

**MUTUALISTIC INTERACTION BETWEEN
MEALY BUGS AND ANTS TOWARDS THE
DEVELOPMENT OF A NOVEL SUGAR ANT
BAIT**

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by

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SALING INTERAKSI ANTARA KOYA DAN SEMUT TERHADAP PERKEMBANGAN SUATU UMPAN GULA SEMUT BAHARU

ABSTRAK

Koya merupakan penghasil cecair manisan yang penting dan kebanyakan koya sudah menjalin salingan interaksi bersama semut. Kebanyakan species semut tertarik dengan cecair manisan dan ia membekalkan karbohidrat berterusan untuk memenuhi keperluan metabolik semut. Penerokaan interaksi bersama dan kandungan honeydew menambakan lagi ilmu baru mengenai interaksi dan mengenal pasti potensi kawalan semut melalui kandungan penarik dalam cecair manisan. Oleh itu, tesis ini menumpu kepada saling interaksi antara semut dan koya terhadap perkembangan suatu umpan gula semut yang baru. Semut memainkan peranan dalam memanipulasi rembesan cecair manisan daripada koya-koya. Koya nanas, *Dysmicoccus brevipes* (Cockerell, 1893) menghasilkan jumlah isi padu cecair manisan yang sama apabila berinteraksi dengan semut mayat, *Tapinoma melanocephalum* (Fabricius, 1793) dan semut firaun, *Monomorium pharaonis* (L., 1758). Sebaliknya, apabila koya betik *Paracoccus marginatus* Williams & Granara de Willink, 1992 berinteraksi dengan semut mayat, ia menghasilkan jumlah isipadu cecair manisan yang lebih tinggi berbanding interaksi dengan semut firaun. Antara empat pasangan interaksi semut-koya, kejayaan dalam pemerolehan cecair manisan yang paling tinggi dicapai semut mayat apabila berinteraksi dengan koya nanas. Ini adalah hasil usaha penuaian yang aktif oleh semut mayat untuk mendapatkan cecair manisan dari koya nanas. Analisis kromatografi cecair prestasi tinggi (HPLC) telah mengenal pasti sebanyak lapan jenis gula dalam

keuda-dua sampel cecair manisan yang dihasilkan oleh *D. brevipipes* dan *P. marginatus*. Cecair manisan dari koya nanas mengandungi kepekatan melezitose sebanyak tiga kali ganda lebih tinggi daripada koya betik. Dalam kajian pemilihan cecair manisan, semut mayat lebih tertarik dengan cecair manisan yang dihasilkan oleh koya nanas berbanding dengan koya betik. Kajian pilihan gula secara serentak menunjukkan semut mayat lebih tertarik kepada melezitose, sukrosa, fruktosa dan glukosa. Tiada perbezaan yang signifikan ($P > 0.05$) dari segi tarikan antara empat gula tersebut. Kajian pilihan gula secara pasangan menunjukkan gula kegemaran semut mayat mengikuti urutan seperti berikut: melezitose > sukrosa > glukosa > fruktosa. Dalam kajian deria tahap sensitif (EC_{50}) terhadap jenis gula, melezitose mencatatkan kepekatan yang terendah diperlukan untuk merangsang semut mayat untuk minum. Semut mayat masih dapat mengesan melezitose walaupun melezitose telah dilarutkan dengan sukrosa hingga ke nisbah 1:19 dengan molar yang sama. Walaupun diberi larutan gula yang berterusan, semut mayat masih gemar atas larutan gula yang mengandungi melezitose berbanding larutan gula lain. Dalam kajian makmal umpan tunggal yang mengandungi 0.0001% fipronil, tiada perbezaan yang signifikan ($P > 0.05$) yang ditunjukkan antara prestasi umpan fipronil yang menggunakan 0.5 M campuran melezitose-sukrosa (1:19) dan 0.5 M sukrosa. Kedua-dua umpan fipronil mengurangkan pekerja semut mayat > 75% dalam tiga hari, > 80% pengurangan larva dan pupa dalam 7 hari dan 96% pengurangan ratu dalam 9 hari selepas rawatan. Walau bagaimanapun, kemunculan gula saingan dalam kajian pilihan dalam makmal menjejaskan prestasi umpan fipronil sukrosa berbanding dengan campuran melezitose-sukrosa (1:19). Pada hari ke-3 selepas penggunaan umpan fipronil, umpan campuran melezitose-sukrosa (1:19) mengurangkan semut pekerja sebanyak 70% manakala umpan sukrosa cuma mengurangkan sebanyak 50%. Umpan campuran

melezitose-sukrosa (1:19) mencapai 90% pengurangan ke atas larva, pupa dan ratu semut sementara umpan sukrosa cuma mencapai ~70% pengurangan di akhir kajian. Dalam kajian umpan di kawasan luar, umpan 0.0001% fipronil melezitose-sukrosa (1:19) berjaya menghapuskan *Anoplolepis gracilipes* Smith, 1875 dalam 2 minggu, *T. melanocephalum* dalam 4 minggu dan *Paratrechina longicornis* (Latreille, 1802) dalam 8 minggu. Sementara itu, umpan sukrosa juga berjaya menghapuskan *A. gracilipes* dalam 2 minggu tetapi beberapa ekor *T. melanocephalum* dan *P. longicornis* masih terlihat di akhir kajian pada minggu ke-8 selepas rawatan. Kajian kami telah menjelakan fenomena kekhususan pasangan spesies dalam saling interaksi bersama antara koya dan semut melalui interaksi tingkah laku kedua-dua serangga. Melezitose dalam cecair manisan yang dihasilkan oleh koya mempunyai daya tarikan yang tinggi terhadap semut dan kajian kami menunjukkan melezitose telah meningkatkan keberkesanan umpan gula terhadap semut.

**MUTUALISTIC INTERACTION BETWEEN MEALY BUGS AND ANTS
TOWARDS THE DEVELOPMENT OF A NOVEL SUGAR ANT BAIT**

ABSTRACT

Mealy bugs are important honeydew producers, and many have developed mutualistic interaction with ants. Honeydew appears to be attractive to many ant species and it supplies constant carbohydrates to meet ant vast metabolic requirement. The exploration of the mutualistic interactions and the honeydew content provide new insights on the interactions and potential control of ants through the attractant in honeydew. Hence, this thesis focuses on the mutualistic interaction between mealy bugs and ants in conjunction with the development of a novel sugar ant bait. Our study found that honeydew excretions by mealy bugs were mediated by attending ant species. Pineapple mealy bug, *Dysmicoccus brevipes* (Cockerell, 1893) excreted similar total volume of honeydew when attended by both ghost ant, *Tapinoma melanocephalum* (Fabricius, 1793) and pharaoh ant, *Monomorium pharaonis* (L., 1758). In contrast, papaya mealy bug, *Paracoccus marginatus* Williams & Granara de Willink, 1992 discharged higher overall volume of honeydew when attended by ghost ant than pharaoh ant. Among four ant-mealy bug associations, ghost ant displayed highest successful honeydew acquisition when associated with pineapple mealy bug. Most honeydew excretions by pineapple mealy bug were the result of the active solicitation behavior of ghost ant. High-performance liquid chromatography (HPLC) analysis identified eight sugars in both honeydew produced by *D. brevipes* and *P. marginatus*. Honeydew produced by pineapple mealy bug contained melezitose concentration three

folds higher than that of papaya mealy bug. Honeydew produced by pineapple mealy bug was visited more by ghost ant over papaya mealy bug. Concurrent multiple sugar preference tests showed ghost ants highly visited melezitose, sucrose, fructose, and glucose with no significant difference ($P > 0.05$). Pair choice test differentiated the gustatory preference of ghost ant on the sugars as such: melezitose $>$ sucrose $>$ glucose $>$ fructose. In the gustatory sensitivity test on ghost ant, melezitose recorded with the lowest half efficiency concentration (EC₅₀) compared to other sugars. Ghost ant could detect the presence of melezitose at a very low portion when being mixed up to 1:19 with sucrose at equal molar. Despite continuous provisioning of carbohydrate, ghost ant still had higher feeding acceptance towards melezitose and melezitose-containing sugar mixtures (1:9 and 1:19) compared to other sugars. In laboratory non-choice baiting bioassay, performances of both baits incorporated with 0.0001% fipronil: 0.5 M melezitose-sucrose mixture (1:19) and 0.5 M sucrose had no significant differences ($P > 0.05$). Both baits reduced ghost ant workers $>$ 75% in just 3 days; $>$ 80% brood reduction in 7 days and 96% queen reduction in 9-day post-treatment. However, the presence of competing sugars in choice bait test differentiated the performances of the two attractants in fipronil bait as melezitose-sucrose mixture (1:19) and sucrose reduced the workers up to 70% and 50% on 3-day post-treatment, respectively. At the end of the test at 8-week post-treatment, melezitose-sucrose mixture (1:19) reduced about 90% of the broods and queens while sucrose achieved reduction about 70%. Field study demonstrated that 0.001% fipronil bait with melezitose-sucrose mixture (1:19) completely reduced *Anoplolepis gracilipes* Smith, 1875 within 2 weeks, *T. melanocephalum* in 4 weeks and *Paratrechina longicornis* (Latreille, 1802) in 8 weeks. Meanwhile, when baited with sucrose, only *A. gracilipes* was completely reduced in 2 weeks while few foragers of *T. melanocephalum* and *P. longicornis* were still sighted

at the end of the test in the 8-week post-treatment. Our study has provided new insights on the species-specific phenomena in ant-mealy bug associations through the behavioural interaction of both insects. Melezitose in honeydew produced by mealy bug has very high attractiveness towards ants and our study demonstrated that melezitose improved effectiveness of sugar-based bait against tramp ants.

CHAPTER 1

GENERAL INTRODUCTION

The gustatory carbohydrate preference of ants lays a crucial foundation for the mutualistic interaction between ant and mealy bug. Such preference in ants creates dynamic associations with the honeydew producer. The level of association between different species of tending ant and mealy bug holds the crucial key to the stability of the association (Eubank and Styrsky, 2006). Stable association drive both groups of insects to co-evolutionary interactions. If the interaction is consistently positive in both species over evolutionary time, the emergence of mutual coadaptation is likely to take place (Stadler and Dixon, 2005).

Positive interactions between these two groups of insects play a widespread role in facilitating biological invasions of both insects (Abbott and Green, 2007). Evidence demonstrated that the invasive red imported fire ant *Solenopsis Invicta* Buren, 1972 protect mealy bugs against the attack of natural enemies (Zhou et al., 2014). Association of ants with mealy bug also causes economic loss in pineapple through the spread of the mealy bug wilt disease of pineapple (Jahn et al., 2003). In urban settings, ant infestations cause issues such as food contamination (Lee et al., 2001), stings, bites, and allergies (Goddard, 1993; Williams et al., 2001) as well as contamination of surgical instruments in hospitals (Beatson, 1972). A wide range of bacteria was isolated and identified from the external body surface of household ants collected in food preparation premises (Alharbi et al., 2019; Lim, 2001). This further supports that ants are highly capable of transmitting human diseases mechanically

(Alekseev et al., 1972; Beatson, 1972; Edwards and Baker, 1981; Eichler, 1990; Bueno et al., 1994).

Although many studies on the association between mealy bug and ant were conducted, detailed information and knowledge on the behavioural response of both insects in the mutualistic interaction are limited. Many studies instead focus on associating ant alone in mutualistic interactions. This does not reflect the complete interactive behaviours between the two insect groups, which could offer a comprehensive explanation of the specific mutualistic associations between ant and mealy bug species. Based on the paradigm that honeydew as the keystone currency used in ant-mealy bug mutualistic interaction, it will be an experimental breakthrough to be able to use the honeydew content in development of a more potent ant bait.

This study aims to explain the specificity in ant-mealy bug association as well as to explore on the honeydew attractant that potentially enhance the ant bait palatability. The study adheres to the following objectives:

- i. to study the behavioural characterization in honeydew excretion by mealy bug and honeydew acquisition by tending ants in multiple ant-mealy bug associations,
- ii. to identify the most attractive sugar through profiling and preference assays of sugar composition in honeydew of mealy bugs as a phagostimulant for ghost ant, *Tapinoma melanocephalum* and;
- iii. to examine the performance of the attractant in toxic ant bait against *T. melanocephalum* in laboratory and field evaluation; *Paratrechina longicornis* and *Anoplolepis gracilipes* in field evaluation.

CHAPTER 2

LITERATURE REVIEW

2.1 Mealy bugs

Mealy bugs (Hemiptera) are important vineyard insects under the subfamily Pseudococcidae (Hardy et al., 2008). They are named for their cottony appearance, small oval, and soft-bodied. They are unarmoured scale insects that usually found in moist and warm habitats. They fed on a wide range of plants and regarded as pests of horticultural crops and ornamental plants (Ben-Dov, 1994). Mealy bugs are abundant and diverse sap-feeding insects which closely related to scale insects and aphids. Due to their ability to lay many eggs, mealy bugs can rapidly build up in population density that cause debilitation of their host plants. Just like other sap-feeding hemipterans, mealy bugs actively excrete large quantities of honeydew upon feeding. This accumulated honeydew often allows the growth of sooty mold that disrupts photosynthesis of host plants, which subsequently affect the yields (Mansour et al., 2011). Besides, the accumulation of honeydew also caused the producers themselves to be vulnerable to bacterial infections. Mealy bugs form close mutualistic interactions with ants which maintains good hygiene services by regular honeydew removal that prevent honeydew buildup (Wimp and Whitham, 2001) Mealy bugs also regarded as vectors to several plant-affecting viruses such as the well-known mealy bug wilt disease (German et al., 1992). Plant viruses cannot penetrate the intact plant cuticle and the cellulose cell wall, and this can be overcome through the penetration through a wound in the surface layers, as in mechanical inoculation by mealy bugs (Hull, 2013) Insecticidal control on mealy bug infestation is challenging owing to their waxy

cuticles (Watson and Kubiriba, 2005) Hence, biological control against mealy bugs surfaced by using natural enemies such as parasitoids, predators, and pathogens.

2.1.1 Biology of Mealy bugs

Biology of mealy bugs are generalized and derived from studies on common mealy bug species. The biology of mealy bugs varies among the species. Female mealy bugs have three larval instars and males have four instars (Ben-Dov, 1994; Wakgari and Giliomee, 2005). The first instar or known as crawler is extremely active and moves quickly to find a feeding spot and thus, this stage is considered as the dispersal stage. The subsequent stages or instars resembles the former but increase in size and amount of wax secretion. As the females mature, they become more sessile. A female mealy bug does not possess wings and is longer than a male mealy bug. Adult females resemble immature stages in appearance, whereas males metamorphose into winged forms at the fifth instar. The male mealy bugs go through a cocoon or prepupal stage before becoming the wings adults.

As the ovaries develop, the mature adult female begins to grow in size and look far less dorso-ventrally flattened. Most economically important species lay several clusters of eggs within a white mass of wax threads which is known as an ovisac (Downie and Gullan, 2004). The adult male is long winged and develop into brown coloured body and two multi-segmented antennae that are about half of the body length. Sex determination in mealy bugs is unusual and it varies with species and this phenomenon has been studied in detail as it impacts pest management programs. These mealy bugs have the lecanoid type of the paternal genome elimination system, where both sexes develop from fertilized eggs, but during early development of the male the

paternal half is deactivated through heterochromatinization (Ross et al., 2010). This system suggests females would produce a male-biased sex ratio when alone, and a more female-biased sex ratio when crowded with other females. However, opposite effect of crowding effect was found with *Planococcus citri*, suggesting that some mealy bug species may adjust their sex ratio selectively (Ross et al., 2010).

Many mealy bug species have males and females and they produce sexually meanwhile some are parthenogenetic, which mating with males is not necessary to produce offspring. Females emit a sex pheromone to attract adult males for sexual reproduction. Females mate multiple times and number of mating influences egg production (Waterworth et al., 2011). Most mealy bugs place their eggs in ovisacs but species such as *Pseudococcus longispinus*, *F. gilli*, *D. brevipes*, and *Heliococcus bohemicus* Sulc deposits live first instars (Ovoviviparous). The number of offspring produced varies with species, environmental conditions, and food supply (Zaviezo et al., 2010). It ranges from about 50 to over 800.

2.1.2 *Dysmicoccus brevipes*

Dysmicoccus brevipes (Cockerell, 1893) is also known as the pineapple mealy bug. It is highly polyphagous as it attacks plant species belonging to more than 100 genera in 53 families (Ben-Dov, 1994). Among the important affected crops are groundnut, oil palm, rice, soybean, gram, potato, *pandanus* palm and a range of grasses and weeds (Clausen, 1978, Dove and Williams, 1971; Kalshoven, 1981; Khan, 1984; Khoo et al., 1991; Rajagopal et al., 1982). When infesting on pineapple, this mealy bug species commonly infests on the roots of pineapple, and large colonies develop on the stems above ground level. As the infestation prolongs, the mealy bugs may spread upwards to feed in the floral cavities on both fruit and crown leaves. The infestation

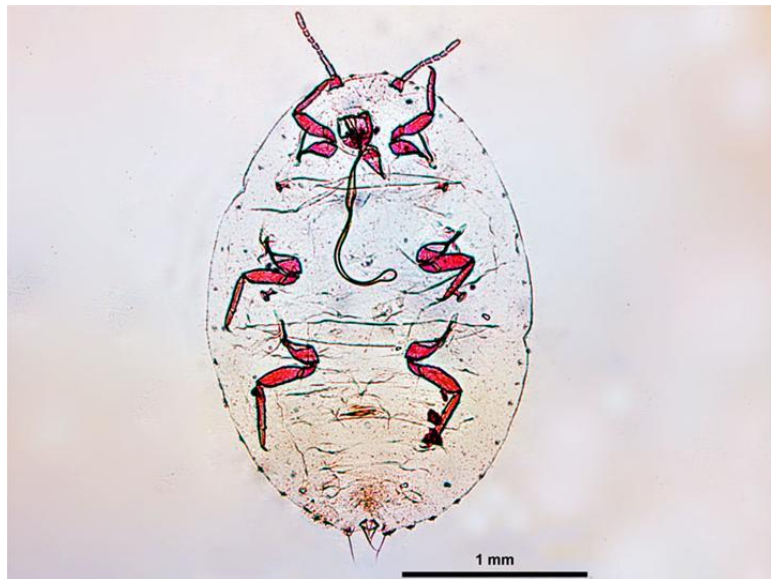


Plate 2.1: *Dysmicoccus brevipes*

causes wilting in the plant, which affects the growth such as reduced weight, leaf surface area, number of leaves, leaf length, and breadth as well as root length (Rohrbach et al., 1988). The infestation of the mealy bug also occurs on other parts of pineapple plants, such as on the foliage, stems, and fruits. This causes the vigor reduction and incapacity of the host plant, yellow spotting on the undersides of leaves, which may be shed prematurely, dieback of stems, and wilting. Honeydew deposition on the leaves leads to the growth of black sooty molds, which reduce the photosynthetic area on leaves. It is often associated with specific plant diseases such as the Cocoa Trinidad virus (Williams and Granara de Willink, 1992) and the Banana streak virus (Kubiriba et al., 2001).

In older records, two races or “strains” of this species were reported in Hawaii. The most differentiating feature between the two is one reproduces non-sexually and another bisexually. The bisexual “strain” caused green spotting on pineapple, and the bisexual “strain” did not produce a green spot. Later studies (Beardsley, 1959) revealed that the two “strains” were separate species: *D. brevipes*, which reproduce through parthenogenesis and *Dysmicoccus neobrevipes* Beardsley, which was bisexual. To distinguish two species morphologically, *D. brevipes* has longer dorsal setae on segment VIII than on segment VII and VI (Williams and Watson, 1988).

The species are ovoviviparous, and it does through three larval stages before becoming an adult (Lim, 1973). The larvae, also known as “crawlers,” have flattened bodies with long hairs that aid in their dispersal by wind or animals. Before the crawlers develop waxy covering on their bodies, they remain underneath the mother’s body for protection for a short period. Larvae molt three times before becoming an adult. In these series of studies, a bisexual race of *D. brevipes* was used as the subject species. This bisexual *D. brevipes* was recorded and described in detail for the first

time after its discovery in West Malaysia (Lim, 1973). Lim recorded that the female had three nymphal instars while the male had two nymphal instars, a prepupal and pupal stage. It took about 24 days for both sexes to develop from a crawler to an adult. The adult males were short-lived, which last 1-3 days while the females lived for 17-49 days. A gravid female could give rise to 19-137 crawlers with a sex ratio of 1:1. After the deposition of the nymphs, the females survived for a further 4.3 days. The adult male is a delicate creature with only a pair of membranous wings. The adult male does not feed as it has no externally developed mouthparts. It is an ephemeral creature that usually lives for only a day after copulation.

2.1.3 *Paracoccus marginatus*

The papaya mealy bug, *Paracoccus marginatus* Williams & Granara de Willink, 1992 are believed to be native to Mexico or Central America (Miller et al., 1999). The infestation of this mealy bug species is easily indicated by clusters of cotton-like masses on the above-ground portion of plants. Its greenish-yellow body colour can be distinguished with considerable amount of white waxy secretion. Like many other mealy bug species, *P. marginatus* is polyphagous and caused havoc in agricultural and horticultural crops. There are over 60 species of plants that serve as a host plant to this species (Chen et al., 2011, Seni and Chongtham, 2013). Excreted honeydew serves as a medium for sooty mold covers the leaves, fruits, and stems, impeding photosynthesis and gaseous exchange. *Paracoccus marginatus* has been recorded to only feed on above-ground parts of its hosts, particularly on leaves and fruits (Miller et al., 1999). Deformation of new growth, leaf yellowing, leaf curl, and early fall of fruits are among the symptoms of heavy infestation by *P. marginatus*

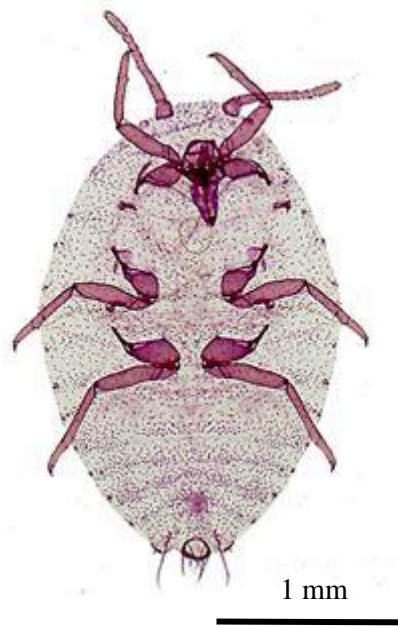


Plate 2.2: *Paracoccus marginatus*

(Miller et al., 1999). Fouled fruit resulted from its infestation, such as papaya, may face value reduction or even unmarketable.

As for most mealy bugs, the first-instar crawlers of *P. marginatus* disperse short distances by walking. Some on exposed parts of the plant could be carried to other host plants of greater range facilitated by the wind. Other than natural dispersal, the crawlers of *P. marginatus* could be carried by animals as the infested plants were being brushed on. Examples of such vectors are crows and mynah, and bats play an important role in disseminating *P. marginatus* from infested plants to new host plants when feeding on ripe fruits (Sarma, 2013).

This mealy bug species reproduce sexually, each female lays up to 600 eggs in a white, waxy ovisac. The ovisac is developed ventrally on the adult female. According to Thangamalar et al. (2010), egg-laying usually lasts for 1 to 2 weeks. Eggs are hatched in about 3-4 days, and the crawlers immediately search for a feeding site after hatching. With papaya as the host plant, the duration of the first instar nymph took 4-5 days. The second instar female nymph and male nymph took around 3-5 days. At third instar, female nymph took a development duration of 4-6 days while the male nymph (pre-pupa) only took 2-3 days. Compared to females, the male of *P. marginatus* has to go through an additional nymphal stage - the fourth instar nymph (pupa), which lasts 3-5 days. Female has three larval stages before emerging to the larviform adult stage. Meanwhile, the male has two immature larval stages (first and second instar) that feed, followed by non-feeding prepupal and pupal stages before emerging into a short-lived, winged adult. In favorable conditions, *P. marginatus* could produce up to 15 generations in a year.

Two common predators which are *Apertochrysa* sp. and *Cryptolaemus montrouzieri* Mulsant, 1853 were recorded to feed on egg masses and nymphs of *P. marginatus*. There were few parasitoids known to parasitize *P. marginatus*, which are *Acerophagus papaya* Noyes and Schauff, *Anagyrus Loecki* Noyes & Menezes and *Schauff* parasitoids. These natural enemies were very successful biological control agents against *P. marginatus* (Meyerdirk et al., 2004; powell et al., 2006).

2.1.4 Economic Importance of Mealy Bugs

Out of approximate 2000 described mealy bug species worldwide, only 158 species of mealy bugs are recognized as pests (Miller et al., 2002). Mealy bug post significant negative economic impacts, particularly on crops and ornamentals. Most mealy bug species associated with economic injuries are polyphagous. There are four main ways that mealy bug incurs economic damage (Mani and Shivaraju, 2016). First, plant infested with a high population of mealy bug can lead to a drop of fruit, flower, and leaf, as well as deformation and development of discolored welts on the rind of the fruits and flowers. Second, the copious amount of honeydew excreted by mealy bug causes the growth of black sooty mold that may stain the fruits and flower, resulted in reduced harvest percentage as well as delay in fruit color development. Growth of young trees may be stunted if sooty mold infection is severe. Third, the presence of mealy bugs on fruit or flower is a phytosanitary issue for export markets with the consequence of the rejection of the consignment. Fourth, mealy bugs are also known as vectors to several plant virus diseases, which cause heavy agricultural losses. Few mealy bug species are vectors of viral infections of several crops, for instance, banana, pepper (Bhat et al., 2003) and grapevine (Tsai et al., 2008). In cases of plant virus

disease transmission, mealy bugs can be important economic pests even at low densities.

Despite a wide range of economic damages caused by mealy bugs, some mealy bug species could be manipulated to play an important role in conservative biological control strategies. Cupress mealy bug, *Planococcus vovae* (Nasonoc, 1909) serves as an alternative host for natural enemies of the mealy bug pest in surrounding cocoa plantations and citrus orchards (Cox, 1989; Franco et al., 2004; Ho and Khoo, 1997). Due to the specificity of mealy bug in host plant selection, certain mealy bug species have been used as a biological control agent against weeds. For instance, *Hypogeococcus pungens* Granara de Willink, 1981 from Argentina was introduced into Queensland, Australia, to control *Harrisia martinii* (Labour.) Britton and related plants (Williams and Granara de Willink, 1992).

2.1.5 Mode of the Spread of Mealy Bugs

Concern and understanding of the spread of mealy bugs help to develop comprehensive preventive approaches against the establishment of invasive status of certain mealy bug species. There are several modes of dissemination been confirmed, investigated, and discussed. Among the mode of spread include planting material and equipment, trade and commerce, irrigation water, air currents, animals, and farming equipment (Mani and Shivaraju, 2016). The purchase of infested plant material facilitates the establishment of mealy bugs at a new place. They are hardly noticed as they usually hide at a protected spot such as crack and crevices in bark, roots, stems, and leaves. In an agricultural setting, the dispersal of mealy bugs is facilitated by farming transports and equipment during farm operations. Besides, dispersal over

longer distances can be facilitated rapidly by trade and commerce (Mani and Shivaraju, 2016). For example, the papaya mealy bug, *P. marginatus* was aided by the transport of infested papaya from one state to another in India (Macharia et al., 2017). Flood irrigation also carries fallen leaves and other infested parts of the host plant from one place to another (Tanwar et al., 2010). Besides, the first instar nymphs of mealy bugs (crawlers) may be dispersed over long distances by wind (Parsa et al., 2012).

Aerial dispersal plays a crucial role in the quick establishment of mealy bugs in new areas. It is suggested that *P. marginatus* was spread very quickly in India through the wind dispersal of the crawlers from Tamil Nadu to others (Muniappan et al., 2008). Furthermore, mealy bugs are known to cling on nectar-feeding animals as a means of transportation. For instance, mynah and bats are among the animals that transfer papaya mealy bug, *P. marginatus* to new host plant through phoretic method (Sarma, 2013). Ants are also known as the culprit to the dispersal of mealy bug due to their mutualistic associations. It was reported that *Pseudolasius* spp. was responsible for the continuous transfer of mealy bugs during colony fission of the ant (Malsch et al., 2001). Moreover, the *Pseudolasius* species adopted the adults and immature instars of their mealy bug partners within their nests.

2.1.6 Management of Mealy Bugs

Mealy bugs are challenging to be killed using insecticides due to their waxy protective covering, which forms a physical barrier against chemical penetration. Due to the hydrophobic property of the cuticle, wetting agent in the insecticide spray is often required to enhance the effectiveness. Systemic insecticides are commonly used as a therapeutic method in attempt to suppress mealy bug population. However, a

recent study by Herrick and Cloyd (2017) showed unsatisfactory control using systemic insecticides against citrus mealy bug, *Planococcus citri*. Two methods using systemic insecticides were tested and compared: therapeutic and preventive applications. The therapeutic applications using dinotefuran, imidacloprid, and thiamethoxam resulted not more than 35% mortality of the mealy bugs, whereas preventive applications only yielded 50% mortality. Ironically, with four times the label rate, none of the systemic insecticides provided more than 61% mortality. The failure of systemic insecticides is attributed to several factors. The active ingredient may not be translocated uniformly to every part of the host plant (Cloyd and Bethke, 2011). Hence, the mealy bugs were not ingesting lethal concentrations of the active ingredient (Cloyd et al., 2012).

On the contrary, the drench application of systemic insecticides with imidacloprid successfully suppressed whiteflies populations above 94% mortality (Bethke and Redak, 1997). A few similar systemic insecticidal studies on whitefly were proved to be highly effective in suppressing the population (Cloyd et al., 2012; Horowitz et al., 1998; Schuster and Morris, 2002). The disparity between the effectiveness of systemic insecticides against citrus mealy bug and whiteflies are due to the different feeding behaviors. This is due to the translocation of systemic insecticides that occur within the xylem instead of phloem. Hence, citrus mealy bug might not be exposed to the lethal concentrations of the active ingredients (Pillai, 2016; Sur and Stork, 2003). According to Ng and Falk (2006), mealy bugs only feed in the phloem tissues. Similar findings by Franco et al. (2009) also indicated that mealy bugs feed within phloem, mesophyll, or both.

Cultural control approaches, such as crop sanitation, is useful in suppressing the mealy bug populations. This approach works best with insecticides application

integratively. Crop sanitation is an example of a cultural method which includes removal of weed, burning, and plowing crop after harvest. Removal of weeds was proved to successfully control *Brevinnia pulverarius* (Newstead, 1892) (Dietz and Harwood, 1960) and *Heterococcus nigriensis* Williams (Harris, 1961). Discarding heavily infested plants and pruning of infested parts of plants are also helpful in reducing the mealy bug population.

Biological control against mealy bugs remains one of the most classical biological control that remains relevant and frequently used as an alternative to pesticides to control mealy bug infestation (Moore, 1988). Among the agents used in biological control are parasitoid, predator, and pathogen. However, biological control of mealy bugs remains challenging by taxonomic misidentification that leads to the failure of the establishment of the biological control agents. A combination of morphological and molecular characterization are necessary steps taken before embarking on the biological control program (Beltra et al., 2015).

Parasitic wasps lay eggs insides mealy bug's body, and when the larvae hatch, they eat the way out of that mealy bug host, slowly mummifying and killing them. In many cases, one parasitoid species is useful to control several mealy bug species. For instance, *Chrysoplatycerus splendens* (Howard, 1888) is reported to control several mealy bug species such as *D. brevipes*, *Dysmicoccus ryani* Cockerell, 1893, *Ferrisia virgate* Cockerell, 1893, *Formicococcus njalensis* (Laing, 1929), *Planococcus ficus* (Signoret, 1875), *Planacoccus citri* (Risso, 1813), *Pseudococcus* sp., *Pseudococcus calceolariae* (Maskell, 1879), *Pseudococcus maritimus* (Ehrhorn, 1900), *Pseudococcus viburni* Signoret, 1875 and *Pseudococcus comstocki* (Kuwana, 1902). Other than that, generalist predators such as ladybugs, lacewing, and mealy bug destroyer (*C. montrouzieri*) are widely used to control mealy bugs. However, the

presence of several prey may compromise the efficacy of biological control using generalist predators as they may switch to more abundant or more preferred alternative preys instead of feeding on the targeted prey (Murdoch, 1969). Another biological control approach uses entomopathogenic fungi such as *Beauveria bassiana* (Bals.-Criv.) Vuill (1912) to suppress mealy bug population. When spores of fungus contact with the cuticle of mealy bugs, they germinate and grow directly through the cuticle to the inner body of the host. Subsequently, the fungus proliferates throughout the mealy bug's body, producing toxins and draining nutrients from the body, eventually killing it. The fungus then produces millions of new infective spores that are released to infect nearby mealy bug individuals.

However, the use of insecticidal control, especially contact insecticides, should be avoided when the biological control program is implemented. It is shown that contact insecticides are highly toxic to mealy bug parasitoid, *Coccidoxenoides peregrinus* (Timberlake, 1919), causing 98-100% mortality in just 6 hours (Wakgari and Giliomee, 2003).

2.2 Ants

Ants are a group of eusocial insects from the order Hymenoptera, belong to the family Formicidae. There are a total of 20 subfamilies in Formicidae recorded with 17 extant subfamilies and 3 extinct subfamilies (Borowiec et. al., 2021). Ants are known as a terrestrial animal with ecological success with their abundance. For instance, measurements done by Beck (1971) suggested that one-third of the entire animal biomass of the Amazonian terra firme rain forest is made up of ants and termites, with each hectare of soil containing more than 8 million ants and 1 million termites. The

ecological impact of ants on the environment is considered significant. They are among the leading predators of many insects and small invertebrates. These are the attributes of their high degree of polymorphism and the complex associations with a wide range of animal groups, including humans (Wheeler, 1910). Some ant species have adapted well to disturbed habitats. Most cities in the tropics are habitat to “tramp ant” species, which have been carried worldwide by human commerce.

However, associations of ants with plants and hemipterans have formed a paradigm of variety interactions for research. Mutualisms involving ants, honeydew-producing insects, and host plants are incredibly widespread in terrestrial ecosystems, including agriculture settings, and may alter the communities’ structure of organisms involved. The dynamism of the interaction mostly dependent on several factors such as food, shelter, and protection.

Diversity of ants renders various aspects of ants to be explored. Through extensive knowledge of biology and behaviour of certain ant species and their associates, we can further enhance the effort and knowledge in better pest management.

2.2.1 Biology of Ants

An ant life cycle encompasses four stages: egg, larva, pupa, and adult. In the growing process, the larvae grow through four to five molts before entering the pupal stage. The later differentiate into queens and workers through caste determination. One detailed study demonstrated several parameters responsible for influencing the caste determination in Florida harvester ant (Smith et al., 2008). It showed that caste determination began during the larval stage based on the nutrients they receive. It was found genetic differences also decide the larva’s developmental pathway. Therefore,

nutritional, social, and genetic factors contribute in different ways to the growth of the individual despite the caste is determined. This is one example where caste determination involves both nature and nurture.

Due to caste differentiation, each caste of ant is responsible for specific tasks and roles, which is also known as caste polyethism (Iwasa and Yamaguchi, 2020). Males in an ant colony is known to contribute only in reproduction and present within a colony for only a short time in which they mostly die soon after mating. Queen in ant colony is the principal female reproductive type that appears anatomically distinguishable from the worker caste who responsible for reproduction. The worker is the ordinarily sterile female who possesses reduced ovaries or none at all. This caste of ants engaged in foraging, defense, and nursing, depending on the age of the workers (Mirenda and Vinson, 1981). It was reported that late instar larvae in *M. pharaonis* involved as the critical social player and effectors of colony regulatory dynamics due to their ability and role in processing solid protein for the entire colony (Warner et al., 2016). Hence, the stimulatory effect on queen production is the result of their role in nutrient regulation.

Temporal polyethism, also known as age-related task performance, occurs in most social insects, including ants (Calabi and Traniello, 1989). In a colony with temporal polyethism, less risky tasks are assigned to younger workers such as nursing or nest maintenance. (Enzmann and Nonacs, 2021). In contrast, older workers are assigned to more dangerous tasks that take place outside of nests, such as foraging, defense, and raiding. This is beneficial to the colony growth as it maximized each worker's longevity and breed fewer workers (Tofilski, 2009). One would assume that younger worker is more of a specialist or expert in brood care and queen care than old workers. This assumption was proven wrong, at least in the case of *Pheidole dentate*

Mayr 1886. Muscedere et al. (2009) found that larvae reared by old workers gained significantly more mass compared to those raised by the young workers. In addition to that, the older workers were more attentive in queen care, which suggests older workers retain the ability to perform these tasks and even at high productivity.

2.2.1.1 Trophallaxis

In ants, trophallaxis is the mutual exchange of food among nestmates. Other nestmates freely consume solid food such as prey and seeds brought into the nest by few foragers. Meanwhile, liquid diet is stored in the crops of foragers will be regurgitated to nestmates, and through this food transmission, liquid food is distributed over the colony members. Hence, the crops of all the ant workers together known as “social stomach,” in which the whole colony draws nourishment without having all individuals to forage (Hölldobler and Wilson, 1990). A series of behaviors by solicitor stimulate the donor during stomodeal trophallaxis. An exchange of liquid food by regurgitation was illustrated in detail by Hölldobler (1985). However, another unusual food exchange behavior known as abdominal trophallaxis was recorded for *Zacryptocerus varians* Smith (Cole, 1980; Wilson, 1976) and *Procrpytocerus scabriusculus* Forel 1899 (Wheeler, 1984). This form of trophallaxis occurs when nestmates consume excreted droplets of rectal liquid.

Other than food sharing purposes, trophallaxis also plays a role in information sharing during trophallactic interactions. A recent study by Hayashi et al. (2017) displayed that ants, *Tetramorium tsushimae* Emery, 1925 learn to recognize their associated aphids through trophallaxis. Ants that had interacted with aphid-experienced nestmates reduced their aggressiveness toward aphids, although they had

never directly experienced them. This is an example of the co-evolution result from the ant-hemipteran association.

Trophallaxis was proven to be the underlying mechanism to the social facilitation of disease resistance among ants. Evidence demonstrated that trophallactic behaviour among immunized workers of *Camponotus pennsylvanicus* (De Geer, 1773) enhances the survival of recipient ant through the regurgitate droplet (Hamilton, 2011). They have identified protein related to cathepsin D, a lysosomal protease, as a potential contributor to such antimicrobial activity. This suggests that prophylaxis through food regurgitation is an essential strategy for social immunity continuity, allowing the colony to thrive better in high pathogenic pressure.

Moreover, the keystone success in ant control using ant baiting programs is by targeting the food exchange behaviour among adults, larvae, and queens. The foraging workers carry the bait material in which usually incorporated with minute amount of insecticide back to the nest. Due to the inability of adult ant in digesting solid foods, the baits are fed to the larvae to digest before the bait regurgitate back to the adults. The baits are subsequently fed to other nestmates, and therefore, the insecticide spread throughout the targeted colony.

2.2.2 Foraging and Recruitment Strategies

Ant recruitment on food resources was reported to be linked with the colony size (Planqué et al., 2013). The method of ant recruitment is the subject of efficiency at a particular colony size. There are three categories of foraging and recruitment strategies demonstrated by ants. Individual ants do not recruit nestmates during

foraging are known as solitary foragers. Another approach is tandem running in which one ant leads a single nestmate to a target.

Meanwhile, in group recruitment, one ant worker may lead a group of individuals of ants to the resources. Species with small mature colonies such as ponerines and primitive ant genera such as *Nothomyrmecia* and *Myrmeica* seem to predominantly use solitary foraging methods (Hölldobler and Wilson, 1990; Ito, 1993; Traniello, 1987). Species with small to medium colony sizes use tandem running (Beckers et al., 1989). Species with larger colony sizes such as *Eciton* and *Dorylus* army ants use group recruitment (Franks et al., 1991; Franks et al., 1999). Recruitment methods are closely associated with aspects such as resource distribution and ecological factors. For instance, Wehner and et al. (1983) suggested desert living *Cataglyphis sp.* forage solitarily instead of group recruitment using pheromone trails as the pheromones would evaporate quickly under high temperature. Besides, small arthropods can be easily retrieved by solitary workers. Franks and Richardson (2006) have demonstrated one classic definition of teaching in animal behavior through the tandem running in *Temnothorax albipennis* Curtis, 1854. This method not only allows the tandem follower to teach others, but it is also a reliable recruitment method to lead a nestmate to a food source or new nest. Tandem running supposedly occurs among primitive and highly derived ant genera, and it was reported to have evolved independently multiple times (Brady et al., 2006; Moreau et al., 2006). The attribute to reliability and success of group recruitment by large colonies in the maintenance of the pheromone trails by copious ant traffic. The finding of a study was in agreement with this hypothesis by showing that the minimum number of *M. pharaonis* is required for these trails to function (Beekman et al., 2001).

Mass-recruitment communication in ants is governed exclusively by odor trails. In this method of recruitment, the number of workers employed to the goal depends on the all-or-none trail-laying response of the individual workers. Besides, the decision to reinforce the existing odor trail is determined by the thresholds of individual foragers towards the quality and quantity of the discovered food. For instance, *Lasius niger* (Linnaeus, 1758) only returns to the nest with trail laying behavior when the desired volume of solution was ingested (Mailleux et al., 2000). Hence, the level of starvation influences the threshold as more starved foragers have a lower trail-laying threshold (Mailleux et al., 2006). The type of food, proteinaceous, or sucrose droplets was found to change the proportions of *L. niger* foragers that laid trails instead of the intensity of trail-laying by an individual (Portha et al., 2004). Workers of *Solenopsis geminate* Fabricius, 1804 demonstrated the correlation of the continuity of their pheromone trail with colony starvation, food quality, and decreasing distance to the resource (Hangartner, 1969). The intensity of the trail laid by *Acanthomyops interjectus* (Hangartner, 1970) and *Monomorium pharaonis* (Jackson and Châline, 2007) could be modified concerning the food quality.

Besides, the lasting duration of the pheromone trails is the crucial key factor in their function. For instance, ants that forage on stable long-term food resources such as aphid populations and foliage of particular trees will employ pheromones with decay rates that last for several days (Howard, 2001; Quinet and Pasteels, 1991; Simon and Hefetz., 1991). On the contrary, opportunistic scavenging ant, *S. invicta*, instead use rapid-response recruitment pheromones, which decay within minutes (Wilson, 1962). Functions of pheromones vary with ant species, and they are known to work interactively to optimize foraging efficiency. In the foraging and recruitment strategy of pharaoh ant, three types of pheromone were used. Two are attractive pheromones:

first one forms a stable trail network and is long-lasting to allow the trails to be re-located and reused even not being used for several days (Jackson et al., 2006); the second one is to guide workers to current food sources and decays in minutes if not reinforced (Jeanson et al., 2003). The third pheromone is repellent, function to signal “No Entry” to cause avoidance behaviour in ants (Robinson et al., 2005). A detailed study (Robinson et al., 2008) was later conducted to explain the role and interactions of the three trail pheromones.

Interestingly, a repellent pheromone has a more significant initial effect than the attractive pheromone to provide information to foragers at a bifurcation which one trail branch is rewarding and one unrewarding. The short-lasting attractive pheromone was marked from nest to food. Volatility is the subject of the short-term pheromones, and it has significant effects on its function. The author also suggested that volatile pheromones can be detected from a distance without the ants touching their antennae on the substrate. Thus, the repellent pheromone can be detected up to 30mm in advance, and foragers could increase zigzagging behaviour, which helps them to locate the correct trail at a bifurcation (Robinson et al., 2005).

In contrary to *L. niger*, which modulates trail strength by an all-or-nothing individual response to food quality, *M. pharaonis* uses a different mechanism where trail-marking intensity correlates with the quality of food (Jackson and Chaline, 2007). A significantly higher concentration of pheromone trail marks was observed in ants fed with a high-quality food source (1.0 M sucrose) than unfed ants. However, when the ant fed with low-quality food (0.01 M sucrose), no significant difference in terms of the pheromone trail marking intensity was observed compared with unfed ants. The challenges to these recruitment strategies and abilities arise when it comes to best resource selection in a changing, competitive environment. One may expect foraging

ants to switch to collect better resources available when several food sources present simultaneously. Though, when better support was introduced, *L. niger* showed either very slow or no switching at all from the primary resources, which was selected first (Beckers et al., 1990). This is due to the reliance of *L. niger* on visual memory in foraging that requires more time and effort to switch from one resource to another.

Furthermore, there is evidence of the influence of the presence of brood on the proportion of trail-laying ants (Portha et al., 2002). At the same time, this study also indicates the decision of forager to consume the food droplet is influenced by the food type and not the brood. In particular, few workers of *L. niger* encountered proteinaceous droplets that did not ingest it and did not go back to nest. Proteinaceous food was found to elicit weak feeding, followed by a weak recruitment trail that affects the recruitment of nestmates and the accuracy of the trail to these foods (Pasteels et al., 1987). This finding could be due to the preference of *L. niger* towards carbohydrate-based food and may not reflect the trail laying behaviour of ant species that prefer proteinaceous based food. Critical volume rule (Mailleux et al., 2000) also suggested governing the decision of the ants to return to the nest. Hence, factors which known to influence the volume of food ingested such as viscosity (Josens et al., 1998), concentration (Bonser et al., 1998; Josens et al., 1998), distance (Bonser et al., 1998) of the food and starvation level (Josens and Roces, 2000) are likely to influence the decision of forager to return to the nest with recruiting pheromone trail.

2.2.3 Food Preference

Food preference governs the decision of foraging ants to collect and ingest discovered food to the extent that it determines the type of association with the sap-