.00 and 94.99 % respectively. Pupa stage showed the lowest survival rate of 68.64 %

Artificial diet of wheat-yeast formula: The highest mortality of 9.36 % was found in the 2nd instar larva. Other stages mortality were 9.31 % in the 1st instar larva, 9.06 % in pupal stage, 8.75 % egg stage and 8.00 % in the 3rd instar larva. The highest survival rate in the 3rd instar larva was 92 %, 91.25 % in egg stage, 90.94 % in pupal stage, 90.69 % in the 1st instar larva and 90.64 % in the 2nd instar larva were also encountered. Sex ratio was 1 male : 1 female (Table 7).

Srikachar *et al*. (2005) studied the partial life table of *B. correcta* on guava fruit in laboratory conditions. They found the 1st instar larva to have the highest percentage of mortality (33.99 %). The other mortality rates were 13.86 % in pupal stage, 8.20 % in egg stage, 3.30 % in 3rd instar larva and 3.14 % in 2nd instar larva.

The experiment showed that different mass rearing diets affected mortality in various developmental stages due to the diets of different nutrients. Humidity and physical environments also had influence over the difference.

Comparing survival rate of *B. correcta* among 3 kinds of diet, mango, papaya and artificial diet of wheat yeast formula, artificial diet was found to have the highest survival rate. It might caused by suitable artificial diet containing all essential nutrients for their development. For example 100 g artificial diet contains 3.6 g yeast (protein), 12 g sugar and 26 g wheat bran (carbohydrate) while 100 g papaya contains 0.5 g protein, 0.1 g fat, 24 g calcium, 22 g phosphorus, 0.6 g iron, 4 g sodium, 0.04 g diamin, 0.04 mg riboflavin, 0.4 mg niacin and 70 g ascorbic (vitamin c) (Anonymous, 2009)

There were advantages of the mass rearing of *B. correcta* on fruits as easiness to find the fruits, inexpensive with less time consuming compared to artificial diet preparation. On the other hand, the fruits could be rotten and the liquid oozing from the rotten fruits caused some problem as well thesea factor that caused mortality in the larval stage and the surviving larvae could not develop into pupae. Moreover, mass rearing of *B. correcta* on the artificial diet was quite convenient with no need to worry about the stale or the growth of fungi or bacteria as anti-fungi and anti-microorganisms such as sodium benzoate, methyl-p-hydroxybenzoate and HCl (conc.) being a part of the recipe. This resulted in the high number and good quality of fruit fly produced for the requirement of the experiments and sterile insect techniques.

Survivorship curve of *B. correcta* in laboratory conditions showed that the highest survival was found in the egg stage and then declined until the adult stage. The study started with 400 eggs on mango, papaya and artificial diet resulting in the adults 185 (46.25 %), 221 (55.25 %) and 251 (62.75 %) adults respectively (Figure 3).

Table 5 Partial life table of the guava fruit fly, *Bactrocera correcta* (Bezzi) fed on mango under laboratory conditions

Age interval	No. alive at the beginning of x	No. dead during x	Percent mortality	Percent survival	Probability of survival
Egg	400	29	7.25	92.75	1.00
Larvae					
1st instar	371	19	5.12	94.88	0.92
2nd instar	352	9	2.58	97.45	0.88
3rd instar	343	168	48.98	80.18	0.85
Pupae	175	39	22.28	67.28	0.44
Adult	136				0.34
Male	43	-	-		
Female	93	-	-		
Ratio M: F= 1 : 2					

Table 6 Partial life table of the guava fruit fly, *Bactrocera correcta* (Bezzi) fed on papaya under laboratory conditions

Age interval	No. alive at the beginning of x	No. dead during x	Percent mortality	Percent survival	Probability of survival
Egg	400	20	5.00	95.00	1.00
Larvae					
1st instar	380	11	2.89	97.17	0.95
2nd instar	369	30	8.13	91.87	0.92
3rd instar	339	17	5.01	94.99	0.85
Pupae	322	101	31.36	68.64	0.80
Adult	221				0.55
Male	93	-	-		
Female	128	-	-		
Ratio M: F= 1 : 2					

Table 7 Partial life table of the guava fruit fly, *Bactrocera correcta* (Bezzi) fed on artificial diet of wheat-yeast formula under laboratory conditions

Age interval	No. alive at the beginning of x	No. dead during x	Percent mortality	Percent survival	Probability of survival
Egg	400	35	8.75	91.25	1.00
Larvae					
1st instar	365	34	9.31	90.69	0.91
2nd instar	331	31	9.36	90.64	0.83
3rd instar	300	24	8.00	92.00	0.75
Pupae	276	25	9.06	90.94	0.69
Adult	251				0.62
Male	122	-	-		
Female	128	-	-		
Ratio M: F = 1:1					

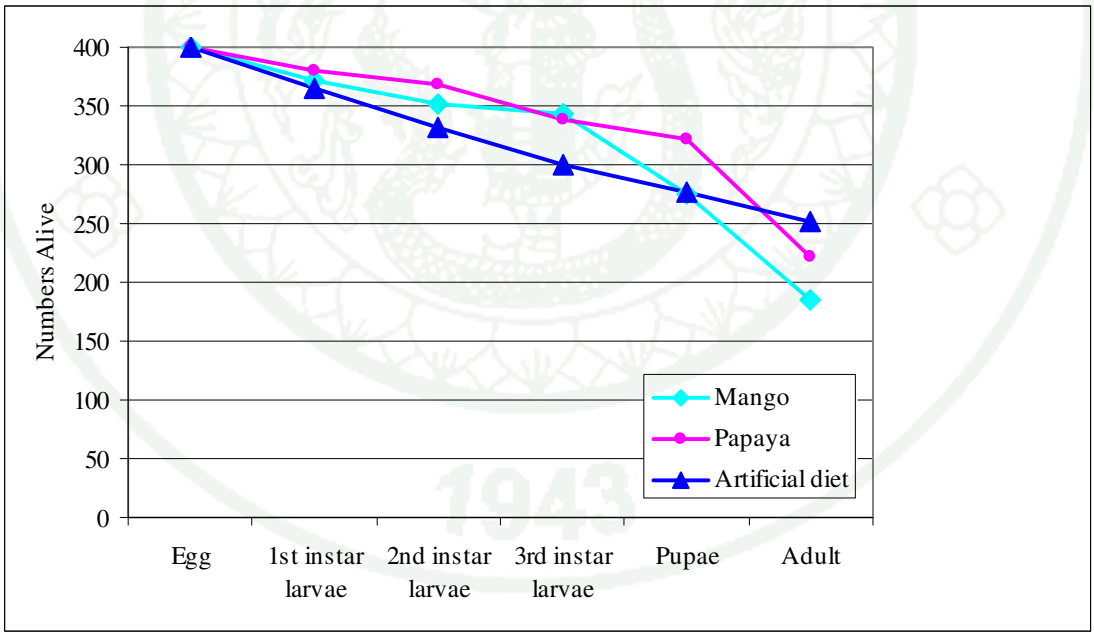

Figure 3 Survivorship curve of the guava fruit fly, *Bactrocera correcta* (Bezzi) at each growth stage fed on different diets

Radiation effects on mature pupae

Adult emergence

Adult emergence following irradiation of mature pupae at 0, 5, 10, 15 and 30 Gy were 97.00, 96.67, 98.33, 97.33 and 96.33% respectively while adult deformation were 0.67, 0.33, 1.00, 1.00 and 1.33 % respectively (Table 8). Adult emergence and adult deformation were not significantly different from the control or from one another.

The results were similar to those reported by Draz *et al*. (2008) who studied the effects of radiation on the peach fruit fly, *B. zonata.* At 0, 10, 30 and 50 Gy, they found that the percentages of emergence were 94.50, 93.40, 89.01 and 86.20 % respectively while the percentages of deformed pupae were 1.40, 3.7, 5.5 and 7.4 % respectively. The adult emergence percentages decreased while deformed pupae increased with increasing gamma radiation doses. Resilva *et al*. (2007) reported the effects of different doses of gamma radiation on the pupae of the *B. philippinensis*. They found doses of 0-100 Gy to be not significantly affect percentage adult emergence. Draz *et al*. (1997) mentioned that the irradiated Mediterranean fruit fly pupae one day before eclosion with doses ranged between 100 and 150 Gy seemed to have no deleterious damage. The results indicated that pupal irradiation might be a viable option if pupal harvesting techniques compatible with other mass rearing procedures could be developed.

Figure 4 Deformed of guava fruit fly, *Bactrocera correcta* (Bezzi) emerged from irradiated pupae

Longevity

The longevity of adult males from irradiated mature pupae decreased gradually as the dose increased. The male longevities were 31.30, 30.05, 31.55 and 30.60 days when mature pupae were exposed to 5, 10, 15 and 30 Gy, respectively as compared with 37.70 days for normal males (Table 8). The longevity of flies from irradiated pupae was less than those of untreated flies (the control) at every dose. There was no significant difference between the longevity of the control and irradiated adults.

Similar results were reported by many authors. Pransopon and Sutantawong (2005) studied the effects of gamma radiation at 0-80 Gy on pupal stage of *B. correcta* and found that the percentage of survival of flies 17 days after adult eclosion did not show significant difference. Cendra *et al*. (2004) reported that the survival rates in field cages of both non-irradiated and irradiated by laboratory-reared of *A. fraterculus* were compared with that of the wild flies. Both type of laboratory-reared flies survived longer than their wild counterparts over 8 days under the experimental conditions. The irradiation doses (70 Gy) did not affect survival of the laboratory-reared flies.

Sterility

The results demonstrated the effects of gamma irradiation on *B. correcta* male sterility when treated males (TM) were crossed with untreated virgin females (UTF). The sterility of unirradiated (the control) and males irradiated at 5, 10, 15 and 30 Gy were 23.85, 21.78, 59.10, 72.57 and 98.34 % respectively (Table 8). The sterility increased with increasing gamma radiation doses. The percent sterilities at 5 Gy was not significantly different from the control (0 Gy), while they differed from 10, 15 and 30 Gy.

Present results indicated that 30 Gy was the suitable gamma radiation dose to obtain the highest sterility of males of *B. correcta* when applied on pupae 48 h before adult emergence. Pransopon and Sutantawong (2005) reported that the dose of gamma radiation for sterilization of *B. correcta*, was found to be 60 Gy which gave a high percentage of sterility in males but caused no egg laid in females. Draz *et al*. (2008) reported that the effects of radiation of the peach fruit fly, *B. zonata* at 30 Gy applied to pupae 48 h before adult emergence induced 98.60 % sterility in males. Nahar *et al*. (2006) irradiated *B. cucurbitae* pupae before emergence with 30 Gy and found that the mating of unirradiated females with those of irradiated males did not affect the production of eggs but egg viability was reduced to 0.93 %. The male irradiated at a dose of 40 Gy produced 100 % sterilization. The exposure of female to gamma irradiation sharply reduced the production of eggs by the latter. Irradiation of females had an effect on both fecundity and fertility. Calkins *et al*. (1988) reported that lower irradiated dose (30 Gy) applied 24-48 h before emergence of *Anastrepha suspense* (Loew) induced high levels of sterility. Allinghi *et al*. (2007) found that the low doses of gamma radiation (20-40 Gy) 48 h before emergence caused 90-97 % sterility of *A. fraterculus*. Zumreoglu and Akman (1987) reported that the level of sterility in males of *C. capitata* increased with the increasing doses of radiation. Prasad (1992) found that the ideal dose of gamma radiation for male sterilization of Mediterranean fruit fly, *C. capitata*, was found to be 90 Gy when applied to mature pupae. However, the high level of sterility probably is due to the detrimental effect of gamma radiation on the less developed reproductive system of immature pupae of *C. capitata* (Akman and Zumneoglu, 1978).

Generally, adults are more radiation-resistant than pupae, which in turn are more resistant than larvae. Fully grown pupae are more resistant to gamma radiation than developing pupae (Ahmad *et al*., 1990, Dongre *et al*., 1997). Mansour (2003) and Hallman (2003) found that sterilizing dose of a species depending on developmental stages and ages at irradiation. In general, the sterility dose seems to be differ from laboratory. Within Diptera, Coleoptera and Hymenoptera, radiation doses vary widely among families and ranged from 20-200 Gy, with a mean dose for sterilization ranges from 20 to 160 Gy in Diptera (Bakri *et al*., 2005). Results of

Resilva *et al*. (2007) suggested that the best irradiation range to achieve complete sterility of *B. philippinensis* with a Gamma-cell 220 should be between 67 and 74 Gy. However, present results highlight the need for further efforts to standardize experimental dosimetry and irradiation procedures for guava fruit fly and provide a suitable platform for guiding future research on this serious pest, the newly species of fruit flies.

Table 8 Effects of gamma radiation on adult emergence, adult abnormality, age and male sterility of the guava fruit fly, *Bactrocera correcta* (Bezzi) pupae irradiated at various doses

Dose (Gy)	Emergence (%)	Deformation (%)	Longevity (day)	Sterility (%)
0 [1/]	97.00 a	0.67 a	37.70 a	23.85 a
5	96.67 a	0.33 a	31.30 a	21.78 a
10	98.33 a	1.00 a	30.05 a	59.10 b
15	97.33 a	1.00 a	31.55 a	72.57 b
30	96.33 a	1.33 a	30.60 a	98.34 c

[1/] Means within column not followed by the same letters are significantly different at 0.05 % level as determined by DNMRT

Mating competitiveness

The male flies were irradiated with 30 Gy as mature pupae for mating competitiveness study. Normal male flies and normal female flies were together caged in oviposition cages. Table 9 shows the ratio of irradiated male : normal male : normal female to be 0:1:1, 1:0:1, 1:1:1 and 3:1:1. with the average egg hatch of 89.43, 0.66, 30.98 and 10.94 % respectively.

The competitiveness value from the ratios (irradiated male : normal male : normal female) of 1:1:1 and 3:1:1 were 1.45 and 2.09 respectively indicating irradiated males to be fully competitive with normal males.

The same observations were reported by Nahar *et al*. (2006) for *B. cucurbitae* males irradiated as mature pupae with dose of 30 Gy. The competitiveness value from different ratios of 1:1:1 and 3:1:1 were 0.91 and 0.74 respectively. Steiner *et al*. (1965), Feron (1964) and Katiyar and Ramirez (1969) also made similar observations and reported that gamma irradiation at 60-100 Gy applied to matured pupae had little or no effect on the mating ability of irradiated male *C. capitata*. On the other hand, Ohinata *et al*. (1977) reported that when male *C. capitata* was irradiated 2 days before eclosion, the competitiveness of male was significantly reduced. However, when the same dose was applied 2 days after eclosion of the flies, it had no adverse effect on mating competitiveness. Teruya and Zukeyama (1979) estimated the competitiveness of *D. cucurbitae* by direct counting method. They stated that doses from 10 to 100 Gy did not reduce competitiveness significantly while Fried's method was applied. Islam and Gordon (1992) stated that the greater mating competitiveness of the treated males did not always correspond to the greater total competitiveness value of the same males as revealed from their study with *Musca domestica* sterilized by hempa. Technological methodology for the attainment of induced sterility may be the cause of their variation.

Fried (1971) and Hooper and Horton (1981) proposed that the procedures to quantify sterile-insect total competitiveness was based on egg-hatch resulting from

competitive mating tests. Based on those calculations, C-values of X-ray sterilized European corn borer males, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) were found to be 0.3-0.4 (Fried, 1971), those of irradiated (50 Gy) males of *C. capitata* 0.52 and irradiated (70 Gy) cucumber fly, *D. cucumis* French 0.68 (Hooper and Horton, 1981). Brower (1982) calculated C-value (0.91-0.93) of irradiated males of the tobacco moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) and found it to be as good as that of the untreated males. While C-value of chemo sterilized *Culex quinquefasciatus* male was 0.96 (El-Gazzar and Dame, 1983), that of the greater wax moth males, *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae), sterilized by radiation was 0.91 (Eischen *et al*., 1984). These findings were in good agreement with the present results at the different ratios from 1:1:1 to 3:1:1.

The competitive mating tests gave an estimate of mating competitiveness of radio-sterilized males and the percentage hatching data obtained from three ratios of treated and untreated males gave a measure of the total competitiveness of the treated males. A large number of sterilized males are usually released in order to compensate for their sexual-reduced competitiveness caused by laboratory rearing and irradiation. The consequences of the strategies of sterile insect release method will need much more attention and warrant through further study.

Table 9 Mating competitiveness of irradiated males of guava fruit fly, *Bactrocera correcta* (Bezzi) with 30 Gy

Cross ratio TM : UTM : UTF [1/]	Egg hatch (%)		Competitive value [2/] (CV)
	Observed	Expected [2/]	
0 : 1 : 1	89.43	-	-
1 : 0 : 1	0.66	-	-
1 : 1 : 1	30.98	45.04	1.45
3 : 1 : 1	10.94	22.85	2.09

[1/] TM for treated males, UTM for untreated males, UTF for untreated females

[2/] The competitiveness value and expected egg hatch rates were computed as described by Fried (1971)

Effect of gamma radiation on melanization process of *B. correcta* larvae

Figure 5 presents the color of *B. correcta* at different doses of gamma irradiation. When the first instar larva were treated with various doses (0-30 Gy), the color variation of the larvae ranged from black to creamy white. The control larvae (0 Gy) were extensively melanized and then turned black. Larvae irradiated with 5, 10 and 15 Gy showed similar melanization but the black color developed more slowly than the control. Larvae irradiated at 30 Gy were just creamy white.

The results were similarly was reported by Nation *et al*. (1995a) who stated that gamma radiation induced melanization and phenoloxidase changes in Caribbean fruit fly larvae, *Anastrepha suspens*a (Loew). At ≥20 Gy, larvae failed to show typical melanization. Supawan (2005) found that melanization occurred in untreated larvae of azuki bean weevil after being killed by freezing. The colors of larval body appeared black, light gray and creamy white color. In treated larvae at 100, 300 and 500 Gy, the degree of melanization decreased with the increasing doses. Surisan (2004) also reported the colour changes in irradiated cuticle of the *H. armigera* larvae which did not distinctly occur compared to the control due to its variation in colour. Only the appearance of black spots on the dorsum was used in the identifications. There were less spots found in the irradiated insects than in the control. Kongrat-arpon (2002) showed the degree of melanization in non-irradiated cigarette beetle larvae to be significantly different from the irradiated larvae and decreased with the increasing doses. Banasik-solgala and Stanislaw (1997) found that the degree of melanization differed significantly between treated and untreated Indian meal moth (*Plodia interpunctella* HBN), the Mediterranean flour moth (*Ephestia* (*Anagasta*) *kuehniella* ZELL) and the almond moth (*Cadra cautella* WLK). Ignatowicz and Banasik-Solgala (1997) reported the melanization process to be significantly inhibited in cold-killed young larvae after irradiation from 0.3 to 0.5 kGy. Old larvae of khapra beetle melanized so slowly that after of khapra beetle death, some visible in the body color were noted as late as after 24 h. However, the changes in melanization of the khapra beetle larvae can not be used for indicating previous exposure of the insects to irradiation because of the great variability in response of the melanization process to

the irradiation treatment. Ignatowicz and Ibrahim (1996) demonstrated the melanization in irradiated young larvae of the confused flour beetle, *Tribolium confusum* DUAVL., to be reduced in the first week after treatment and was completely inhibited in the second week. Great variation of melanization in the untreated old larvae partially obscured the effects of gamma radiation on this process. However, the melanization was considerably reduced in all experiments involving old larvae.

The changes in the production of one or more enzymes responsible for the melanization induced by irradiation are not inactive by the doses treating the first instar and evaluating as the third instar larvae. The change in melanization of the guava fruit fly, *B. correcta* can be used for indicating previous exposure of these insects to irradiation treatment.

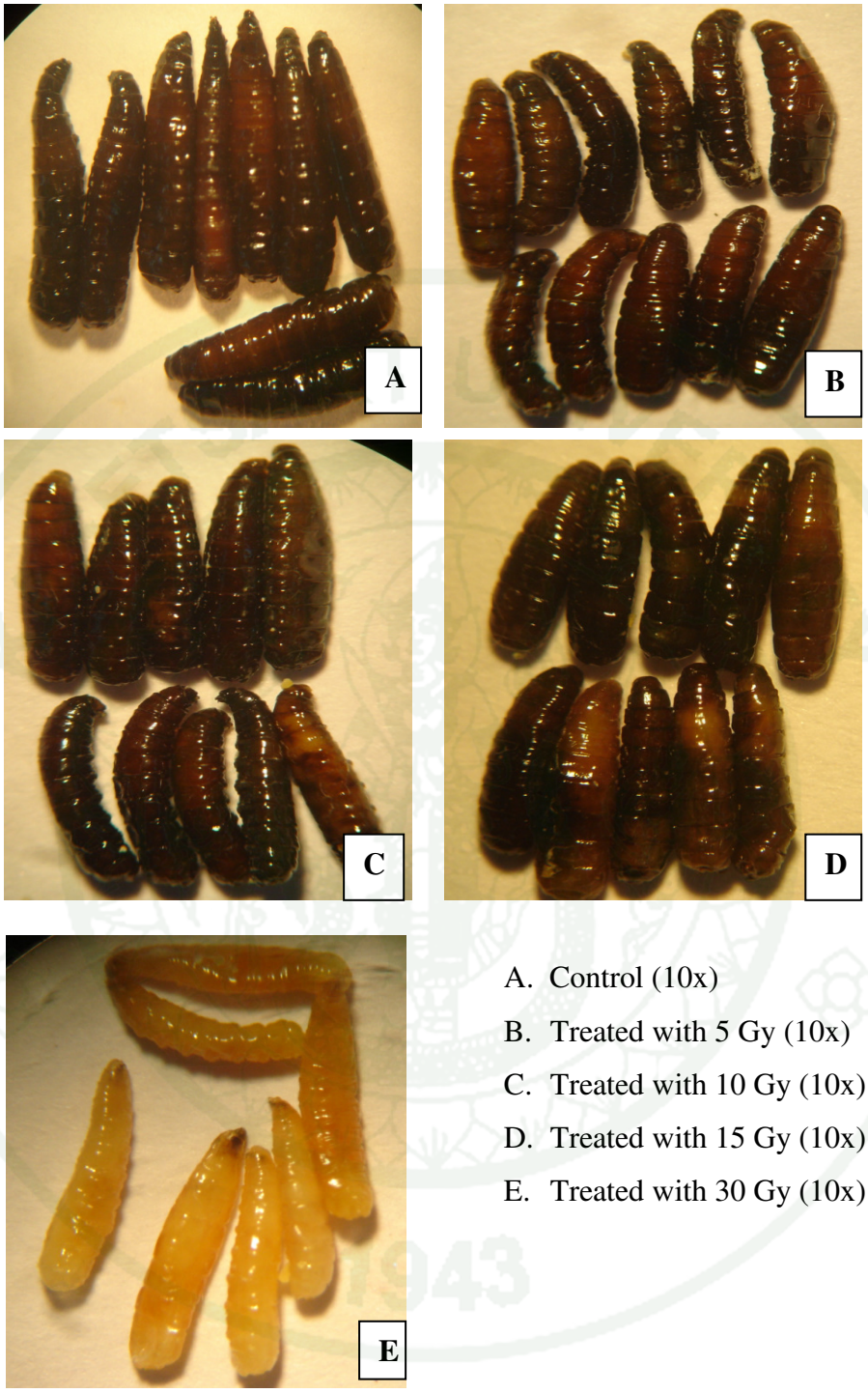

Figure 5 Degrees of darkening in the larvae of guava fruit fly, *Bactrocera correcta* (Bezzi) after gamma irradiation at the first instar larvae

Changes of total haemocyte count (THC)

The total haemocyte counts (THCs) was made on individuals of the larvae irradiated at the 1st and observed in the 3rd instar of *B. correcta* from a haemocytometer grid using a haemocytometer. The biometric measurements of haemocyte counts in the unirradiated (the control) and irradiated larvae are shown in Table 10. It was demonstrated that irradiation had an effect on the haemocyte number present in the haemolymph of larvae. The values obtained for THCs showed a decrease in the number of haemocytes. Irradiated at 0, 5, 10, 15 and 30 Gy, haemocyte amounts were found to be 3150 $\pm$ 95.74, 2200 $\pm$ 336.65, 700 $\pm$ 191.49, 550 $\pm$ 50.00 and 350 $\pm$ 50.00 h/mm^3 respectively which resulted in % reduction increases of 30, 78, 83 and 89 % at 5, 10, 15 and 30 Gy respectively. There were no significant differences among THCs of larvae irradiated at 10, 15 and 30 Gy and those at 5 Gy. THCs at every dose were also found to be significantly different from that of the control.

The result was similar to the report of Nation *et al*. (1995b) which indicated the effects of gamma irradiation with 50 Gy on the 1st instar and observed in the 3rd instar larvae of the Mediterranean fruit fly, *C. capitata*. They found 30,000 h/mm^3 hemolymph in unirradiated larvae compared with 500 h/mm^3 hemolymph in irradiated larvae.

The THCs difference between the efficacy of unirradiated and irradiated insects could also be compared to the experimental results of Elbadry (1964) who demonstrated the effects of gamma irradiation with 30 Gy and 90 Gy on the potato tuberworm on both morphological changes and changes in total haemocyte count. The THCs were found to decrease in the treated larvae. The same result was reported by Hoffmann (1972) who studied the X-irradiation of 250 Gy to *Locusta migratoria* larvae and adults on the dorsal haemocytopoietic tissue. The THCs fell by approximately 50%. Tu *et al*. (2002) found the effects of heavy-ion radiosurgery on the haemopoietic function of a silkworm, *Bombyx mori.* The haemocyte densities of the irradiated larvae at different developmental stages compared to the later stages had

a significant suppressive effect on haemocyte densities. The percentage of dead haemocytes was obviously higher for irradiated larvae than unirradiated controls during the late 5th instar. Surisan (2004) reported the changes in the effect of radiation on haemocyte on each instar of cotton bollworm, *H. armigera*. Irradiated at 75 and 150 Gy, the 1st, 2nd, 3rd and 4th instar larvae were found to have THCs decreased in the number of haemocytes during the larval development. At each dose, THC of each larval instar was significantly lower than of that of the control (12921 h/mm^3).

The decreased haemocyte counts of the irradiated compared to the unirradiated larvae could possibly be explained as follow: Insects, in general, are sensitive to radiation. During the larval period of insects, very little cell differentiation occurs (Molin, 2001). According to Tubiana *et al*. (1990), the cell cycle of the treated insects was delayed after irradiation. The accumulation of cells was connected with a surveillance mechanism blocking the cell with radiation-reduced lesion which prevented cell division. The same theory could be applied with this study resulting in the small amount of haemocytes in the irradiated larvae. In addition, factors known to affect the absolute number of haemocytes in insects are developmental status, stress, infection by foreign organism and wounding (Romoser and Stoffolano, 1994). The last one could be caused by radiation, hence, giving haemocyte decrease.

By law of Bergonie and Tribondeaus, the most radiosensitive cellular populations are cells that 1) are primitive in their relative degree of maturity, 2) are rapid division during irradiation, and 3) have the ability to divide for long period of time. Because the blood cells were considered as primitive cells, the results were well explained as the blood cells were damaged by irradiation (Tubiana *et al*., 1990)

In addition, Eppensteiner and Karp (1989) reported the effect of gamma irradiation on the American cockroach, *P. americana* exposed to 50 Gy which depleted 1/3 haemocytes from normal. Grégioire (1974) also found that after irradiation by X-rays of *L. migratoria* adult (100 Gy), the alterations decreased in the numbers and sizes of haemocyte.

Table 10 Total haemocyte counts (THCs) and percent reduction of the 3rd unirradiated and irradiated larvae at the 1st instars of *Bactrocera correcta* (Bezzi) at different gamma radiation doses

Dose (Gy)	**Mean ± S.E. of THCs (h/mm^3) (% reduction)**
0 (control)	3,150 ± 95.74a
5	2,200 ± 336.65b (30.00)
10	700 ± 191.49c (78.00)
15	550 ± 50.00c (83.00)
30	350 ± 50.00c (89.00)

1/ Means within column not followed by the same letters are significantly different at 0.05 % level as determined by DNMRT

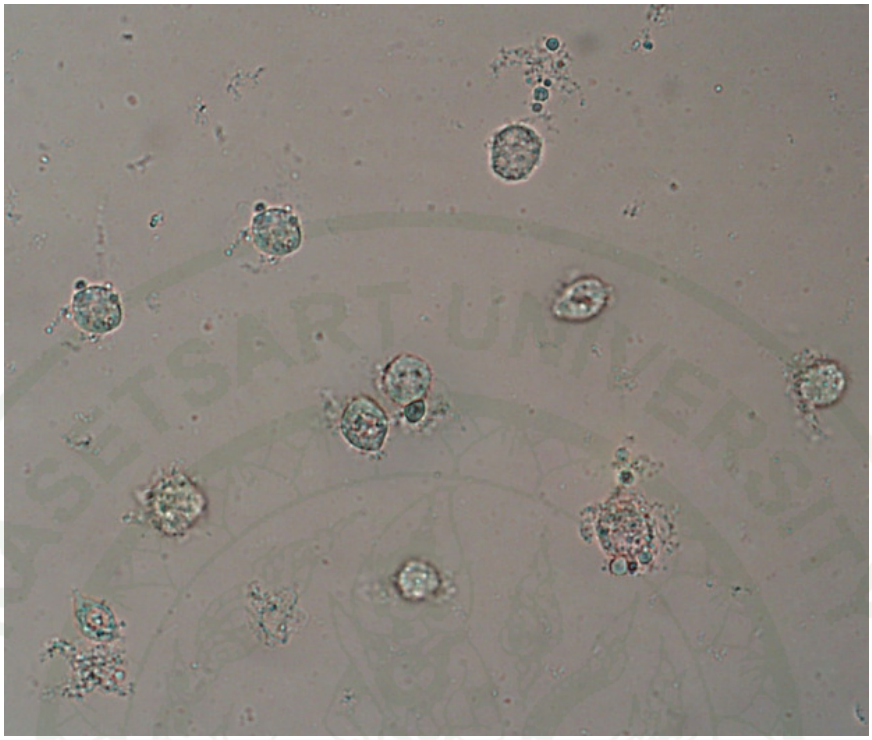

Figure 6 The haemocyte (800x) of *Bactrocera correcta* (Bezzi) fixed with versene, inspected through phase contrast light microscope

CONCLUSION

According to the results of this study, the biological development and the effects of differently gamma irradiated doses against *B. correcta* were as follow:

1. The female guava fruit fly, *B. correcta* laid egg singly or mass in a hole under fruit skin. Percent egg hatch equaled 92.95 % and the fruit fly had three instars. The longevity of adult female was longer than those of adult male. The life cycle from egg to adult was 93.10 days.

The analysis of the biological life table of *B. correcta* revealed the following population statistics: the net reproductive rate of increase (R_o) = 197.2200, the capacity for increase (r_c) = 0.0910, the cohort generation time (T_c) = 58.1235 days, and the finite rate of increase (λ) = 1.0952.

From partial life table in mango, the 3rd and 2nd instars larvae had the highest percentages of mortality and survival of 48.98 and 97.45 respectively. In papaya, the highest percentages of mortality and survival of 31.36 and 97.17 were found in the pupal and the 1st instar larval stages respectively whereas those of 9.36 and 92.00 were encountered in the 2nd and 3rd instar larvae respectively in the artificial diets.

2. Effects of gamma radiation on mature pupae after irradiation with the doses of 0, 5, 10 15 and 30 Gy.

2.1 Emergence, deformation and longevity of male flies were not significantly different.

2.2 Sterilities of irradiated males mating with untreated females were significantly different between 0 Gy and other doses but not significantly different from 5 Gy.

2.3 The melanization of irradiated larvae decreased with the increasing doses and could be used for indicating the response of melanization process to the irradiation treatment with $\geq$ 30 Gy.

2.4 Total haemocyte count (THC) decreased with the increasing doses.

3. A competitiveness value of the ratio 1:1:1 and 3:1:1 of irradiated males with 30 Gy were 1.45 to 2.09 which indicated full competitiveness of the irradiated males.

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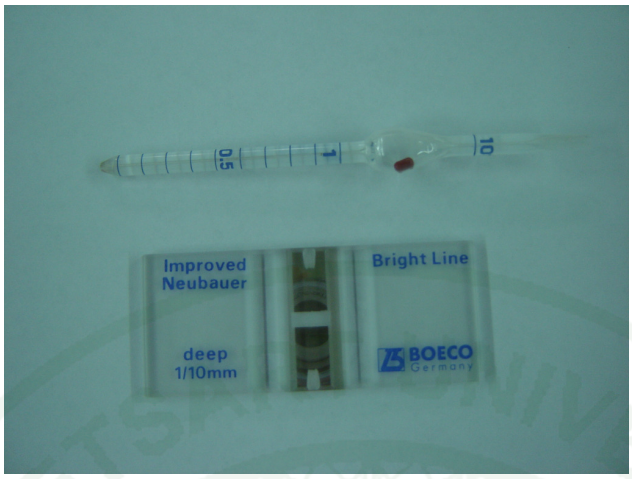
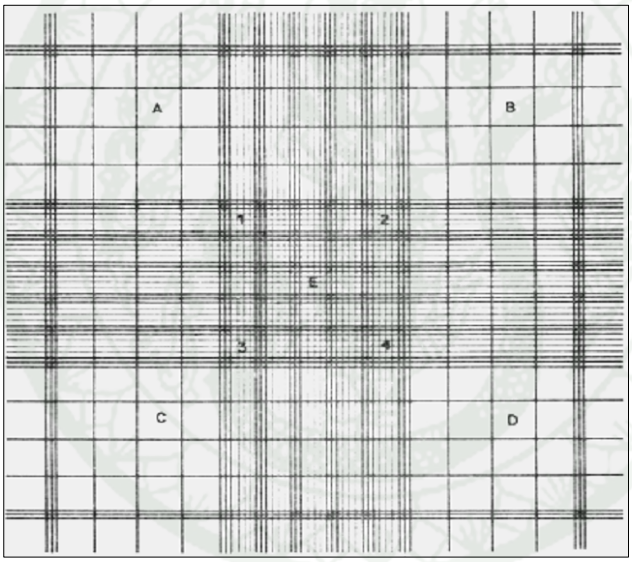
APPENDIX



A



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Appendix Figure 1 A. Thoma white blood cell pipette (up)
Haemocytometer (down)
B. Haemocytometer grid

Appendix Figure 2 Gamma irradiator Model Mark I with Cesium 137 source.

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