

DIVERSITY AND POPULATION OF THRIPS SPECIES ON
LEGUMES WITH SPECIAL REFERENCE
TO *Megalurothrips* sp.

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**DIVERSITY AND POPULATION OF THRIPS SPECIES ON
LEGUMES WITH SPECIAL REFERENCE
TO *Megalurothrips* sp.**

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WITH SPECIAL REFERENCE TO *MEGALUROTHRIPS* SP.**

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ABSTRACT

Thrips (Thysanoptera) are a common pest on legume plants, yet little is known about their ecology or diversity in Peninsular Malaysia. In legumes, thrips are typically found in the flowers, where their feeding activity causes malformations that eventually lead to crop damage. The aim of this study is to examine the diversity and population of thrips species, particularly *Megalurothrips usitatus*, in three selected legume farms around Peninsular Malaysia (Janda Baik, Pahang; Bestari Jaya, Selangor and Jelevu, Negeri Sembilan). Depending on growing season, legume flowers were inspected monthly for thrips in five random plots from each farm for 6 to 12 months. Sampling was performed six times in Bestari Jaya and Jelevu, and twelve times in Janda Baik. The most abundant thrips species on legumes was *M. usitatus* (89.97%) followed by *Thrips parvispinus* (9.77%), *Thrips hawaiiensis* (0.13%) and *Ceratothripoides brunneus* (0.12%). Differences in legume species, which is i.e. long bean, French bean and winged bean, did not affect the abundance of *M. usitatus*, and the species was equally distributed among different arbitrary strata on legume plants. Temperature and light intensity were found to be positively correlated with the abundance of *M. usitatus*, but relative humidity showed negative relationship. *M. usitatus* was found in large numbers during the hot and dry months, but in lower numbers during the raining season. This suggests that the wet season may help to reduce populations of *M. usitatus* on legume plants. The doubtful female individuals of *M. usitatus* were obtained from the field and from the unmated female of lab rearing. There are 15.4% of doubtful female of *M. usitatus* were produced from the unmated female. The molecular analysis showed the doubtful females with several *M. usitatus* females belong to the sub clade 2 which is in the same clade of *M. typicus*. Hence, at this stage, the doubtful female could be considered as a cryptic species and a by-product from the unmated female. Thus,

the present study is important for obtaining more information of *M. usitatus* on legume crops in Malaysia, and thus to improve effective management strategies for this pest.

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ABSTRAK

Jengking Daun (Thysanoptera) adalah perosak yang biasa ditemui pada tumbuhan kekacang, namun sedikit yang diketahui tentang ekologi atau kepelbagaian mereka di Semenanjung Malaysia. Pada tumbuhan kekacang, Jengking Daun kebiasaannya dijumpai di dalam bunga, di mana aktiviti pemakanan mereka menyebabkan kecacatan dan kerosakan pada tanaman. Tujuan kajian ini adalah untuk mengkaji kepelbagaian spesies Jengking Daun, terutamanya *Megalurothrips usitatus*, di tiga ladang kekacang terpilih sekitar Semenanjung Malaysia (Janda Baik, Pahang; Bestari Jaya, Selangor; dan Jelevu, Negeri Sembilan). Bunga kekacang diperiksa setiap bulan untuk dikumpul Jengking Daun dalam lima plot secara rawak dari setiap ladang selama 6 hingga 12 bulan bergantung kepada musim penanaman. Kerja pensampelan dilakukan sebanyak enam kali di Bestari Jaya dan Jelevu, dan dua belas kali di Janda Baik. Spesies thrips paling banyak pada kekacang adalah *M. usitatus* (89.97%) diikuti oleh *Thrips parvispinus* (9.77%), *Thrips hawaiiensis* (0.13%) dan *Ceratothripoides brunneus* (0.12%). Perbezaan dalam spesies kekacang iaitu kacang panjang, kacang buncis dan kacang botor tidak menjejaskan kelimpahan *M. usitatus* dan spesies ini terbahagi sama rata antara strata yang berbeza pada tumbuhan kekacang. Suhu dan keamatan cahaya didapati berkorelasi positif dengan kelimpahan relatif *M. usitatus*, manakala kelembapan relatif menunjukkan hubungan yang negatif. *M. usitatus* didapati dalam jumlah yang banyak semasa musim panas dan kering tetapi boleh didapati dalam jumlah yang sedikit semasa musim hujan. Ini menunjukkan bahawa musim hujan boleh membantu mengurangkan populasi *M. usitatus* pada tumbuhan kekacang. Individu betina *M. usitatus* yang meragukan diperolehi dari lapangan dan daripada induk betina tidak mengawan yang dipelihara di makmal. Sebanyak 15.4% betina yang meragukan terhasil daripada induk betina tidak mengawan. Keputusan analisa molekul menunjukkan betina yang meragukan bersama dengan beberapa betina *M. usitatus*.

tergolong dalam subklad 2 dan sekumpulan dengan kumpulan *M. typicus*. Oleh itu, pada peringkat ini betina yang meragukan boleh dianggap sebagai spesies 'cryptic' dan satu hasilan dari betina tidak mengawan.

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LIST OF SYMBOLS AND ABBREVIATIONS

'	Minute
"	Second
%	Percentage
<	Less than
=	Equal to
>	More than
°C	Degree Celsius
°	Degree
♀	Female
♂	Male
ANOVA	Analysis of variance
<i>C. b</i>	<i>Ceratothripoides brunneus</i>
cm	Centimeter
COI	Cytochrome c oxidase subunit I
CSIRO	Commonwealth Scientific and Industrial Research Organization
D	Dominance
df	Degree of freedom
DNA	Deoxyribonucleic acid
Dntp	Deoxyribonucleotide triphosphate
e.g.	Latin phrase <i>exempli gratia</i> (for example)
<i>et al</i>	Latin phrase <i>et alia</i> (and other)
F	Fisher
H	Shannon entropy
HSD	Honest significance difference
i.e.	Latin phrase <i>id est</i> (that is)
m	Meter

min	Minute
mm	Millimeter
<i>M. t</i>	<i>Megalurothrips typicus</i>
<i>M. u</i>	<i>M. usitatus</i>
P	Possibility value
PCR	Polymerase chain reaction
ppm	Part per million
r	Correlation coefficient
SD	Standard deviation
SE	Standard error
sp.	Species
<i>T. p</i>	<i>Thrips parvispinus</i>
<i>T. h</i>	<i>T. hawaiiensis</i>
vs.	Versus
μl	Microliter
μm	Micrometer

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CHAPTER 1: INTRODUCTION

Thrips (Insecta: Thysanoptera) are small insects (>1 mm) that commonly infest crops and flowers (Palmer, 1987). Despite their small size, thrips infestations can cause 80–100% losses in legume crops, particularly from the genus of *Megalurothrips* (Oparaeke, 2006). Thrips damage on flower buds usually leaves behind distorted, malformed and discoloured flowers that affect pod development and eventually cause crop failure (Ogah, 2011). In Malaysia, legumes, especially long beans, are the most popular vegetable planted (3,663 ha), producing approximately 42,623 metric tonnes of bean in 2011 (Anon., 2015). Knowledge of the spatial and temporal distribution of thrips on plants is critical for formulating an effective thrips management strategy (Ananthakrishnan *et al.*, 1982; Kasina *et al.*, 2009). Checklists on thrips in Peninsular Malaysia recorded 86 known Thripidae species, including 17, 6 and 57 species from subfamilies Panchaetothripinae, Sericothripinae and Thripinae, respectively (Mound & Azidah 2009; Azidah 2011; Ng & Mound, 2015). Other than these checklists, there are also several studies on the spatial distribution and population density of Thysanoptera species that were done in Malaysia by Aliakbarpour & Che Salmah (2011) and Aliakbarpour & Che Salmah (2012). There is thus a poverty of information regarding the ecology and population dynamics of thrips and their relation to legume crops. Additionally, none of these studies were focused on the diversity of *Megalurothrips* and their associated host plant. According to Palmer (1987) *Megalurothrips* are commonly recorded on legumes, although the adults can be found on flowers of many other plant species. Surveys on thrip diversity by Azidah (2011) and Fauziah & Wan (2004) noted that *M.usitatus* was found more abundantly than other thrips species on legumes. The only previous study focusing on this genus in the region was a taxonomic study using morphometrics to distinguish species by Palmer (1987). However, the common species *Megalurothrips sjodjesti* has been quite widely studied in Africa, in

terms of toxicology (Abtew *et al.*, 2015; Opraek, 2006; Omo-Ikerodah *et al.*, 2000) and ecology (Salifu & Hodgson 1987). Even though they are considered one of the most common thrips in Malaysia, studies on the diversity and ecology of *Megalurothrips* species in Malaysia are scarce. Thus, the present study is important for obtaining more information of *M. usitatus* on legume crops in Malaysia, and thus to improve effective management strategies for this pest.

Therefore, the aims of this study are:

1) to determine thrips diversity on legumes, including long bean, French bean and winged bean, from multiple sites in Peninsular Malaysia; 2) to investigate the spatial and temporal distribution and abundance of *M. usitatus* and 3) to elucidate the relationship between abiotic factors such as temperature, humidity and light intensity, on the abundance of *M. usitatus* 4) To verify the identification of *Megalurothrips* species through morphology and life history studies.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION TO THYSANOPTERA

The Thysanoptera is an order of insects, commonly known as thrips which is related to the Psocoptera (book lice) and the Hemiptera (bugs) (CSIRO, 2006; Grimaldi & Engle, 2005). The word Thysanoptera is derived from two Greek words meaning a fringe, and a wing (Bhagat, 2011). Their common name, thrips, is also derived from the Greek meaning a “wood louse”, other names are bladder feet because of their peculiar terminal tarsal segment (Lewis, 1973). Many species frequently inhabit flowers and leaves of angiosperms and sheaths of Graminae, while others are known to occur beneath bark of living trees, fallen leaf litter and within plant galls. By feeding habits they are phytophagous or mycophagous, comparatively rarely predaceous (Ananthakrishnan, 1990).

2.2 GEOGRAPHICAL DISTRIBUTION AND HISTORICAL BACKGROUND OF THYSANOPTERA

There are about 5000 known species worldwide (Mound, 1996) with the habitats range through forests, grasslands, scrub, desert, most cultivated crops and gardens (Lewis, 1973). There are vast numbers of tropical species, many temperate ones, and even a few species extending to arctic regions (Lewis, 1973). The Thysanoptera comprises an array of beautiful and diverse creature, brightly colored or somberly shaded, often intricately sculptured and occasionally of bizarre shapes and proportion (Lewis, 1973). Some species of the order Thysanoptera are distributed widely throughout the world (Mound, 1983). Thripidae and Phleothripidae are the two largest families that comprise more than 90% of Thysanoptera and they are often found worldwide. Compared to the least advance family, Merothripidae, they are generally less distributed. The distribution of thrips at different

level species seems confusing. The faunal comparisons have been confused previously by faulty species taxonomy, such as description of single species under different names from different parts of the world (Mound, 1983). According to Mound (2014), Thysanoptera continues to be poorly sampled, with the tropical and subtropical areas of the southern continents inadequately surveyed. The number of described thrips species worldwide increased only by 1.3% between 2011 and 2013 and, mostly reported from China and South America (Mound, 2011). There are two factors contributing mostly to the geographical distribution of thrips; human activities and wind dispersion (Mound, 2011). Human activities had caused more disruption in natural patterns of thrips distribution to a considerable extent. It may accidentally happened where men carried food as immediate consumption or future crops in the form of seeds, roots and stems where dormant eggs of thrips were stored in it. Transporting thrips infested planting materials such as bananas and orchids also would accidentally distribute them worldwide (Mound, 2011).

2.3. GENERAL STRUCTURE AND CLASSIFICATION OF THYSANOPTERA

Most thrips are tiny and visible. They are ranging from 1 to 2 mm long with the smallest are about 0.5 mm and the largest are about 14 mm (Lewis, 1973). Their bodies are elongate, which are often flattening dorso-ventrally (Nakahara, 1991). The head comprises of three ocelli arranged in a triangle between the two compound eyes. For adult the antenna is often 7 or 8 segments (Figure 2.0). The most remarkable feature of all adult thrips is their mandible, which is only the left part is developed. The pointed mandible is used to puncture plant tissue and the two maxillary stylets are used to insert through the hole to make a tube, where thrips will suck the plant sap (Reyes, 1994). Thrips has different number, size, position, shape and color of the body setae either in adults or the immature. Depending upon the kind of setae present they are variously referred to as hair, setae, flagellum, bristle,

spine and cilia (Ananthakrishnan, 1990). These setae could be found in the most part of their body especially on sternite and tergite. Well-developed cuticular appendages on tergite IX in males of some genera of terebrentia for example, are notable characters for species determination, these being in form of dark, stout, chitinous, thorn-like setae or lateral horn-like productions. Dense rows of microtrichia on tergites IX of both male and female on the posterior margin provided with comb or hair-like continuous or discontinuous have significant taxonomic implications besides the number and the nature of the accessory setae of tergites and sternite. The number of setae on the dorsum of tergite IX as well as the three pairs of lateral setae are also equally significant, particularly of their shape, size and arrangement (Ananthakrishnan, 1990). Living specimens, seen with the naked eye are slender, shiny and often pigmented. However, the mounted thrips slightly has different general appearance. The head, thorax and abdomen become flattened to display the separate segments and components. The wings and legs are spread and extended to reveal the complex part of setae, spines and tubercles. The body color also slightly changed and become slightly paler due to the usage of chemical substances such as alcohol during soaking.

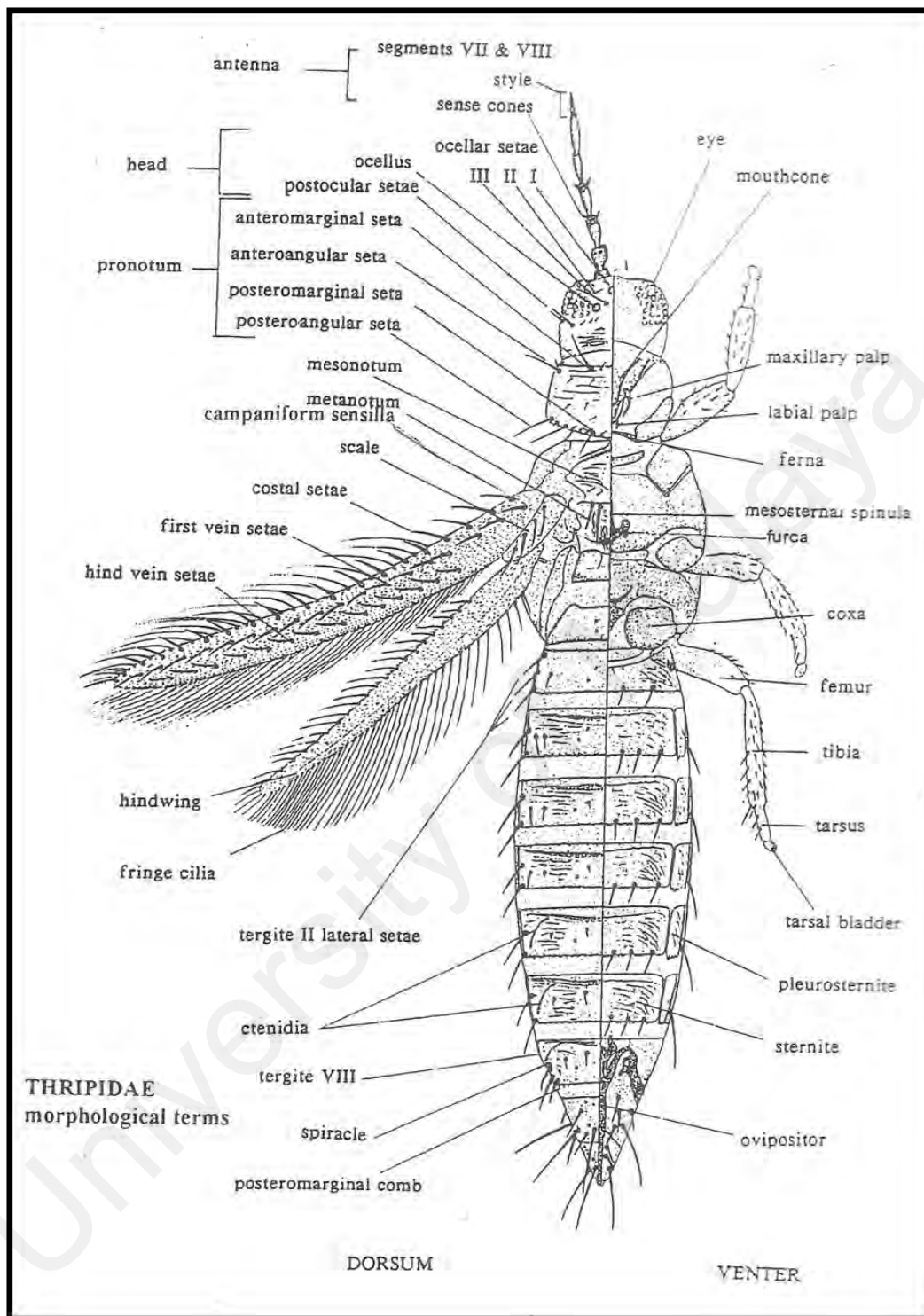


Figure 2.0: Thrips' body structure (Mound, 2007)

Thysanoptera is divided into two suborders: Terebrantia and Tubulifera (Mound *et al.*, 1980). According to Mound & Minaei (2007) based on the widely accepted and traditional classification of Thysanoptera, the suborder of Terebrantia comprises 2400 species in eight families with the largest, Thripidae, whereas suborder of Tubulifera comprises a single family, the Phleothripidae with about 3500 described species. The available classifications of Thysanoptera are all morphological based (Mound & Morris, 2007) and never been evaluated formally with a comprehensive molecular phylogenetic analysis (Buckman *et al.*, 2013). Many of the thrips species being described under many different names due to intraspecific phenotypic variation that is widespread in Thysanoptera (Tyagi *et al.*, 2008). Adults of Thysanoptera are commonly monomorphic, and sexual dimorphism continues to be a major limitation in identification of thrips (Tyagi *et al.*, 2008). Based on the morphological data, Mound *et al.* (1980) summarized that there are two possibilities: either the Tubulifera is a sister-group to the Terebrantia or it is a sister group to a subgroup within the Thripidae. From 1980 until 2007, there were varied classifications of Thysanoptera among the researcher, for example, Bhatti (1988) recognized Superorder Thysanopteroidae, with two orders; Terebrantia and Tubulifera as sister group within a single lineage. Whereas, Zherikhin (2002) recognized order Thripidae with two suborders, the Lophoneurina and the Thripinae (Table 2.0).

The families in suborder of Tubulifera and Terebrantia are all included in Table 2.1 and known as currently accepted classification (Mound & Morris, 2007). For Terebrantia; family Merothripidae are all fungus – feeding on dead branches and in leaf litter, Aeolothripidae comprises mainly predator thrips and most of them found in temperate parts, Heterothripidae is found only in America, where the species live in the flowers of native plants while the most serious pest species are members of the family Thripidae

(Mound, 2007). Sartiami & Mound (2013) reported that members of subfamily Thripinae under Thripidae include pests of legume crop (*Megalurothrips*), pests of cereal crops, rice and grasses (*Anaphothrips*, *Aptinothrips*, *Arorathrips*, *Chirothrips*, *Fulmekiola* and *Stenchaetothrips*), tea and coffee (*Scirtothrips*) and many vegetable crops (*Bathrips*, *Ceratothripoides*, *Frankliniella* and *Thrips*) According to Palmer (1987), species of *Megalurothrips* are widespread and common throughout the old world tropics. There are twelve species that are currently listed, all from the old world areas between Africa, China and the Pacific (Palmer, 1987). However, the validity of some of these species is open to doubt, including some of those most recently redescribed; *M. usitatus* and *M. typicus* which is common in northern Australia (CSIRO, 2006). This genus all breed in the flowers of Fabaceae and some are pest of cultivated legumes (Masumoto, 2010).

Megalurothrips generally is distinguished by following characters; the head is wider than long with three pairs of ocellar setae present. They have developed postocular setae and anterocellar setae including the longest pair, introcellar setae. Their eyes are large and antennal III color varies from yellow to light browns with large forked sense cones attach on it. Two pairs of developed and long posteroangular setae are present at their pronotum. Forewing is banded with a long row of setae present at first and second vein. Their abdominal tergite VIII have patch of microtrichia anterior to the spiracle, and tergite IX is not completely divided longitudinally. There are no discal setae present on their sternites (Mirab-bolou *et al.*, 2011; Palmer, 1987).

Table 2.0: Classification of Thysanoptera according to several taxonomists (Mound & Morris, 2007).

Mound <i>et al.</i>, 1980	Bhatti, 1988	Zherikin, 2002
		Order Thripidae
		Suborder Lophioneurina
Order Thysanoptera	Superorder Thysanoptera	Suborder Thripina
Suborder Terebrantia	Order Terebrantia	Infraorder Thrimorpha
Suborder Tubulifera	Order Tubulifera	Infraorder Phloeothrimorpha

Table 2.1: Classification of Thysanoptera according to Mound & Morris (2007).

SUB-ORDER	FAMILIES	SUB-FAMILIES	GENERA	SPECIES
Tubulifera	Phlaeothripidae	Phlaeothripinae	350	2500
		Idolothripinae	80	700
Terebrantia	Uzelothripidae		1	1
	Merothripidae		3	15
	Melanthripidae		4	65
	Aeolothripidae		23	190
	Fauriellidae		4	5
	Adiheteropidae		3	6
	Heterothripidae		4	70
	Thripidae	Panchaetothripinae	35	125
		Dendrothripinae	10	90
		Sericothripinae	10	90
		Thripinae	235	1700

2.4 THRIPS DIVERSITY

Pest in Southeast Asia is not being studied widely with the absence of identification literature and general overviews especially for the order of Thysanoptera (Mound & Azidah, 2009). According to Talekar (1991), researchers mainly from Thailand, Indonesia, Philippine, Taiwan and Malaysia made a general overview that thrips is not well studied in their own countries and the list of recorded thrips species is relatively less compared to the other regions such as in temperate countries (Alves-Silva & Del-Claro, 2010). Even though thrips are among the highly destructive pest to crops, farmers may have not complained about them, due to either thrips occur in negligible numbers or they are not observing their crops very closely to notice the presence and abundance of the insect pests (Bernado, 1991). There are 78 known thrips species including 17 species from the family of *Panchaetothripinae*, 6 species from family *Sericothripinae* and 55 species from family of *Thripinae* in Peninsular Malaysia (Mound & Azidah, 2009). In latter study, Azidah (2011) documented another two newly-recorded species of *Thripinae* that can be found on common plants in Peninsular Malaysia. The study on diversity of thrips was further performed by Ng & Mound (2015), where another new species and six newly-recorded species from *Thripinae* were added to the list. There are also few studies on thrips conducted in Malaysian mango orchards by Aliakbarpour *et al.* (2010), Aliakbarpour & Che Salmah (2011) and Aliakbarpour & Che Salmah (2012). Five common different genera had been recorded in the mango orchard; *Thrips*, *Scirtothrips*, *Frankliniella*, *Megalurothrips* and *Haplothrips* (Aliakbarpour & Che Salmah, 2011). According to Mound & Azidah (2009), there remains a need for a broad introduction to the Thysanoptera fauna of Malaysia and field studies are needed on particular species to determine the extent of their variation in color, structure and their precise host plants, for example *Megalurothrips* species. However, there are several studies being done elsewhere on

Megalurothrips such as demographic study on *M. sjodjesti* and *M. usitatus* on legume in Africa and China regions such as by Omo-ikerodah *et al.* (2008), Sani & Umar (2017) and Tang *et al.* (2015).

2. 5 THRIPS POPULATION

Population of thrips always fluctuates depending on many factors (Premachandra *et al.*, 2005; Pankeaw *et al.*, 2011; Atakan *et al.*, 1996; Pearsall, 2000; Sedaration *et al.*, 2010). In general, thrips density is affected by several factors such as temperature and humidity (Sedaration *et al.*, 2010), migration and predators (Pickett *et al.*, 1988; Atakan *et al.*, 1996), weather and season and other factors including plant characteristics, specific microhabitat preference or vegetation system (Premachandra *et al.*, 2005). However, according to Taylor (1963) and Pearsall (2000), temperature is the most important factor compared to other physical factors. According to Ananthakrishnan *et al.* (1982) and Kasina *et al.* (2009) knowledge of thrips population is critical and important especially from the aspect of spatial and temporal distributions. There are several studies that have described the spatial distribution and population density of Thysanoptera species (Duchovskiene, 2006; Kasina *et al.*, 2009) but there was none of such study done in Malaysia. According to Mound & Teulon, (1995) thrips are opportunistic and enable to accumulate large host range. They adapt quickly and establish large population in rapidly changing environments (Samler, 2012). They can be found in almost part of the host; the leaf buds, leaves, flower buds, flowers and fruits (Sedaration *et al.*, 2010). However, according to Ugine *et al.* (2006) all stages of thrips (male and female adults and immature) are detected mostly at the bud sepals. Their population is difficult to control and chemical applications against thrips are rarely warranted as they become resistant towards insecticides (Ellington *et al.*, 1984). Biological control also does not guaranty 100% success in combating them. A serious

research need to be done if natural enemy wanted to be practiced in controlling their population. According to Van Houten *et al.* (2005), the effect of natural enemies on different species of thrips at different area will be vary. For example, the predatory mites *Amblysius cucumeris* (Oudemans) is successfully used for controlling thrips on Dutch sweet pepper in Northern Europe but they were not effective in Southern Europe (Van Houten, 1996). However, there is no study on controlling thrips by using the natural enemy in Malaysia.

2.6 LEGUME TAXONOMY

Legumes are flowering plants in the leguminosae family and the word legume is derived from the latin verb 'legere' which means to gather. This family is also known as Fabaceae and can be classified into three common sub families; Papilionidae, Caesalpinioideae and Mimosoideae. There are 690 genera and 18000 species within this family. However, the most prominently cultivated are the genera of *Phaseolus*, *Vigna*, *Vicia* and *Glycine* (Tobias, 2004). Each family is identified by its floral characters, particularly in petal aestivation patterns (imbricate ascending in Caesalpinioideae vs. imbricate descending in papilionoideae vs. valvate in Mimosoideae) and floral symmetry (variable in Caesalpinioideae vs. radially symmetric in Mimosoideae vs. bilaterally symmetric in papilionoideae) (LPWG, 2017). Sub-family papilionidae is literally resembles the most edible legume in the world (Morris, 2003) and the three species of *V. unguiculata*, *P. tetragonolobus* and *P. vulgaris* are categorized in this group. *V. unguiculata* which is known as long bean or cow pea is originally come from Africa (Schipper, 2002). However, it has been cultivated worldwide with an estimated cultivation area of about 14.5 million metric tons (Singh *et al.*, 2002). Africa and South East Asia are known as the main producers of long bean. This legume exhibits different morphological forms; some are

prostrate, erect or climbing. The leaves are trifoliate and inflorescences are axillary with few crowded flowers near the tip in alternate pairs. The anthers bear sticky and heavy pollen grains (Purseglove, 1968). They can grow quickly and rapidly in the drier regions where rainfall is scanty and soil is sandy (Singh *et al.*, 1997). *P. tetragonolobus* in the other hand is known as winged bean or four angled bean originated from a tropical or subtropical area. In Asia, it is grown predominantly in the high altitude zones (Yanagi, 1984). The potential yield of the grain has been shown to be more than 4500 kg/ha in northern Australia and Malaysia (Enriquez, 1978). The special characteristic of this legume is on the pods. They can be straight or slightly curved, short, thick or very thin with the width varying from 2 to 4 cm. They have four prominent longitudinal wings corresponding to the four corners of the pod, with curved indentations of the border of the wings. The color ranges from white to green to purple with many combination and shades of basic color (Enriquez, 1978). *P. vulgaris* or French bean tolerates most environmental conditions in tropical and temperate zones (Duke, 1992). French bean grows best in well drained, sandy loam, silt loam or clay loam soils and rich in organic content. Asia had the largest hectarage (11 697 000) of yield followed by South America. They are erect and bushy, about 20 to 60 cm tall or twining with stems of 2 to 3 m long. The leaves are alternate, colored green or purple. The pods are slender sometimes cylindrical or flat measure from 8 to 20 cm. The color are also varied from green to purple (Duke, 1992).

2.7 ECONOMIC IMPORTANCE OF LEGUME

Legumes are grown on 180 million Ha, or 12% to 15% of the earth's arable surface. They account for 27% of the world's primary crop production, with grain legumes alone contributing 33% of the dietary protein nitrogen (N) needed by human (Vance *et al.*, 2000). Legume has been used traditionally and industrially by human. Other than as a food source, it had been processed as biodegradable plastics, paper, thickener, gums, dyes, inks and pill formulation (Duke, 1992; Kindsher & Lawrence, 1992; & Morris, 2003). Legume is being traded worldwide and the rate growth of legume imports in the South East Asian market is spectacular (Hill, 1986). South East Asia is listed as one of the main producer's countries of legume lead by Myanmar (Sing *et al.*, 2002). In Malaysia, long bean is listed as one of the ten vegetables that has highest harvested area, valued 4306.6 Ha with production value of 69 294.7 Mt which equivalent to RM 162 843 margin (Anon., 2015). However despite of this high production, legume faces numerous harvesting constraints (Asare-Bediako, 2012) such as insect pest, plant disease, parasitic flowering plants and drought that cause major reduce in yielding (Terao *et al.*, 1997). According to Diffie & Funderburk, (2009) *M. sjodjesti* and *M. usitatus* are the most important pests of legume in Africa and Asia. The legume yield losses cause by this thrips in Africa as stated by Ngakou *et al.* (2008) could be 20% and 70% depending on severity of infestation. According to Singh & Taylor (1978), plant parts mainly attacked by thrips are flower buds and flower themselves. Flower abortion is one the normal damage symptoms cause by thrips infestations. Before drop off, the flowers may get distorted, malformed and discolored (Lewis, 1973). However, no detailed studies were conducted in Malaysia regarding losses caused by thrips on legume plantation. Perhaps, due to minute size of thrips and farmers even did not realize on their presence as pest.

CHAPTER 3.0: STUDY SITES, MATERIALS AND METHODS

3.1 STUDY SITES

Three agricultural farms in Peninsular Malaysia were chosen as study sites; Janda Baik [East Coast region], Bestari Jaya [Central region] and Jelevu [Southern region]. None of the study sites used pesticides that targeted thrips. Janda Baik is located at 3°21'33.1" N 101°48'29.5" E, with a maximum day time temperature of 28°C. It is surrounded by pristine natural tropical forest at an altitude of 600 to 800 m above sea level. Bestari Jaya is located at 3°23'03.2" N 101°25'20.3" E, with a day time temperature that occasionally reaches 32°C. It is surrounded with oil palm plantation. Finally, Jelevu is located at 3°04'35.1"N 101°52'46.5"E. It is the driest location in Peninsular Malaysia and receives the least amount of rainfall in a year, with a day time temperature that may reach 34°C. Like Janda Baik, Jelevu is also surrounded by hills covered by tropical forest (Jelevu, 2015). Sampling was conducted from April, 2013 until May, 2014. To reduce temporal variation, sampling was done between 08:00 until 11:00.



Figure 3.0: Study site in Janda Baik, Pahang.



Figure 3.1: Study site in Bestari Jaya, Selangor.



Figure 3.2: Study site in Jelevu, Negeri Sembilan.

3.2 FIELD SAMPLING

3.2.1 THRIPS DIVERSITY STUDY

In Janda Baik, sampling was done on all three legumes species. While in Bestari Jaya and Jelevu, the sampling was done on long bean only. According to (Duke, 1992) French beans, is not cultivated in warm regions unlike long beans which is more tolerate to high temperature. Thus, long beans are widely distributed in warm and dry regions. Khan (1976) also reported that winged bean is not tolerated to excessive high growing temperatures. For this reason, farmers do not plant French bean and winged bean in Jelevu and Bestari Jaya. Due to availability of legumes being planted through the whole year by farmers, monthly sampling was conducted 12 times in Janda Baik. Of these 12 months, we sampled seven times on winged bean, two times on long bean and three times on French bean. By contrast, in Bestari Jaya and Jelevu, monthly sampling was done six times on long bean only. The abiotic factors of temperature, relative humidity and light intensity were recorded for every sampling in Janda Baik by using whirling hygrometer (Casella London) and lux meter (Extech Instruments) respectively. From our observation, there was slight difference in the composition of plantation among the three study sites. Janda Baik was classified as mix crop plantation while Bestari Jaya and Jelevu as a single crop plantation.

3.2.2 THRIPS POPULATION STUDY

In the selected study site, five plots were constructed, each with measurement of 10m x 10m. In each plot, five legume plants were randomly chosen and each plant was divided into three stratus; above, middle and lower. Each stratum is approximately 0.5m. Five flowers from each stratum were then randomly sampled. Abiotic factors such as temperature, relative humidity and light intensity were recorded in every sampling by using

whirling hygrometer and lux meter respectively. Reading of each abiotic factor were taken from each five plots respectively and averages of the measurement were used for this study.

3.2.3 THRIPS COLLECTION

Thrips were collected by beating the flowers over a white tray (Figure 3.3) and which thrips fell on the tray were then removed with a fine brush into collecting vials contains 95% alcohol (Figure 3.4). The vial is labeled with the information such as date, time, number of plot, strata, species of legume and location.



Figure 3.3: White tray and fine brush used for collecting thrips.

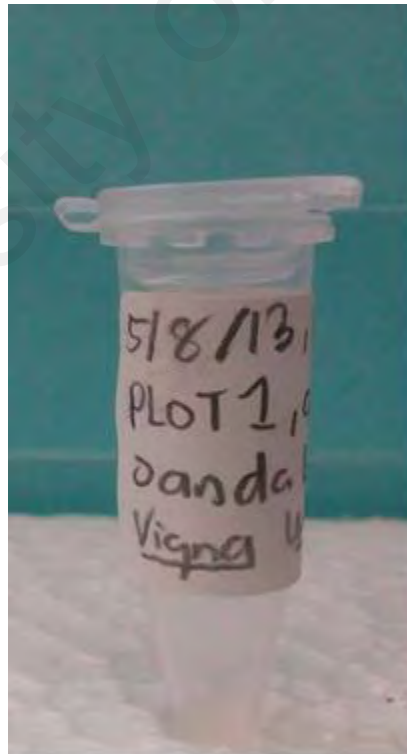


Figure 3.4: Vial containing 95% alcohol with data collection label.

3.3 SLIDE PREPARATION FOR THRIPS IDENTIFICATION

The collected thrips were then slide mounted based on the methodology by Mound (2007). The objective is to prepare specimens onto slides with their shape and color retained in a condition as close as possible to their natural, living state with the body cleared so that surface detail is visible. Under compound microscope, specimen from a vial were separated roughly to a different species such as species A, B, C and so on according to visual observation. There are four major steps involved in the slide preparation i.e. i) maceration, ii) dehydration, iii) mounting and iv) labeling.

3.3.1 MACERATION

The objective of maceration is to remove the body contents. About 20 thrips was put in an excavated block at one time. First the specimens were soaked for at least an hour in distilled water. The specimens were then removed to another excavated block that contained 5% sodium hydroxide (NaOH). This step is important as NaOH would macerate the body content and this process would take 24 or 48 hours depending on the body color of thrips. Darker thrips would need longer time. Specimens were then transferred and soaked for a few hours in distilled water. Every single thrips would be gently massaged to expel the body content and this process was done under dissecting microscope by using a loop and a very fine bent needle. And then the specimens were transferred into 60% alcohol solution and were stored for 12 to 24 hours.

3.3.2 DEHYDRATION

The objective of dehydration is to remove water from the specimen. Unmacerated specimens need to be punctured to speed the entry of alcohols by using a fine needle and they are soaked in 70% alcohol for about an hour. The specimens were then transferred into 80% alcohol (20 minutes) followed by 95% alcohol (10 minutes), absolute alcohol (5 minutes) and another fresh absolute alcohol (5 minutes) before being put into clove oil for 30 minutes.

3.3.3 MOUNTING

A small mounting block is prepared to facilitate this process. This is done by fixing a 2 mm deep layer of 1-inch square white card to the center of microscope slide. The center of the white card is marked with crossed lines and securely covered with plastic tape to provide a clean and shiny surface. A 13 mm diameter of cover slip was placed onto the mounting block. By using a dropper, one drop of Canada balsam was placed at the center of the cover slip and a specimen with ventral side uppermost then was gently placed onto the Canada balsam. The legs and wings of specimens were spread and the antenna was straightened by pressing on the basal segments with a fine needle. A clean microscope slide was gently lowered onto the specimen in balsam on the cover slip. As soon as the surfaces touch, the slide was re-inverted with the cover slip adhering. This is crucial technique, as it would avoid the trap bubble which may ruin the slides. The slide was then placed onto a hot plate at 50°C to dry off the Canada balsam as quickly as possible before putting it in an oven at 50°C for several weeks for hardening process.

3.3.4 LABELING SLIDES

On the right hand label, information such as host plant, country (in capital letters), locality, date and collector's name are stated. Meanwhile, on the left hand label, genus and species names with author and sex setae are added into the label (Figure 3.5).

3.4 IDENTIFICATION OF THRIPS SPECIES

Thrips were identified under the compound microscope until species level by using the revised taxonomic key for female and male by Palmer (1987) and Oz Thrips (2015) an on-line identification key.

List of characters used for *M. usitatus* identification is quite different between female and male.

Female of *M. usitatus* according to Palmer (1987)

1. Abdominal sternite VII with posteromarginal setae situated anterior to the posterior margin (Figure 3.6)
2. Forewing usually with sub apical pale area, rarely completely dark, always with a short gap in the row of fore vein setae; antennal segment III usually yellow.
3. Antennal segment IV brown; forewing with a distinct sub-apical pale area; ocellar setae pair III (usually about 2.5) times as long as the distance between their bases.

Male of *M. usitatus* according to Palmer (1987)

1. Posterior margin of tergite IX without a pair of setae – like projections (Figure 3.7).
2. Abdominal sternites without spine – like setae or glandular areas.
3. Pronotum pale, yellow; antennal segments rounder.



Figure 3.5: Slide with data collection label.

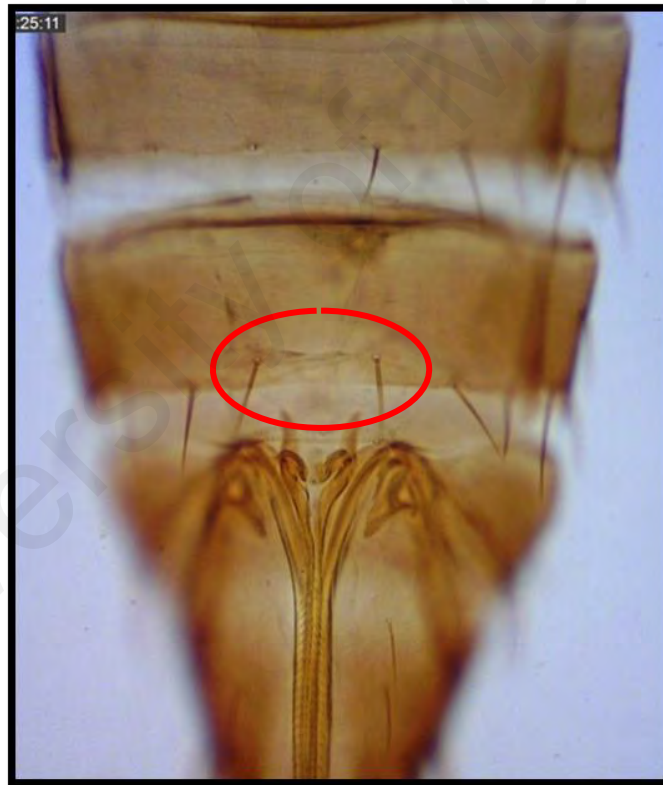


Figure 3.6: A pair of median setae is in front of the posterior margin of sternite VII.

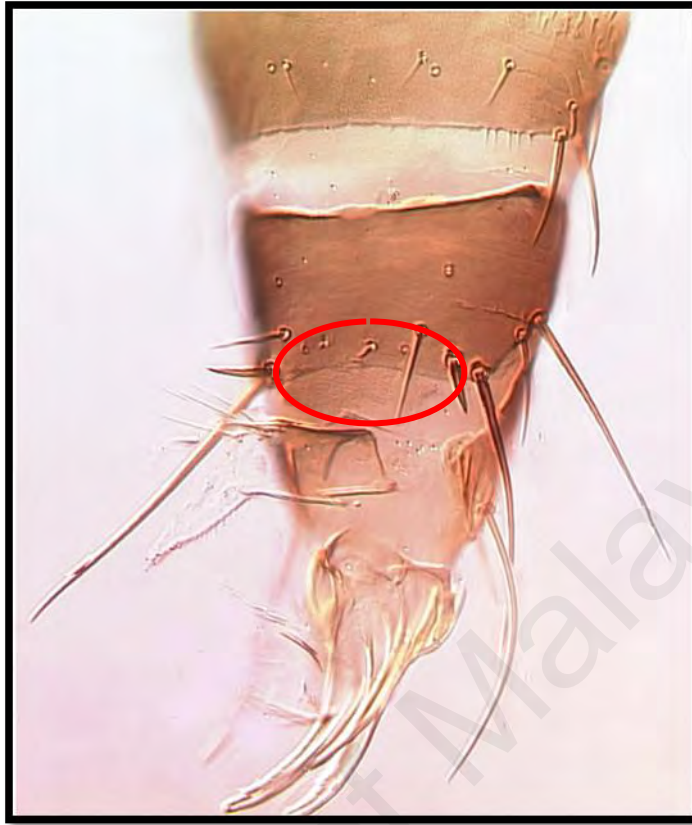


Figure 3.7: Tergite IX: posterior margin without paired setiform processes, posterolaterally with a pair of short and very stout setae.

Female of *M. usitatus* according to Oz thrips (2015)

1. Body brown; tarsi, apices of mid and hind tibiae, also most of fore tibiae yellow.
2. Antennal segment III yellow to light brown.
3. Hind tibiae with two stout dark apical setae.
4. Fore wings brown with basal quarter pale and an extensive pale area sub-apically.
5. Antennae 8-segmented, III–IV with constricted apical neck, sensorium forked.
6. Head as wide as long; 3 pairs of ocellar setae present, pair III on anterior margins of ocellar triangle and longer than distance between compound eyes; postocular setae small.
7. Pronotum with 2 pairs of long posteroangular setae; posterior margin with 3 pairs of setae; anteroangular setae well-developed.
8. Metanotum with weak sculpture; campaniform sensilla present, median setae arise at anterior margin.
9. Fore wing first vein with long row of setae before distinct subapical gap followed by 2 setae; second vein with complete row of setae.
10. Tergites without sculpture medially, ctenidia absent; tergite VIII with irregular group of microtrichia anteromesad of spiracle, posteromarginal comb of slender microtrichia but broadly absent medially.
11. Sternites without discal setae; median pair of marginal setae on sternite VII arise in front of margin (Figure 3.6).

Male of *M. usitatus* according to Oz thrips (2015)

1. Similar to female but smaller and paler.
2. Legs sometimes almost yellow.
3. Tergite IX posterior margin without paired setiform processes, posterolaterally with pair of short very stout setae (Figure 3.7)
4. Sternites without pore plates.

In this study, the characters used for identifying the female of *M. usitatus* are the abdominal sternite VII with median posteromarginal setae situated anterior to the posterior margin (Figure 3.6); ocellar setae pair III presents on anterior margins of ocellar triangle and its length is usually longer than the distance between the compound eyes.

Meanwhile, for the male, the posterior margin of tergite IX has no pair of setiform processes, posterolaterally with a pair of short and very stout setae. For a comparison, the posterior margin of tergite IX with paired setiform processes, posterolaterally with a pair of setae not short and stout is shown in Figure 3.8. The male that has this character is known as *M. typicus*.

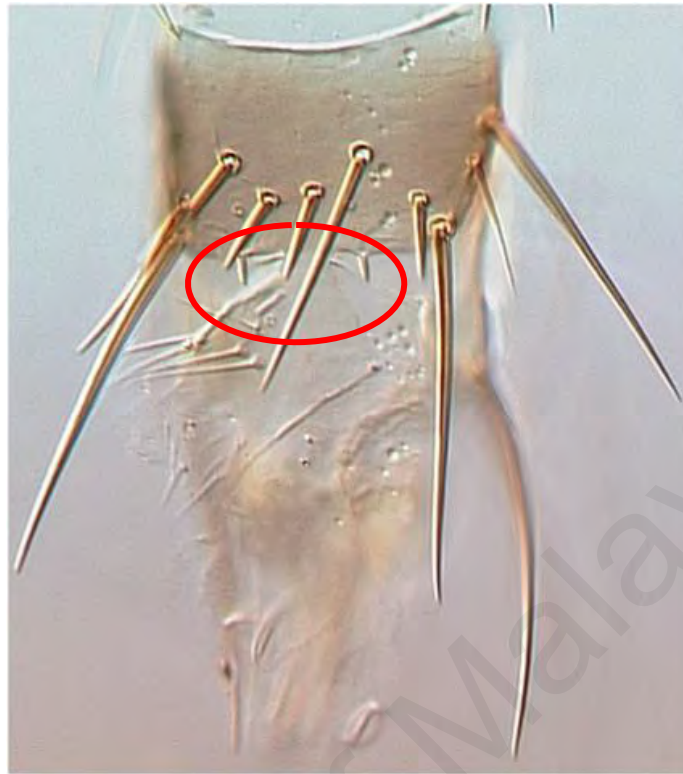


Figure 3.8: Tergite IX: posterior margin with paired setiform processes, posterolaterally with a pair of setae not short and stout.

3.5 STATISTICAL ANALYSIS

The abundance of each thrips species found in each site was tabulated. Shannon and Simpson's diversity indices, and Shannon evenness were then calculated for each site. The mean abundance for the most dominant species, *M. usitatus*, was calculated for each study site. A one-way ANOVA was then used to determine if there was a significant difference among the different study sites. We also used a one-way ANOVA to determine if there was a significant difference in the abundance of *M. usitatus* in legume flowers among the different legume plant strata. Finally, the collected samples of male and female of *M. usitatus* were subjected to one-way ANOVA with post-hoc Tukey HSD test to analyse their abundance among different months. We assessed the Pearson correlation between the abundance of male and female of *M. usitatus* and the different abiotic factors (temperature, light intensity and relative humidity) for collected sample from Janda Baik. Finally, we used one-way ANOVA to determine if the abundance of male and female of *M. usitatus* varied among the different species of legumes (long bean, winged bean and French bean). All analyses were conducted using STATISTICA (StatSoft Inc., 2001).

3.6 REARING OF *M. usitatus* IN LABORATORY

This study was conducted to verify the species identification of the doubtful *Megalurothrips*. The rearing was carried out from January until December 2014 with nine replications (Every replication started with 100 female and 50 male adults of *Megalurothrips*). A plastic cup measured 5cm x 9cm with ventilated cover lid was used to rear the thrips. For feeding and ovipositing purposes, *Cucumis sativus* or Japanese cucumber was chosen because it is less soggy and does not rot quickly. The Japanese cucumber was cut into small pieces measured approximately 3 cm each and was sterilized

in 400 ppm sodium hypochlorite solution for 4 minutes. It was then rinsed twice with water, dried with paper towel and placed in the plastic cup. Due to extreme sensitivity of thrips towards humidity, extra care was taken especially from the aspect of air ventilation. Cloth was used to cover the ventilation lid instead of gauze because the larva can escape themselves from the plastic cup through the tiny gap of the gauze. We also avoid using the wet cotton rolls, honey wick and bean pollen as proposed by DeGraaf & Wood (2009) because those materials accelerated fungus infection.

3.6.1 REARING OF MATED FEMALE OF *M. usitatus* IN LABORATORY

Approximately 100 female and 50 male adults of *Megalurothrips* were put into a plastic cup which contained Japanese cucumber. A fresh cucumber was replaced after three to four days and the adults that present on the former cucumber were gently blown back into the plastic cup. This starts the next batch of eggs and subsequent larvae that would be three to four days younger than the batch. The cucumbers with eggs then were removed from the plastic cup and placed into a newly prepared plastic cup for larval emergence. One fresh washed cucumber was then added as the extra food for emerging larvae. Pupae were removed from the cups and placed singly into another new plastic cup until it turned into an adult.

3.6.2 REARING OF UNMATED FEMALE OF *M. usitatus* IN LABORATORY

A single pupa was reared until it became an adult female and the rearing procedure is similar to the mated female except without the male species. The larvae emerged from this single virgin mother was then removed from the old cucumber and was put into new plastic cup until it turned into an adult.

3.6.3 IDENTIFICATION OF EMERGED ADULT FROM REARING

The emerged adults from mated female were then identified through morphological characters. While, the emerged adults from unmated female were identified through morphological characters and molecular technique for species verification.

3.7 MOLECULAR IDENTIFICATION

This experiment acted as a tool in order to confirm the identification of *Megalurothrips* species in this study. The DNA sequences were generated from the reared offspring of the unmated female and the wild specimens of *M. usitatus* from the three study sites. The former was chosen because to ensure that the offspring were produced parthenogenically. According to Havelka (2009); Nakao & Yabu, (1998), this parthenogenic offspring tend to be either similar or different morphologically from their mother and after certain generation of isolation in the reproduction, new species could be produced. These sequences were then compared with the sequences deposited in the gene bank. Thus, this gene sequences comparison is to ensure the status of *Megalurothrips* species especially for the certain individuals that possess the intermediate characters.

3.7.1 DNA EXTRACTION

DNA was extracted from each specimen using XytXtract Insect (ANDE) nondestructive DNA extraction kit. This kit was chosen because the specimens need to be slide mounted after the DNA analyses for the morphological identification.

All isolation steps were performed according to the instructions of the manufacturer.

3.7.2 POLYMERASE CHAIN REACTION (PCR)

The amplification of extracted genomic DNA was conducted using partial mitochondrial primers of COI from Brunner *et al.* (2002): forward primer 5' -ATT AGG AGC HCC HGA YAT AGC ATT -3' and reverse primer 5'-CAG GCA AGA TTA AAA TAT AAA CTT CTG-3'. The amplification of extracted genomic DNA was conducted using mitochondrial primers of COI regions performed in a final volume of 50µl containing 1x buffer, 0.2µm Dntp, 0.2µm of each forward and reverse primer, 2 U Bio-X-Act Long DNA polymerase (Bioline, London, UK) and 1µL genomic DNA of *Megalurothrips*. PCR was carried out using Bio-rad MyCycler™ Thermal Cycler Serial Number: 580BR 7200 (CA, USA). The PCR conditions of COI included an initial denaturation of 95°C for 5 min, followed by of 95°C for 1min (denaturation), 50°C for 1min (annealing) and 72°C for 1min (extension) and 72°C for 10min (final extension).

3.7.3 DNA PURIFICATION

The amplified fragments were electrophoresed on 2% agarose gel pre-stained with SYBR Safe™ (Invitrogen, USA). PCR products were sent to commercial company (First Base Co., Selangor, Malaysia) for DNA sequencing.

3.7.4 DNA SEQUENCES ALIGNMENT

Sequencing data were analyzed and edited by using BioEdit 7.0.9.0® (Hall, 1999). While for the homology, insertion and deletions, stop codons, and frameshifts were using NCBI BLAST. (Jalali *et al.*, 2015).

3.7.5 PHYLOGENETIC ANALYSES

COI sequences of *M. usitatus* were aligned with other sequence of *M. usitatus* obtained from gene bank and subjected to Maximum likelihood (ML), Neighbor Joining (NJ) and Maximum Parsimony (MP) analyses. All these analyses were performed using Mega 6 (Tamura *et al.*, 2004) and was performed with 500 bootstrap replicates. Bootstrap percentage was computed with kimura-2-parameter model of substitution (K2P distance) evolution model. Two other species from Thripidae family were used as the outgroup in the phylogenetic tree namely *M. distalis* and *F. occidentalis*. *M. typicus* was included in the phylogenetic analyses because it is suspected that the doubtful *Megalurothrips* species is an interbreed species between *M. usitatus* and *M. typicus*.

CHAPTER 4.0: RESULTS

4.1 DIVERSITY OF THRIPS ON LEGUMES

Generally, there are three genera of thrips that can be found on the legumes crop. They are *Megalurothrips*, *Thrips* and *Ceratothripoides*. One species of *Megalurothrips* (i.e. *M. usitatus*), two species of *Thrips* (i.e. *T. hawaiiensis* and *T. parvispinus*) and one species of *Ceratothripoides* (i.e. *C. brunneus*) were normally sampled. However, there are certain females of *Megalurothrips* species that possess intermediate character between *M. usitatus* and *M. typicus*. They are also included in the analyses as *M. usitatus* until their species identification is verified. Although *M. typicus* was sampled in this study, they were not included in the analyses since their number was too small. Janda Baik had the highest Shannon and Simpson diversity compared to Bestari Jaya and Jelevu (Table 4.0). This is due to the high proportion and dominance of *M. usitatus* at both Bestari Jaya and Jelevu.

4.2 THRIPS POPULATION

4.2.1 OVERALL THRIPS POPULATION ON LEGUMES

M. usitatus had the highest mean abundance at each site, followed by *T. parvispinus* (Table 4.1). In contrast, *T. hawaiiensis*, which was found in low numbers in all sites, and *C. brunneus*, which was found only in Janda Baik, were the two thrips species found least frequently on the legumes (Table 4.1). Overall, in Janda Baik, 64% of the collected thrips species were *Megalurothrips* species, whereas in both Bestari Jaya and Jelevu, 95% of collected thrips were *Megalurothrips*.

Table 4.0: Species diversity indices at selected study sites.

	Janda Baik	Bestari Jaya	Jelevu
Evenness e^{H_s}	0.5142	0.6139	0.3056
Shannon H	0.7211	0.2052	0.2009
Simpson 1-D	0.4701	0.0991	0.0932
Dominance D	0.5299	0.9009	0.9067

Table 4.1: Mean number of thrips species on three different species of legumes flowers; long bean, French bean and winged bean, at three different selected agricultural farms from April 2013 until May 2014.

	Janda Baik	Bestari Jaya	Jelevu
<i>M. usitatus</i>	23.83 ± 14.73	85.78 ± 77.47	81.87 ± 70.41
<i>T. parvispinus</i>	21.68 ± 20.11	11.56 ± 9.74	10.01 ± 8.67
<i>C. brunneus</i>	0.85 ± 0.43	0	0
<i>T. hawaiiensis</i>	0.94 ± 0.53	0.53 ± 0.31	0.1 ± 0.00

4.2.2 OVERALL POPULATION OF *M. usitatus* ON LEGUMES

4.2.2.1 ABUNDANCE OF *M. usitatus* AT THE STUDY SITES.

Overall, 64% of the collected thrips species in Janda Baik comprised of the *M. usitatus* while in Bestari Jaya and Jelevu, 95% respectively. The total number of the *M. usitatus* female is always more than the total number of the male at the three study sites. In Janda Baik, 97% is female and 3% is male. While in Bestari Jaya and Jelevu, 82% and 91% are females respectively, and the males are 18% and 9% respectively.

There was a significant difference in overall abundance of *M. usitatus* at the different sites ($F = 9.6$, $df = (2, 88)$, $p < 0.05$), with Bestari Jaya having the most females (141 ± 72.3) and males (30.6 ± 25.9), while Janda Baik having the fewest females (41.9 ± 27.5) and males (1.5 ± 2.3) (Fig. 4.0).

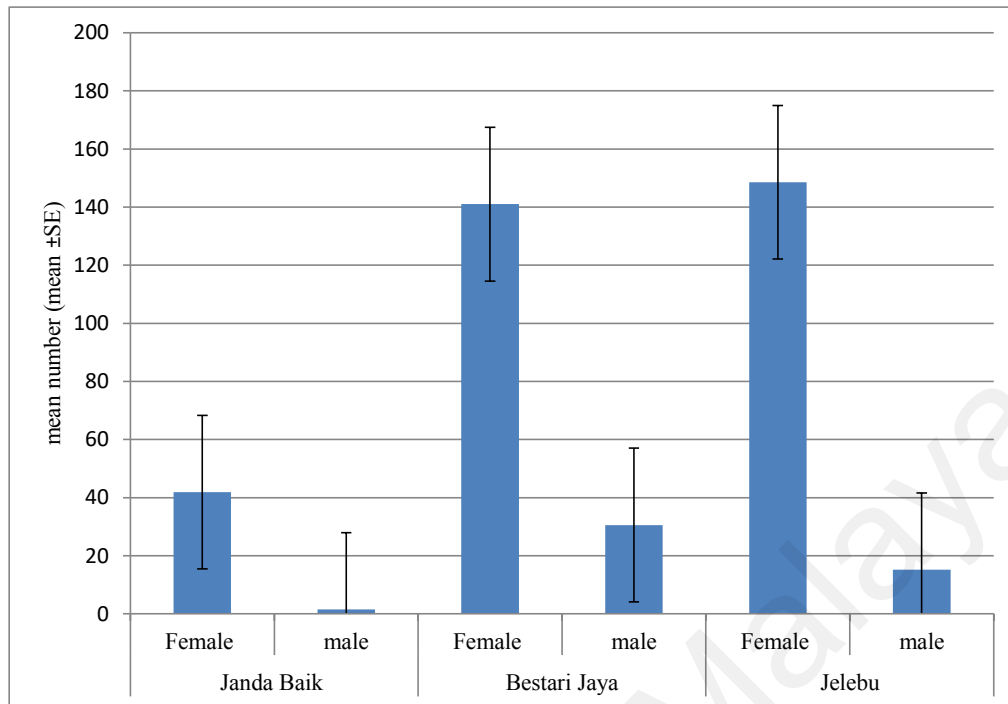


Figure 4.0: Mean numbers of female and male of *M. usitatus* on legumes at three different selected three different selected agricultural farms from time period of April 2013 until May 2014.

4.2.2.2 SPATIAL DISTRIBUTION OF *M. usitatus* ON LEGUMES ACROSS STRATA.

There was no significant difference in the distribution of *M. usitatus* at the three different strata in the legume plants (Table 4.2).

4.2.2.3 TEMPORAL DISTRIBUTION OF *M. usitatus* ACROSS SAMPLING PERIODS.

There were significant differences between the number of females [$F = 15.7$, $df = (11, 48)$, $p < 0.05$] and males [$F = 7.389$, $df = (11, 48)$, $p < 0.05$] of *M. usitatus* among different months. The number of females was highest in January and February (110.6 ± 40.8 and 111.4 ± 39.0), with a temperature of 27°C and 29°C and lowest in December (1 ± 1), where the temperature was 24°C (Fig. 4.1). Similarly, the abundance of males was highest in January and February (9.8 ± 3.5 and 7.8 ± 10.0) but lowest in March, July, August and December, where no male specimen was collected at an average temperature of 25°C (Fig. 4.1).

4.3 CORRELATION BETWEEN *M. usitatus* POPULATION WITH THE ABIOTIC FACTORS

A correlation analysis between *M. usitatus* population and abiotic factors was done only in Janda Baik since the sampling was complete a year cycle. There was a moderate correlation between the female abundance and temperature [$r = 0.626$, $df = (1, 11)$, $p < 0.05$] (Fig. 4.2), but a strong correlation between the total number of males and temperature [$r = 0.753$, $df = (1, 11)$, $p < 0.05$] (Fig. 4.3). Thus, temperature appears to influence the total number of both females and males of *M. usitatus*.

Relative humidity in Janda Baik fluctuated between 79% and 100%, with an average of 88.17%. There was a negatively weak correlation between both the total number of females

[$r = -0.068$, $df = (1, 11)$, $p < 0.05$] (Fig. 4.4) and males [$r = -0.34$, $df = (1, 11)$, $p < 0.05$] (Fig. 4.5) with relative humidity. There were two outliers in figure 4.5 indicating the higher numbers of *M. usitatus* in January and February coincide with hot season and high temperature within that both months.

Light intensity also fluctuated in Janda Baik, ranging from 180×10^2 cd to 700×10^2 cd, with an average of 470×10^2 cd. Light intensity was moderately correlated with the total number of females [$r = 0.5$, $df = (1, 11)$, $p < 0.05$] (Fig. 4.6), but weakly correlated with the total number of males [$r = 0.4$, $df = (1, 11)$, $p < 0.05$] (Fig. 4.7). Hence, the light intensity may also have influenced the total number of both females and males of *M. usitatus*.

Table 4.2: Results of One Way ANOVA for the relative abundance of *M. usitatus* at three different stratum of legume plants at three different selected agricultural farms from April 2013 until May 2014.

.^{NS} Not significantly different.

Location	stratum	N	Mean	F	DF	p-value
Janda Baik	Above	6	98.5±68.09	0.9009	2,15	0.4271 ^{NS}
	Middle	6	65.83±40.16			
	Lower	6	64.83±32.88			
Bestari Jaya	Above	6	355±174.40	1.569	2,15	0.2405 ^{NS}
	Middle	6	293±154.51			
	Lower	6	209.83±81.05			
Jeledu	Above	6	384.17±191.43	2.128	2,15	0.1536 ^{NS}
	Middle	6	253±150.00			
	Lower	6	180.0±177.60			

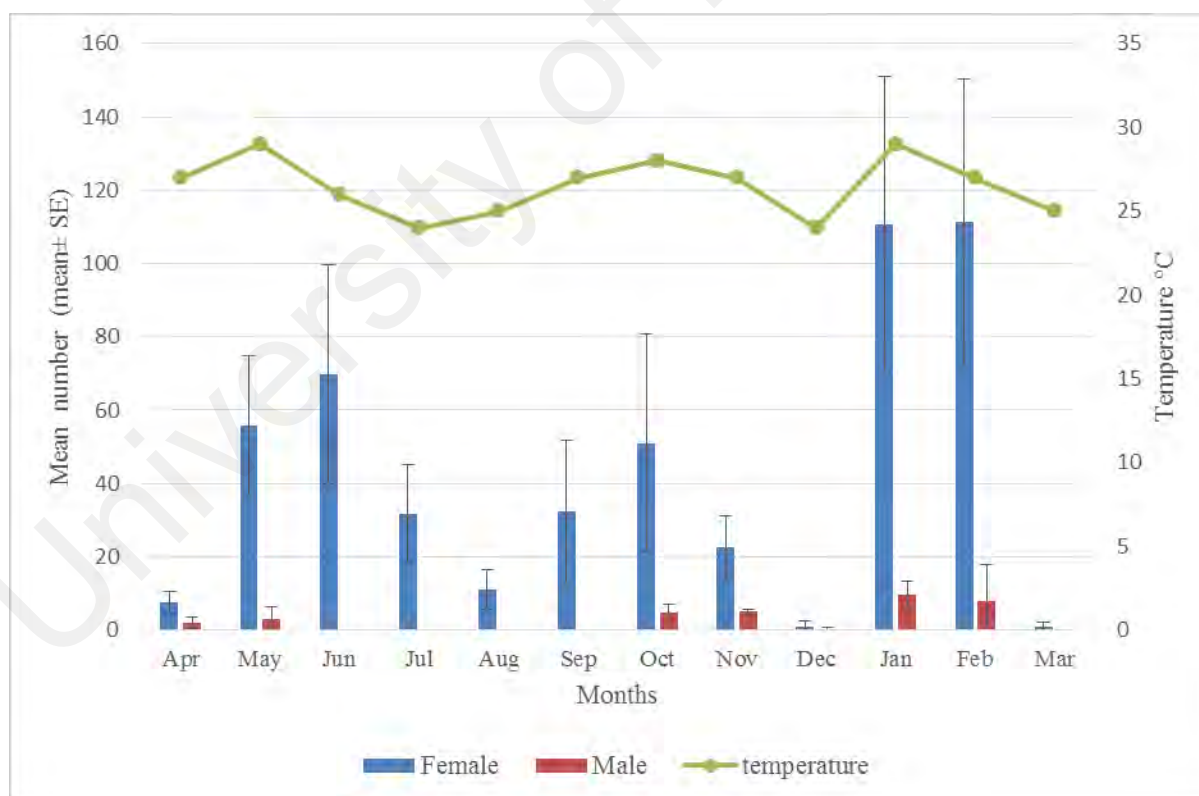


Figure 4.1: Mean numbers of the female and male of *M. usitatus* in Janda Baik with respective of months and temperature sampled from April 2013 until May 2014.

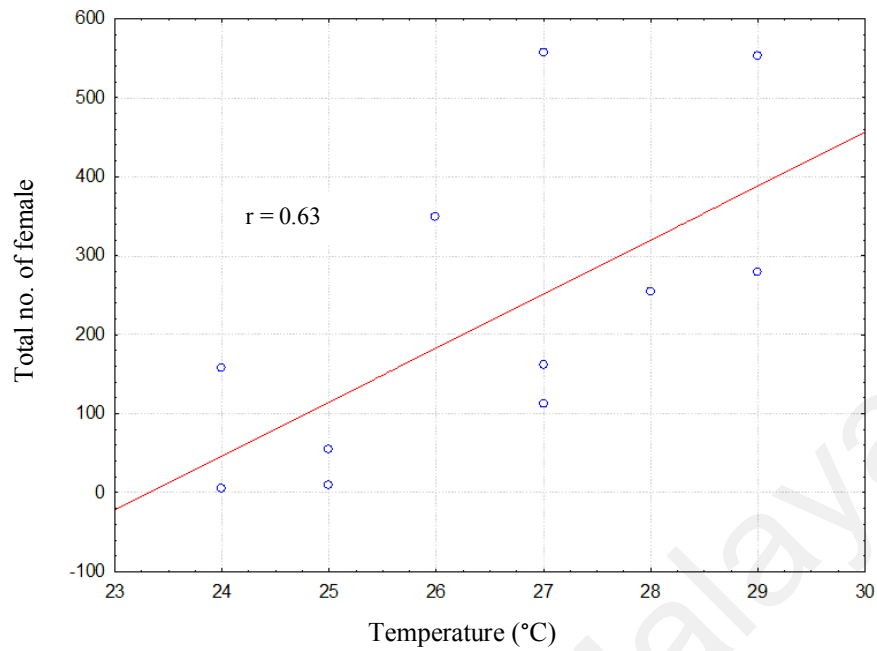


Figure 4.2: The correlation between the total number of female of *M. usitatus* in Janda Baik and the temperature from April 2013 to May 2014.

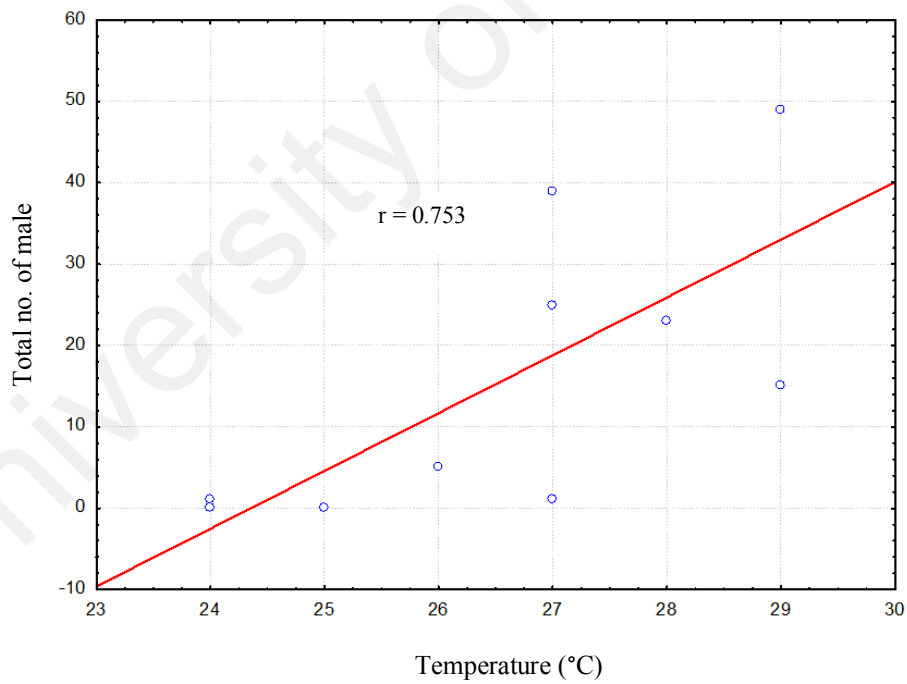


Figure 4.3: The correlation between total number male of *M. usitatus* in Janda Baik and temperature from April 2013 to May 2014

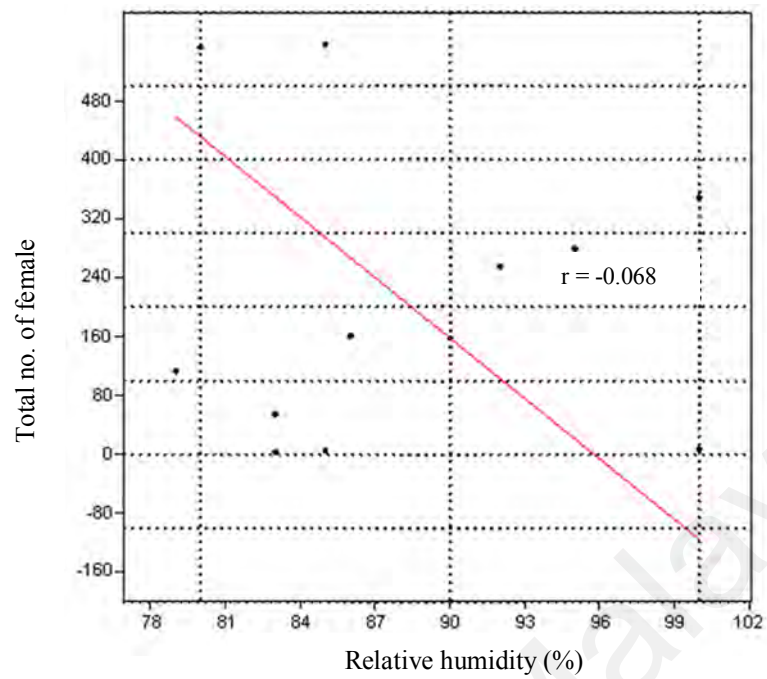


Figure 4.4: The correlation between numbers of female of *M. usitatus* in Janda Baik and the relative humidity from April 2013 to May 2014.

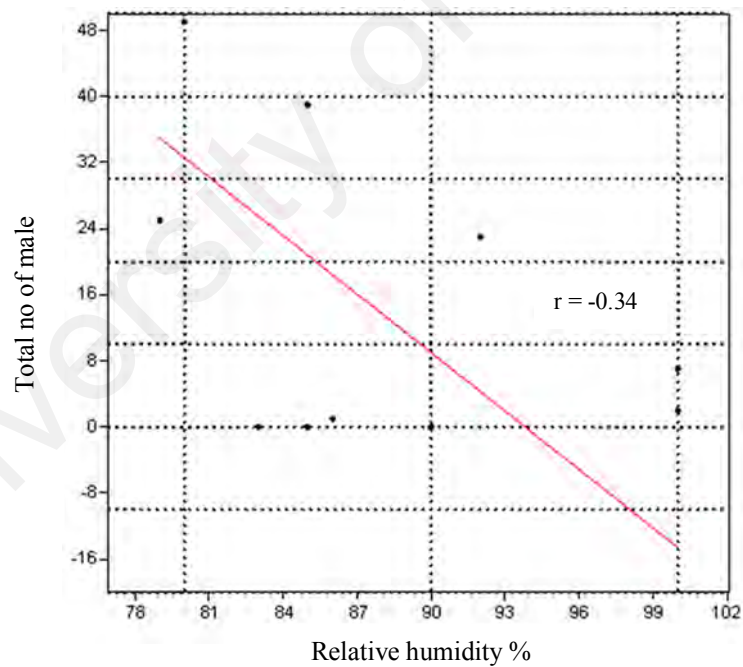


Figure 4.5: The correlation between total number of male of *M. usitatus* in Janda Baik against the relative humidity from April 2013 to May 2014.

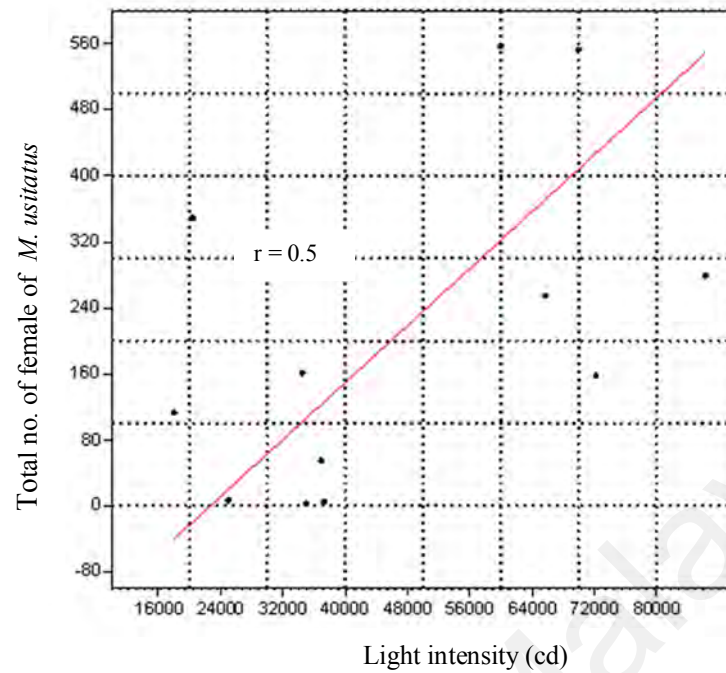


Figure 4.6: The correlation between total number of female of *M. usitatus* in Janda Baik and the light intensity from April 2013 to May 2014

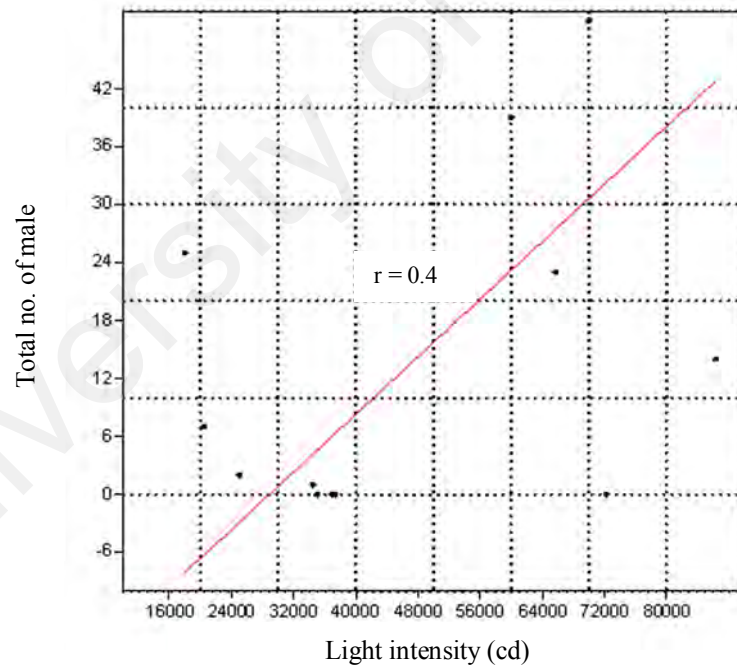


Figure 4.7: The correlation between total number of male of *M. usitatus* in Janda Baik and the light intensity from April 2013 to May 2014.

4.4 *M. usitatus* POPULATION ON LEGUMES SPECIES

There was no significant difference in the total number of female [F = 27.67, df = (2, 57), p > 0.05] or male [F = 8.67, df = (2, 57), p > 0.05] *M. usitatus* on different legume species (i.e. *T. teragonolobus*, *P. vulgaris* and *V. unguiculata*).

4.5 REARING OF *M. usitatus* IN THE LABORATORY FOR VERIFYING SPECIES IDENTIFICATION

4.5.1 REARING OF MATED FEMALE OF *M. usitatus* IN THE LABORATORY

In this rearing, survived adults were not being obtained in every replication especially for the 1st, 2nd, 4th and 5th replicates (Table 4.3). This is due to thrips sensitivity to diet, temperature and humidity especially in artificial environment. It is conceded that rearing of thrips is legitimately difficult (Grundy *et al.*, 2000). Thrips and their food resources are easily being attacked by fungus which led to death and had proven through our observation in laboratory. On average, about 5.0 female and 2.7 male of adults per replicate manage to survive. The total number of survived female adults per replicate fluctuated from 0% until 86%. While for the male, it fluctuated from 0% to 67%. The highest percentage of survived female adults which was 86% was obtained in the 8th replicate. While for the male, it was in the 7th replicate which was 67%. All survived female (45 individuals) and male (24 individuals) adults in this rearing were morphologically identified as *M. usitatus*.

Table 4.3: Total number and percentage of adults emerged from mated female of *M. usitatus* in the laboratory and their species identification.

Replicate	Date of rearing	Sex	N	Adult survived per replicate (%)	Species identification
1	9/1/14	♀	0	0	-
		♂	0	0	-
2	23/1/14	♀	0	0	-
		♂	0	0	-
3	18/2/14	♀	2	67	<i>M. usitatus</i>
		♂	1	33	<i>M. usitatus</i>
4	16/3/14	♀	0	0	-
		♂	0	0	-
5	3/4/14	♀	0	0	-
		♂	0	0	-
6	18/4/14	♀	3	60	<i>M. usitatus</i>
		♂	2	40	<i>M. usitatus</i>
7	23/5/14	♀	1	33	<i>M. usitatus</i>
		♂	2	67	<i>M. usitatus</i>
8	28/10/14	♀	6	86	<i>M. usitatus</i>
		♂	1	14	<i>M. usitatus</i>
9	25/11/14	♀	33	65	<i>M. usitatus</i>
		♂	18	35	<i>M. usitatus</i>

4.5.2: REARING OF UNMATED FEMALE OF *M. usitatus* IN THE LABORATORY

This section is continuous from the rearing of the mated female. On average about 1.4 female and 1.1 male of adult per replicate survived (Table 4.4). The total number of survived female adults per replicate fluctuated from 0% until 100%. While for the male, it fluctuated from 0% to 100%. The highest percentage of survived female adults which is 100% was obtained in the 6th and 8th replicates. While for the male, it was in the 3rd replicate which is 100%. Overall, 13 female and 10 male adults survived in this rearing.

All of the male individuals were morphologically grouped into as *M. usitatus*. Whereas, 84.6% of the female individuals were confirmed as *M. usitatus* and 15.4% were doubtful '*Megalurothrips usitatus*'

The morphological characteristics of the doubtful species '*M. usitatus*' can be identified as possessing possess one of the median setae (on the right) located very near (i.e. a diameter of the basal pore) to the posterior margin of sternite vii. (Fig. 4.8) or near (i.e. one and the half to two time the diameter of the basal pore) to the sternite posterior margin (Fig.4.9).

Thus, this phenomenon shows the intermediate form of this particular character between *M. usitatus* and *M. typicus*. Normally, the pair of median setae on sternite vii for *M. usitatus* is in front of the posterior margin (Fig. 5.0) while for *M. typicus* it is situated along the posterior margin (Fig. 5.1).

Table 4.4: Total number and percentage of adults emerged from unmated female of *M. usitatus* in the laboratory and their species identification.

Replicate	Date of rearing	Sex	N	Adult survived per replicate (%)	Species identification
1	8/1/14	♀	0	0	-
		♂	0	0	-
2	22/1/14	♀	0	0	-
		♂	0	0	-
3	17/2/14	♀	0	0	-
		♂	1	100	<i>M. usitatus</i>
4	15/3/14	♀	0	0	-
		♂	0	0	-
5	2/4/14	♀	0	0	-
		♂	0	0	-
6	17/4/14	♀	1	100	<i>M. usitatus</i>
		♂	0	0	-
7	22/5/14	♀	0	0	-
		♂	0	0	-
8	27/10/14	♀	1	100	' <i>M. usitatus</i> '
		♂	0	0	-
9	24/11/14	♀	11	55	10 individual are <i>M. usitatus</i> & 1 individual is ' <i>M. usitatus</i> '.
		♂	9	45	<i>M. usitatus</i>



Figure 4.8: One of the median setae (left) located very near (i.e. a diameter of the basal pore) to the posterior margin of sternite VII.

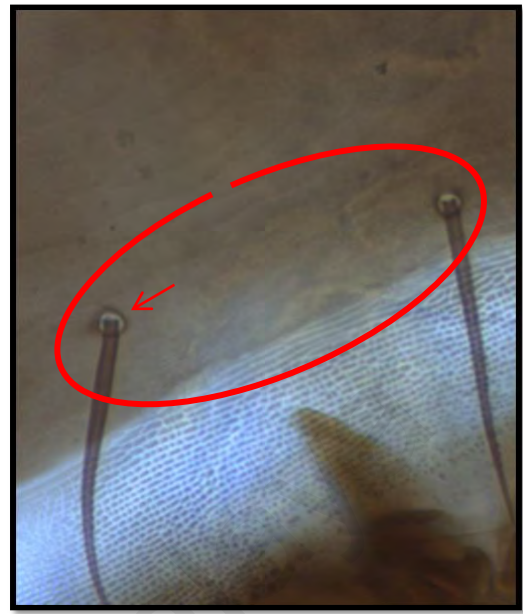


Figure 4.9: One of the median setae (right) near (i.e. one and the half to two time the diameter of the basal pore) to the posterior margin of sternite VII.

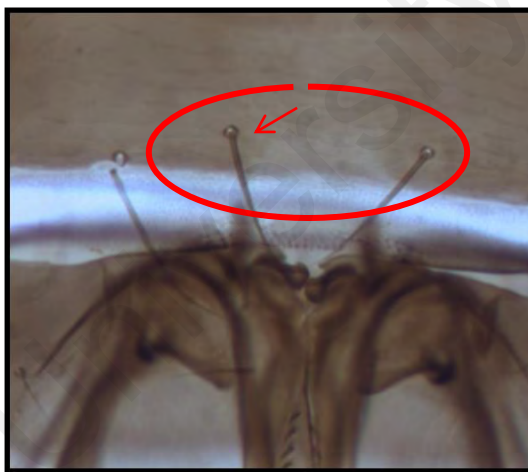


Figure 5.0: The pair of median setae is in front of the posterior margin of sternite VII.

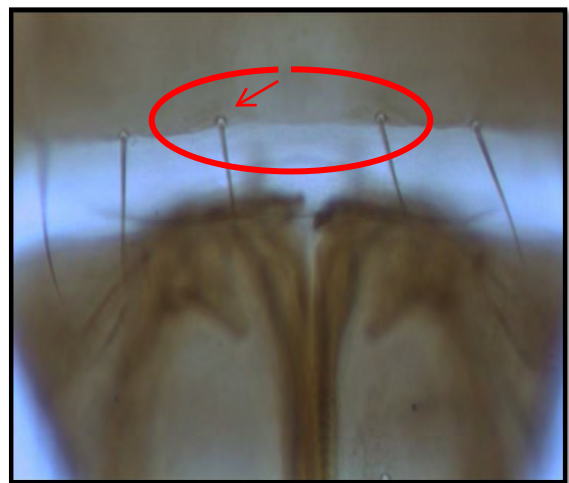


Figure 5.1: The pair of median setae is situated along the posterior margin of sternite VII

4.6 MOLECULAR IDENTIFICATION

Two major clades are formed within the phylogenetic tree of *Megalurothrips* taxa, i.e. clade 1 and clade 2 (Fig. 5.2). For the clade 1, the bootstrap value is 0.53/56/53 (MP/ML/NJ) while clade 2 is 0.98/100/94. There are two subclades within the clade 1. The subclade 1 comprised of *M. typicus* and *M. distalis* (Kenya) with bootstrap value of 1.00/94/87. While, the subclade 2 constituted of *M. usitatus* and '*M. usitatus*' which were all females from Janda Baik and Bestari Jaya with bootstrap value of 0.50/98/85. The clade 2 consisted of both males and females of *M. usitatus* from Janda Baik, Jelevu, Bestari Jaya, India and China. The reared of both males and females of *M. usitatus* were also in this group with bootstrap value of 0.98/100/94. The bootstrap value is to show how well the node is supported to generate the phylogenetic analysis. High bootstrap value which is close to 100% mean uniform support, i.e. if the bootstrap value for a certain clade is close to 100%, nearly all of the characters informative for individuals is almost similar and they are considered as a group (Berry & Gascuel, 1996). In this study, clade 1 has lower percentage and the value is quite distant from 100% showing that subclade 1 and 2 is not grouped together. However, within the subclade 1 and 2 the bootstrap value is nearly 100%, confirming that each individual within subclade is in its own group, i.e. subclade 1 is for *M. typicus* and *M. distalis*. While individuals in subclade 2 is confirmed as *M. usitatus* and '*M. usitatus*'. For clade 2, the value generated was high, nearly 100% confirming that individuals in clade 2 is *M. usitatus*.

F. occidentalis and *M. distalis* were chosen as the outgroup for the phylogenetic analyses (Fig. 5.1). According to Baum & Smith (2012), outgroup serve as reference group when determining the evolutionary relationship among monophyletic groups of organism. The chosen outgroup is hypothesized to be related to the ingroup and has common ancestor. The

outgroup also could be a sister group to the ingroup or maybe more distantly related. In this study *F. occidentalis* and *M. distalis* are related to *M. usitatus* because they are from the subfamily of thripinae. *F. occidentalis* is from the genus of *Frankliniella* while *M. distalis* is similar to *M. usitatus* which is from the genus of *Megalurothrips*. *M. typicus* was included in the phylogenetic analyses because it is suspected that the doubtful *Megalurothrips* species is an interbreed species between *M. usitatus* and *M. typicus*.

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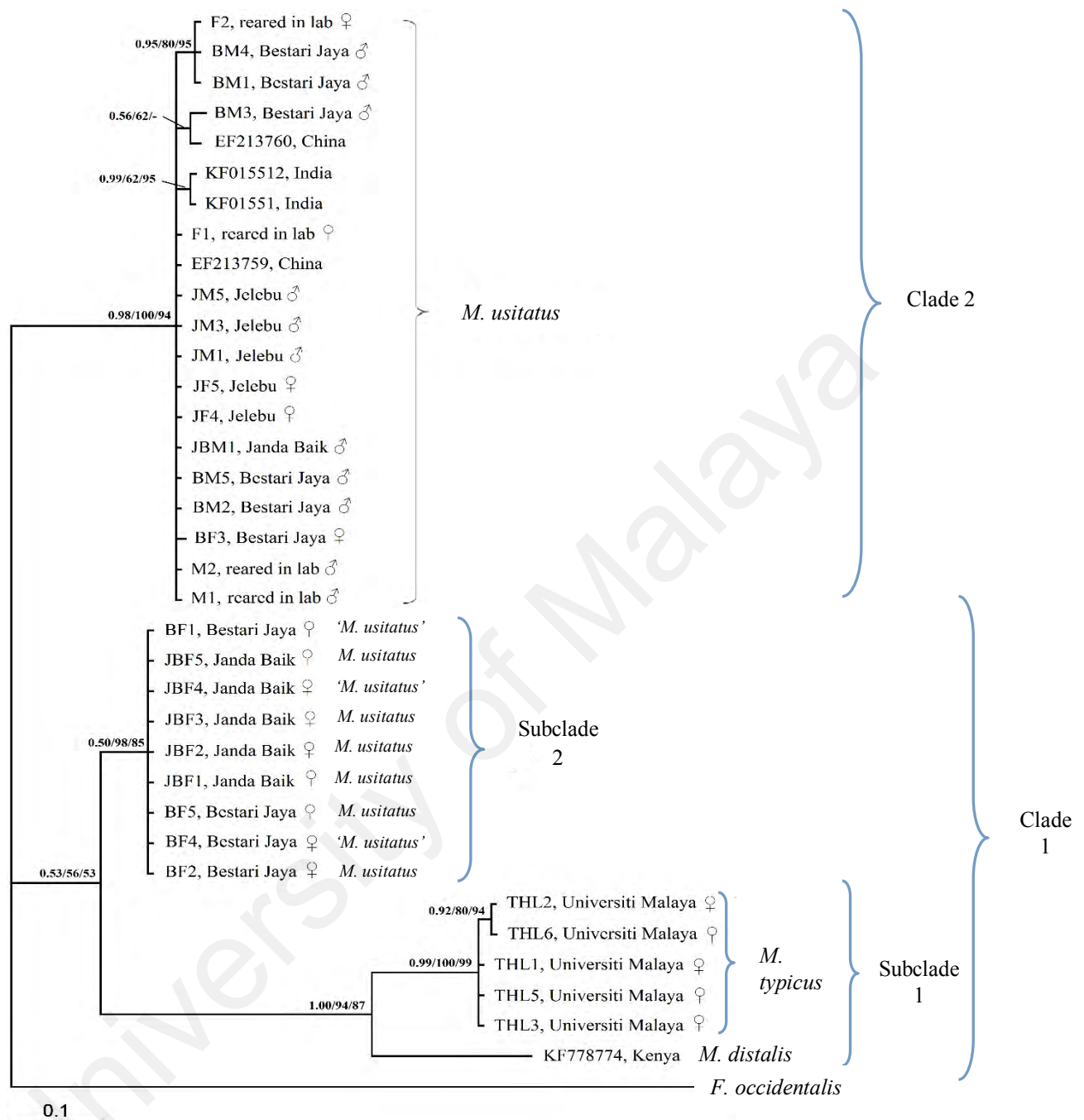


Figure 5.2: The phylogenetic tree of *Megalurothrips* taxa based on COI gene marker. The bootstrap values [Maximum Parsimony (MP)/ Maximum likelihood (ML)/ Neighbor are Joining (NJ)] are shown on the branches.

CHAPTER 5: DISCUSSION

5.1 DIVERSITY OF THRIPS ON LEGUMES

The closest study to this research was conducted by Fauziah & Wan (2004), who reported *M. usitatus*, *T. palmi* and *T. taiwanus* were sampled from legumes. However, in this study the thrips species which were collected from the legumes are *M. usitatus*, *M. typicus*, *T. parvispinus*, *T. hawaiiensis* and *C. brunneus*. Thus, there is a slight difference in the composition of the thrips species on legumes from both studies. However, *T. taiwanus* has been reported by Mound & Collin (2000), as a synonym species of *T. parvispinus*. Although *T. parvispinus* is widespread in south East Asia, its misidentification is due to unclear identity in most of the current literatures (Mound & Collin, 2000).

The presence of *T. parvispinus* could be due to the existence of other host plants which were planted alternately between the legumes such as chili especially in Janda Baik. According to Johari *et al.* (2014) and Azidah (2011), *T. parvispinus*, *T. hawaiiensis* and *C. brunneus* were reported to be the pest for chili. However, there is a possibility that these thrips might fly from their host and landed on the legumes. According to Aliteri (1986), this phenomenon could happen due to the difficulty and misallocation of the real host by thrips especially for feeding and breeding as the odors released by some plants might mask the effect of those released by other plants. Furthermore, Parker *et al.* (2013) stated that the companion plants may disrupt the host location by thrips. It was obvious that the thrips diversity among the three study sites was different and this could be due to the differences in the cropping system which was practiced by the farmers in Janda Baik, Bestari Jaya and Jelebu. The cropping system/pattern in Janda Baik can be classified as mixed cropping and the alternate plants can be grouped into two types; 1) small tree such as chili, and 2) big trees such as Durian and Rambutan. Researchers classify this as a mixed cropping system

or polyculture system which is also known as diverse planting system. This system builds up a complex interaction between host, non-host and pest (Parker *et al.*, 2013). According to Aliteri & Letourneau (1982), the complex crop habitat can support more insect species than a single crop habitat. They attributed this to the diverse chemical environment, varied in microclimate patterns and disparate in wind circulations.

5.2 OVERALL THRIPS POPULATION ON LEGUMES

The variability in thrips populations on crops is influenced by climate and weather variables (Ananthakrishnan, 1993). Further, Palumbo (2011) stated that weather can either promote insect population growth or cause populations to decline. Generally, Janda Baik has the lowest abundance of thrips while Bestari Jaya has the highest. Taburan Hujan Malaysia (2015) stated that Janda Baik is located at the highest altitude (about 600 – 800 meter above the sea level) and has the lowest temperature, valued 28°C on the peak day (Riverside Janda Baik, 2015). While Bestari Jaya and Jelevu has higher temperature which can reach 32°C at day time respectively (Jelevu, 2015). Clearly, these three study sites have different and varied environmental conditions. According to Waiganjo *et al.* (2008), different climate trend and varied weather variables may affect the thrips population differently. He also claimed that thrips occurred in all crop season, however, dryer weather with higher temperature increased the thrips number. This could explain why thrips are more abundant in Bestari Jaya and Jelevu compared to Janda Baik. Iklim Malaysia (2015) also reported that Janda Baik has highest rainfall (2500 – 3500 mm), followed by Bestari Jaya (2000 – 2500 mm) and the driest is Jelevu (1190 mm). According to Lorini & Junior (1990), high temperature and lack of rainfall increased the thrips population. While heavy

rain with low temperature had been reported causing sharp declines on the thrips population (North & Shelton, 1986).

Resource concentration hypothesis by Root (1973) could also explain on how the abundance of *Megalurothrips* at the three study sites is different. He explained that mono cropping system which is similar to Bestari Jaya and Jelevu can cause the thrips population to increase. It was hypothesized that in monoculture fields where the same plant species is cultivated over the large areas, the herbivores may find a concentrated source of food that support their uninterrupted population to build up (Root, 1973). Moreover, the available food plants is in pure stands and is easy to be detected and colonized (Pankeaw *et al.*, 2011). While in mix crop system, the different microenvironments produced from the interactions among different varieties of plants could suppressed the thrips population (Pankeaw *et al.*, 2011). Tall trees such as rubber and durian trees usually produce canopy that create shade. Shading in intercropping system reduce the air circulation, leading to high humidity and increase in disease incidence (Sileshi, 2007). In Janda Baik, the shade covered almost the entire legume plot, blocking the sunshine and created a humid environment for the legume trees. Few *M. usitatus* were found dead in the wet flowers. Thus, in this occurrence Janda Baik has less abundance of *M. usitatus* compared to Bestari Jaya and Jelevu. This phenomenon is congruent with the hypothesis. However, in Jelevu, *C. brunneus* and *T. hawaiiensis* were present in certain plot but in a very small number. Their presence could be due to the two plantation areas (i.e. chili and legume) that were closely planted side by side. Several studies have shown that the abundance of thrips is higher in the monocrop plantation (Hook & Johnson, 2003; Pankeaw *et al.*, 2011). Thus, further study is needed regarding on thrips population dynamics in different types of cropping system. Besides, Elmstrom *et al.* (1988) reported that most studies failed to

question how changes in the plant quality caused by the companion plants could affect the insect population dynamics. However, the farmers in Janda Baik are basically practiced the alternate planting purposely for space saving in plantation area. They did not realized that their action actually could help them in combating the pest especially thrips in their legume plantation area.

Knowledge of the dispersal patterns of insect pest species is fundamentally important for integrated pest management studies (Pearsall, 2000). This enables to forecast pest outbreaks and is useful for creating pest sampling plans and management tactics (Pedigo & Rice, 2009). One of the factors that accelerates the infestation and makes thrips difficult to control is their dispersion and spatial distribution potential (Helyer & Brobyn, 1992). According to Alves-Silva & Del-Claro (2010) thrips prefer the microhabitats in plant due to the plant phenology and climatic conditions. Abiotic factors such as light intensity, humidity, wind velocity and temperature that vary from near ground to upper canopy and from the interior of tree crown to the tips of leaves provide series of unique environment for plants and animal to adapt (Nieder *et al.*, 2000). The upper canopy for instance, experience extreme temperature and humidity fluctuation, stronger winds and heavier rainfall compared to the understory. Thus, different abiotic condition within plant canopy subsequently affects the distribution of resources and the spatial composition of insect (Wardbhaugh, 2014). Nevertheless, in this study, *M. usitatus* were spatially distributed among different stratum of the legume plant. The height of the legume plant which was only 1.5m probably was not sufficient enough to create different layers of canopies with different climate conditions. According to (Mohanlall, 2002), standard height canopy layers that could contribute to this climate variation are 0 to 65m.

According to Pickett *et al.* (1988) thrips prefer younger flowers that are usually located at the upper stratum. This is due to food availability such as floral nectar and pollen that is represents an additional food sources for the adult thrips. The older flowers which are usually located at the lower stratum of the plant on the other hand had already shed their pollen and have fewer resources (Kasina *et al.*, 2009). However, based on our observations, younger flowers are smaller in size and offer less space for protection, but older flowers have bigger space and provide more protection for thrips against predators. Thus, the equal distribution of *M. usitatus* found among strata in the legumes sampled in this study probably as consequences of *M usitatus* preference on both, protection and food resources. It is unclear what are the definite factors contributing the thrips distribution on plant vertically. Previous research done by Pearsall (2000) on *F. occidentalis* adults, showed there was significant differences in the vertical distribution within nectarine orchards, with more adults found at lower levels than at higher levels, possibly due to a preference of low lying plant hosts. However on cotton plants, more adult being found in the middle section (Atakan *et al.*, 1996). Climate and weather factors are also probably play important role as *F. occidentalis* fly higher in the summer than in the spring in nectarine orchards as reported by Pearsall & Myers (2001). A further study in future is needed to determine the exact factors contributing to the spatial distribution of *M. usitatus* and perhaps research can be extended on distribution of different level of life stages of this species on legume.

5. 3 CORRELATION BETWEEN *M. usitatus* POPULATION WITH THE ABIOTIC FACTORS

Seasonality is common phenomenon among insect (Wolda & Wong, 1988). However, this insect seasonality is better known in temperate regions where food availability and tolerable

climatic conditions are restricted to become the seasonal factors (Hunter, 1992; Forkner *et al.*, 2008). However, studies that have documented the population dynamics of insects within tropical rainforest have found that the abundance or activity periods of most species show strong seasonal changes (Wagner, 2001). Wolda (1988) and Pinheiro *et al.* (2008), proposed two hypotheses to explain the variability in abundance of tropical insect; climate predictability and seasonal variation of food resources. According to Wolda (1988) the tropics seasonal changes in temperature tend to be minimal or absent but this does not mean that there is no fluctuation in the thrips population. Insect in tropical area may fluctuate from year to year just like the temperate insects (Gray, 1972; Wolda, 1979). Even at the same sites or in the same localities with relatively non seasonal climates, some species behave as if their environment is highly seasonal (Wolda, 1988). According to Lowman (1982) and Gangloff (1999), the seasonality of Thysanoptera is unpredictable. In fact, the causes of fluctuation in insects abundant generally are still not completely understood (Pinheiro *et al.*, 2008).

According to Taburan Hujan Malaysia (2015), this country has a tropical weather with uniformly high temperatures throughout the year. In most area the average maximum and minimum temperature per month vary less than 2°C annually (McGinley, 2011). It receives rainfall throughout the year but with two monsoons seasons that bring heavy down pour; from November to February and between Aprils to October. In this study both female and male of *M. usitatus* are fluctuate within temperature coincide with rainfall and dry or hot conditions. The total number of both females and males of *M. usitatus* are highest in the months of January and February, while lowest in December. This is due the hot season in January and February and heavy rain season in December. This occurrence is supported by Ananthakrishnan (1990), who stated the number and density of thrips fluctuated between

months in relation to their life histories and environmental factors such as precipitation, humidity and temperature. Further, Lewis (1973) reported that the reproduction and survival of most species of thrips are encouraged by the warm, sunny and dry climate. However, when the rainfall is heavy, the population dropped drastically. This phenomenon is supported by Harrison (1963) and Ibrahim & Adesiyun (2010) where driving rain with hail washed away the population of *T. tabaci* from onion crops. Further, when the burrowing larvae were in the ground, the crevices in the soil were drowned when got washed off by heavy rain.

Relatively high temperature and lack of rainfall have been associated with increase in thrips population while high relative humidity and rainfall reduce thrips population (Hamdy & Salem, 1994). Most insect prefer conditions of higher temperatures (above 25°C) and higher relative humidity (above 70%) although some can tolerate different conditions (Child, 2007). However in this study, negative correlation was found between thrips population and relative humidity. This finding is similar with several previous researches (Nyasani *et al.*, 2013; Mailhot *et al.*, 2007). According to Hamdy & Salem (1994), relative humidity is related directly to the rainfall and has frequently been demonstrated as a decisive thrips mortality factor.

Aliakbarpour & Che Salmah (2011) reported that changes in thrips density on the crop are related to their responses to sunlight. In general, light intensity will increase thrips activity (Lewis, 1973 & Johansen *et al.*, 2011). According to Kirk (1996), thrips prefer high light intensity instead of dark for flight and feeding. These statements supported the finding of this study where the populations of both female and male of *M. usitatus* are correlated to the light intensity. However, light intensity was not the only key factor that governing the

abundance of thrips, temperature stands out as the most important factor in constraining the abundance and distribution of insect (Pankeaw *et al.*, 2011). Furthermore, it is well documented that abiotic factors, especially temperature, regulate the ecology of insect communities (Savopoulou – Soultouki *et al.*, 2012). This is also shown in this study where the total number of *M. usitatus* fluctuated with temperature (Figure 4.2). This phenomenon is also supported by Palumbo (2011) where they stated that high temperature can promote insect population growth and low high temperature can cause populations to decline.

Nevertheless, human factor also played a role on the abundance of *M. usitatus* on legume. For example, the least population of *M. usitatus* was found in January and February in Bestari Jaya. In that month, farmers did an open burning near the plantation area, resulting an extreme hot condition affecting the population decline for *M. usitatus*. The legume flowers were dried, the leaves curled and caused burning scar on most of the legume's plant. Due to the less number of flowers and extreme condition, the number of *M. usitatus* also decreased.

5.4 *M. usitatus* POPULATION ON LEGUMES SPECIES

In Malaysia, very little study has been done on thrips with their associated host plants (Ahmad & Ho, 1980). However, compared to other countries especially from temperate zones more published records of thrips hosts were available (Alves-Silva & Del-Claro, 2010; Trdan *et al.*, 2003; Zilahi-Balogh *et al.*, 2006; Messelink *et al.*, 2005). According to Ananthakrishnan *et al.* (1982), thrips recognized their host plants through stigmas with pollen. However, due to thrips migratory and host shifting activities, it is often difficult to separate the true host with non-true host (Silva *et al.*, 2013; Marullo, 2009; Lewis, 1973). It is possible that, where species which are considered as host specific to one type of plant

have been reported as host-specific on different plant type in another region (Samler, 2012). For example, a study on *Frankliniella pest* species, has found that they always migrating from their natural hosts to other crops or plants and vice versa (Chellemi *et al.*, 1994; Carrizo, 1998). In this study, it is shown that the host plant preference of *M. usitatus* is generally a legume species but there is no specific preference of *M. usitatus* on the different species of legume. This study has proved that the abundance of *M. usitatus* on *P. tetragonolobus*, *P. vulgaris* and *V. unguiculata* is similar. Thus, leguminous in general is the specific host for *M. usitatus* and different species of legume does not affect their abundance.

5. 5 VERIFICATION OF *Megalurothrips* SPECIES IDENTIFICATION

5.5.1 THE IMPORTANCE OF VERIFYING THE *Megalurothrips* SPECIES IDENTIFICATION

Traditionally taxa are distinguished by using the morphological characters. However, not all species lend themselves to this approach because of insufficient phenotypic variation (Brunner *et al.*, 2002). According to Hawksworth & Kalin-Arroyo (1995), taxonomist can critically identify only more than 0.01% of the estimated 10 to 15 million species. 15 000 of taxonomist will be required to identify species if our reliance on morphological diagnosis is to be sustained. There are certain significant limitations of this traditional species identification. First; phenotypic plasticity in character employed for species recognition can lead to incorrect identification. Second; this approach overlooks morphologically cryptic taxa which are very common in many groups. Third; morphological keys are often effective only for a particular life stage or gender, many individuals cannot be identified because of varied lifestages and sex. Finally; the use of

keys often demands such a high level of expertise that misdiagnoses are common. For thrips, the traditional method basically requires expert knowledge of the genus (Mehle & Trdan, 2012). Technical problems in slide preparations may lead to wrong identification, for example, without fully cleared and expertly slide-mounted specimens, the minute structural details that used to diagnosed thrips species cannot be studied accurately (Rugman - Jones *et al.*, 2006). According to Mound & Kibby (1998) and Mehle & Trdan (2012), appearance can vary in many ways within species.

DNA barcoding by using the cytochrome oxidase I (COI) gene has then become a popular technique to identify insect species precisely (Glover *et al.*, 2010). Hebert *et al.* (2003) reported that this COI has two important advantages. First; the universal primers for this gene are robust, enabling recovery of its 5' end from representatives of most of all animal phyla (Zhang & Hewitt, 1997). Second; COI appears to possess a greater range of phylogenetic signal than any other mitochondrial gene (Knowlton & Weight, 1998). It is believed that COI is enabling to differentiate and reveal the presence of cryptic species within several morphospecies (Kosakyan *et al.*, 2015).

However, according to Smith *et al.* (2008), analyzing a complex cryptic species is actually requiring a combination of both traditional morphological and molecular technique. It is important to have collaboration between taxonomist and molecular specialist (Mound *et al.*, 2010). The integration of both identification methods could produce double confirmations for validating identification of various thrips species (Kumar *et al.*, 2013). Possessing ability in species identification is fundamental. Apart from the concern for biological research strategy, countering pest from severe infestation is another priority in order to have accurate species identification (Brunner *et al.*, 2002). Misidentification of thrips species

can lead to the misapplications of management practices, resulting in wasting of money, resources and time. Selection of the wrong biological control agents due to ambiguous identification of the target pest also discourages farmers to adopt chemical free pest management strategies (Kumar *et al.*, 2013). In this regard, classical taxonomy has its own strength; however, DNA barcoding employing COI has the added advantage of not being limited by polymorphism, sex, and life stage of the target species (Rebijith *et al.*, 2014).

5.5.2 VERIFICATION OF THE *M. usitatus* IDENTIFICATION (THROUGH REARING AND MOLECULAR ANALYSES)

According to Brunner *et al.* (2002), thrips is notorious in taxonomic problem and their identification is challengeable due to several factors such as their minute size, complex life cycles, parthenogenic mode of reproduction, scarcity of solid morphological characters and the coexistence of the same species on the same plant. Females of *Megalurothrips* especially, are difficult to distinguish from several other named species due to their high similarity in morphology (Palmer, 1987). The validity of several species is open to doubt including *M. usitatus* and *M. typicus* (Palmer, 1987). Through this study, there are certain doubtful females of *Megalurothrips usitatus* (i.e. '*M. usitatus*') that possess intermediate character between *M. usitatus* and *M. typicus* particularly on the median setae of sternite vii. Intermediate form in certain group of species as according to Emelianov *et al.* (1995); Parson & Shaw (2001) and Dres & Mallet (2002) is also the process that lead to full speciation represent by cryptic or sibling species, host races, biotypes and ecological races. According to Bickford *et al.* (2007) cryptic refer to recently diverged, separable only with molecular data, occurs in sympatry or be reproductively isolated. Brunner *et al.* (2002), stated that cryptic species can also be in three conditions; 1st, the insect species is polyphagous. 2nd, they possess a complex of host race with partial genetic differentiation

and 3rd, a complex of morphologically cryptic species with no longer joined via gene flow. In this study, *M. usitatus* can be classified as polyphagous species and own a different host race in one particular area, if referring to leguminous as the plant species. Thus, '*M. usitatus*' can be named as biotypes. According to Claridge & den Holder (1983); Diehl & Bush (1984); Saxena & Barrion (1987); Menken & Raijmann (1996), biotypes is defined as a population of the same pest species that differ in performance on different plant species or varieties. They are simply phenotypically similar but with unknown number of different genotypes (Via, 1989). According to Menken & Raijmann (1996), adaptation to host plants will lead to a decrease in genetic exchange between biotypes and this may ultimately result in speciation. For *Megalurothrips* there are no data available on biotypic variation with regard to differential performance on host plants. However, other species of thrips such as *F. occidentalis* which has been known as polyphagous insect herbivore for a long time, their biotypes status has been stated several times in the literature (Bryan & Smith, 1956; De Vries, 2010). Through these previous studies, biotypes of thrips regarding different species of host plants resistance have been described. Plant size, shape, coloration, leaf hair, cuticle thickness, and natural chemicals (attractants and repellents) can all affect the pest susceptibility. Plants have developed a variety of resistance mechanisms to deter feeding and damage by insects. Insects, at the same time, have evolved ways of circumventing the defense mechanisms in plants (Zehnder, 2010). According to De Kogel *et al.* (1997), biotypic variation should be one of major concern in studying insect species, as aggressive biotypes can lead to pest outbreak.

This study has shown that both '*M. usitatus*' (the doubtful individuals) and *M. usitatus* (the typical individuals) were produced from the unmated female. The parthenogenetic mother produced different form of morphology among the offsprings especially on the character of

sternite vii. After generation of isolation in reproduction, separate species could be formed (Toda & Murai, 2007). This theory is supported by previous research done by Nakao & Yabu (1998), where they suggested that parthenogenesis in thrips such as *Thrips nigropilosus* corresponded to a separate species due to the existence of apparent genetic differences and reproductive isolation. However, there is also certain case where the phylogenetic relationship between the two reproductive forms of thrips species such as *T. tabaci* which is unclear and their taxonomical relationship remains obscure. This phenomenon is similar to *M. usitatus* in this study. Thus, further study is needed to verify their taxonomic relationship status.

Since the study site was done in three different geographical areas, a prediction proposed by Brunner & Frey (2010) is relevant to the current work. They predicted that genetic distance among population is correlated with geographical isolation. According to Mittelbach *et al.* (2007), different environmental conditions such as climate, habitat and availability of resources due to latitudinal variation including different altitudinal level resulting in speciation. Moreover, Kay & Schemske (2008), Francis & Currie (2003) claimed that there is genetic differentiation among insect based on the geographical site correlated mainly with temperature. This supported the findings of this study where Janda Baik, Jelevu and Bestari Jaya are geographically separated and the temperatures are different. Janda Baik has lower temperature compared to Jelevu and Bestari Jaya due to the differences in the altitudinal level and a number of *Megalurothrips* female species from here were found to have slightly different in the morphological character (in particular the position of the median setae on the sternite vii). This phenomenon is supported by Hodkinson (2005), who stated that insect species response differently to the changing environments along altitudinal gradients. Some of them response in the form of

polymorphism and variation in size, color or genetic. Furthermore, harsh environmental condition combined with the isolated or fragmented nature serve a microevolution for insect adaptation (Haslett, 1997). In this study, *M. typicus* was included in the molecular analyses to counter check our hypothesis that there might a possibility of interbreeding between *M. usitatus* and *M. typicus*, since there is an intermediate character shown on certain individuals of the '*M. usitatus*' female. However, the phylogenetic analyses showed that '*M. usitatus*' was not in the same cluster as *M. typicus*. Thus, presumably there is no interbreeding occur between *M. typicus* and *M. usitatus*.

CHAPTER 6: CONCLUSION

To date the diversity and population study of thrips species in particular *Megalurothrips* on legume were not being studied in depth especially in Malaysia. However, this study has documented several findings regarding these matters where majority of the thrips species on the legumes is *M. usitatus*. The presence of other thrips species such as *T. hawaiiensis*, *T. parvispinus* and *C. brunneus* are probably because of the presence of the alternate plants in between the legume plants. It is believed that the alternate host plants interfered the true host location and disturbed their landing. Similar to thrips diversity, thrips abundance was also affected by the cropping systems or pattern. This study also discovered that *M. usitatus* was equally distributed among different stratum on legume plant. Presumably, they were present throughout the study duration and were found numerously at low altitudinal area during dry season. It is also shown that the abiotic factors such as temperature and light intensity influenced positively the *M. usitatus* population, but not the relative humidity. Further, the differences of legume species do not affect their population. In this study, several individuals of *Megalurothrips* females with doubtful identification (i.e. '*M. usitatus*') were found in the wild. Their identification which was verified through rearing showed that they were produced from the unmated female i.e. 15.4%. While, the molecular analysis showed the doubtful females along with several *M. usitatus* females belong to the sub clade 2 which is in the same clade of *M. typicus*. Hence, at this stage, the doubtful female could be considered as a cryptic species and a by-product from the unmated female. Nevertheless, further studies on morphological characters, coupled with the cross breeding experiments among the morphoforms/lineages, and the use of additional molecular markers for phylogenetic inference, are required to verify their species status ultimately. So far, more than 5500 species of thrips had been identified. Some are beneficial and some are considered as pest in agriculture. Treatments against thrips are also often very specific,

especially for thrips that have acquired insecticide resistance and these treatments require the correct and unambiguous identification of species. Thus, this study act as a fundamental step in order to understand better on the *Megalurothrips* species classification, including their ecology and biology to empower the integrated pest management strategies. Perhaps in the future, minimal usage of insecticides, the discovery of natural enemies, including practical farming practices such as water sprinkler that stimulate the rain water system could help Malaysian farmers in increasing the yield without raising the production cost.

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LIST OF PUBLICATION AND PAPER PRESENTED

1. **Zafirah, Z.** & Azidah, A. A. (in press). Diversity and population of thrips species on legumes with special reference to *Megalurothrips sp.* *Sains Malaysiana Jurnal*.
2. **Zafirah, Z.** & Azidah, A. A. (2014). Diversity and Ecological Studies of thrips Species (with special reference on *Megalurothrips* on Various Host plants (particularly the Fabaceae or leguminose). Poster presented at the 19th Annual Biological Science Graduate Congress conducted on 12 - 14 December 2014 in National University of Singapore.

APPENDIX A: Abstract for seminar presented

BioD-P-22

Diversity and Ecological Studies of Thrips Species (with special reference on *Megalurothrips*) on Various Host Plants (particularly the Fabaceae or Leguminose).

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Megalurothrips are among most common thrips in orchard in Malaysia and they become pest to legumes. Previous study mostly concern on ecological study of *Thrips* and there is not much discoveries on *Megalurothrips*. Main objectives of this study are to study the diversity of thrips (particularly *Megalurothrips* spp.) on legumes plantation and other plants and to study the spatial and temporal distribution of *Megalurothrips* spp. On legumes. This study will be conducted in Pahang, N. Sembilan and Selangor. Samples will be collected monthly from each plantation. Five plots are set up in each plantation, each with 10x10m area. Five trees are randomly sample in each plot. Sampling will be done between 8.30 am until 11.30 am. Thrips rearing also will be conducted in order to prove that sexual dimorphism occurs. There are four expected collected species; *Megalurothrips usitatus*, *Megalurothrips typicus*, *Thrips parvispinus* and *thrips hawaiiensis*. The most expected abundant species is *M.usitatus* followed by *T.parvispinus*, *T. hawaiiensis* and *M.typicus*. Batang berjuntai should have the most abundant *Megalurothrips*. They will be found mostly at the peak hour; 8.30 am until 11.30 am. *Megalurothrips* is abundant on the upper part of the tree on the flower and the least on the lowest part. Sexual dimorphism does occur where *M. usitatus* and *M. typicus* are actually derived from the same species. The finding of this study could help in controlling and managing the *Megalurothrips* systematically. Consequently, wasting of time, energy and money can be avoided especially among the farmers who strive to eliminate the *Megalurothrips* from their crops.

APPENDIX B: Total number of *Thrips* sp. collected in sampling site

JANDA BAIK, PAHANG 18/04/2013	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	20	0	8	0	0	5	1	9	0	0	6	1	3	0	1
PLOT 2	22	0	9	0	0	35	2	4	0	0	25	2	8	1	2
PLOT 3	20	0	3	0	0	18	0	5	0	0	14	1	1	0	0
PLOT 4	47	6	5	0	0	1	1	8	0	0	1	1	0	1	1
PLOT 5	25	0	5	0	0	21	0	4	0	0	19	0	0	0	0
TOTAL	134	6	30	0	0	80	4	30	0	0	65	5	12	2	4

JANDA BAIK, PAHANG 23/05/2013	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	48	0	20	0	0	34	0	23	0	0	29	0	19	0	2
PLOT 2	11	0	6	0	0	22	2	16	0	0	25	0	15	0	0
PLOT 3	12	0	9	0	0	20	0	17	0	0	9	1	2	0	0
PLOT 4	16	0	7	0	0	14	0	12	0	0	18	0	13	1	3
PLOT 5	22	1	16	0	0	41	0	17	0	0	28	1	11	0	0
TOTAL	109	1	58	0	0	131	2	85	0	0	109	2	60	1	5

JANDA BAIK, PAHANG 21/06/2013	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	10	0	15	0	0	7	0	5	0	0	13	0	28	0	2
PLOT 2	6	0	2	0	0	12	0	9	0	0	5	0	20	0	0
PLOT 3	12	0	10	0	0	6	0	5	0	0	21	0	18	0	0
PLOT 4	22	0	9	0	0	19	0	11	0	0	9	0	35	0	3
PLOT 5	2	0	1	0	0	6	0	6	0	0	8	0	7	0	0
TOTAL	52	0	37	0	0	50	0	36	0	0	56	0	108	0	5

JANDA BAIK, PAHANG 09/07/2013	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	1	0	5	0	0	0	0	1	0	0	1	0	2	0	0
PLOT 2	1	0	0	0	0	3	0	6	1	0	6	0	4	2	0
PLOT 3	6	0	6	0	0	6	0	6	0	1	4	0	13	2	0
PLOT 4	9	0	4	1	1	4	0	3	0	0	0	0	1	0	0
PLOT 5	7	0	3	2	0	0	0	0	0	0	7	0	9	1	0
TOTAL	24	0	18	3	1	13	0	16	1	1	18	0	29	5	0

JANDA BAIK, PAHANG 10/08/2013	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	25	0	2	0	0	18	0	4	0	0	18	0	4	0	0
PLOT 2	13	0	11	0	0	16	0	4	0	0	11	0	7	0	0
PLOT 3	7	0	15	0	0	6	1	10	0	0	2	0	6	0	0
PLOT 4	12	0	5	0	0	8	0	15	0	0	11	0	7	0	0
PLOT 5	7	0	9	0	0	4	0	13	1	0	3	0	7	0	0
TOTAL	64	0	42	0	0	52	1	46	1	0	45	0	31	0	0

JANDA BAIK, PAHANG 14/09/2013	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	21	1	2	0	0	16	2	1	0	0	23	0	2	0	
PLOT 2	25	4	19	0	0	1	0	3	0	0	9	3	0	0	0
PLOT 3	24	2	21	0	0	11	0	4	0	0	0	2	2	0	0
PLOT 4	34	0	0	0	0	23	0	5	0	0	42	2	4		
PLOT 5	9	7	1	0	0	9	0	13	0	0	8	0	0	0	0
TOTAL	113	14	43	0	0	60	2	13	0	0	82	7	8	0	0

BESTARI JAYA , SELANGOR 22/01/2014	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	78	25	14	0	0	64	22	4	0	0	54	13	7	0	0
PLOT 2	92	34	11	0	0	68	23	12	0	0	16	15	2	0	0
PLOT 3	67	26	13	0	0	77	16	1	0	0	68	12	7	0	0
PLOT 4	42	21	6	0	0	116	50	10	0	0	5	3	0	0	0
PLOT 5	111	18	11	0	0	80	20	6	0	0	58	19	6	0	0
TOTAL	390	124	55	0	0	405	131	6	0	0	201	62	22	0	0

BESTRAI JAYA, SELANGOR 06/02/2014	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	48	9	24	0	0	53	3	7	0	0	48	7	2	0	0
PLOT 2	10	1	4	0	0	45	5	5	0	0	2	0	0	0	0
PLOT 3	10	1	7	0	0	12	1	10	0	0	7	11	2	0	0
PLOT 4	2	0	2	0	0	3	1	4	0	0	12	3	15	0	0
PLOT 5	34	3	10	0	0	17	0	2	0	0	28	1	4	0	0
TOTAL	104	14	47	0	0	130	10	2	0	0	97	22	23	0	0

BESTARI JAYA, SELANGOR 17/03/2014	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	82	1	4	0	0	68	4	4	0	0	48	8	8	0	0
PLOT 2	53	2	9	0	0	113	9	3	0	0	12	2	6	0	0
PLOT 3	88	4	11	0	0	81	10	3	0	0	58	3	15	0	0
PLOT 4	61	5	4	0	0	0	0	0	0	0	21	5	3	0	0
PLOT 5	68	3	1	0	0	61	1	2	0	0	77	1	5	0	0
TOTAL	352	15	29	0	0	323	24	2	0	0	216	19	37	0	0

BESTARI JAYA, SELANGOR 15/04/2014	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	71	43	1	0	0	24	5	3	0	0	32	19	1	0	0
PLOT 2	51	35	1	0	0	68	7	1	0	0	8	8	0	0	0
PLOT 3	71	30	0	0	0	39	19	1	0	0	12	7	0	0	0
PLOT 4	36	6	3	0	0	0	0	0	0	0	33	14	0	0	0
PLOT 5	7	2	0	0	0	48	3	1	0	0	132	18	2	0	0
TOTAL	236	116	5	0	0	179	34	6	0	0	217	66	3	0	0

BESTARI JAYA, SELANGOR 02/05/2014	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	55	1	1	0	0	53	7	1	0	0	24	7	0	0	0
PLOT 2	32	5	1	0	0	11	2	0	0	0	9	4	0	0	0
PLOT 3	4	2	0	0	0	12	7	0	0	0	8	0	0	0	0
PLOT 4	68	12	0	0	0	42	3	0	0	0	23	7	0	0	0
PLOT 5	22	4	0	0	0	9	2	0	0	0	9	5	1	0	0
TOTAL	181	24	2	0	0	127	21	1	0	0	73	23	1	0	0

BESTARI JAYA, SELANGOR 08/06/2014	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	117	29	6	0	0	90	39	4	0	0	48	27	1	0	0
PLOT 2	106	29	4	0	0	59	8	2	0	0	52	5	3	0	0
PLOT 3	140	7	10	0	0	50	5	3	0	0	64	5	2	0	0
PLOT 4	42	3	0	0	0	52	3	1	0	0	36	2	0	0	0
PLOT 5	67	34	5	0	0	57	11	0	0	0	19	5	2	0	0
TOTAL	472	102	25	0	0	308	66	10	0	0	219	44	8	0	0

JELEBU, N. SEMBILAN 03/11/2013	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	59	1	5	0	0	33	7	18	0	0	7	1	2	0	0
PLOT 2	25	3	2	0	0	15	2	2	0	0	5	1	0	0	0
PLOT 3	28	0	7	0	0	17	0	2	0	0	13	4	2	0	0
PLOT 4	35	3	12	0	0	19	1	4	0	1	15	2	5	0	1
PLOT 5	33	1	3	0	0	12	0	0	0	0	0	0	0	0	0
TOTAL	180	8	26	0	0	96	10	26	0	1	40	8	9	0	1

JELEBU, N. SEMBILAN 05/12/2013	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	49	1	0	0	0	68	0	0	0	0	41	0	2	0	0
PLOT 2	65	4	0	0	0	33	1	2	0	0	2	0	0	0	0
PLOT 3	107	2	0	0	0	17	3	0	0	0	2	0	0	0	0
PLOT 4	81	0	0	0	0	76	0	0	0	0	77	0	0	0	1
PLOT 5	56	5	0	0	0	18	0	0	0	0	6	0	0	0	0
TOTAL	358	12	0	0	0	212	4	2	0	0	128	0	2	0	1

JELEBU, N. SEMBILAN	UPPER					MIDDLE					LOWER				
08/01/2014	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	62	2	0	0	0	97	0	0	0	0	49	5	0	0	0
PLOT 2	95	3	0	0	0	48	2	0	0	0	53	3	0	0	0
PLOT 3	77	5	0	0	0	98	15	0	0	0	115	7	0	0	0
PLOT 4	137	2	0	0	0	97	4	0	0	0	71	5	0	0	0
PLOT 5	187	25	0	0	0	154	10	0	0	0	223	30	0	0	0
TOTAL	558	37	0	0	0	494	31	0	0	0	511	50	0	0	0

JELEBU, N. SEMBILAN	UPPER					MIDDLE					LOWER				
05/03/2014	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	25	2	8	0	0	9	2	3	0	0	7	2	0	0	0
PLOT 2	54	17	3	0	0	15	21	2	0	0	52	0	5	0	0
PLOT 3	67	22	7	0	0	30	16	7	0	0	4	10	4	0	0
PLOT 4	28	5	4	0	0	38	17	4	0	0	19	6	0	0	0
PLOT 5	68	2	8	0	0	74	4	4	0	0	22	28	6	0	0
TOTAL	242	48	30	0	0	166	60	20	0	0	104	46	15	0	0

JELEBU, N. SEMBILAN 17/04/2014	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	43	1	4	0	0	48	1	11	0	0	44	2	6	0	0
PLOT 2	85	21	13	0	0	63	12	13	0	0	7	2	5	0	0
PLOT 3	82	3	4	0	0	79	4	7	0	0	50	5	6	0	0
PLOT 4	309	28	16	0	0	32	5	11	0	0	1	0	0	0	0
PLOT 5	96	9	4	0	0	81	7	20	0	0	66	5	0	0	0
TOTAL	615	62	41	0	0	303	29	62	0	0	168	14	17	0	0

JELEBU, N. SEMBILAN 22/05/2014	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	32	1	1	0	0	40	3	1	0	0	0	2	0	0	0
PLOT 2	32	5	0	0	0	36	1	1	0	0	0	0	1	0	0
PLOT 3	29	7	1	0	0	16	4	0	0	0	2	0	0	0	0
PLOT 4	34	7	0	0	0	1	1	1	0	0	0	0	0	0	0
PLOT 5	50	5	1	0	0	10	0	0	0	0	0	0	0	0	0
TOTAL	177	25	3	0	0	103	9	3	0	0	2	2	1	0	0

JANDA BAIK, PAHANG 8/10/2013	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	36	0	2	0	0	33	1	0	0	0	35	3	2	0	1
PLOT 2	46	5	5	0	0	25	0	2	0	0	16	5	3	0	0
PLOT 3	50	13	5	0	0	16	0	8	0	0	1	0	0	0	0
PLOT 4	73	6	1	0	0	59	1	1	0	0	41	3	0	0	0
PLOT 5	77	2	0	0	0	37	6	2	0	0	8	4	0	0	0
TOTAL	282	26	13	0	0	170	8	13	0	0	101	15	5	0	1

JANDA BAIK, PAHANG 17/11/2013	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	41	0	1	1	0	63	0	0	0	0	51	0	1	0	0
PLOT 2	61	8	8	0	0	37	9	1	0	0	37	8	2	0	0
PLOT 3	38	4	5	0	0	10	1	0	0	0	28	2	0	0	0
PLOT 4	33	1	0	0	0	46	0	0	0	0	47	1	0	0	0
PLOT 5	2	1	2	0	0	32	1	0	0	0	31	3	0	0	0
TOTAL	175	14	16	1	0	188	11	1	0	0	194	14	3	0	0

JANDA BAIK, PAHANG 27/12/2013	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	0	0	4	0	0	0	0	4	0	0	0	0	6	0	0
PLOT 2	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0
PLOT 3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PLOT 4	0	0	2	0	0	0	0	5	0	0	0	1	2	0	0
PLOT 5	0	0	1	0	0	0	0	0	0	0	0	6	1	0	0
TOTAL	2	0	7	0	0	1	0	10	0	0	0	7	10	0	0

JANDA BAIK, PAHANG 11/01/2014	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	0	0	0	0	0	2	0	1	0	0	0	0	1	0	
PLOT 2	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0
PLOT 3	0	0	3	0	0	0	0	4	0	0	0	0	0	0	0
PLOT 4	2	0	1	0	0	0	0	2	0	0	0	0	1	0	0
PLOT 5	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	3	0	6	0	0	2	0	7	0	0	0	0	4	0	0

JANDA BAIK, PAHANG 08/02/2014	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	28	4	0	0	0	1	1	0	0	0	4	3	1	0	0
PLOT 2	33	11	1	0	0	15	6	1	0	0	8	5	0	0	0
PLOT 3	22	2	0	0	0	13	5	0	0	0	14	12	0	0	0
PLOT 4	40	4	2	0	0	9	2	1	0	0	17	2	0	0	0
PLOT 5	25	8	0	0	0	29	18	0	0	0	2	2	1	0	0
TOTAL	148	29	3	0	0	67	32	2	0	0	45	24	2	0	0

JANDA BAIK, PAHANG 31/03/2014	UPPER					MIDDLE					LOWER				
	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>	<i>M.u</i>	<i>M.t</i>	<i>T.p</i>	<i>C.b</i>	<i>T.h</i>
PLOT 1	5	0	0	0	0	3	3	1	0	0	2	0	1	0	0
PLOT 2	2	0	0	0	0	2	0	3	0	0	0	0	1	0	0
PLOT 3	3	0	0	0	0	8	3	0	0	0	1	0	2	0	0
PLOT 4	7	3	0	0	0	4	1	0	0	0	4	1	0	0	0
PLOT 5	8	1	0	0	0	3	0	1	0	0	1	0	0	0	0
TOTAL	25	4	0	0	0	20	7	5	0	0	8	1	4	0	0