CHAPTER 1: GENERAL INTRODUCTION

1.0 INTRODUCTION

Fruit fly are flies lay their eggs in immature or ripening fruits so that their larvae can feed and grow within the fruit. These flies are placed in the taxonomic family Tephritidae. The great majority of species in this family are placed in one of four subfamilies: Dacinae; Trypetinae; Ceraitinae; and Tephritinae (seed flies).

The species in subfamily Dacinae are found predominant in tropical and subtropical regions and are associated with soft fruit from a vast range of plants. There more than 800 plant species in which Dacinae are found {in Africa (200 species), Asia/South East Asia (300 species) and the south Pacific (300 species)}.

The papaya fruit fly, Bactrocera papayae (Drew 1989) is placed in the family Tephritidae with in the order Diptera (true flies). The flies are small to medium size, the wings are usually banded and the bodies are brightly colored.

Adults of this family are identified by apical part of the sub costa which abruptly bend forward and then becomes weak. Legs dark colored; abdominal terga III - V with a narrow medial longitudinal dark band forming a T with a transverse dark band a cross anterior margin of tergum III, anterolateral corners of terga IV and V with triangular dark marking. Ovipositor with a culeus needle sharpened; spicules long and narrow with 10-24 dentations.

Order: Diptera
Family: Tephritidae
Subfamily: Dacinae
Genus: Bactrocera
Species: Papayae
A. Wing

B. Leg

C. Abdomen

Ovipositor (apex of aculeus)
The oriental fruit fly *Bactrocera dorsalis* (Drew, 1989) Diptera: Tephritidae, is distributed throughout tropical and subtropical region in countries such as Indonesia, India, Thailand, Singapore, Hawaiian (Newel and Haramoto, 1968; Hardy and Adachi; 1954; Hardy, 1973), East Malaysia and Peninsular Malaysia. It is one of more dominant fruit flies in many areas in Malaysia and the most destructive crop pests (Corbett, 1928). It damaged many varieties of commercial fruits (local fruits) such as chilli (*Capsicum annul* L.), guava (*Psidium guajava*), starfruit (*Averrhoa Carambola*), papaya (*carica papaya*) and tomato (*Lycopersicum esculenta*). (Yunus and Ho, 1970)

*Bactrocera dorsalis* is also known to be the most damaging insect pest to many fruit cultivation in the tropical and subtropical regions of the world. It was first found in Hawaii in 1945 (Van Zwaluwenburg, 1947). Within 3 to 5 years after it was detected, it developed into an agricultural problem of major proportions (Bess and Haramoto, 1961; Newell and Haramoto, 1968). During the peak years (between 1947 to 1950) of its abundance in Hawaii, the intensity of attack on edible fruits was so great that often no saleable or edible fruit could be harvested. More than 120 species of fruits become its hosts. Of this 15 species grown on the major islands were often heavily damaged. In Taiwan, 53 fruit species were reported damaged by this pest (Liu, 1982); 36 in India (Kapoor and Argawal, 1982); and more than 10 species in Malaysia (Tan and Lee, 1982).

In Peninsular Malaysia, cultivation of fruit trees are mostly limited to villages. Unfortunately, the highest population of *B.dorsalis* among all ecosystems in the peninsular is also found in villages and the incidence of damage by this pest on harvestable fruits is close to near-100% (Tan and Lee, 1982; Serit and Tan, 1986). However, spraying of insecticides is not a common practice (and therefore had never been applied to control agricultural pests) in villages because each household grows a
few fruit trees and some villagers also keep livestock. Occasional sprayings of insecticides, if any were applied in some mixed orchards where most fruit are cultivated for sale.

There were three common types of economic losses incurred by *B.papayae* in Peninsular Malaysia. First, ovipositional punctures may cause various types of surface defects of the fruits including discoloration or formation of abnormal growth around the puncture. Punctures may also provide entry points for secondary pathogens which further aggravate the disease in fermentation and decomposition of the fruit. Second, maggots feeding on the tunneling fruits fleshy tissue can damage the entire fruit. The third, losses incurred indirectly by the establishment and enforcement of quarantine regulations that prevent fruit fly *B.dorsalis* into uninfested areas. These quarantine restriction hinder the expansion of export market of Malaysian fruit crops.

Behaviour and life history of *B.dorsalis* have many similarities with other tephritids (Nishida and Bess, 1957; Bess and Haramoto, 1958; Christenson and Foote, 1960). Laboratory mating and courtship in *B.dorsalis* were observed and described by Roan *et al.* (1954). In the field, *B.dorsalis* adults feed on a variety of materials such as extra floral glandular secretion nectar, honey-dew, damaged ripe and decomposed fruits, and animal faces. Nishida, 1958 and Drew *et al.*, 1983 reported that the type and abundance of leaf and fruit surface bacteria appear to be the most important factor in life history of tropical fruit flies.

Various control methods were employed to combat *B.dorsalis*. The integrated pest management approach for controlling fruit flies is likely to offer the best control strategies. However fruits that are grown for exportation to uninfested areas requires a perfect product. There is a strong preference for eradicative measures rather than the
integrated control. Over the past few years, both biological and chemical control had been explored and tested extensively but with little successes.

Natural enemies collected from many countries (Clancy et al., 1952) were released in Hawaii to combat *B. dorsalis*. The trials resulted in the establishment of four of parasitoids of *B. dorsalis* (Van de Bosch et al., 1951) but only 3 i.e. *Biosteres arisanus*, *B. vandenbosh*, and *B. longicaudatus* had contributed significantly to the biological control of *B. dorsalis*. The history of the field establishment and evaluations of these 3 the parasitoids were reviewed by Newell and Haramoto (1968). However, the used of parasitoid as control method had not achieved a high degree of effectiveness (Bateman, 1972). Other control methods which were also tested and developed against *B. dorsali*. This include the use of fruit resistant varieties (Macion et al., 1968; Seo et al., 1970 and 1973b; Armstrong et al., 1979 & 1983).

Chemical control includes the use of insecticides and sterilants. Since 1952, over a period of 20 years, almost all insecticides were evaluated at Honolulu, Hawaii against *B. dorsalis* and other tephritids (Keiser, 1968; keiser and Tomikawa, 1970; keiser et al., 1973). Insecticides were applied mainly though bait spraying in which they were mixed with food attractants such as sugar (Myburgh and Stubbings, 1950), protein hydrolysates (Seiner, 1952b) and methyl eugenol (Steiner, 1952a). However methyl eugenol mixed with 5% technical naled were widely used with successes in eradication of *B. dorsalis* (Steiner et al., 1965). Insecticides used as fumigants were also reported by Seo et al., (1979).

The information on the various environmental components that determine the abundance of *B. dorsalis* has been accumulated. The range of enviromental components is broad and so far, these were area that had independently contributed to the abundance
of *B. dorsalis*. The main component are food, moisture, temperature, light natural enemies and symbionts (Bateman, 1972).

Like many other insect, food is one of or otherwise, the most important components of environment that influences the abundance of fruit flies (Nishida, 1980). General relationship has been observed between the amount of larvae food available to local population and the rate of production of new individuals (Bateman and Soleitner, 1967, Newll and Haramoto, 1968).

Some stage of life cycle of fruit flies are susceptible to dessication, especially larvae and the newly emerged adult (Bateman, 1972). However, Newell and Haramoto (1968) did not include dryness amongst the seven major mortality factors affecting larvae and pupae of *B. dorsalis* in Hawaii. They found that the mortality of pupae in the soil was only significant in narrow range from (0.0 - 09 %) water content except in extremely dry and hot condition, which are also unfavourable to most hosts.

The role of temperature as a determinant of abundance in tephritids are mediated either directly or indirectly on rates of development, mortality and fecundity. The rates of increase or decrease are dependent upon the values of these parameters, which in turn are determined by the multiple influences impinging upon the individuals from within the population life system (Clark et al., 1967). However, in subtropical areas such as Hawaii, there is a distinct seasonal pattern of abundance of *B. dorsalis* (Bess and Haramoto, 1961; Newell and Haramoto, 1968; Haramoto and Bess, 1970). This was probably due to the availibility of suitable hosts rather than directly to changes in temperature. However, the effect of temperature on seasonal abundance of univoltine species is understood.

Light plays an important role in the determination of fecundity in fruit flies, but has less direct effects on rate of development and mortality (Bateman, 1972). The
fecundity is affected, first through the general activity of adult females (especially feeding and ovipositional activities) and secondly through the synchronization of mating behaviour. *B.dorsalis* females reach sexual maturity earlier, mate sooner and lay eggs earlier when kept in bright light (Flitter 1964). For many species of tephritids, falling illuminance at dusk act as a stimulant for the initiation of sexual activity. These include *B.tryoni, B.ciliatus, B.zontus* and *B.cucurbitae* (Roan et al., 1954; Bess and Haramoto, 1961; Arakaki et al., 1984).

In the life of *B.dorsalis*, the eggs, larvae and young in the fruits are relatively well protected against the extreme changes in weather conditions and many other mortality factors. However, several natural enemies of the immature has been reported. They are basically the parasitic wasps that include *Biosteres vandenboschi* (Fullaway), *B.longicaudatus* (Sona) and *B.arisanus* (Ashmead). Fullaway (1951), Bateman (1972), and Wharton and Gilstrap (1983) had also reviewed many other species of parasitoids. The fruit associated immatures are also exposed to pathogenic microorganisms and saprophytic fungi and their toxic substances though these mortality factors are not particularly important in the life history of *B.dorsalis* (Bess and Haramoto, 1961; Newell and Haramoto, 1968).

The soil-associated immatures of *B.dorsalis* (i.e., the matured larvae, pupae and the newly emerged adults) are very vulnerably to extreme changes in weather condition and to natural enemies. *B.dorsalis* are also exposed to the pathogenic microorganisms which include the fungi (*Penicillium, Mucor* and *Serratia*) and bacteria (Bess and Haramoto, 1961, Newell and Haramoto, 1968).

The behaviour of communication has evolved in similar manner as morphological characters (Lorenz 1968, Tinbergen, 1951). Primarily there are two different mechanisms through which animal communication system may have been
shaped. Some animal signals were derived from an adaptation to physical environment (Bennet, Clark 1970, Lall et al., 1980, Paul and Walker, 1979). For example females of several species of fireflies locate males during twilight by producing a yellow flash, while those that call after dark gave a brilliant green flash. It is thought to be an advantage for the species to produce certain flash color light to avoid masking by the environment (Lall et al., 1980).

Intersexual selection is the process in which individuals of one sex, females in most cases exhibit mating preference for individuals of the other sex possessing particular traits. The intrasexual selection occurs through the competition among member of one sex usually male for access to individuals of other sex. The mode of communication is classified by the sense organs. Among mode of communication in insects include chemical (both airborne substances chemical and deposition on substrates), auditory, visual, and tactile signals. In most cases combination of two or more of these senses contribute to the communication system of a species. However in many species one mode of communication is dominant over others. Each communication mode carries with it some advantages and disadvantages (Alcock, 1979). In active diurnal species in open habitats, visual signals provide easy communication between moving individual. On the other hand pheromones provide persistent signals over a greater distance than the visual signals. Olfactory signals are important in nocturnal insects whose visual cues may be limited. Among various modes, auditory signals usually detailed communication over a longer distance and are not constrained by wind conditions like pheromones.

In Diptera, the drosophilids and tephritids are the best known in terms of their sexual communication (Burk, 1981). The complexity of signaling in these flies appears to have evolved because individuals compete in producing advertisement signals in
order to gain matings. Among various modes of communication signals used in tephritid species visual signals are suggested to be important in only few cases. For example particular movements such as spinning or circling of their shiny gold body and their clear wings with distinctive brown patterns used during courtship in caribbean fruit fly *Anastrepha suspensa* are described as visual signals (Dodson, 1978). For very close range communication tactile signals produced through licking of the female’s genitalia or probing with the eggs during courtship are important in gall formers in Tiphritidae of the genus *Valentinubula*, (Wangberg, 1978). Olfactory and acoustic signals, however, are two major modes of potenially long-range signals that play significant roles in sexual communication of most tephritids and therefore deserve special emphasis.

Olfactory signals a form of chemical communication is important in several groups of flies including fruit flies (Chambers, 1977 and Fletcher, 1977). They include both long distance and contact or short range pheromones. In some species such as the olive fruit fly, *B. oleae* the female produce a pheromone that attracts the male (Baker *et al.*, 1980; Mazomenos *et al.*, 1985). While in *B. ryoni* and *C. capitata* the male attracts female (Chnbers, 1978 and Pritchard, 1967). In these cases long distance pheromones which are produced and dispersed by males attract both receptive females and also other males. Among the species in which the males release pheromones long range pheromones consist of different chemical compound distinctive of short-range pheromones, e.g. *B. Tryoni* (Fletcher 1977).

Many species of Tephritidae produce complicated stereotypic auditory signals as part of sexual communication. Males of *A. suspensa* produce characteristic acoustic signals calling aggressive and pre copulatory songs each with a distinct pulse pattern during rapid wing vibrations (Webb *et al.*, 1976). The calling song stimulates female
activity (Burk, 1981) and with the presence of pheromones attracts females (Webb, 1973). The precopulatory song causes a prolonged copulation and also increases the female’s sexual motivation towards their mate (Burk, 1981). Other tephritid species such as *B. tryoni*, *B. oleae* and *C. capitata* also produce sound signals which are species specific (Monro, 1953 and Rolli, 1976).

In mating behaviour of *B. dorsalis* various channels of sexual communication have been studied in different species of tephritid flies. However only few reports have been published for *B. dorsalis* [Kobayashi et al., 1978 and Roan et al., 1954]. *B. dorsalis* *mate* only at dusk under low light intensity (Arakaki et al., 1984). Decreasing light intensity at twilight is also reported as stimulus for initiation of sexual activity. As twilight approaches, sexually active *B. dorsalis* males produce a characteristic high pitched buzzing sound by copulation which lasted for 2 to 12 hours (Roan et al., 1954).

It has not been on recorded whether a particular type of sound produced during the wing vibration of male’s results from stridulation. Keiser et al., (1973) noted that the wing of *B. dorsalis* males have deeper and more sharply incised wing margins at the distal tip of Cu1 (1A (anterior cubitus + first anal vein according to the Comstock and Needham system referred by Borror et al., 1981) than the females. Two rows of large cilia are found along the abdominal tergites of males only (Hardy, 1955). These sexually dimorphic structures were hypothesized to be the stridulatory organs. However Keiser et al., (1973) failed in their attempt to demonstrate that stridulation exist in *B. dorsalis*. They failed to locate actual brushing of the wings against the abdominal cilia. However, they suggested that the males sound are not necessarily produced for successful copulation because the wingless males mated as effectively as normal winged males. Therefore the role of sound stridulation activity in the mating behaviour *B. dorsalis* has not been determined. However sound believed to be produced along with
the distribution of male pheromones in the sexual communication offer on area worth to be explored.

Live male and the male rectal gland complex are reported as highly attractive to virgin females (Kobayashi et al., 1978; and Schultyz & Bouch, 1971). This gland complex was suspected to be the source of olfactory pheromones. The substance emitted as visible smoke by males at dusk was collected and analysed by Ohinata et al.,(1982). It consisted of large amounts of trisodium and potassium phosphates which may act as carrier for the volatile components of pheromone. The main chemical compound of the smoke was found to be amide group which are less attractive to females in laboratory tests.

The published information related to the mating behaviour of B.dorsalis, showed that sexual behaviour may include sound as well as release of pheromone, which are associated with the wing fanning of males. However the roles of both signal modalities in the two stages of sexual connection attracting a female or eliciting copulation are still not clear. It also noted that most of the studies done so far have been emphasized on close range sexual activities including courtship and mating. To date the most detailed study of its courtship behaviour is largely descriptive in nature (Arakaki et al., 1984).

Two important aspects of behavioural quality i.e. (1) searching ability for mating sites or sexual partners and (2) mating behaviour are affected in mass reared insects (Bolero, 1972). Related example included the alteration of female responsiveness to male pheromone in screw worm flies Cochliomyia hominivorax (Fletcher et al., 1968) and the unsynchronized mating period of laboratory and wild population in codling moths Carpocapsa pomonella (Boller, 1972). Tephritid specific example of laboratory effects are reported in only two species. Laboratory males of
*Ceratis capitata* have a lower insemination rate than recently established laboratory male (Rossler, 1975). Moreover both sexes of the laboratory strain reach sexual maturity much earlier than the wild (Wong and Nakahara, 1978). From the laboratory rearing sterilization with gamma radiation there were some problems related to mating in *S. suspense* pupa increase the frequency of signaling behaviour of males (Sharp and Webb, 1977). In *C. capitata* the ability of sterilized males to compete successfully with non sterilized males in mating is reduced by 50 % and the peak of mating activity of both sexes is delayed as a result of sterilization causing unsynchronized sexual activity (Holbrook and Fujimoto, 1970). Therefore it is not surprising that deviation in sexual activities of sterilized laboratory reared flies from that of wild ones has suggested as a primary cause for the failure of several Sterile Insect Release Method (SIRM) programs (Ito and Kawamoto, 1979). Successful eradication of *B. dorsalis* using effective SIRM program requires an indepth knowledge of its complex sexual behaviour in order to develop the laboratory strain which can either mate in the field or prevent normal mating of wild males. In the addition any basic research on the sexual behaviour of any pest has recently recognised to be an important component in formulating insect control strategies. However, in case of *B. dorsalis* most of the information on communication behaviour, role of different sexual signals and their significance in mating behaviour are either missing or generally misunderstood. Therefore the present study was undertaken in order to understand the complete sexual behaviour of *B. papayae* and their significance insexual communication. A complete knowledge and understanding on this economically important species will be of paramount importance for Malaysian economy. Furthermore, a complete understanding of sexual behaviour will be helpful in devising or developing a species-specific pesticide or formulating a program for its integrated pest management strategies.
1.1 Culture of insect: Laboratory culture of *B.papayae* was established from fresh free from insecticide and infested chillies (*Copsisum annuum*) were collected from MARDI INSTITUTE, Serdang were used for oviposition and larval development. However this method failed to produce consistent samples for the experiments. An alternative medium, i.e. chillies supplemented with larval medium madeup of chilli or guava (*Petidium guajava*) similar to that developed for *Bactrocera tryoni* (Heather and Corcoran, 1985). Larval was maintained on papaya (*Carica papaya*) as based medium.

1.1.2 Preparation of larval medium.

The formulation consisted of 94.6% by weight fresh fruit papaya (seed and skin removed), 4.1% Torula yeast (sanitarium), 0.4 Nipagin Mr (methyl P- hydrobenzoic acid) and 0.8 ml concentrated hydrochloric acid. The ingredients were blended using an electric blender and the media kept in refrigerated until needed.

Chillies were used as host for oviposition. The eggs were dissected out, filtered through Whatman filter paper No, 1 and kept in sterilized petridishes. Fifty eggs were transferred aseptically to separate petridish (6x6x1.5 cm) containing 20 g larval medium (previous preparation). Subsequently after egg hatching and larval development, the larvae were transferred to containers (9 x 9 x 10 cm) filled with sterile sand for pupation. The pupa were transferred to a large cage (a wooden cage having 2cm frame thickness and volume, 30 x 30 x 30 cm. The top, base and back of the cage were fitted with glass to monitor larvae, while the front side of the cage had a small window (10 x10 cm²) for accessible by. The half of lateral side had a wire netting aeration). The adults were fed with 3:1 mixture of sugar and peptone (soy hydrolysate), honey and water in plastic cup. A typical rearing cage is shown in (Plate 1) Rearing conditions were at temperature 25 - 27 C⁰ and 79 - 80 % RH.
Plate 1: A typical laboratory cage (30 × 30 × 30 cm) for maintaining adult *Bactrocera papayae* in the laboratory; adult food, distilled water were provided in the cage