

PHEROMONES OF FRUIT FLIES (*Bactrocera spp.*) AND
THE GLANDULAR ANATOMY OF WILD ORCHIDS (*Bulbophyllum spp.*)
THAT ATTRACT THE FRUIT FLIES.

by

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*To my late parents & grandmother,
"..... for the love and wisdom. "*

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CONTENTS

	<u>PAGE</u>
List of Tables	vi
List of Figures	vii
List of Plates	ix
List of Appendices	xii
Abstrak	xiv
Abstract	xvi
1.0 INTRODUCTION AND REVIEW OF LITERATURE.	1
1.1 Insect semiochemicals.	1
1.2 The ecology of fruit flies (<i>Bactrocera spp.</i>).	5
1.3 Control and eradication of fruit flies.	7
1.4 Pheromones and attractants.	12
1.5 Plants - insects relationships.	17
1.6 Wild orchids (<i>Bulbophyllum spp.</i>)	21
2.0 OBJECTIVES.	24
3.0 MATERIALS AND METHODS.	26
3.1 Accumulation and identification of pheromones.	26
3.1.1 Breeding of fruit flies.	26
3.1.2 Isolation of males.	27
3.1.3 Treatment and control males.	27
3.1.4 Extraction of rectal glands.	28
3.1.5 Identification of pheromones (GC / GC-MS).	30
3.1.6 Quantification of pheromones	31

3.2	Anatomical study of the floral parts and leaves of <i>Bu. cheiri</i> , <i>Bu. macranthum</i> and <i>Bu. patens</i> .	33
3.2.1	Histology	34
3.2.2	Scanning Electron Microscope (SEM).	37
4.0	RESULTS AND DISCUSSION	46
4.1	General structure of the sex pheromone gland in males of <i>Bactrocera</i> spp.	46
4.2	Identification of pheromones produced by males of <i>Bactrocera</i> spp.	54
4.2.1	Identification of pheromones produced by <i>B. papayae</i> males.	55
4.2.2	Identification of pheromones produced by <i>B. dorsalis</i> males.	57
4.2.3	Identification of pheromones produced by <i>B. carambolae</i> males.	60
4.2.4	Identification of pheromones produced by <i>B. umbrosa</i> males.	61
4.3	Glandular anatomy of the floral parts and leaf surfaces.	84
5.0	SUMMARY	111
5.1	Conclusion	111
5.2	Suggestions	113
6.0	REFERENCES	114
6.1	Bibliography	114
6.2	Appendices	125

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1.1 Classification of chemicals according to the types of behaviour induced in living organisms (Dethier, 1970).	4
1.2 Summary of insect attractants.	11
1.3 Attraction of ethyl ether extracts of 232 botanicals to Oriental fruit flies, Melon flies and Mediterranean fruit flies (Keiser <i>et al.</i> , 1975).	16

LIST OF FIGURES

Figure	Page
3.1 Typical orchid flower (frontal view).	39
4.1 Sex pheromone gland (Fletcher, 1969).	49
4.2 Gas chromatogram of the rectal gland complex of <i>Bactrocera papayae</i> males unfed (as controls).	63
4.3 Gas chromatogram of volatiles in the rectal gland complex of <i>Bactrocera papayae</i> males fed with methyl eugenol [1 day post - treatment].	64
4.4 Relationship between the pheromones excised from the male rectal glands of <i>Bactrocera papayae</i> fed with methyl eugenol and the time of analysis.	65
4.5 Gas chromatogram of volatiles in the rectal gland complex of <i>Bactrocera papayae</i> males fed with eugenol [30 minutes post - treatment].	66
4.6 Relationship between the pheromones excised from the male rectal glands of <i>Bactrocera papayae</i> fed with eugenol and the time of analysis.	67
4.7 Gas chromatogram of the rectal gland complex of <i>Bactrocera dorsalis</i> males unfed [as controls].	68
4.8 Gas chromatogram of volatiles in the rectal gland complex of <i>Bactrocera dorsalis</i> males fed with methyl eugenol [1 hour post - treatment].	69
4.9 Gas chromatogram of volatiles in the rectal gland complex of <i>Bactrocera dorsalis</i> males fed with methyl eugenol [1 day post - treatment].	70
4.10 Relationship between the pheromones excised from the male rectal glands of <i>Bactrocera dorsalis</i> fed with methyl eugenol and the time of analysis.	71
4.11 Gas chromatogram of volatiles in the rectal gland complex of <i>Bactrocera dorsalis</i> males fed with eugenol [6 hours post - treatment].	72
4.12 Relationship between the pheromones excised from the male rectal glands of <i>Bactrocera dorsalis</i> fed with eugenol and the time of analysis.	73
4.13 Gas chromatogram of the rectal gland complex of <i>Bactrocera carambolae</i> males unfed [as controls].	74

LIST OF FIGURES

Figure	Page
4.14 Relationship between the pheromones excised from the male rectal glands of <i>Bactrocera carambolae</i> controls (unfed) and the time of analysis.	75
4.15 Gas chromatogram of volatiles in the rectal gland complex of <i>Bactrocera carambolae</i> males fed with methyl eugenol and the time of analysis.	76
4.16 Relationship between the pheromones excised from the male rectal glands of <i>Bactrocera carambolae</i> fed with methyl eugenol and the time of analysis.	77
4.17 Gas chromatogram of volatiles in the rectal gland complex of <i>Bactrocera carambolae</i> males fed with eugenol [1 day post - treatment].	78
4.18 Relationship between the pheromones excised from the male rectal glands of <i>Bactrocera carambolae</i> fed with eugenol and the time of analysis.	79
4.19 Gas chromatogram of the rectal gland complex of <i>Bactrocera umbrosa</i> males unfed [as controls].	80
4.20 Gas chromatogram of volatiles in the rectal gland complex of <i>Bactrocera umbrosa</i> males fed with methyl eugenol [3 hours post - treatment].	81
4.21 Relationship between the pheromones excised from the male rectal glands of <i>Bactrocera umbrosa</i> fed with methyl eugenol and the time of analysis.	82
4.22 Gas chromatogram of volatiles in the rectal gland complex of <i>Bactrocera umbrosa</i> males fed with eugenol [1 day post - treatment].	83
4.23 Structure of gland on the leaf surface of <i>Bulbophyllum cheiri</i> , <i>Bulbophyllum macranthum</i> and <i>Bulbophyllum patens</i> .	90
4.24 Structure of gland on the lip of <i>Bulbophyllum cheiri</i> , <i>Bulbophyllum macranthum</i> and <i>Bulbophyllum patens</i> .	95
4.25 Structure of gland on the column of <i>Bulbophyllum cheiri</i> , <i>Bulbophyllum macranthum</i> and <i>Bulbophyllum patens</i> .	99
4.26 Structure of gland on the sepals and petals of <i>Bulbophyllum cheiri</i> , <i>Bulbophyllum macranthum</i> and <i>Bulbophyllum patens</i> .	108

LIST OF PLATES

Plate		Page
3.1	Storage of the extracted rectal glands for GC and GC-MS analysis.	40
3.2	Gas Chromatograph (SHIMADZU GC-14A).	41
3.3	Gas Chromatograph (5890 Series II, HEWLETT PACKARD) - Mass Spectrometer (589 A HEWLETT PACKARD) / [GC - MS].	42
3.4	Freeze - dryer (EMITECH K750).	43
3.5	SEM Coating System, Sputter Coater (POLARON SC 515).	44
3.6	Scanning Electron Microscope (LEICA CAMBRIDGE 360).	45
4.1	Extracted rectal gland of <i>Bactrocera papayae</i> (unfed / control).	50
4.2	Extracted rectal gland of <i>Bactrocera papayae</i> (methyl eugenol fed).	50
4.3	Extracted rectal gland of <i>Bactrocera papayae</i> (eugenol fed).	50
4.4	Extracted rectal gland of <i>Bactrocera carambolae</i> (unfed / control).	51
4.5	Extracted rectal gland of <i>Bactrocera carambolae</i> (methyl eugenol fed).	51
4.6	Extracted rectal gland of <i>Bactrocera carambolae</i> (eugenol fed).	51
4.7	Extracted rectal gland of <i>Bactrocera umbrosa</i> (unfed / control).	52
4.8	Extracted rectal gland of <i>Bactrocera umbrosa</i> (methyl eugenol fed).	52
4.9	Extracted rectal gland of <i>Bactrocera umbrosa</i> (eugenol fed).	52
4.10	Extracted rectal gland of <i>Bactrocera dorsalis</i> (unfed / control).	53
4.11	Extracted rectal gland of <i>Bactrocera dorsalis</i> (methyl eugenol fed).	53
4.12	Extracted rectal gland of <i>Bactrocera dorsalis</i> (eugenol fed).	53
4.13	<i>Bulbophyllum patens</i> King.	88
4.14	<i>Bulbophyllum cheiri</i> Lindl.	89

LIST OF PLATES

Plate		Page
4.15	Histological sections of glands on the leaves of <i>Bu. cheiri</i> , <i>Bu. macranthum</i> and <i>Bu. patens</i>	91
4.16	Scanning Electron Micrograph of the glands and stomata on the leaves of <i>Bu. cheiri</i> , <i>Bu. macranthum</i> and <i>Bu. patens</i> .	92
4.17	Scanning Electron Micrograph of the glands on the leaves of <i>Bu. cheiri</i> , <i>Bu. macranthum</i> and <i>Bu. patens</i> .	93
4.18	Scanning Electron Micrograph of the stomata on the leaves of <i>Bu. cheiri</i> , <i>Bu. macranthum</i> and <i>Bu. patens</i> .	94
4.19	Histological sections of glands on the lip of <i>Bu. cheiri</i> , <i>Bu. macranthum</i> and <i>Bu. patens</i> .	96
4.20	Scanning Electron Micrograph of the glands on the lip of <i>Bu. cheiri</i> , <i>Bu. macranthum</i> and <i>Bu. patens</i> .	97
4.21	Scanning Electron Micrograph of the glands on the lip of <i>Bu. cheiri</i> , <i>Bu. macranthum</i> and <i>Bu. patens</i> .	98
4.22	Histological sections of glands on the column of <i>Bu. cheiri</i> .	100
4.23	Histological sections of glands on the column of <i>Bu. macranthum</i> .	101
4.24	Histological sections of glands on the column of <i>Bu. patens</i> .	102
4.25	Scanning Electron Micrograph of the cup where the pollinarium is deposited in the column of <i>Bu. cheiri</i> .	103
4.26	Scanning Electron Micrograph of the glands on the column of <i>Bu. cheiri</i> .	104
4.27	Scanning Electron Micrograph of the glands on the column of <i>Bu. patens</i>	105
4.28	Scanning Electron Micrograph of the glands on the column of <i>Bu. macranthum</i> .	106
4.29	Scanning Electron Micrograph of the glands on the column of <i>Bu. macranthum</i> .	107

LIST OF PLATES

<u>Plate</u>		<u>Page</u>
4.30	Histological sections of glands on the sepals and petals of <i>Bu. cheiri</i> , <i>Bu. macranthum</i> and <i>Bu. patens</i> .	109
4.31	Scanning Electron Micrograph of glands on the sepals and petals of <i>Bu. cheiri</i> , <i>Bu. macranthum</i> and <i>Bu. patens</i> .	110

LIST OF APPENDICES

<u>Appendix</u>	<u>Page</u>
1. <i>Bactrocera dorsalis</i> Hendel.	125
2. <i>Bactrocera carambolae</i> Drew and Hancock.	126
3. <i>Bactrocera papayae</i> Drew and Hancock.	127
4. <i>Bactrocera umbrosa</i> Fabricius.	128
5. Males of <i>Bactrocera cucurbitae</i> feeding on the sepals and petals of the orchid <i>Dendrobium anosmum</i> .	129
6. Males of <i>Bactrocera papayae</i> feeding on the spike of <i>Spathiphyllum cannaefolium</i> .	130
7. Gas chromatograph - mass spectrometry analysis of the chemical compounds in <i>Bulbophyllum cheiri</i> Lindl.	131
8. Preparation of Formol Acetic Alcohol (FAA) fixative.	132
9. Preparation of Ethanol : TBA solutions.	132
10. Preparation of Cacodylate Buffer.	132
11. Identification of A : N-3-methylbutyl acetamide.	133
12. Identification of B : 2-allyl-4,5-dimethoxyphenol (Compound Z).	134
13. Identification of C : <i>cis</i> -3,4-dimethoxycinnamyl alcohol.	135
14. Identification of E : eugenol	136
15. Identification of G : 4-(3-hydroxy-1-propenyl)-2-methoxyphenol (Coniferyl alcohol).	137
16. Identification of P : 6-oxo-1-nonanol.	138
17. Identification of ME : methyl eugenol.	139
18. Concentration of pheromones excised from the male rectal glands of <i>Bactrocera papayae</i> fed with methyl eugenol / eugenol and the time of analysis.	140

LIST OF APPENDICES

Appendix	Page
19. Concentration of pheromones excised from the male rectal glands of <i>Bactrocera dorsalis</i> fed with methyl eugenol / eugenol and the time of analysis.	141
20. Concentration of pheromones excised from the male rectal glands of <i>Bactrocera carambolae</i> controls (unfed) / methyl eugenol fed / eugenol fed and the time of analysis.	142
21. Concentration of pheromones excised from the male rectal glands of <i>Bactrocera umbrosa</i> fed with methyl eugenol and the time of analysis.	143
22. Types of stomatal complexes (Metcalf and Chalk, 1950; Mauseth, 1988).	144
23. Types of leaves [i] and floral parts [ii] (Vermeulen, 1991).	145
24. Shapes of leaves and floral parts (Vermeulen, 1991).	146

**FEROMON LALAT BUAH, *Bactrocera* spp. DAN ANATOMI KELENJAR
PADA BUNGA ANGERIK LIAR, *Bulbophyllum* spp. YANG
MENARIK LALAT BUAH.**

ABSTRAK

Komponen kelenjar rektum lalat buah *Bactrocera dorsalis*, *Bactrocera papayae*, *Bactrocera carambolae* dan *Bactrocera umbrosa* yang telah diberi makan metil eugenol dan eugenol telah diisolat dan dikenalpasti. Hasil dari kajian ini juga mendapati metil eugenol adalah bahan penarik lalat buah yang lebih cekap berbanding dengan eugenol. Komponen utama kelenjar rektum lalat buah *Bactrocera* spp. yang tidak diberi makan metil eugenol atau eugenol adalah asid - asid lemak yang tepu dan tidak tepu serta berantai panjang, spiroacetal, ketol, ester dan amida.

Dua fenilpropanoid, iaitu 2-alil-4,5-dimetoksifenol (Sebatian Z) dan 4-(3-hidroksi-1-propenil)-2-metoksifenol atau koniferil alkohol, serta satu amida, N-3-metilbutil acetamida, telah dicamkan sebagai komponen utama kelenjar rektum keempat - empat spesis lalat buah *Bactrocera* dalam kajian ini apabila lalat - lalat buah ini diberi makan metil eugenol. Sebatian 6-oxo-1-nonanol pula di dapati hanya wujud dalam kelenjar rektum *Bactrocera carambolae*, dan ianya dijumpai dalam kuantiti yang banyak di dalam kelenjar rektum lalat buah jantan yang matang. Dengan ini, ianya telah dicamkan sebagai feromon yang dihasilkan secara semula jadi di dalam kelenjar rektum *B. carambolae* jantan. Fenilpropanoid yang seterusnya, *cis* 3,4-dimetoksisinamil alkohol pula, hanya dijumpai di dalam kelenjar rektum *B. dorsalis* dan *B. umbrosa*. Hanya 2-alil-4,5-dimetoksifenol telah dikenalpasti apabila lalat buah *B. papayae* diberi makan eugenol. Ketiga - tiga spesis lalat buah yang lain di dalam kajian ini tidak menghasilkan sebarang feromon apabila diberi makan eugenol, kecuali untuk peningkatan di

dalam feromon yang dihasilkan secara semula jadi seperti pada *B. carambolae*. Dalam erti kata lain, metil eugenol dan eugenol berperanan sebagai prekursor dalam penghasilan dan pengeluaran feromon di dalam kelenjar rektum keempat - empat spesies lalat buah dalam kajian ini .

Suatu pemerhatian terperinci anatomi kelenjar pada tiga spesies bunga anggerik liar, *Bulbophyllum cheiri*, *Bulbophyllum patens* dan *Bulbophyllum macranthum* telah dijalankan. Kelenjar - kelenjar dengan ciri yang sama dan taburan yang spesifik telah dicamkan pada bahagian - bahagian tertentu pada bunga dan daun ketiga - tiga bunga anggerik ini. Kelenjar yang pertama hanya dijumpai pada bahagian daun dan jenis kedua pula dijumpai pada bahagian bibir (lip) bunga manakala jenis kelenjar yang ketiga di dapati pada bahagian lajur (column). Bahagian sepal dan petal (kelopak) bunga pula mempunyai jenis kelenjar yang keempat .

ABSTRACT

The rectal gland secretions of males of *Bactrocera dorsalis*, *Bactrocera papayae*, *Bactrocera carambolae* and *Bactrocera umbrosa* that were fed with methyl eugenol and eugenol were isolated and identified. Through this work methyl eugenol has been established as a more potent and efficient attractant compared to eugenol. The major glandular components that occur naturally in these species are long chain saturated and unsaturated fatty acids, spiroacetals, ketols, esters and amides.

Two phenylpropanoids, 2-allyl-4,5-dimethoxyphenol (Compound Z) and 4-(3-hydroxy-1-propenyl)-2-methoxyphenol or coniferyl alcohol, and one amide, N-3-methylbutyl acetamide were identified as the major glandular components of all four *Bactrocera* species in this study when they were treated with methyl eugenol. A compound, 6-oxo-1-nonanol, was identified only in the rectal secretions of *Bactrocera carambolae* and it was found to be the major component in the rectal glands of sexually mature males, and thus it is characterised as a naturally occurring pheromone in *Bactrocera carambolae*. A phenylpropanoid, *cis* 3,4-dimethoxycinnamyl alcohol, was also identified in the rectal secretions of *B. dorsalis* and *B. umbrosa*. Only 2-allyl-4,5-dimethoxyphenol was detected in the rectal glands of males of *B. papayae* that were fed with eugenol. None of the other three species in this study produced any form of pheromones when fed with eugenol, except for the increase in the naturally occurring pheromone in *B. carambolae*. In other words, both methyl eugenol and eugenol take on the role of precursor in the manufacture and production of pheromones in the male rectal glands of all four species.

A detailed study on the glandular anatomy of *Bulbophyllum cheiri*, *Bulbophyllum macranthum* and *Bulbophyllum patens* is also described. Glands with similar characteristics

were found to occur with an even and restricted distribution on the vegetative and reproductive parts of all three species. The first type of gland exists primarily on the leaves of all three species and the second type of gland is found on the lip, whereas the third type of gland is found on the column. The sepals and petals possess a similar gland, the fourth type.

1.0 Introduction and Review of Literature

1.1 Insect semiochemicals

In the early evolution of animal communication, chemical messages were probably the first signals put to use (Wilson, 1970). Today, chemical communication appears to be the primary mode of information transfer in most groups of animals (Birch, 1974; Shorey, 1977). These chemicals are used for such diverse functions as location of prey, avoidance of predators, signalling to conspecific mates prior to mating, warning to other individuals of the same species in times of danger, or even during location of nests or habitats. Behavioural patterns for all of these functions are strongly modulated by chemical cues which are major sources of information used by most insects to interpret environmental stimuli and, their reliance on chemical stimuli undoubtedly stems from the development of chemosensory organs and cells early in evolutionary history (Nation, 1977).

Generally, these chemical stimuli are known by their common term of semiochemicals, and they are usually volatile and affect the behaviour of a receptive animal or insect at submicrogram or nanogram levels (Tan, 1993). Most of these chemicals used in communication have not been satisfactorily defined and most investigators now classify them as pheromones, allomones, kairomones, synomones, or apneumones. These terms should not be confused with, or misinterpreted as insect hormones or insect juvenile hormones.

A kairomone is a chemical or a mixture of chemicals that is released from one organism and that induces a response by an individual of another species and the response is adaptively favourable to the recipient (Brown *et al.*, 1971).

An allomone is a chemical or a chemical mixture that is released by one organism and induces a response by an individual of another species where the response is adaptively favourable to the emitter.

A pheromone is a chemical or a mixture of chemicals which are secreted into the environment by an organism to influence the behaviour or physiological processes of other members of its species. Pheromones, stems from the Greek, *pherein* (to carry) and *horman* (to excite, to stimulate) and are the actual chemical language of insects and their means of communication (Tahori 1971). These chemical messengers are also called assembling scents (Kettlewell, 1961). The term pheromone was first coined by Karlson and Luscher (1959) and Karlson and Butenandt (1959), and modifications to the definition were proposed by Kalmus (1965).

Kairomones and allomones are used at the inter-specific level while pheromones are used for intra-specific communication. However, there is considerable overlap between these classes of chemicals, and often the same compounds serve both intra and inter-specific functions. The response induced by these chemicals may be immediate behavioural reactions or long-lasting physiological changes. These chemicals may also be synthesised *de novo* or acquired intact by the organisms from their food or other aspects of the environment.

Certain plants, have been seized upon by certain herbivores and insects that possess an ability to detoxify chemicals produced by the plants and use these chemicals as kairomonal stimulants for aggregation or feeding. Females of *Bactrocera papayae*, a common species of the *dorsalis* complex in Malaysia, was found to oviposit on banana (*Musca paradisiaca*), starfruit (*Averrhoa carambola*) and papaya (*Carica papaya*) in order of favourability (Tan, 1993).

Examples of allomones are the defensive secretions that are released by many insects and that are poisonous or repugnant to attacking predators. Many plant species secrete allomones (Dethier, 1970; Whittaker, 1970; Whittaker *et al.*, 1971). These substances, which usually have no other apparent role in the physiology of the plant, have presumably evolved as defense mechanisms against insects, birds and other vertebrate predators (Tan and Nishida, 1992).

Toward this end, many scientists have been intrigued by the possibility of combating insect pests through the use of pheromones. These highly selective chemicals pose no hazard to man and are manufactured by the insects themselves and are relied upon by the insects for the sake of survival.

A wide range of compounds have been identified as insect pheromone components. While carbon, hydrogen, and oxygen are the usual atoms incorporated into these molecules, nitrogenated and chlorinated compounds have also been identified (Tumlinson *et al.*, 1987). Usually, small molecules are used for communication when rapid dispersal of the signal is needed, while larger, less volatile compounds tend to function in attraction and stimulation when prolonged exposure is necessary. In addition to the different molecular sizes which reflect behavioural functions, pheromone structures vary greatly between different orders of insects, and even within orders, tremendous diversity in molecular structure exists.

Another class of chemicals discovered to be attractants or repellents through chemical screening, is also known to occur and these chemicals are categorised according to the types of behaviour that they induce from the organisms. Dethier (1970) erected the following six categories that encompass most behaviourally active chemicals (Table 1.1).

Table 1.1 : Classification of chemicals according to the types of behaviour induced in living organisms (Dethier, 1970).

<u>Classification</u>	<u>Behaviour / Reaction</u>
<u>Locomotory stimulant</u>	Causes kinesis reactions that, in the absence of orientation cues, cause the animals to disperse from an area by increasing the speed of locomotion.
<u>Arrestant</u>	Causes kinesis reactions that, in the absence of orientation cues, often cause the animals to aggregate near the chemical source by decreasing the speed of locomotion.
<u>Attractant</u>	Causes animals to make orientated movements towards its source.
<u>Repellent</u>	Causes animals to make orientated movements away from its source.
<u>Feeding, mating or ovipositional stimulant</u>	Elicits one of these behavioural reactions.
<u>Feeding, mating or ovipositional deterrent</u>	Inhibits one of these behavioral reactions.

Insects have managed to persist in hostile surroundings because they have developed extraordinary adaptations or abilities, one of which is a highly specialised sense of smell. Because many insects depend on their sense of smell for survival, they can frequently be attracted to a trap by a chemical for detection purpose, to a toxicant that destroys them, or to a substance that makes them incapable of fertile mating (Jacobson and Beroza, 1963). Attractants may be classified as sex, food or oviposition lures. A chemical is classified as a sex attractant if it attracts an insect, which then assumes a mating position or attempts to mate with the chemical or with an object on which the chemical has been placed. Sexual odours

released by males are primarily for the purpose of sexually exciting the female, thus making her more receptive to the male's advances.

Certain volatile components from the male salivary glands of the South American fruit fly, *Anastrepha fraterculus* have been found to act not only as an attractant at close range, but also has an arrestant effect on sexually mature females of this species (De Lima *et al.*, 1996).

There is now widespread interest in insect sex pheromones that aggregate one or both sexes (Jacobson, 1972). A trap baited with the sex pheromone of a particular species is a highly specific trap, and under some circumstances, it could be more effective than a food trap. Thus, pheromone-baited traps should be excellent for detection of old and new pest and to assess the progress of eradication attempts.

1.2 The ecology of fruit flies (*Bactrocera* spp.)

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 tropical and temperate regions of the world. The tropical Tephritidae are relatively long-lived, polyphagous with a very wide and expanding host range and they also have a high capacity for dispersal which makes the monitoring and control of these species very difficult.

These fruit flies are serious pests and the damage caused by them are multiple in nature. They infest more than 150 different species of fruit and vegetable crops such as guava, mango, papaya, peach, banana, starfruit, jack fruit, tomato and avocado (Christenson and Foote, 1960).

The ovipositional punctures caused by the female flies lead to sap oozing, discolouration and abnormal growth of the infested fruit. These punctures also provide

entrance holes for various microorganisms (Bess and Haramoto, 1961). The larvae growing inside the infested fruit, feed on the pulp and their tunneling behaviour provide entry into the fruit for other pest organisms. There are more than 700 described species of Dacinae fruit flies, whose immature stages develop in many species of fleshy fruits found in tropical and subtropical rain forests. Most of these species are monophagous or stenophagous, but numerous are polyphagous. The presence of these very destructive species in some regions inhibit the economic development of potential fruit and vegetable crops.

The *Bactrocera dorsalis* complex of fruit flies comprises of at least 52 species (Drew and Hancock, 1994) bearing a taxonomic resemblance to *Bactrocera 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 and Hancock, 1994) and tissue enzyme analysis as well as morphological and biological data, and these advance technologies have enabled taxonomists such as Drew and Hancock (1994) to further unravel the complexities and mysteries of the *dorsalis* complex in Asia. Currently, the latest approach to the determination of species limits has been based on the male rectal gland pheromone analysis (Perkins *et al.*, 1990a, 1990b; Fletcher and Kitching, 1995).

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 Oceania region, and into Australia. There are very few species recorded in Africa and only *Bactrocera oleae* is found in Southern Europe.

Bactrocera dorsalis (Hendel), [Appendix 1], is restricted in habitat to India, Southern China, Taiwan and Hawaii (Drew, 1989), and is not found throughout South East Asia as reported by Newell and Haramoto (1969). *Bactrocera carambolae* Drew and Hancock (previously called Mal. A) [Appendix 2], and *Bactrocera papayae* Drew and Hancock (previously called Mal. B) [Appendix 3] are two of its sibling species (Drew and Hancock, 1994) that occur across Malaysia, Thailand, Indonesia and Singapore. Their status as two separate species has been questioned by Khoo, S.G, Tan, K.H and Yong, H.S., independently (personal communications, 1995). This is because they are able to mate and produce viable offsprings. Furthermore, intermediate forms of these species have also been regularly collected in the field (Tan, personal communication, 1996). *Bactrocera umbrosa* (Fabricius), [Appendix 4], commonly infests jack fruit and other *Artocarpus spp.*, is widely distributed throughout Malaysia, Indonesia, Thailand and Southwest Pacific areas (Hardy, 1983).

1.3 Control and eradication 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. Mechanical methods such as bagging fruits to prevent access of ovipositing females is labour intensive and this can be a limitation (Fullaway, 1948). Besides, in the event of rain, the bags are easily damaged. Although chemical control with pesticides and insecticides was favoured in the early days, the hazardous effects on the environment and living organisms including man himself, have prompted a search for alternative methods of control. Currently integrated pest management, with the combination of several control methods together with biological control is the most favoured approach. This was demonstrated in an epidemic of *Bactrocera dorsalis* Hendel infestation in Hawaii in 1946 (Clancy *et al.*, 1952). Eradication was difficult due to the

continual reinfestation from vast areas of wild hosts, until the discovery of natural enemies such as the larval parasites *Diachasmimorpha longicaudata* (Ashm.), *Biosteres persulcatus* (Silv.), *B. incisi* Silv., and *Syntomosphyrum indicum* Silv. After the success with the larval parasites, alternative predators were also introduced in other cases. This included the staphylinid beetles (Fullaway, 1948) and frugivorous predators such as *Macropygia phasianella* (Temminck), the brown pigeon, and rodents that have been found to be the major natural enemies of larvae of tropical Tephritidae in their endemic habitat (Drew, 1987).

Nevertheless, it cannot be denied that the use of an effective lure and trap are still essential for the control of fruit flies. Mixtures of fermenting food materials, and particularly protein hydrolysates, are routinely used as lures in many parts of the world. In July 1972, an eradication campaign of *Bactrocera tryoni* (Froggatt) infestation in Easter Island was launched using protein hydrolysate bait sprays mixed with insecticides (Bateman *et al.*, 1972). These food lures however, are not specific and normally end up attracting other insects as well. In Hawaii, South Africa and Italy (Christenson, 1958) a stomach poison such as arsenic combined with a food attractant such as molasses were used for the control of the Mediterranean fruit fly. Wheat bran was used in baited containers by Newman (1926) in Australia while sugar, syrup and molasses sprays containing arsenicals were employed in Florida in 1929 to combat the introduced Mediterranean fruit fly (Rohwer, 1958). Kerosene as well as fermenting solutions were employed in traps for the Mediterranean fruit fly in Florida in 1929 and in Australia (Rohwer, 1958).

A lure that was attractive to both sexes of the Mediterranean fruit fly and the Queensland fruit fly, *Bactrocera tryoni* (Froggatt) was made up of a mixture of imitation vanilla essence, household ammonia and water, and said to be effectively used in many orchards

(Jarvis, 1931). Terpinyl acetates were used in a male annihilation attempt against the natal fruit fly, *Ceratitis rosa* (Karsch) in South Africa (Ripley and Hepburn, 1935).

McPhail (1937) had also used in Mexico an invaginated glass trap, still commonly used as a standard for trap design, and a lure for the Mexican fruit fly, *Anastrepha ludens*. This trap uses brown sugar and brewer's yeast to produce an attractive fermenting substance to lure the flies. Among the lures discovered were protein hydrolysate for *Anastrepha spp.* (McPhail, 1939), linseed oil soap and corn oil soap for the melon fly, *Bactrocera cucurbitae* (Coquillett) (McPhail, 1943) and a various mixture of sugar, molasses, brewer's yeast, casein and clensel.

This invaginated glass trap was originally used to trap house flies in China and is better known as the wet trap, and is the standard trap design for liquid lures (McPhail, 1937). These wet traps however have limitations as they normally contain fermenting sugar or decomposing protein solutions, and the captured flies become difficult to discern from the many decomposing blow flies, bottle flies, moths and other insects that also come to the trap. Steiner (1957) developed a low-cost plastic dry trap which has proved to be a most promising trap, where the addition of an insecticide to the lure, kills the flies that enter the trap upon contact thus preserving them intact. The useful sticky trap designs developed by Harris and co-workers (1971) also has drawbacks. The effectiveness of the adhesive declines as it becomes dirty. Tan (1984) designed a fruit fly attractant trap to trap fruit flies without the use of an insecticide and this can be used both for pest surveillance, monitoring, and also mass trapping or for capture-mark-release-recapture studies.

As a move to improve formulations for liquid traps, Lopez and Hernandez (1967) added borax to PIB-7 and to enzyme-hydrolysed cottonseed proteins to discourage putrefaction. Lopez and co-workers (1971) later went on to develop pellets of cottonseed

hydrolysate with borax which are now widely used in the United States detection programmes as they are not only more attractive in solution but also greatly facilitate trap servicing. In using baited traps, it is important to remember that the host tree is the primary attractant, whereas the baited trap is the secondary attractant, and as such, for most effective use in a detection program, the traps should be placed in host trees whenever possible (Farias and Nakagawa, 1970). Proteinaceous food baits in water traps were twenty times more efficient in trapping Oriental fruit flies, medflies and melon flies in a dry climate than in a wet climate (Cunningham *et al.*, 1978a). Traps with different colours and odours (bait) were also found to influence tremendously the number of flies caught (Economopoulos, 1979; Haniotakis and Skyrianos, 1981) although the colour of traps have recently been discovered not to influence the number of flies caught when placed under tree canopies (Tan, personal communication, 1995).

Food-bait spray techniques have been improved tremendously and in 1956, a full coverage bait spray was applied at a rate of 4.5ℓ / 4000m² as part of the Mediterranean fruit fly control campaign in Florida (Steiner *et al.*, 1961). Prior to this, Steiner (1952a) conducted preliminary tests in Hawaii with insecticidal sprays supplemented with sugar and certain protein hydrolysates that were highly effective in controlling infestations of the Oriental fruit fly and the medfly.

Attractants have played an important role in control programmes (Steiner *et al.*, 1961). Research in this field has brought forth the discovery of a few highly effective and specific attractants. Specimens of Dacini were found to be specific in their response to the types of attractants (Drew, 1974; Drew and Hooper, 1981). A summary of insect attractants are shown in Table (1.2) .

Table 1. 2 : Summary of Insect Attractants

<u>Lure</u>	<u>Insect attracted</u>
Sig lure	^(a) Medfly.
Butyl sorbate	^(a) European chafer, <i>Amphimallon majalis</i> (Razoumowsky)
Trimed lure	^(a) males of the Mediterranean fruit fly and the natal fly.
Cue-lure	^(a) males of the melon fly, <i>Bactrocera cucurbitae</i> Coquillett.
Methyl eugenol	^(b) males of the Oriental fruit fly, <i>Bactrocera dorsalis</i>
Eugenol	^(a) males of the Oriental fruit fly.
Willison's lure	^(a) the Queensland fruit fly.
Protein hydrolysates	^(g) both sexes of virtually all tephritids.
Med lure	^(a) Medfly
Gyplure	^(a) gypsy moth, <i>Porthetria dispar</i> (L.)
Anisylacetone	^(c) male melon fly.
Verture	^(d) <i>Dacus vertebratus</i> Bezzi
Methyl(E)-6-Nonenoate	^(e) males of the Mediterranean fruit fly.
Angelica seed oil (<i>Archangelica officinalis</i> Hoffn.)	^(f) medfly

^(a) McGovern *et al.*, (1966) ; ^(b) Steiner, L.F. (1952b); ^(c) Barthel *et al.*, (1957) ;

^(d) Hancock, D.L. (1985); ^(e) Ohinata *et al.*, (1979) ; ^(f) Steiner *et al.*, (1957); Steiner *et al.*, (1958); ^(g) Matsumoto *et al.*, (1985)

Drew (1974) reported that 79 species of fruit flies in the South Pacific area and in northeast Australia were attracted to either methyl eugenol or cue-lure combined with Willison's lure. No attractants for females of any tephritid species are yet in use except for general food attractants. In recent years, these attractants have become more acceptable in both an economic and environmental sense through formulation developments. After six weeks of weathering in Southern California, a standard survey confirmed that a viscous formulation of methyl eugenol with a pesticide applied to exposed surfaces killed more males of *Bactrocera dorsalis* than an equal amount of unthickened bait freshly applied to the wick (Cunningham *et al.*, 1978b):

Steiner and co-workers (1955; 1965) eradicated the Oriental fruit fly from some localities in the Mariana Islands by aerial distribution of fibreboard blocks treated with methyl eugenol and an insecticide. Cunningham and Steiner (1972) reduced the population of the melon fly in a 2 square mile plot in Hawaii by more than 99% with fibreboard blocks treated with cue-lure and an insecticide. Capilure® an English commercial product that contains a mixture of trimedlure and extenders, was as effective or more effective than trimedlure alone in capturing male *Ceratitis capitata* (Wiedemann) and the mixture also prolonged the effective life of the attractant from 1 to 3 months (Nakagawa *et al.*, 1981). Thickened foliar sprays of methyl eugenol and a toxicant applied aerially at 5-10 lb / mile to 8 mile plots reduced trap catches of males of the Oriental fruit flies over 98%, indicating their potential usefulness in male-annihilation programs (Cunningham *et al.*, 1972). In tests conducted on large plots on the island of Hawaii in 1965 to 1966, aerial foliar applications of specific male lures mixed with insecticide and extenders, showed promise for use in male annihilation programs against the melon fly and medfly (Cunningham *et al.*, 1970). Although the initial performance of aerial spray applications are very good over a wide range of dosages, other factors such as the costs of reapplication must be taken into consideration in any actual eradication program (Cunningham *et al.*, 1975).

1.4 Pheromones and Attractants

In recent years, more attention has been given to the possible use of pheromones-based attractants in fruit fly control. Male tephritids store the pheromone in a reservoir and secrete it from a sac. The release of the pheromone is generally accompanied by courting behaviour, and females generally respond to it. The pheromone aids both long and short range orientation of the female to the male and also induces sexual excitation. Most of these

pheromones are released by the males at fairly specific times of the day (Fletcher, 1968; Fletcher, 1969; Nation, 1972; Nation, 1974). Studies on pheromones have not had the same impetus in Diptera as they have had in some of the other economically important groups, namely Lepidoptera, Coleoptera and Hymenoptera. As such, very few Dipteran pheromones have been characterised and identified.

Although the volatile components of the male rectal gland secretion of *Bactrocera cucurbitae* were identified, the biological significance of phenylbutanoids in the association with *B. cucurbitae* has not been clarified yet (Nishida *et al.*, 1990). Nevertheless, N-(3-methylbutyl) acetamide, an amide present in the male rectal gland of *B. cucurbitae* was shown to elicit activation and increased flight activity of female *B. cucurbitae* (Baker *et al.*, 1982). Methyl eugenol when consumed by males of *Bactrocera dorsalis* (taxon A) and *Bactrocera dorsalis* (taxon B) was found to be converted to 4-(3-hydroxy-1-propenyl)-2-methoxyphenol (Coniferyl alcohol) in both the species, and also converted to 2-allyl-4,5-dimethoxyphenol and Z-3,4-dimethoxycinnamyl alcohol in *Bactrocera dorsalis* (taxon B) (Nishida *et al.*, 1988a, 1988b). Coniferyl alcohol and 2-allyl-4,5-dimethoxyphenol are attractive to conspecific males, while coniferyl alcohol attracts conspecific females and stimulates ovipositor extrusion at close range (Tan and Nishida, 1996).

There are several ways of using pheromones or related compounds for controlling Diptera. Short range sex pheromones which by themselves may not be very effective, can be combined with already existing trapping techniques to improve the trap's efficiency. A spiroacetal was identified as the major component of the sex pheromone of *Bactrocera oleae* (Gmel.), and this compound was used effectively as a lure in the development of a monitoring trap for this species in an olive grove near Granada, Spain (Jones *et al.*, 1983).

Male sex pheromones that are isolated, identified and characterised can be developed as attractants for the capture of female fruit flies of the same species. Sex pheromones produced by the male *Ceratitis capitata* Wiedemann were collected, separated and identified as methyl(E)-6-nonenoate and (E)-6-nonen-1-ol and both these pheromones were synthesised and shown separately to be attractive and sexually excitatory to females (Jacobson *et al.*, 1973; Baker *et al.*, 1990). As studies on the pheromone system of the fruit flies continue, new pheromones will be discovered and other methods of using them in control programs will be developed. Pheromones of fruit flies are under intensive investigation and hold promise of providing us with additional capabilities in detecting and managing these important pests.

While methyl eugenol and eugenol only attract the male flies, the sex pheromones could be used to attract the female flies. To date, there is still no one single attractant available that has yet to attract the female flies of the Oriental fruit fly. The male lures, trimedlure and cue-lure (McGovern *et al.*, 1966) and methyl eugenol (Steiner, 1952b) usually catch more than 100 times as many male as female fruit flies. But surprising results were obtained when males were scarce or absent, and sperm-deficient, sexually mature female Mediterranean fruit flies were attracted by the three male medfly lures (Nakagawa *et al.*, 1970). Out of 61 extracts, 31 extracts of 232 ethyl ether extracts of air-dried botanicals from 196 plant species from 73 different families were found to be attractive to the female Mediterranean fruit flies and the female melon flies respectively, and female Oriental fruit flies were not much attracted although many of the extracts were attractive to males of all three species (Keiser *et al.*, 1975).

Since it is found that methyl eugenol is a liver carcinogen for the mouse, the search for alternatives to this lure should be pursued even more strongly (Miller *et al.*, 1983). It is

hoped that this work will be able to support as well as extend and contribute to the understanding of the pheromone profile of these economically important tephritids, namely the Oriental fruit fly, and its two siblings.

Table 1.3 is a summary of the work done by Keiser and co-workers (1975) on the attraction of ethyl ether extracts of 232 botanicals to *Bactrocera dorsalis* Hendel (Oriental fruit fly), *Bactrocera cucurbitae* Coquillett (melon fly) and *Ceratitis capitata* (Wiedemann) (Mediterranean fruit fly).

Table 1.3 : Attraction of Ethyl Ether Extracts of 232 Botanicals to Oriental Fruit Flies, Melon Flies and Mediterranean Fruit Flies (Keiser et. al., 1975).

<u>Sex and Species</u>	<u>Attracted to plant extracts of,</u>
<u>Males and females of Mediterranean fruit flies</u>	<i>Alangium salviifolium</i> , <i>Helianthemum lavendulifolium</i> , <i>Elephantopus elatus</i> , <i>Tagetes minuta</i> , <i>Coriaria ruscifolia</i> subsp. <i>microphylla</i> , <i>Doryonium rectum</i> , <i>Lotus crassifolius</i> var. <i>subglaber</i> , <i>Montezuma speciosissima</i> , <i>Clematis virginiana</i> , <i>Paeonia suffruticosa</i> , <i>Bouvardia ternifolia</i> , <i>Borreria verticillata</i> , <i>Cephananthus occidentalis</i> , <i>Coffea arabica</i> , <i>Coffea robusta</i> , <i>Morinda royoc</i> , <i>Rubia tenuifolia</i> , <i>Haplphyllum buxbaumii</i> , <i>Toddalia asiatica</i> , <i>Populus heterophylla</i> , <i>Solanum brachystachys</i> , <i>Clerodendron fragrans</i> , <i>Vitis girdiana</i> , and <i>Kallstroemia maxima</i> .
<u>Males and females of melon flies</u>	<i>Alangium salviifolium</i> , <i>Asclepias curassavica</i> , <i>Arnica chamissonis</i> var. <i>incana</i> , <i>Iva axillaris</i> , <i>Calystegia soldanella</i> , <i>Ipomea pandurata</i> , <i>Lepidium virginicum</i> , <i>Diospyros discolor</i> , <i>Juglans nigra</i> , <i>Sidalcea oregana</i> , <i>Bersama abyssinica</i> , <i>Nyssa sylvatica</i> , <i>Eriogonum annuum</i> , <i>Paeonia suffruticosa</i> , <i>Bouvardia ternifolia</i> , <i>Coffea robusta</i> and <i>Boehmeria cylindrica</i> .
<u>Males and females of Oriental fruit flies</u>	<i>Iva axillaris</i> , <i>Licaria salicifolia</i> , and <i>Coffea robusta</i>
<u>Female melon and Mediterranean flies</u>	<i>Alangium salviifolium</i> , <i>Iva axillaris</i> , <i>Lepidium irginicum</i> , <i>Diospyros discolor</i> , <i>Juglans nigra</i> , <i>Brongniartia benthamiana</i> , <i>Bersama abyssinica</i> , <i>Paeonia suffruticosa</i> , <i>Physocarpus capitatus</i> , <i>Sorbus arbutifolia</i> , <i>Bouvardia ternifolia</i> , <i>Coffea robusta</i> , <i>Haplphyllum buxbaumii</i> , and <i>Solanum brachystachys</i> .
<u>Female Mediterranean and Oriental flies</u>	<i>Kallstroemia maxima</i> , <i>Tagetes minuta</i> , and <i>Licaria salicifolia</i>
<u>Female melon and Oriental flies</u>	<i>Sidalcea oregana</i>
<u>Females of all three species</u>	<i>Coffea robusta</i> and <i>Iva axillaris</i> .

1.5 Plants - insects relationships

Plants form the focal point for the ecological behaviour of fruit flies, which involves host seeking, adult feeding, mating behaviour, oviposition, and egg development (Drew, 1989). Plant scents are actually relatively volatile substances, and are formed by complex, metabolic processes in at least 30% of all the higher plant species. Because of their potential toxicity, they are stored as essential oils in special cells at the periphery of flowers, leaves or even roots. Their volatility allows these essential oils, to be detected by the human or animal sense of smell. Humans generally experience them as a fragrant scent, although some plants can give off an offensive odour. Several varieties of *Ocimum sanctum* (Labiatae) and *O. basilicum* contain varying amounts of methyl eugenol in their essential oils (Tan, 1982; Toong and Tan, 1992). Naturally, it is assumed that insects interpret all plant scents as semiochemical messengers with a significant role to play in their own survival. The ecology of physiological coadaptation is exemplified in the appreciation insects have for their environment e.g. their appreciation of the attractive features of the flowers such as colour, motile floral parts (as shown by the lip and column of numerous orchids), and the odour generated by the specific flowers, (Kevan and Baker, 1983) as observed in Appendix 5 .

The scent of *Reseda odorata*, which humans also perceive as a very pleasant fragrance, is associated by its pollinator, the honey bee, with food in the form of nectar and pollen. The odouriferous flowerspike of *Spathiphyllum cannaefolium* (Appendix 6) is found to contain benzyl acetate which attracts males of *Bactrocera cucurbitae*, *Bactrocera dorsalis* and *Ceratitis capitata*, and methyl eugenol which attracts *B. cacuminatus*, *B. dorsalis* and *B. occipitalis* (Lewis *et al.*, 1988). The formation of the pollen in the white flower spike is characterised by a sweetish odour, and as the flower ages, the pollen is lost along with the odour, the colour changes from white to green and the aromatic compound present

afterwards is methyl eugenol. The Golden Shower Blossom, *Cassia fistula* L. (Leguminosae) has no fragrance, but contains only one volatile component which is methyl eugenol, a highly attractive lure to the male Oriental fruit flies (Kawano *et al*, 1968).

Essential oil from *Piper betle* leaves, *Piper sarmentosum* (the whole plant), *Couroupita guianensis* flowers, *Michelia alba* flowers and *Asarum sieboldi* Miq. roots have also shown positive reaction towards the Oriental fruit fly, where headspace analysis of the active substance in the essential oil were isolated and identified as methyl eugenol in *P. betle*, *M. alba*, *P. sarmentosum* and *A. sieboldi*, and eugenol for *C. guianensis* (Lim, 1993).

A feeding stimulant of the male medfly was discovered in the stem extract of its host plant, *Litchi chinensis* Sonn. (Sapindaceae) and also on milky exudates from stems of *Ficus retusa* L. and *F. benjamina* L. (Moraceae) (Warthen and McInnis, 1989). Extracts from the spadix and bracts of *Colocasia esculenta* blossoms were found to contain methyl eugenol, eugenol, methyl ester of fatty acid and high molecular weight alcohol that were highly attractive to the male Oriental fruit fly (Sinchaisri *et al.*, 1985). In *Magnolia spp.*, the beetles imbibe floral secretions (nectar), eat pollen, and chew on floral parts or special food bodies (Kevan and Baker, 1983). The extract of *Coffea robusta* Linden (family Rubiaceae) is found to attract both sexes of the Oriental, melon and Mediterranean fruit flies (Keiser *et al.*, 1975). The flower of *Nicotiana glauca*, which only release their scent during the night, are pollinated by a particular type of moth being guided, by the scent, to the nectar in the corolla tube. The scent, colouring and shape of the flower of *Stapelia gettleffii* resemble carrion to female blowflies, which therefore deposit their eggs on the flower, serving as pollinators at the same time.

Host odours are particularly significant in attracting fruit flies to plants that are typically scattered through dense forest canopy and attract flies only when fruits reach a particular

stage of development. Generalizations about odour as an attractant for pollinating insects are more difficult than for colour because the insects' power of olfaction appear to be more diverse than their powers of vision which is normally from ultraviolet at ca. 300 nm to yellow orange at ca. 650nm (Kevan and Baker, 1983). The flowers of *Ophrys insectifera* mimic the appearance and smell of the sexual attractant of the female sphecoid wasp, thereby inducing the male to copulate with the flower and in turn resulting in pollination.

Five species of *Bulbophyllum* (Orchidaceae), namely *Bu. cheiri*, *Bu. patens*, *Bu. macranthum*, *Bu. ecornutum* and *Bu. vinaceum* are known to attract the males of the Oriental fruit fly, *B. dorsalis*, complex species and *B. umbrosa* where a synomonal relationship exist among the orchids and the fruit flies (Tan, 1993). These examples illustrate most of the pollination methods in which scent plays the major role, as we are about to see in the relationship and co-evolution between the fruit flies, *Bactrocera* spp. and the wild orchids, *Bulbophyllum* spp.

The insect-plant relationships may be basically categorised to three types and the most frequent are plants providing food and/or shelter for insects, whilst the mobility of the insects in the distribution of pollen and propagules have led to the evolution of the plants. Extrafloral secretions of some common plants in Hawaii are found to be food sources for certain insects (Nishida, 1958). These include Cattleya orchids, *Cattleya* spp.; cotton, *Gossypium barbadense* L.; wild euphorbia, *Euphorbia geniculata* Ortega; yellow passion fruit, *Passiflora edulis* f. *flavicarpa* Degener; peach, *Prunus persica* L.; castor bean, *Ricinus communis* L.; and ground orchids, *Spathoglottis* spp.

Anthophilous insects are described as many different kinds of insects that can be found on flowers such as coleopterans and dipterans, which with their suctorial or lapping mouthparts, are considered to be two of the most primitive pollinators based on their

importance in the evolution of the Angiosperms (Kevan and Baker, 1983). Most of the major orders of angiosperms are largely or entirely insect pollinated and floral structures and insect pollinators exhibit remarkable co - evolution (Leppik, 1957). Other insects described by Kevan and Baker (1983) as anthophiles are the Lepidoptera, Hymenoptera, Holometabola, Collembola, Plecoptera, Orthoptera, Heteroptera, Homoptera, Neuroptera, Mecoptera and Trichoptera.

Plants produce a wide variety of secondary metabolites to cope with various kinds of herbivorous attacks (Kaiser, 1993). Some insects have not only overcome such chemical barriers after a long evolutionary history but also have developed the ability to sequester allomonal components originating from their specific host plants (Blum, 1985).

A number of insects are strongly associated with specific chemicals produced by plants and by sequestering these plant metabolites, the insects gain protection from predators and precursors for the male pheromones (Nishida and Fukami, 1990). *Zieria smithii* (Andrews), an Australian plant from the family Rutaceae consists of ca. 85% o-methyl eugenol and it is this single constituent that attracts the males of *Dacus (Strumeta) cacuminatus* (Hering) to it (Fletcher *et al.*, 1975). Although o-methyl eugenol is not an essential ingredient of the diet and plays no known role in the biology of the fruit flies, a simple explanation for the attractancy of the flies towards this plant could be that there is immense similarity between that of o-methyl eugenol and some compound, such as a male aggregation or sex pheromone used by the flies. Flath and Ohinata (1982) observed that the male melon fly *Bactrocera cucurbitae* Coquillett. is attracted to the blossoms of the orchid *Dendrobium superbum* Rchb. because the scent of the orchid flower is somewhat similar to aromas of certain male melon fly attractants such as cue-lure (McGovern *et al.*, 1966), and

anisylacetone (Barthel *et al.*, 1957). Further analysis of the orchid floral fragrance revealed that the orchid emits raspberry ketone as part of its floral fragrance (Nishida *et al.*, 1993).

Flower bud and leaf extracts of *Ocimum sanctum* contain methyl eugenol as a major constituent and this indicates that *O. sanctum* might contain a component which synergised the effect of methyl eugenol and strongly attracts the Oriental fruit fly (Tan, 1982). Tan and Toong (1993) have also reported that essential oils of more than 200 plant species from 32 families contain methyl eugenol in varying amounts.

Such is the relationship between the *Bactrocera* spp. and the wild orchids, *Bulbophyllum* spp. *Bulbophyllum cheiri*, for instance, is known to secrete methyl eugenol, an attractant for males of *Bactrocera* spp., and 2-allyl-4,5-dimethoxyphenol (Compound Z), (Toong and Tan, 1992; Appendix 7) which happens to be a pheromone of the fruit fly. The fruit flies that feed on these two compounds, when disturbed have been found to ejaculate rectal contents containing allomone to escape from predators and also mate gregariously with the females during the courtship period (Tan, 1992). It is also found that the sex pheromone is released in order to attract the female fruit flies during the courtship and mating period normally during dusk (Ohinata *et al.*, 1982). The fruit flies in return help to distribute the pollinium from this orchid by visiting the column where the pollinium is deposited in the orchid. This is a simple case of mutual benefit, pollination for the flowers, food and stimulants for the insects (Kaiser, 1993; Tan, 1996).

1.6 Wild orchids (*Bulbophyllum* spp.)

There are over 25,000 known orchid species in the world and the orchid family easily comprises 7 - 9% of all flowering plants making it the family with the greatest number of species, and they also exceed all other plant groups in respect of variety of floral designs

(Van Der Pijl and Calaway, 1966). Almost 94 - 95% of these species are concentrated in the tropics and they exist primarily as epiphytes on trees. Most *Bulbophyllum* species as we shall come to know, are found as epiphytic plants of the virgin forest (Vermeulen, 1991).

With about 2500 proposed names, the genus *Bulbophyllum* is probably the largest genus within the orchid family, Orchidaceae. *Bulbophyllum* is a pantropical genus and the species occur in the tropical parts of South and Central America, Madagascar, mainland Asia, Australia, the Pacific islands and South East Asia. New Guinea has the highest number of species, close to 400 or more species occurring there and almost all are endemic to the area. The second richest area is the island of Borneo with about 200 species (Vermeulen, 1991).

The *Bulbophyllum* complex is characterised by its balanced see-saw labellum which is hinged by a narrow, springy claw and tips over when its equilibrium is disturbed. The plants have a sympodial rhizome with the last node swollen into a pseudobulb. The lip is mobile and attached to the column by a thin ligament (Van Der Pijl and Calaway, 1966).

These species favour certain habitats such as the misty montane forests and the mixed lowland forests. Very few species are found in mangrove forest, or forest on limestone or ultrabasic soil. The genus is also absent in very dry forests or forests with a strongly seasonal rainfall.

The species is currently fading away fast from the face of the planet due to numerous reasons. Forest destruction such as selective or non-selective logging damages most trees where these species thrive on as epiphytes. In most areas, the ensuing exposure of the habitat to drought and to the sun will cause most species to disappear quickly. The natural habitat of these orchids are also being disturbed in the name of development such as the clearing of forests for the making of bridges and roads, housing developments and crop plantations. Individuals that have an eye for exotic orchids too tend to have the habit of

removing these orchids from their natural habitats and replanting them at another location which may not be suitable for them, thus making that particular species extinct!

In view of the recent development between the *Bactrocera spp* and the wild orchids, *Bulbophyllum spp.*, it is interesting to note that the fruit flies known throughout their lives as destructive and damaging pests are finally being looked upon as pollinators of these orchids and instead of eradicating the fruit flies, we should try to delimit and conserve these pests in the hope that they shall not allow our rare and endangered *Bulbophyllum spp.* to go extinct.

2.0 Objectives

- 1) To confirm and extend published observations on the structure of the male - specific glands in *Bactrocera papayae*, *Bactrocera carambolae*, *Bactrocera umbrosa* and *Bactrocera dorsalis*.
- 2) To establish and support methyl eugenol as a more efficient and potent attractant compared to eugenol for *Bactrocera papayae*, *Bactrocera carambolae*, *Bactrocera umbrosa* and *Bactrocera dorsalis*.
- 3) To isolate and identify the chemical volatiles of male rectal gland secretions from *Bactrocera papayae*, *Bactrocera dorsalis*, *Bactrocera carambolae* and *Bactrocera umbrosa* after the males of all four species were fed with either methyl eugenol or eugenol.
- 4) To characterise the pheromones identified based on previous studies and preliminary testing. Although the existence of a male produced sex pheromone in *Bactrocera dorsalis* has already been studied (Kobayshi *et al.*, 1978), only preliminary testing of several chemicals for biological activity has been done so far (Ohinata *et al.*, 1982). It is hoped that the identification of pheromones for the four species would help to give an insight into the intraspecific communication system of these species as well as provide a future tool in the control of these damaging pests. The availability of a sex pheromone for monitoring as well as control purposes would be very welcome especially in this age of biological control.
- 5) On the observation that *Bulbophyllum cheiri* (Toong and Tan, 1992 - Appendix 7), *Bulbophyllum patens* (Tan and Nishida, unpublished data), and *Bulbophyllum macranthum* emit certain chemical compounds that attract the *Bactrocera spp.*, a