POPULATION DYNAMICS AND MOVEMENT OF *Bactrocera umbrosa* (FABRICIUS) IN RELATION TO *B. papayae* (DREW & HANCOCK) (DIPTERA: TEPHRITIDAE) IN PENANG ISLAND, WEST MALAYSIA

by

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ABSTRAK

Buah nangka hanya dirosakkan oleh *Bactocera umbrosa(Fabricius)* pada tahap pertengahan, buah yang masak, matang yang berwarna hijau serta berada pada tahap buah masak yang gugur. Nilai min tertinggi larva per buah adalah dikaitkan dengan kadar buah masak yang gugur. Nisbah bagi jantan dan betina adalah tetap 1:1 bagi semua peringkat usia buah. Parameter fizikal bagi buah tersebut memainkan peranan yang signifikan dalam menentukan bilangan larva dan dewasa *B. umbrosa* pada buah tersebut. Nilai min yang tertinggi bagi parasitoid braconid bagi setiap buah dikaitkan dengan penghasilan buah nangka tersebut. Tiga parasitoid utama *B. umbrosa* pada buah nangka di Balik Pulau ialah *Fobius arisanu*s > *Diachasmimorpha longicaudatus* > *Biosteres vandenboschi*

Dibawah keadaan makmal, jantan hidup selama 152 hari manakala betina mampu hidup sehingga 146 hari sahaja. Jantan yang tertarik kepada umpan metil-eugenol (ME) di lapangan menunjukkan 2 tahap masa dalam kitar harian iaitu puncak tertinggi pada awal pagi dan puncak yang rendah pada tengah hari. Tindak balas terhadap ME menurun secara signifikan menjelang senja

Bagi populasi asal jantan, penambahan bilangan dan kadar kemandirian *B. umbrosa* dan *B. papayae* telah dianggarkan dengan menggunakan teknik tanda-lepas-tangkap semula menggunakan umpan ME yang dijalankan di kampung Sungai Burung dan kebun Teluk Kumbar di Pulau Penang, Malaysia. Saiz populasi dan penambahan bilangan spesies adalah lebih tinggi secara signifikan di kawasan kampung berbanding kawasan kebun. Ini adalah disebabkan kadar kerosakan buah-buahan di kampung adalah lebih tinggi berbanding di kebun.

Faktor persekitaran yang terpenting yang mempengaruhi populasi dewasa *B. umbrosa* di kawasan tropika adalah kehadiran perumah yang sesuai. Di antara semua kategori buah, kategori 'buah yang rosak' adalah yang paling penting. Anggaran populasi serta penambahan jantan *B. umbrosa* berubah dan lagged 4-6 minggu dan 3-5 minggu, masing-masing. Walaupun kehadiran *B. umbrosa* dewasa dan perumah iaitu buah nangka lebih banyak ketika musim hujan berbanding musim kering namun tidak terdapat korelasi yang signifikan di antara taburan hujan, kelembapan bandingan dan suhu. Pada musim kering, populasi lalat adalah berkurangan kerana kurangnya sumber makanan.

Sebanyak 39.39% daripada jumlah keseluruhan larva matang pada buah nangka yang rosak di kampung Sungai Burung dalam jangka masa 17 ½ bulan telah hilang disebabkan pelbagai faktor mortaliti yang terjadi di kampung berkenaan. Populasi larva yang tinggi telah dilihat 1-3 minggu selepas telimpahan musim nangka di kampung tersebut

Tahap matang populasi jantan *B. umbrosa* dan *B. papayae* di 3 ekosistem di Teluk Kumbar telah dianggarkan selama 31 minggu dengan mengunakan teknik -tanda-lepas-tangkap semula dengan umpan ME. Kepadatan populasi spesies jantan yang matang *B. papayae* di 3 kawasan menunjukkan perbezaan yang signifikan. Perbandingan bagi min jumlah lalat jantan *B. umbrosa* ditangkap setiap kali penyampelan menunjukkan perbezaan yang signifikan bagi ketiga-tiga kawasan yang dikaji. Kadar pergerakan dewasa antara 3 ekosistem itu adalah sangat perlahan. Hanya 65 ekor lalat jantan ditangkap di kawasan hutan dalam masa kajian dijalankan adalah berkemungkinan pendatang baru kerana di kawasan tersebut tidak terdapat pokok *Artocarpus. Bactocera umbrosa dan B. papayae* pada keadaan semulajadi secara relatifnya akan tinggal tetap di satu kawasan dan hanya akan bermigrasi jika kekurangan dan kehilangan perumah yang sesuai. Jumlah lalat yang bermigrasi (10) yang berjaya ditangkap adalah suatu nilai yang sangat kecil berbanding jumlah yang dilepaskan dan yang boleh ditangkap semula (3180). Kadar rendah pergerakan lalat dewasa diantara ekosistem adalah tidak sesuai untuk menyebabkan fluktuasi yang signifikan bagi populasi lalat.

ABSTRACT

Jack fruits were only damaged by *Bactrocera umbrosa* at the intermediate, mature green, ripe and the dropped ripe stages. The highest mean larva per fruit was associated with the dropped ripe fruits. The ratio of males to females was consistently 1:1 for all fruit stages. The physical parameters of the fruit played a significant role in determining the number of larvae and adults the fruit produced. The highest mean of braconid parasitoids per fruit was associated with dropped fruits. The 3 major parasitoids of *B. umbrosa* in jack fruit in Balik Pulau were *Fobius arisanus* > *Diachasmimorpha longicaudatus* > *Biosteres vandenboschi*.

Under laboratory conditions, males lived for 152 days while females survived up to 146 days. Males attracted to methyl eugenol (ME)-baited traps in the field showed two peaks in a daily cycle, a high peak in the early morning and a low peak in the afternoon. The response to ME declined significantly at dusk.

Native male populations, new recruits and survival rates of *B. umbrosa* and *Bactrocera papayae* were estimated by mark-release-recapture technique using ME –baited traps, in Sungai Burung village and Teluk Kumbar orchard in Penang Island, Malaysia. Population size and new recruits were significantly higher in the village than in the orchard. This was caused by the higher number of fruits damaged in the village.

The strongest component of the environment influencing adult populations of *B. umbrosa* in the tropics was the availability of its suitable host fruits. Among all fruit categories "ripe fruits and total damaged fruits" were the most important. Its numbers fluctuated and lagged behind the estimated populations and new recruits of *B. umbrosa* males by 4-6 and 3-5 weeks, respectively. Although *B. umbrosa* adults and its host jack

fruit were more abundant during wet than dry season of the year, they had no significant correlation with the daily rainfalls, relative humidities, and temperature. During dry seasons, the fly populations declined because of less food.

About 39.39 % of the initial number of matured larvae in the damaged jack fruits in Sungai Burung village within a 17 ½ months period were lost to the various mortality factors operating in the village. Peak larval populations were observed 1 to 3 weeks after an abundance of jack fruits in the village

Mature male populations of *B. umbrosa* and *B. papayae* in three ecosystems at different elevations in Teluk Kumbar were estimated weekly over a period of 31 weeks by the mark- release-racapture technique using methyl eugenol -baited traps. The *B. papayae* mature male population densities in the three areas were significantly different ($pc<0.05$) from one another. The highest adult population and new recruits of *B. papayae* were observed in Teluk Kumbar orchard, followed by Teluk Kumbar village and Teluk Kumbar native forest. Comparisons of means of total male flies captured per sampling week showed significant differences between the three areas. Rates of adult movement between the three ecosystems were very low. The few male flies (65) captured in the forest during the study period were probably immigrants because there was no *Artocarpus* tree. *Bactrocera umbrosa* and *B. papayae* in nature were relatively very residential and tend to migrate only when suitable host is lacking or diminishing. Low rates of adult movements between ecosystems were insufficient to cause significant fluctuations in the fly population.

Chapter 1 GENERAL INTRODUCTION

1.1 Economic losses and problems associated with fruit flies

The family Tephritidae (true fruit flies) is one of the largest families of Diptera (Drew, 1989). A significant number of these species are extremely destructive pests of many economically important crops in the temperate, subtropical, and tropical regions of the world. Losses are related to direct destruction of fruit and vegetables by the immature or larval forms of the flies, costs of materials and labor for preventive treatments, costs of monitoring the possible presence of flies even in fly-free regions, and costs of quarantine and fruit shipment fumigation. The presence of very destructive species in some regions may also inhibit the economic development of potential fruit and vegetable crops.

These fruit flies are serious pests and the damages caused by them are multiple in nature. They infest more than 150 different species of fruit and vegetable crops (Christenson & Foote, 1960). The ovipositional punctures caused by the female flies lead to sap oozing, discoloration and abnormal growth of the infested fruit. The young larvae tunnel and feed inside the infested fruit. The feeding activity of the larvae could also accelerate the rate of fermentation and decomposition of fruit which are usually caused by secondary microorganisms that might enter through the puncture.

There are a number of aspects to the problem created by fruit flies.First, a number of species may be involved in reducing the quantity or quality of fruit and/or vegetable production and these losses, together with the cost of any control, are a direct cost to the community or to the country. Second, there are quarantine problems which result in either loss of actual or denial of potential markets, or the added cost of appropriate disinfestation procedures to farmers.

The economic threshold value for fruit fly control in succulent fruit cultivation is very low. Tolerance to fruit fly damage is extremely low. A few or even a single larva in a fruit is often sufficient to render it unsuitable for storage, sale or human consumption. In situations where fruit is grown for export to uninfested areas, or where consumers demand a “perfect” product, the economic threshold may often be zero, and the strong preference for “eradicative”

rather than “management” procedure is readily understandable. Besides their short life cycle which enables them to multiply very rapidly and their high fecundity, there are several other factors which also contribute to fruit flies being such damaging pests.

1.2 General ecological characteristics and host specializations of tephritid fruit flies

The distribution of the Tephritidae is virtually worldwide. The family is divided naturally into two major groups on the basis of physiological and ecological characteristics (Bateman 1972; Fletcher 1987). (1) The multivoltine species which have no obvious diapause and inhabit warmer regions of the world (e.g., *Bactrocera* and *Anastrepha* spp.). The tropical Tephritidae, particularly those of the Dacinae, are characterized by being relatively long-lived, polyphagous with a very wide expanding host range, multivoltine, having a high potential fecundity and in many species an ability of adults to survive unfavorable periods of the year, highly mobile in the adult stage and have a high capacity for dispersal. The multivoltine species may produce up to six overlapping generations in a single season (Bateman, 1972). These attributes led to many of the species being significant pests of horticulture and make the monitoring and control of these species very difficult. (2) The univoltine species which usually have a winter diapause and inhabit the more temperate regions of the earth (e.g., *Rhagoletis* spp.) (Bateman, 1972).

Based on their degree of host specialization, tephritid fruit flies can be classified as monophagous (utilizing a single larval host); stenophagous (utilizing a few, usually closely related species in a single plant family); oligophagous (a restricted host range, with species in only one or a few plant families); or polyphagous (a wide host range including species in many plant families).

The majority of species in all four economically important genera are either monophagous or stenophagous, although many of the major pest species, e.g. *Ceratitis capitata*, *Bactrocera cucurbitae* and *B. dorsalis* are highly polyphagous.

1.3 General aspects of tephritid taxonomy

Tephritid taxonomic research was pioneered by those forefathers of biology, Linnaeus and Fabricius. Tephritid taxonomy has a long history (over-two centuries) with some 4,500 species having been described since mid-1700s (Drew & Romig, 2000), distributed throughout the temperate, subtropical and tropical areas of the world.

The Dacinae fruit flies, one of the major subfamilies of the Tephritidae, are an economically important group of Diptera. Drew (1988) estimates that there are at least 800 species distributed: in Africa (200 species), the Asian region (300 species) and throughout the South Pacific (300 species). This group is mainly found in subtropical and tropical areas. The rate of discovery of new species suggests that they may be up to a thousand species in total. Economically important species of fruit flies belong to the genera; *Anastrepha*, *Rhagoletis*, *Bactrocera*, and *Ceratitis*. The Dacinae fruit flies have traditionally been divided into two main genera; *Bactrocera* and *Callantra*. Other major pest tephritids of the genera *Anastrepha*, *Rhagoletis* and *Ceratitis* belong to the subfamilies Trypentinae and Ceratinae.

Many *Bactocera* species are known pests of commercial fruits and vegetables. The Oriental fruit fly, *Bactrocera dorsalis* (Hendel), a polyphagous tephritid fruit fly, is one of the most damaging pests to fruit cultivations in the tropical and subtropical regions (Fletcher, 1987).

The *Bactrocera dorsalis* species complex comprises at least 52 species (Drew & Hancock, 1994), bearing a taxonomic resemblence to *B dorsalis* (Hendel), and many are major pests of a wide variety of fruit and vegetable crops. The considerable confusion surrounding the taxonomy of this complex is elucidated with the application of modern technology such as electron microscopy, DNA sequencing, host-plant recordings, morphology of larvae, adult morphometrics (Drew & Hancock, 1994) and tissue enzyme analysis as well as morphological and biological data, and these advanced technologies have enabled taxonomists such as Drew & Hancock (1994) to further unravel the complexities and mysteries of the *dorsalis* complex in Asia.

Based on previous studies, the *Dacus* genus which includes a large number of fruit fly species is now renamed as *Bactrocera* (Drew, 1989). *Bactrocera* as a genus has about 400

species whose members extend throughout Asia, the Oceanic region, and into Australia. There are very few recorded species in Africa and only *B oleae* is found in North Africa and Southern Europe.

1.4 Fruit fly species in Peninsular Malaysia

The equitable tropical climate of Malaysia and the various types of fertile soils in different areas make possible the cultivations of many kinds of fruit trees. Combined with an abundance of host fruits under monoculture situations, the tropical climate of Malaysia enables uninterrupted breeding of fruit flies throughout the year. The fruit harvesting seasons of each fruit variety in Malaysia are overlapping; this condition is favorable for the development of fruit flies.

Peninsular Malaysia has a wide variety of tropical fruits whose cultivation on a commercial scale is rapidly developing and are important from the economic, nutritional and social standpoint. The demand for fruit at local markets is great. Malaysia had for years imported fruits from many countries in order to meet this demand and this had caused an enormous outflow of money from the country. Over the past years, Malaysian Government had encouraged farmers to utilize their waste lands for agricultural purposes, including large scale cultivations of some local fruits for exportation.

The fruit flies jeopardize development of a diversified tropical fruit and vegetable industry in Peninsular Malaysia. Fruit flies are considered pests of economic importance in Malaysia because they infest 15 out of the 17 fruit species that have been recommended by the Ministry of Agriculture for commercial development (Vijaysegaran, 1988).

In villages and orchards, fruits left unprotected to ripen on trees are heavily infested by fruit flies which build up very high numbers and can destroy some fruit crops completely. Damage to young fruits may cause premature fruit drop and oviposition marks blemish the fruit and reduce their market value. Economic losses from fruit flies were estimated at 12.8 million ringgit (3.3 dollars) in the year 1987 (Vijaysegaran, 1988).

In Peninsular Malaysia where endemic field populations of fruit flies are frequently very high, preharvest or field control is a vital component of the overall fruit fly management strategy. Growers currently overcome the fruit problem by picking just before streaks of yellow appear on the fruit (Vijaysegaran, 1983 and1988).

In the light of the seriousness of damage of local fruits by many species of fruit flies, fruit industries may face uncertain future unless a reliable method of control of these pests is available. In the absence of a proper control measure, it will be impossible for the local fruits to pass through quarantine bodies and to convince importers of the safety of these fruits.

The first extensive survey and systematic study of the various fruit fly species, their host ranges and distribution in Malaysia was undertaken from 1986-1991 (Vijaysegaran, 1988; Ferrar, 1990) through trapping and host fruit surveying. From the results of this extensive survey, it was confirmed that the genera *Anastrepha*, *Ceratitis* and *Rhagoletis* are not found in Malaysia.

Studies on the adult taxonomy (Drew, 1988), larval taxonomy (Elson-Harris, 1988) and genetic variation (Ooi, 1988) have revealed that the major pest species in Malaysia is not the oriental fruit fly, *B. dorsalis* (Hendel) and that it is not present in the country as it was thought (Drew & Hancock, 1994). Two closely related sympatric sibling species in the *dorsalis* complex of flies; *B. carambolae* (Drew & Hancock) (Plate 1.1) and *B. papayae* (Drew & Hancock) (Plate 1.2) are the major pest species responsible for much of the damage attributed to the oriental fruit fly in Malaysia.

Plate 1.1 : *Bactrocera carambolae* (Drew & Hancock) – male specimen

Plat 1.2 : *Bactrocera papayae* (Drew & Hancock) – male specimen

A total of 64 species of tephritieds have now been identified from Malaysia of which seven species, all in the genus *Bactrocera*, are recognized as being of economic importance. These are the dominant species of major economic importance which include *B. carambolae*, *B. papayae*, *B. umbrosa* and *B. latifrons* while *B. tau*, *B. caudatus* and *B. albistrigata* are of minor economic importance. According to Tan *et al.* (1994) the tropical rainforest of Malaysia harbors additional species of *Bactrocera.*

In 1994, fifty-two species of fruit flies in Asia were placed in *B. dorsalis* complex. Among them, 8 species were considered of economic importance. In Malaysia, three species belonging to the *B. dorsalis* complex are *B. arecae* (Hardy & Adachi), *B. carambolae* (Drew & Hancock) and *B. papayae* (Drew & Hancock). The latter two sibling species are sympatric species; and are serious pests in Malaysia.

Among the 52 sibling species complex in the Oriental fruit fly, *B. dorsalis* (Hendel), *B. papayae* (Drew & Hancock) is beginning to emerge as an economically important insect pest which poses a severe threat to the fruit cultivation in the South-east Asian region as well as in many subtropical and tropical countries. Recently, the introduction of *B. papayae* into

Queensland, Australia (Fay *et al.* , 1997) has drawn international attention and interest of many entomologists to this species. *Batrocera papayae* is a serious pest of commercial fruits and vegetables in Indonesia, Malaysia and Thailand. Its host range includes over 150 fruit species-but bananas, *Musa* spp. and starfruit, *Averrhoa carambola* L. are the most preferred. If left unchecked and fruits are left to ripen on trees, fruit infestations by *B. papayae* in such situations can reach 100% (Tan & Serit, 1994). *Bactrocera papayae* is more abundant in the northern region of Peninsular Malaysia.

1.5 *Bactrocera umbrosa* (Fabricius)

Bactrocera umbrosa has a narrower host range than *B. papayae* and is known to breed only in *Artocarpus* species Moraceae. This species is commonly a serious pest of jack fruit (*Artocarpus heterophyllus*) and chempedak (*A. integer*) in Peninsular Malaysia (Yunis & Ho, 1980). It is also reported to have been bred from bread fruit; *Artocarpus altilis*. Host records from other families in Asia to be verified. There are old records, requiring confirmation, from giant granadilla (*Passiflora quadrangularis*); pummelo (*Citrus maxima*) and sour orange (*Citrus aurantium*) (Froggat, 1918; Yunis & Ho, 1980). There is a record from *Polyscias* sp. (Araliaceae) in Solomon Island (Vagalo *et al*., 1997). In Vanuatu, it has been recorded from bread fruit only (Stephenson *et al.* , 1997).

Adults mate at dusk. It is very common species causing considerable damage to bread fruit by ovipositing in ripe bread fruit, but also younger fruits, causing premature ripening and drop of fruits. In Solomon Islands, populations peak in December-January, which corresponds to the main bread fruit season (Vagalo *et al.*, 1997).

Damage assessments have shown that it attacks 30% of bread fruits in Vanuatu and up to 75% of bread fruits in Papua New Guinea. It is reported that the losses of jack fruit and cempedak are mainly due to *B. umbrosa*. In 1987, this pest caused a crop loss in Malaysia amounting to RM 845, 000 for jack fruit and chempedak (Vijaysegaran, 1988). The adult of *B. umbrosa* can be differentiated from other species of fruit flies because of traversed marking as on the fore wings (Plates 1.3 and 1.4).

Plate 1.3 : *Bactrocera umbrosa* (Fabricius) – male specimen

Plate 1.4 : Female *Bactrocera umbrosa* ovipositing in red chili in the laboratory.

Quarantine surveilance include methyl eugenol trapping and regular host fruit surveys of bread fruit, jack fruit, and chempedak. If this species has been newly discovered in a country, increased trapping, increased host fruit sampling, restriction of fruit movement, protein bait spraying, and male annihilation will be required to prevent establishment.

Bactrocera umbrosa has a wide distribution in South East Asia and Pasific Islands (Hardy, 1973). This species is widely distributed in Indonesia, Malaysia, Phillipines, Southern Thailand, Palau, Papua New Guinea (much less common in the Highlands), Solomon Islands, Vanuatu, New Caledonia, Bougainville Island (Hardy, 1983; Vagalo *et al.*, 1997; Allwood *et al.* , 1997; Stephenson *et al.* , 1997; McGregor, 2000; Allwood *et al.* , 1999; Allwood *et al.* , 2001).

Bactrocera umbrosa females usually do not lay eggs in captivity (i.e. caged females in the laboratory). This species has been kept in laboratory colonies in New Caledonia, reared on potato-carrot diet (Clare & Lemontey, 1994), and in Vanuatu and Solomon Islands on bread fruit-based diet (Vagalo *et al.*, 1997).

1.6 Management and control of fruit flies

In order to survey for fruit flies and delimit their infestation as well as their control, numerous methods have been developed and are still being perfected. Quarantine surveilance encompasses activities that assist in the early detection of unwanted exotic fruit flies. It is not restricted to trapping systems alone. It includes border inspections, passenger profiling, profiling of airline and shipping routes with respect to level of risk, and host fruit surveys. Various chemical lures may be used to attract flies to traps, but the most effective are male lures, (e.g., methyl eugenol and cue-lure). The species that do not respond to male lures may be sampled only by host fruit surveys and protein bait spraying.

Insecticides have been used to combat fruit flies but the insecticide residues have been harmful to the public and environment. Heavy reliance on insecticides has led to new problems in resurgence of secondary pests, resistance of pest species and undesirable chemical residues and environmental contamination. Horticultural industries in the tropics should minimize the use of insecticides and seek to control flies through supplementary or alternative means.

The use of bait sprays comprising an attractant and a toxicant to attract flies and kill them before they oviposit in fruits dates back to 1889. Protein bait acts as food attractant and its effectiveness relies on the fact that immature females need a protein meal to reach sexual maturity and for developing mature eggs. The bait spray residue on the foliage is ingested by the flies and kills them. A small amount of poison (usually an insecticide) added to the bait will therefore kill them before they reach sexual maturity or lay eggs in the fruit. In Malaysia, the protein source used in bait sprays is a yeast autolysate produced as a by-product of the brewing process in the production of stout. It is marketed under the name of ‘Promar’. Application of

this bait with malathion in spot sprays to the foliage only has provided excellent control for local species of fruit flies (Vijaysegaran, 1989; Mohamed & Bahari, 1993). The implementation of bait spraying for fruit fly control with carambola using the new protein formulation has been very successful, resulting in a doubling of carambola production in Malaysia. Protein bait sprays are less harmful to beneficial insects, making suitable for use in IPM programs. Because of the spot spraying technique, there is less insecticide applied to the crop or tree and non-target species are less harmed. Bait sprays are more environmentally sound because of reduced pesticide usage and less risk of spray drift.

The males of some species of fruit flies are strongly attracted to certain chemical compounds, some of which occur in nature. These compounds have been called "parapheromones". Methyl eugenol (ME) is perhaps one of the best known lures and is a constituent of many common plants. Males of many *Bactrocera* species are strongly attracted to ME and compulsively feed on ME source (Hardy, 1973).

The strong attraction of males to their respective "parapheromones" has been put to good use in male annihilation programmes. Its effectiveness was first demonstrated in isolated islands with the successful eradication of the oriental fruit fly, *B. dorsalis*, from the island of Rota (Steiner *et al.*, 1965). Similar success followed later on the islands of Saipan and Trinian (Steiner *et al.*, 1970). Eradication programmes in Japan (Kawasaki, 1991) have relied on ME assisted male annihilation. However, successes are only limited to "island" populations (Bateman, 1972). Male annihilation may be more effective if carried out over large areas and could be useful for some species that are predominantly found in cultivated areas and not in the rain forests.

The sterile insect technique (SIT) has been successfully used to eradicate fruit flies in several parts of the world. Although it is initially expensive, the one main advantage of SIT is that it is species specific and has practically no side effects and it is safe to the environment.

In nature, fruit flies have many enemies that feed on them. The eggs, larvae and puparia are attacked by a number of parasitic Hymenoptera, particularly by species of Opiinae belonging to the family Braconidae (Christenson & Foote, 1960; Wharton & Gilstrap, 1983).

Commonly used are parasitoids belonging to the family Braconidae which include the genera *Biosteres* and *Opius* (Wharton, 1989). Augmentative release of paraistoids mass reared in the laboratory has been shown to be effective in suppressing native populations of medflies in Hawii (Wong & Ramadan, 1992).

The economic threshold value for fruit fly control in succulent fruit cultivation is very low. This, therefore, does not encourage the use of parasitoids and thus kairomones of parasitoids for control of host populations. A few or even a single larva in a fruit is enough to have it rejected for consumption and the more stringent export market will not tolerate any larvae in fruit. It is unrealistic to expect natural enemies to provide such a high degree of control. Their presence should be encouraged in orchards and supplementary methods that are not deleterious to them should be adopted.

The classical biological control of tephritids suffers from a set of difficulties such as low fecundity of parasitoids compared to fruit flies and poor tracking of fly population growth by parasitoids. The classical approach has been limited to certain conditions of environmental stability and biodiversity which are only found in a few ecosystems (Montoya & Liedo, 2000).

There are ways to overcome these difficulties, e.g., by augmenting the number of parasitoids to certain times and places. Greathead & Waage (1983) defined augmentative biological control as the “strategy in which natural enemies are mass reared for release at critical periods, aiming to suppress a pest population in a short period of time”. According to Knipling (1992) and Barclay (1987), augmentative biological control can be considered as a formal alternative for suppressing pest population and even for use in eradication programmes, after integration with sterile insect technique (SIT).

Breeding of fruit flies in unwanted fruit in orchards is probably the biggest source of damaging populations. It is thus very important to prevent breeding of flies by removing and destroying all unwanted or fallen fruits. In areas where several individual orchards are in close proximity to each other, it is important for all orchards to observe crop hygiene.

Wrapping or bagging of individual fruits to prevent oviposition and produce fruits of high quality appears to be a method somewhat unique to the Asian region. The bag provides a

continuous physical barrier from the time of bagging to harvest that prevents female flies from laying their eggs in the fruits. It is simple to apply, highly effective, has no side effects and is completely safe to the environment. The major constraint is that fruit wrapping is labor intensive and, in the event of rain, the bags may be easily damaged.

Currently, integrated pest management, with the combination of several compatible methods together with biological control is the most favored approach.

The first strategy, which can be called orchard population management consists of each farmer managing the pest in his own orchard according to the means and degrees he sees fit. Single measures such as chemical or cultural control, all the way to various degrees of sophistication within an integrated pest management (IPM) approach can be grouped under this category of coexisting with the pest at the orchard level.

In the second strategy, area-wide population management, similar methods are involved as in the first category; however, these are applied by growers, in a coordinated approach, over wider, mostly commercial areas, and attempt to produce fruit free of fruit fly.

The third strategy, total population management, is also an area-wide approach; however it addresses the pest population of a whole region, including commercial, urban and non-cultivated areas, in a more definite way.

Currently fly control in the region of Peninsular Malaysia is practiced more on an individual orchard basis. Area wide population suppression programmes organized with institutional support should be implemented and will undoubtedly give better control of flies in fruit growing areas. The concept of eradication and area freedom is appealing and has its advantages.

The following studies aim to evaluate the dynamics of *Bactrocera umbrosa* adult populations in relation to the population dynamics of *B. papayae* in a village and an orchard ecosystem of Penang Island, West Malaysia, in relation to its host fruits, temperature, humidity, rainfall, and in relation to its larval stage in a village ecosystem. The effect of immigration and emigration on the population dynamics will also be evaluated. Its natural enemies and sex ratio will be determined. Laboratory studies are also conducted to determine the relationship between

the physical parameters of jack fruit, *Artocarpus heterophyllus*, and the production of *B. umbrosa* immatures and adults. It is hoped that this study will provide a better understanding of the fly's population dynamics and provide information for better decision making and improved control of this noxious pest.

Chapter 2 - LABORATORY STUDIES ON *Bactrocera umbrosa* AND ITS CULTURING FROM ITS HOST JACK FRUIT

2.1 Introduction

Damage to fruits induced by *Bactrocera umbrosa* –has stimulated a need for more information on the biology, demography, population dynamics, movement, management and possible means by which to control this pest species. Despite its major economic importance in Malaysia, few studies on this species have been carried out in this country and in the world as a whole.

Simultaneous population studies of fruit flies and their parasitoids are normally accomplished by collecting infested host fruits in the field, and allowing the insects to develop until adult emergence in the laboratory (Bess & Haramoto, 1961; Besset *et a*l.,1963; Newell & Haramoto, 1968; Haramoto & Bess, 1970; Wong *et al.* , 1983; Ooi, 1984; Vijaysegaran, 1984; Serit *et al.* , 1986; Serit, 1987; Palacio, 1991; Chua *et al.*, 1993; Tan & Serit, 1994).

McDonald & McInnis (1985) stated that oviposition activity of the fruit fly is influenced by fruit volatiles, fruit size and shape, color, humidity and origin of the fly population. The same authors found that the diameter of the host was highly correlated with the number of *Ceratitis capitata* eggs per oviposition. Prokopy & Bush (1973) also observed a close correlation between the size of natural hosts and the size of artificial fruits preferred by certain species of *Rhagoletis pomonella* (Walsh) complex. Fletcher (1987) noted the same relationship between fruit size and clutch size of *B. tryoni* and *B. dorsalis* eggs. Serit (1987) found that in star fruits, the weight, length, and total ovipunctures on fruit did not have significant effect in determining the number of *B. dorsalis* immatures and adults the fruit could produce. Palacio (1991) reported that an equal weight of infested star fruits would yeild equal number of *B. dorsalis* puparia regardless of fruit size and when expressed as average per fruit, big fruits contained significantly more ovipunctures and

immature flies than small fruits. Chua (1993) found that the number of *B. carambolae* eggs and adults per star fruit increased with the number of ovipunctures on the fruit.

The density and percentage infestation of fruits by tephritid fruit flies is frequently influenced by the degree of fruit ripeness during the period of fly oviposition (Boyce, 1934; Dean & Chapman, 1973; Seo *et al.*, 1982; Stoffolano & Yin, 1987; Liquido *et al.*, 1989; Liquido & Cunningham, 1990; Liquido, 1991). Unripe fruits may be too hard for successful penetration by the fly ovipositor (Pritchard, 1969; Averill & Porkopy, 1989), whereas overripe or rotting fruits may be less likely to stimulate egg-laying following ovipositor insertion (Prokopy & Boller, 1971; Smith, 1984; Girolami *et al.*, 1986). The effect of the degree of ripeness on the activity of *B. dorsalis* was reported to differ with the type of host. On papaya, *Carica* sp., the number of oviposition attempts varied with ripeness: 10.8 on ripe and 1.7 on mature green fruits (Seo *et al.* , 1982). But on Japanese plum, *Prunus salicina* (Lindl.), female *B. dorsalis* oviposited the same number of eggs irrespective of the degree of ripeness (Shimada *et al.* , 1981). In both experiments, however, larvae hatching from eggs laid in ripe fruits developed faster than those in unripe fruit. Infestation of papayas by oriental fruit fly has been reported to be highly correlated with fruit ripeness (Seo *et al.* , 1982; Liquido *et al.* , 1989) based on field collection of fruits found to be infested and subsequent ripeness determination based on fruit skin color (Liquido & Cunningham, 1990). In behavioral assays, fruit phenology had only a minor effect on fruit acceptance by the apple maggot, *Rhagoletis pomonella* (Walsh); ovipositing females were less likely to attempt oviposition on immature hawthorn or cherry fruit but failed to discriminate between nearly ripe and ripe fruits (Messina, 1989; Messina & Jones, 1990). In flight tunnel studies, laboratory-reared females of *B. dorsalis* were tested to respond to any three papaya odors (mature green, color-break to one-fourth ripe and one-half to full ripe) emanating from spherical fruit models. Females spent more total time and showed a higher modal fly density, the density of flies which were most often present on the sphere, to ripe fruits than to mature green fruits. The total eggs laid in spheres emitting ripe papaya odor were greater

than the eggs counts for the other two odor classes. In the field, ripe fruits harbored more wild oriental fruit flies than green papayas (Jang & Light, 1991).

The eggs, larvae and pupae of fruit flies are attacked by a number of parasitic Hymenoptera which constitute the majority of natural enemies of the flies. The Family Braconidae ranks first in number with sixteen species. They are composed mainly of the opiines, seven species of which had been recorded from Malaysia (van den Bosch & Haramoto, 1951; Christenson & Foote, 1960; Clausen, 1972; Deulucci, 1976; Wharton & Gilstrap, 1983; Ooi, 1984; Vijaysegaran, 1984; Rohani, 1986; Serit *et. al.* , 1986; Udayagiri, 1987; Wharton, 1989; Palacio, 1991; Ramadan *et al*. , 1995). The species observed parasitizing *Bactrocera dorsalis* in star fruit villages and orchards in Malaysia included; *Fobius* (Synonym = *Biosteres*) *arisanus* (Sonan), *Diachasmimorpha longicaudatus* (Ashmead), *Psytallia* (Synonym = *Opius*) *fletcheri* (Silvestri), *Psytallia* (Synonym = *Opius*) *incisi* (Silvestri), *Biosteres vandenboschi* (Fullaway), *Biosteres skinneri* (Fullaway)(Ooi, 1984; Vijaysegaran, 1984; Rohani, 1986; Serit *et al*. , 1986; Serit 1987; Palacio *et al*. , 1992, Ibrahim *et al.* , 1994). While, the only natural enemy recorded for *Bactrocera umbrosa* was *Pilinothrix* sp. (Hymenoptera :Cynipidae)(Yunus & Ho, 1980).

Fobius arisanus (Plate 2.1) is the only egg-larval parasitoid known in the Opiinae (Wharton & Gilstrap, 1983). All the immatures formed from the parasitized eggs would eventually be killed by *F. arisanus* at the pupal stage and the adult parasitoids emerge. *Boisteres vandenboschi* (Plate 2.2) oviposits in the first instar larvae of *Bactrocera dorsalis*. The parasitized larvae can develop normally but are eventually killed at the pupal stage. The major contribution of *B. vandenboschi* in biological control of tephritid fruit flies is its ability to parasitize and successfully develop in seven different species of tephritid pests (Wharton & Gilstrap, 1983) and its preferential oviposition in vulnerable host stages (first and second instars) that occur near the fruit surface (van den Bosch & Haramoto, 1953; Ramadan *et a*l., 1995). *Diachasmimorpha longicaudatus* Ashmead (Plate 2.3) is a solitary larval-pupal endoparasitoid of a number of economically important tephritid fruit fly species (Clausen *et al.* , 1965; Greany *et al.* , 1976). It parasitizes the second and third larval instars,

normally encountering them in decompositing fruit. Host finding by female D. *longicaudatus* involves attraction to fermentation products liberated from the rotting fruits, a probable site for location of host larvae, rather than to kairomone from the host larvae (Greany *et al.* , 1977). It was observed that the development of *D. longicaudatus* eggs and larvae were inhibited when its hosts contained either *F. arisanus* or *B. vandenboschi*, while *F. arisanus* out-competes *B. vandenboschi* and *D. longicaudatus* inside the larval host (van den Bosch & Haramoto, 1953). *Psytallia fletcheri* (Plate 2.4) was originally recorded on *Bactrocera cucurbitae* in India (Silvestri, 1916; Pruthi, 1937). It became the most important parasitoid of *B. cucurbitae* in Hawaii (Fullaway, 1920; Swezey, 1928). Its presence in star fruit on *Bactrocera dorsalis* in Malaysia was first reported by Vijaysegaran (1984).

Plate 2.1: *Fobius (Biosteres) arisanus* (Sonan) a parasitoid of *Bactrocera umbrosa* eggs

Plate 2.2: *Biosteres vandenboschi* (Fullaway) – a parasitoid of the first instar larvae of *Bactrocera umbrosa*

Plate 2.3: *Diachasmimorpha longicaudatus* (Ashmend)a parasitoid of the second and third instar larvae of *Bactrocera umbrosa*

Plate 2.4: *Psytallia (Biosteres) fletcheri* (Silvestri) – a parasitoid of all instars of *Bactrocera umbrosa*

Species composition and effectiveness of a particular species of opiine parasitoid could vary depending on the locality and type of fruit attacked. Fruit species, size and ripeness influences parasitization of fruit fly larvae (Mainland *et al.*, 1950; van den Bosch & Haramoto, 1953; Hinckly, 1965; Gonzalez, 1975; Wharton *et al*., 1981, Nishida *et al*., 1985; Harris & Lee, 1986; Wong & Ramadan, 1995). Great parasitization levels by *Diachasmimorpha longicaudatus* have been recorded from small fruits such as coffee berries, *Coffee arabia* L. (Harris *et al.* , 1986), loquat, *Ertobtrya japonica* (Lindl.), and peaches *Prunus persica* L. (Wong *et al.* , 1984; Wong & Ramadan, 1987) than from large citrus fruits (Wharton *et al.* , 1981; Harris *et al.* , 1986 and 1988; Harris & Bautista, 1996). Some parasitoids showed a marked preference for certain types of host fruits as reflected by varying levels of parasitization on their tephritid hosts. However, Palacio (1991) found that the consistent of abundance and consequently the level of parasitization among the four species of parasitoids in infested star fruit suggested the absene of species-specific preference for a particular canopy level or fruit size. The levels of parasitization due to each species were also unchanged in all four classes of host fruits.

The opiine parasitoid complex of *Bactrocera dorsalis* in star fruits seemed to vary with habitat and from place to place. In separate field studies on the parasitoid composition of *B. dorsalis*

in different star fruit orchards at Serdang, Selangor, Ooi (1984) recorded *Diachasmimorpha longicaudatus, Biosteres vandenboschi* and *Fobius insici*. Of the three species, he found that *B. vandenboschi* was the predominant parasitoid, followed by (in order of importance) *P. incisi* and *D. longicaudatus*. These differed in composition from those observed by Vijaysegaran (1984) which included *F. arisanus, B. persulcatus* and *P. fletcheri*, in addition to *D. longicaudatus*. In star fruit orchard at Serdang, although the combined parasitism of puparia by the various species ranged from 15.1 to 56.8% with a mean slightly over 28%, no economic control was exerted by these parasitoids (Vijaysegaran, 1984). In a decreasing order of abundance, the four opiine parasitoids in star fruit at Penang, West Malaysia were : *F. arisanus* > *B. vandenboschi* > *D. longicaudatus* > *B. skinneri* (Serit *et al.* , 1986; Serit, 1987). Based on the relative abundance of adults that emerged from puparia reared in a 500 grams sample of infested fruits, four species of parasitoids were associated with *B. dorsalis* in the star fruit orchard of the Universiti Putra Malaysia (UPM) at Puchong, Selangor. These parasitoids were dominated by *B. persulcatus*. The level and relative contribution to parasitization by each species were *B. persulcatus,* 46.53 > *F. arisanus,* 32.82 > *D. longicaudatus,* 15.69 > *P. fletcheri,* 4.95%. Together, these parasitoids caused an average overall parasitization of 36.96% (Palacio, 1991).

Trapping adult flies with the use of methyl eugenol as an attractant is the common procedure for assessing the field populations of *Bactrocera dorsalis, B. papayae, B. carambolae,* and *B. umbrosa* (Batra, 1964; Steiner, 1969; Harris *e. al.*, 1971; Tan & Lee, 1982; Tan, 1984;Tan & Serit, 1988; Serit, 1987; Tan & Serit, 1994).

There are however some problem encountered relating to the use of methyl eugenol in the mark release-recapture (MRR) method. These problems originated from the inability of methyl eugenol to attract both sexes of *B. umbrosa*, *B. papayae*, *B. dorsalis* or *B. carambolae*. Consequently, efforts were made to fully utilize the male estimated populations to predict the female populations. Many researchers and authors just assume that fruit fly males and females exist in equal numbers in natural populations. However, unless males and females of *B. umbrosa* are

produced in similar proportions and exhibit a good 1:1 sex ratio in the field, the above assumption is invalid.

Besides the principal objective, a number of laboratory studies were conducted to provide valuable information on *B. umbrosa* that would assist understanding the most probable factors related to population changes in numbers and serve as guidelines for future studies. Such studies have not been conducted for *B. umbrosa*. Laboratory studies included;

1) Laboratory cultures of the insect from jack fruit and
2) Determination of adult longevity and survival

Laboratory cultures of fruits aimed to assess (1) the proportion of male and female flies of *B. umbrosa* in the field produced by host jack fruit, (2) species composition and abundance of parasitoids associated with *B. umbrosa* larvae in the fruits, and (3) the relationship between the physical parameters of fruit and the production of *B. umbrosa* larvae and adults to provide information on the most succeptible fruit stage to infestation.

2.2 Materials and Methods

2.2.1 Larval infestation of fruits and laboratory cultures of the insect from jack fruit

This experiment was conducted from March 20, 1998 to August 27, 1999 in Sungai Burung village at Balik Pulau in an area measured *ca.* 5 hectars where no insecticides had been used for insect pest control. A description of the village is presented in section 4.2.1. Prior to fruit collection, the maturity of jack fruit was divided into 5 stages, based on fruit size, color and the visual estimation of the degree of ripeness in comparison to a fully grown healthy fruits as follows; small green, intermediate green, mature green, ripe fruits that were still on trees, and dropped but ripe fruits (Chapter 5). Mature green fruits had no apparent yellow color while ripe fruits showed this color.

Sampling of damaged host fruits was done on a weekly basis. On each sampling occasion, a weekly average of 5 infested fruits was randomly collected depending on fruit density and availability. The weekly samples do represent changes in host density over the period of the experiment. Sampling was done on 76 occasions on the same day the mark-release-recapture experiment of adult population estimation was conducted (Chapter 4). A total of 382 infested fruits from 4 different fruit stages; 128 intermediate green, 102 mature green, 133 ripe and 19 ripe dropped fruits recently fallen to the ground as indicated by the relative firmness and color of the skin, were collected over the entire study period. These fruits were infested by *B. umbrosa* eggs and larvae but were free from obvious defects or diseases. Damaged fruits which were not intact, were partially eaten by mammals or birds, or possessed larval exit holes were discarded and not included in the final collection. However, there was some evidence that some larvae had escaped from a few fruits before sampling, which was unavoidable.

The sampled fruits were brought back to the laboratory. Within 2-3 hours of collection, three fruit traits were measured; weight, length and the number of ovipunctures on the fruit which was determined through absolute counting. The fruits werer held individually in plastic containers and kept under the laboratory conditions (27-30^{o}C and 80±5%RH, and a natural photoperiod of 12L: 12 D cycle) separately to allow the viable eggs inside to hatch or the larvae to develop to maturity. Some fruits that contained matured larvae were dissected immediately.

Throughout the fruit culturing process, some precautions were taken to ensure a high survival rate of all stages of *B. umbrosa*. This included occasional moistening of hardened fruits in order to facilitate easy passage of matured third instar larvae while moving out of them. After several days, individual fruits were carefully dissected and the mature third instar larvae (in the interval between emergence from the fruit and pupation) from each fruit were gently removed, counted and allowed to pupate together in a layer of moist sterilized sand provided in a rearing plastic container. In many cases, it was only enough to shake the fruit to get all matured larvae emerge from the fruit. The first and second instar larvae could not be detected and mostly were

inevitably missed since there was uniform larval development within each fruit. Fruits were discarded in the absence of live larvae. Larvae in every fruit were counted separately, and an average number of larvae per fruit was estimated from the total number of larvae over the total number of fruits dissected and contained live larvae (the average number of larvae per fruit was used in chapter 6 to estimate the total larvae in the village for the respective week by multiplying the average number of larvae per fruit times the total weekly number of damaged fruits in the village).

Matured larvae from each fruit pupated separately in the sand which was moistened on different occasions. Duration of the pupal stage was determined under laboratory condition (27-30^{o}C, 80±5%RH and a photoperiod of 12 L: 12 D cycle). Numbers of fruit fly adults and parasitoids that emerged from each fruit were recorded. Identification of parasitoids that emerged was done following the key published by Wharton & Gilstrap (1983). Newly emerged adult flies were transferred to insect cages provided with food and water. Sexing was done 2 to 3 days after emergence and females and males emerging from each of 62 fruits selected randomly were counted.

To determine the longevity of female and male adults of *B. umbrosa* under laboratory conditions, infested jack fruits were collected from Sungai Burung village and adult insects used in this experiment were reared from mature third instar larvae allowed to pupate in plastic containers containing moist sand. Upon adult emergence, 100 females and 100 males were transferred separately to insect cages 45cm x 45 cm x 45 cm provided with distilled water and food and maintained under laboratory condition of 27-30^{o}C, 80±5%RH and natural photoperiod. In the laboratory, adult food was prepared by mixing sucrose, vitamins, yeast extract and distilled water in the proportion of 3:1:3:3 to form a homogenous suspension. Before use, the adult food was dispensed into double-ply filter papers placed on a petri-dish and allowed to air-dry overnight under the room temperature. The food was replaced every 3-5 days. The number of dead flies in each cage was recorded daily. The experiment continued until the last adult insect died in the cages.

2.2.2 Data analysis

For analysis, the average live larvae per damaged jack fruit were determined. In addition, the averages of the following parameters were also determined per damaged jack fruit: (a) *B. umbrosa* adults; (b) adults of parasitoid species; and (c) length, weight and ovipunctures of damaged fruits. The percentage of parasitism of *B. umbrosa* was calculated. The number of live larvae and adults of *B. umbrosa* per gram of jack fruit were determined. The mean number of males and females per fruit were determined for the randomly selected 62 fruit sample. The ratio of male to female flies produced by each fruit was determined. The physical parameters of fruit (weight, length and total ovipunctures per fruit) were correlated with the total number of larvae and adults each fruit produced. Comparisons of means were performed using ANOVA and Tukey's test of unequal sample size.

The longevity of female and male flies were determined in days under the laboratory conditions. Mean daily mortality of male and female flies were calculated and compared using a Student *t*-test. Fifty percent (50%) adult female and male mortalities were determined. Survivorship curves for adult male and female *B. umbrosa* were determined from percent survival of adult flies plotted against their age intervals.

2.3 Results

2.3.1 Laboratory cultures of fruits

Jack fruits were only damaged by *B. umbrosa* at the intermediate, mature green, ripe and the dropped stages. Small green fruits did not have ovipunctures or *B. umbrosa* immatures in them.

Mean weight (gm) per fruit for total jack fruits sampled was 2583.861 ± 59.821. Mean weight per fruit of the different fruit stages were 1375.253 ± 36.382 for the intermediates, 2494.804 ± 47.627 for the mature greens, 3578.308 ± 67.115 for the ripes, and 4242.895 ±