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**TITLE:** Obstructing the Mutualistic Relationship between *Meranoplus bicolor* (Hymenoptera: Formicidae) and Honeydew Producing Hemipterans in Guava Plantation

**NAME:** Miss Duangthip Kantha

**THIS THESIS HAS BEEN ACCEPTED BY**

THESIS ADVISOR

( Associate Professor Intawat Burikam, Ph.D. )

DEPARTMENT HEAD

( Assistant Professor Nantasak Pinkaew, Ph.D. )

APPROVED BY THE GRADUATE SCHOOL ON

DEAN

( Associate Professor Gunjana Theeragool, D.Agr. )

THESIS

OBSTRUCTING THE MUTUALISTIC RELATIONSHIP BETWEEN  
*MERANOPLUS BICOLOR* (HYMENOPTERA: FORMICIDAE) AND  
HONEYDEW PRODUCING HEMIPTERANS  
IN GUAVA PLANTATION



DUANGTHIP KANTHA

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Duangthip Kantha 2014: Obstructing the Mutualistic Relationship between *Meranoplus bicolor* (Hymenoptera: Formicidae) and Honeydew Producing Hemipterans in Guava Plantation. Doctor of Philosophy (Entomology), Major Field: Entomology, Department of Entomology. Thesis Advisor: Associate Professor Intawat Burikam, Ph.D. 65 pages.

Mutualistic relationships between the shield ant, *Meranoplus bicolor* (Guérin-Méneville), and two species of hemipteran, *Aphis gossypii* Glover and *Ferrisia virgata* (Cockerell), were investigated in an unsprayed guava plot at Kamphaeng Saen, Nakhon Pathom, Thailand. The reciprocal benefits were observed in both field and laboratory studies. *M. bicolor* activity coincided with peak seasonal activity of both hemipterans during June–August 2012. There were two sets of support evidence in *M. bicolor* honeydew preference: (i) statistically higher value of adjusted honeydew weight collected by ant workers from *A. gossypii* compared with that from *F. virgata* ( $p$ -value = .005), and (ii) the higher value of the strength of effect ( $\eta^2 = .62$ ) in the total variance of multi-species association. A two-group, ant-tended and ant-excluded, between-subjects multivariate analysis of variance (MANOVA) was used in order to show hemipteran benefits. Both hemipteran populations increased in the ant-tended treatment, together with lesser amounts of their natural enemies: *Menochilus sexmaculatus* (Fabricius) and *Coccinella transversalis* Fabricius, and, *Pseudodorus clavatus* (Fabricius), compared with the ant exclusion treatment ( $p$ -value < .001). The mutualistic relationships were interfered by using liquid ant bait. Three insecticidal materials, i.e. boric acid, imidacloprid, and fipronil, dissolved in 25% sugar water including an untreated control were distributed in a bait dispenser attached to the base of guava tree. Liquid ant baits were evaluated for efficiency on visitation rates of *M. bicolor*, and visual counts of The both hemipterans and their natural enemies, there was statistically significant treatment impact on composite dependent variate consisting of ant visitation rates, hemipteran densities, and abundance of natural enemies ( $p$ -value < .001). Bait dispenser with 3% boric acid was the most effective ant bait followed by untreated control bait and either .0001% imidacloprid or .0001% fipronil. The efficiency of liquid bait was strongly influenced by the persistence of attractive odor over time. Sugary liquid bait with either imidacloprid or fipronil, together with control comprising 25% sugar water were unable to maintain their freshness due to fermentation of the materials at high temperature. Conversely, the durability of sugary smell in boric acid bait came from its antibacterial and antifungal properties.

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Student's signature

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Thesis Advisor's signature

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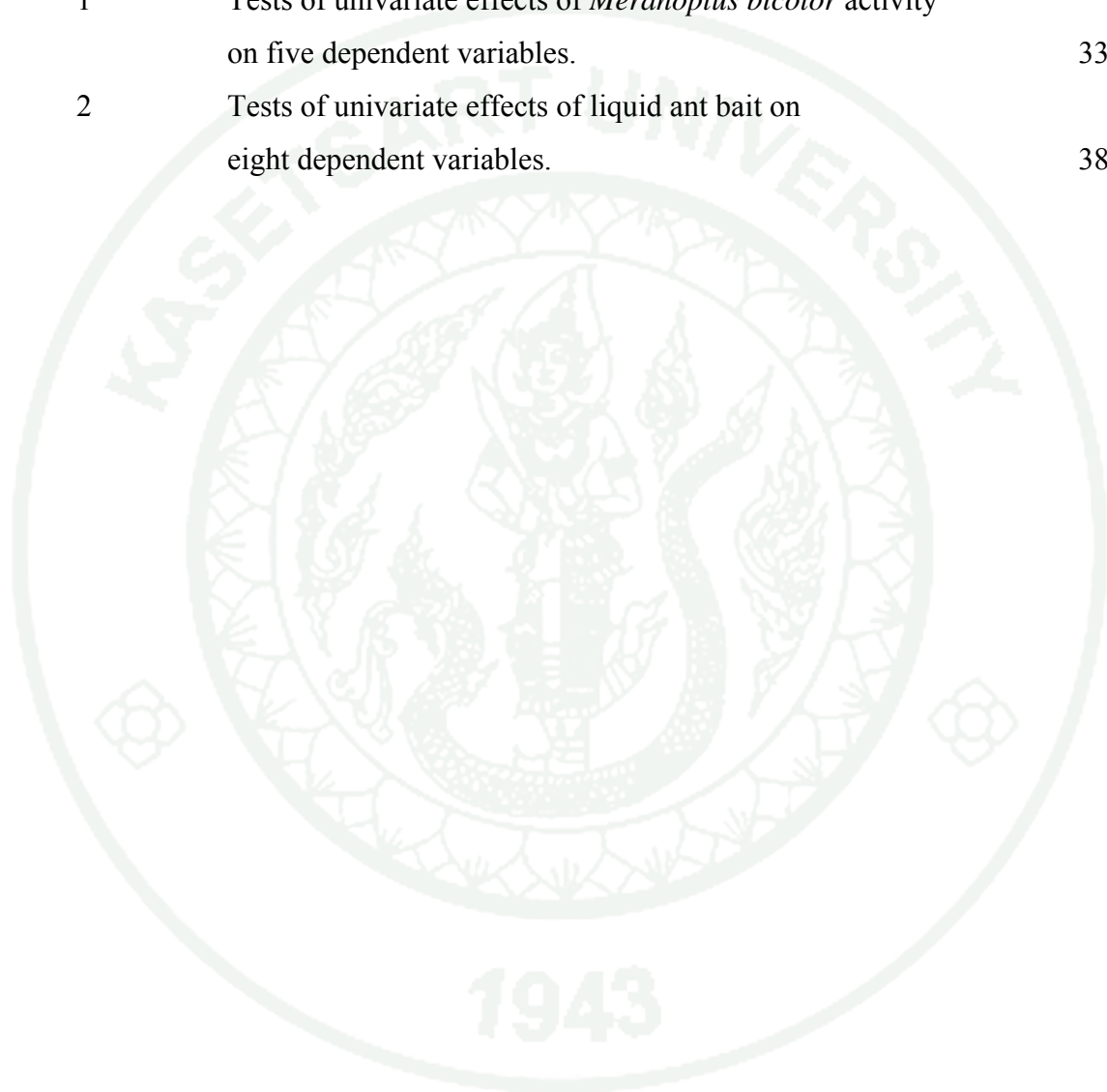
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## LIST OF ABBREVIATIONS

MB5M	=	<i>Meranoplus bicolor</i> entered bait dispenser in 5 minutes
NAG	=	<i>Aphis gossypii</i>
NFV	=	<i>Ferrisia virgata</i>
MSAG	=	<i>Menochilus sexmaculatus</i> on <i>A. gossypii</i>
CTAG	=	<i>Coccinella transversalis</i> on <i>A. gossypii</i>
SFAG	=	<i>Pseudodorus clavatus</i> on <i>A. gossypii</i>
MSFV	=	<i>M. sexmaculatus</i> on <i>F. virgata</i>
CTFV	=	<i>C. transversalis</i> on <i>F. virgata</i> .

**OBSTRUCTING THE MUTUALISTIC RELATIONSHIP  
BETWEEN *MERANOPLUS BICOLOR* (HYMENOPTERA:  
FORMICIDAE) AND HONEYDEW PRODUCING  
HEMIPTERANS IN GUAVA PLANTATION**

**INTRODUCTION**

The shield ant, *Meranoplus bicolor* (Guérin-Méneville) (Hymenoptera: Formicidae), is a common ground nesting species of the subfamily Myrmicinae, and is widely distributed throughout the entire Oriental Region (Schödl, 1998). The workers not only forage on dead arthropods as scavengers, but also collect honeydew as carbohydrate source from hemipterans, e.g. the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphidae) and the striped mealybug, *Ferrisia virgata* (Cockerell) (Hemiptera: Pseudococcidae), in agricultural ecosystem. However, the trophobiotic relationship or mutualism between *M. bicolor* and the honeydew-producing hemipterans are unknown.

*M. bicolor* were frequently associated with the outbreaks of two important hemipterous pest i.e. the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphidae) and the striped mealybug, *Ferrisia virgata* (Cockerell) (Hemiptera: Pseudococcidae) of commercial guava crops throughout Thailand. This interspecific association generally called mutualistic relationship between ants and honeydew-producing hemipterans has been reviewed by several authors, e.g. Way (1963); Delabie (2001); and Stadler and Dixon (2005). In general, ants benefit from associations with hemipterans by obtaining carbohydrate-rich food source in the form "honeydew" excreted from hemipterans (Nixon, 1951; Way, 1963; Hölldobler and Wilson, 1990). In return the benefits, ants may reduce hemipteran contamination of their waste products, removing dead individuals, protecting natural enemies, and transport hemipterans to new feeding site, resulting in the abundance of hemipteran populations (Way, 1963; Nielsen *et al.*, 2010; Stadler and Dixon, 2005). Somehow, ants may

exploit hemipterans not only for their honeydew, but also as a protein source when foraging on them as on them as a common prey (Buckley, 1987; Hölldobler and Wilson, 1990; Delabie (2001).

Many studies indicated that the exclusion of ants can reduce hemipteran attacks in horticulture crops through significant increased in natural enemy pressures on hemipteran populations. Most of them were done in fruit orchard, e.g. cherry (Stutz and Schmidt-Entling, 2011), apple (Stewart-Jones *et al.*, 2008; Miñarro *et al.*, 2010; Nagy *et al.*, 2013), guava (Burikam and Kantha, 2014), and in vineyards (Mgocheki and Addison, 2009). Ant exclusion was achieved mainly by applying stick barrier around the tree trunk or main stem of the plan, providing that no aerial parts touching the ground. However, the sticky barrier might create some practical problems in order to exclude the ant workers as pointed out by Nagy *et at.* (2013). One of this problems appeared in guava plantation of Thailand was the use of multiple stakes to support a single guava tree canopy. The procedure seemed to be complicated and time consuming when dealing with many tree in order to maintain the gluey barrier if need. Another difficulty to control hemipteran pests came from growers regularly wrapped individual guava fruit including its peduncle by using plastic bag, or both for fruit quality and aesthetic value. The infestation of *A. gossypii* and *F. virgata* on fruit peduncle inside the wrapping could not be easily eradicated by insecticide spraying. These ant-tend hemipterans were distributed to the concealed habitats by ants. Interference of ants, primarily *M. bicolor*. using sugary liquid baits either by delayed toxicity or deviated ants from honeydew of hemipterans may reduce the infestation on guava fruits.

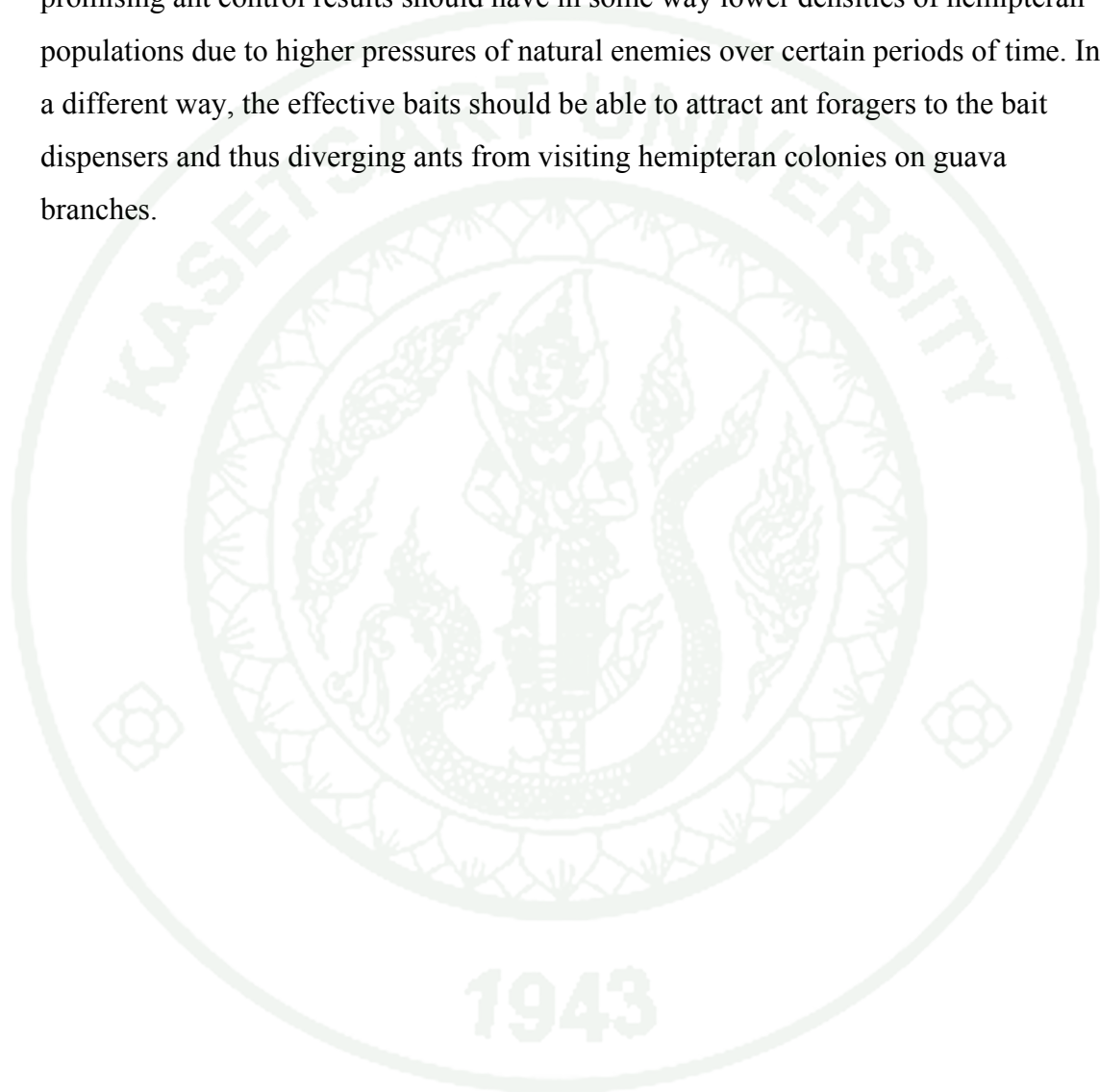
Ants controls using insecticidal sprays as contact poison and barrier treatment offer limited ant population control because they kill or deter foraging workers but have little effect on majority members and queens in the colonies (Rust *et at.*, 1996). Other alternatives to chemical sprays included granular treatments and soil baits are available commercially in agricultural systems with more or less promising result (Costa *et al.*, 2001; Taniguchi *et al.*, 2005; Tollerup *et al.*, 2005; Webb and Hoffmann, 2013). However, many ant species in agricultural systems including

*M. bicolor* in guava plantation mainly forage for honeydew as carbohydrate source. The use of liquid ant bait seems to be promising as evidence in previous studies of other ant-hemipterans relationships (Silverman and Roulston, 2001; Rust *et al.*, 2004; Daane *et al.*, 2006). Liquid ant baits with a slow-performing or delayed toxicity will be collected by foraging ants, and distributed throughout the colony via trophallaxis, grooming, and necrophoresis (Klotz *et al.*, 2002; Rust *et al.*, 2004; Soeprono and Rust 2004), and as a consequence increasing colony mortality. Generally, baits hold a small dose of insecticide, confined in a dispenser or bait station rather than directly applied to the crop or soil. This could minimize undesirable impacts on the environment including non-target species such as pollinators or natural enemies of insect pests (Taniguchi *et al.*, 2005; Daane *et al.*, 2006; Cooper *et al.*, 2008).

The main objective of this study was to investigate, in both field and laboratory experiments, the reciprocal benefits of *M. bicolor* and two species of honeydew-producing hemipterans, *A. gossypii* and *F. virgata*. The study concentrated for over three-month period in the guava plantation of Horticulture Department, Kasetsart University, Nakhon Pathom, Thailand. The mutualism of ant-hemipterans was observed, including the abundances of natural enemies, mainly predators. There were three hypotheses in this session. Firstly, ants received benefits in terms of honeydew from mutualistic associations in guava agroecosystem. Secondly, ants protecting hemipterans from natural enemies, therefore the densities of natural enemies decrease in the presence of ants. Thirdly, in consequence of the two hypotheses mentioned earlier, resulting in the increments of hemipteran densities in ant-hemipteran associations compared with the ant-exclusion arrangement.

The study also investigated the effectiveness of three formulated sugary liquid ant baits for *M. bicolor* control in guava plantation. The sugary liquid bait was placed inside a polyvinyl chloride (PVC) pipe called bait station or dispenser adapted from those of Klotz *et al.*, (2002), Daane *et al.*, (2006), and Cooper *et al.* (2008). The toxicants dissolved in sugary liquid baits for compared were boric acid, imidacloprid, and fipronil. During June-August 2012, the treatment effects were evaluated on eight dependent variables using multivariate analysis of variance (MANOVA) for

consolidate conclusion. Eight variables mentioned consisted of one variable of ant visitation rate at the bait station, two variables of hemipteran densities on plants, and five variables of natural enemies associated with hemipterans. Treatments with effective baits should have comparatively high ant visitation rates, and those with promising ant control results should have in some way lower densities of hemipteran populations due to higher pressures of natural enemies over certain periods of time. In a different way, the effective baits should be able to attract ant foragers to the bait dispensers and thus diverging ants from visiting hemipteran colonies on guava branches.



## OBJECTIVES

By the end of the study on mutualistic relationship between *M. bicolor* and the two honeydew producing hemipterans in guava plantation, we should be able to achieve:

1. General mechanism of mutualistic relationship , i.e. reciprocal benefits between *M. bicolor* and two hemipteran insects (cotton aphids, *A. gossypii*, and striped mealybug, *F. virgata*);
2. Efficacy of three sugary liquid ant baits, i.e. 3% boric acid, .0001% imidacloprid, and .0001% fipronil. All toxicants were dissolved in 25% sugar water and delivered in bait dispensers.

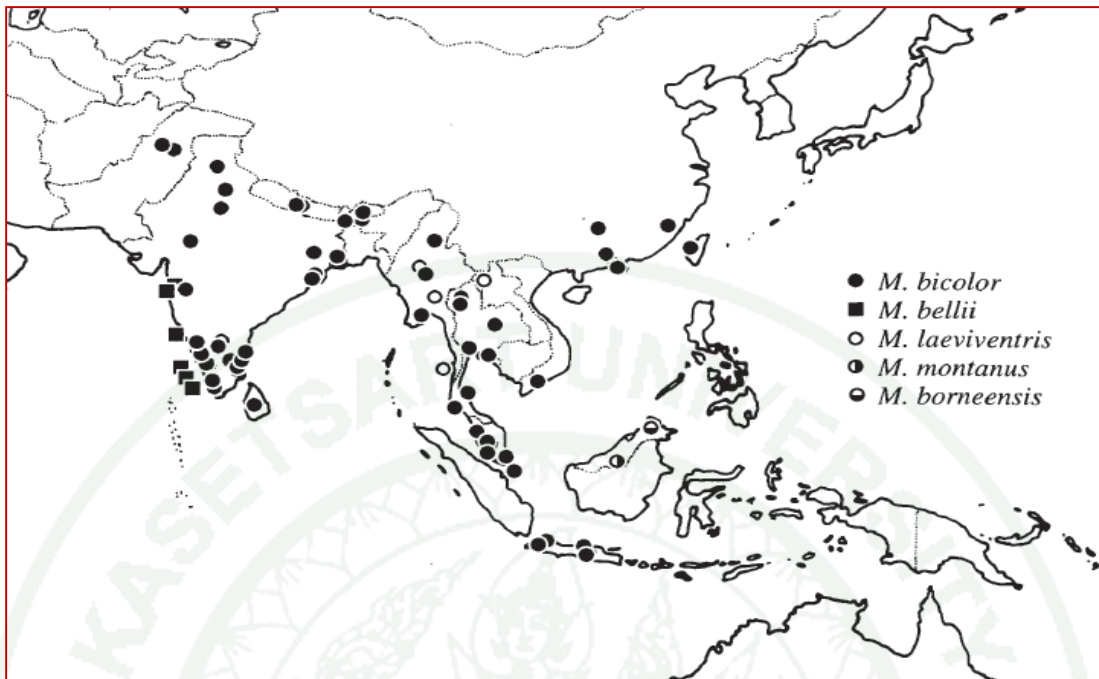
## LITERATURE REVIEW

### Geographical distribution of *M. bicolor*

The distribution of some *Meranoplus* in Oriental Region was studied by Schödl (1998). The distribution range is shown in Figure 1. *M. bicolor* dispersed throughout the entire Oriental Realm. The range of this common *Meranoplus* was from Sri Lanka, passing through the entire Indian Subcontinent, including Pakistan, Nepal and Bhutan, and going farther eastwards to South China, including Taiwan, Hong Kong, and Indonesia. In Thailand Jaitrong and Nabhitabhata (2005) recorded the appearance of *M. bicolor* from all provinces especially in open areas. In the Himalayan regions *M. bicolor* was found at an altitude of 1,750 m above sea level (Bharti, 2008)

*Meranoplus bicolor* Guerin–Meneville (Hymenoptera: Formicidae) is a common ant in grassland and footpaths of India, Pakistan, Nepal, Bhutan and Sri Lanka. It was frequently found throughout Burma and extends to China, Taiwan and Indonesia (Hannan, 2008). *M. bicolor* built their nests in loose and hard soil. The nest entrance is at ground level surrounding by a small crater (Smith, 1875). They were found in agricultural site nearby residential areas in Sri Lanka, (Amarasinghe, *et al.*, 2010). Ghosh *et al.*, 2007 reported that *M. bicolor* together with other three ant species, *Pheidole robertii*, *Tetramorium walshi* and *Monomorium floricola* contribution about 10% or more of the total ant community in West Bengal, India. In Thailand, *M. bicolor* were common found in agricultural lands with histories of pesticide usage.

There were 54 species of the genus *Meranoplus* distributed in Africa through India and south Asia to Australia (Bolton, 1995). Boudinot and Fisher (2013) added 4 more species from Madagascar to the list. However, Andersen (2006) claimed that there would be many species in this genus have not yet been identified, especially those in northern Australia.

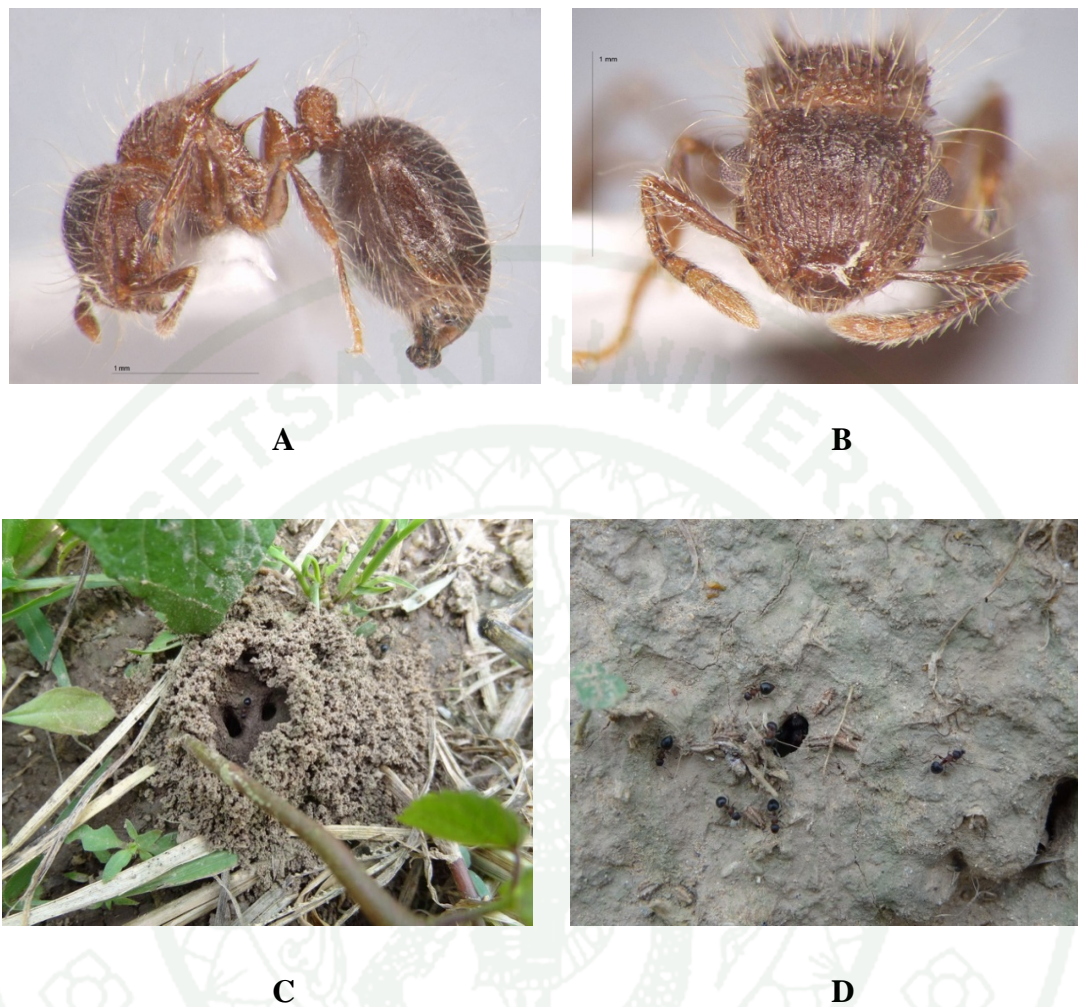


**Figure 1** Distribution map of common species of *Meranoplus* in Asia (after Schodlh, 1998).

### General biology of *M. bicolor*

According to Ghosh *et al.*, 2005, *M. bicolor* is characterized by having promesonotal shield of thorax about as broad as long, anterior angles dentate and posterior sides produced backwards into long, somewhat laminate spines, overhanging metanotum; the latter vertical with a carina on each side ending above in an acute spine. Petiole smooth, triangular with bevelled apex; postpetiole globose; and the shape of abdomen is cordate. The average body length of *M. bicolor* workers is about 4–5 mm. Head, thorax, legs and petiole are ferruginous red and black on abdomen. Head and thorax are coarsely sculptured but in abdomen is finely reticulate. Head is a little longer than broad, broader posteriorly; dentate mandibles obscurely straight, pubescent; clypeus convex, inclined downwards; antennae 9-segmented, antennal furrow lateral and deep. The common name "shield ants" came from having the obvious flat posterior extension of the dorsal of thorax (Bolton, 1995). Moreover Schödlh (1998), reported that *M. bicolor* has promesonotal shield with a single pair of posteriorly directed long spines in the posterior mesonotal corners. Dorsal surfaces and appendages provided with exceptionally long, outstanding hairs as well as shorter pubescence. It is scarcely to be confused with any other species within the range of its occurrence. *M. bicolor* can be distinguished by the long posterior directed spines, and the unique pilosity throughout its body, which are found in no other ant species (Figure 2 A and B).

The average body length of *M. bicolor* worker, female, and male measuring from mandibular apex to posterior end of gaster are 3.7 - 4.5, 7 - 8, and 3.5 mm, respectively (Schödlh, 1998). The color of the body mostly is bicolor, with head, alitrunk, petiole and postpetiole of a pale to darker ferruginous and the gaster piceous. Sometime appearance of entire body is black. The normal colony of *Meranoplus* spp. have male, female and worker or neuter that having imperfectly developed sexual organs (Smith, 1853). During the rainy season nest entrances were seen on an elevated chimney and with no mound during dry season (Figure 2 C and D, respectively).



**Figure 2** Worker of *Meranoplus bicolor*; side view (A), and front view (B) (after Bolton, 1995). Nest entrance in rainy season (C), and in dry season (D).

*M. bicolor* are ground-nesting and slow moving ants. They have unique behavior of death feigning as a defensive response to danger. Boudinot and Fisher (2013) reported that all species of *Meranoplus* are predominantly ground-nesting. When disturbed, will display death feigning or “thanatosis” enhancing by crypsis, i.e., individuals will accumulate dirt in their pilosity and playing dead. Andesen (2006) indicated that when disturbed many species of *Meranoplus* tuck their legs under their dorsal shields, retract their antennae into deep grooves on the sides of their head, and lie motionless until danger passes. Mathew and Tiwari (2000) mentioned that workers of *M. bicolor*'s were slow moving species and they feign dead when touched. Likewise Rastogi *et.al.* (1997) reported that the worker of this species moves very slowly and forages on the ground. In Australia Agosti and Egloff (2009) reported that the workers of *M. bicolor* move very slowly and readily curl up and feign death when handled. They not only appeared to be mainly carnivorous in their diets, but also fond of sugary substance and attending aphids and coccids on plants. In other specie, *M. mucronatus* was described by Boudinot and Fisher (2013) which have a trophobiotic relationship with hemipterans.

### **Mutualism between ants and honeydew producing hemipterans**

The studies on mutualism between ants and hemipteran insects have been reviewed by Way (1963). Ants feed on sugary excretion (honeydew) of sap-tapping hemipterans, accordingly reducing hemipterans contamination of their waste products, removing dead individuals, protecting natural enemies, and transporting to new feeding sites (Nixon 1951; Way 1963)The ant elicits the hemipteran with its antennae in order to obtain a perfect drop of honeydew to collect (Mittler, 1958).

In agriculture, the mutualism between ants and honeydew producing hemipterans has been greatly concerned in crop production. Honeydew producing hemipterans, generally aphids, mealybugs, and coccids are serious pests of many agricultural crops. The cotton aphids, *Aphis gossypii* Glover (Hemiptera: Aphidae) and the striped mealybug, *Ferrisia virgata* (Cockerell) (Hemiptera: Pseudococcidae) are considered to be the most important hemipteran pest species. The cotton aphid has

been reported as serious pest on many economically important crops such as guava, tomato, cotton, okra, egg plant, cucumber, and pumpkin ( Razmjou *et al.* 2010). The cotton aphids damage young guava shoots, while the striped mealybug feed on leaves, calyx and fruits of guava. Most of the ant-attended hemipterans feed on plant phloem (Gullan 1997).

The mutualistic relationships between ants and honeydew producing hemipterans in agriculture system were reported by many entomologist. Kumar and Shekhar (2010) studied the association between papaya mealybug and ants. They indicated that the ants provided the mealybug with protection from parasitoids and predators and aiding in dispersal of the mealybug from plant to plant. The cotton aphid, *A. gossypii* Glover is an important pest on guava. This hemipteran was tented by *Camponotus compressus* Fabricius (Formicidae: Hymenoptera) (Tandon and Verghese, 1987). The ant had a positive relationships with both *A. gossypii*. and the guava scale, *Chloropulvinaria psidii* (Maskell). The effects of ant tending *Lasius niger* (Hymenoptera: Formicidae) on life history of aphid, *Metopeurum fuscoviride* (Hemiptera: Aphidae) was studied by Flatt and Weisser (2000). Several positive effects of the *L. niger* workers existed on the ant-tended aphids, e.g. lived longer, matured earlier, higher rate of reproduction, and a higher expected amount of offspring than aphids not tended by ants. Kaneko (2007) showed that ants prevented hemipteran eggs and nymphs from suffocation and mould growth; and repelled predators and parasitoids approaching the hemipteran colonies.

The volume of liquid that passes through the hemipterans is higher than their needs, and thus the insects excrete large amounts of the liquid by producing droplets of honeydew which is the complete food for ants (Way, 1963). The honeydew is not only the solution of sugar but also consists of twenty-two free amino acids, amides, proteins and vitamins, in which a variety of ant-hemipteran relationships have evolved (Way, 1963; Delabie, 2001). This relationship leads to increased food uptake and larger hemipteran populations (Way, 1963; Kaneko, 2003).

Queiroz and Oliviera (2001) reported the interaction between whitefly *Aleurothrixus aepim*, the economic pest and *Camponotus* ant on the shrub *Croton floribundus* (Euphorbiaceae) in southeast of Brazil. The tending ants, *C. floribundus* can reduce the fungal infection and nymphal parasitoid of whitefly, and enhance whitefly survival. Moreover the whiteflies densities was significantly greater on ant-tended whiteflies nymphs than those on ant-excluded. In the lacking of tending ant the accumulated honeydew from whitefly quickly covered the whiteflies colonies and encouraged infection by fungi (*Cladosporium*), and thus indirectly decreased photosynthesis and leaf amputation (Queiroz and Oliviera, 2001).

Sharma and Sundararaj (2011) reported that the association of ants and honeydew producing in coccids and membracids in sandal tree (*Santalum album* Linn.) plantation, Bangalore, India, they found five species of coccids viz., *Cardiococcus bivalvata* Green, *Ceroplastes actiniformis* Green, *Nipaecoccus viridis* (Newstead), *Parasaissetia nigra* (Nietner) and *Saissetia coffeae* Targioni- Tozzetti and three species of membracids viz., *Leptocentrus longispinus* Dist., *Otinotus oneratus* Walker, and *Oxyrachis tarandus* F. There were several species of ants such as *Camponotus compressus*, F., *Crematogaster* sp., *Meranoplus* sp. *Myrmecaria brunnea* Saunders, *Oecophylla smaragdina* F., *Technomyrmex albipes* Smith and *Tetraponera rufonigra* Jerdon. *Camponotus* sp. was the most dominant ant species associated with maximum number of honeydew-producing hemipterans.

Ant-hemipteran relationships provide good models for the study of mutualism (Delabie, 2001). The two ants, *Pristomyrmex pungens* and *Lasius japonicas*, exclude many species of large predators such as coccinellids, chrysopids, and syrphids from aphid colonies that they attend through aggressive behavior (Kaneko, 2003). Nixon (1951) indicated that the ants do not protect the hemipteran coccids from parasitoids but their active movements hinder the parasitoids in their efforts to lay eggs. Kaneko (2007) reported that the behavioral, chemical, and morphological adaptations of some parasitoids and predators are able to avoid ant aggression and thus can be able to exploit the ant-tended hemipterans. Nixon (1951) indicated that larvae of predators

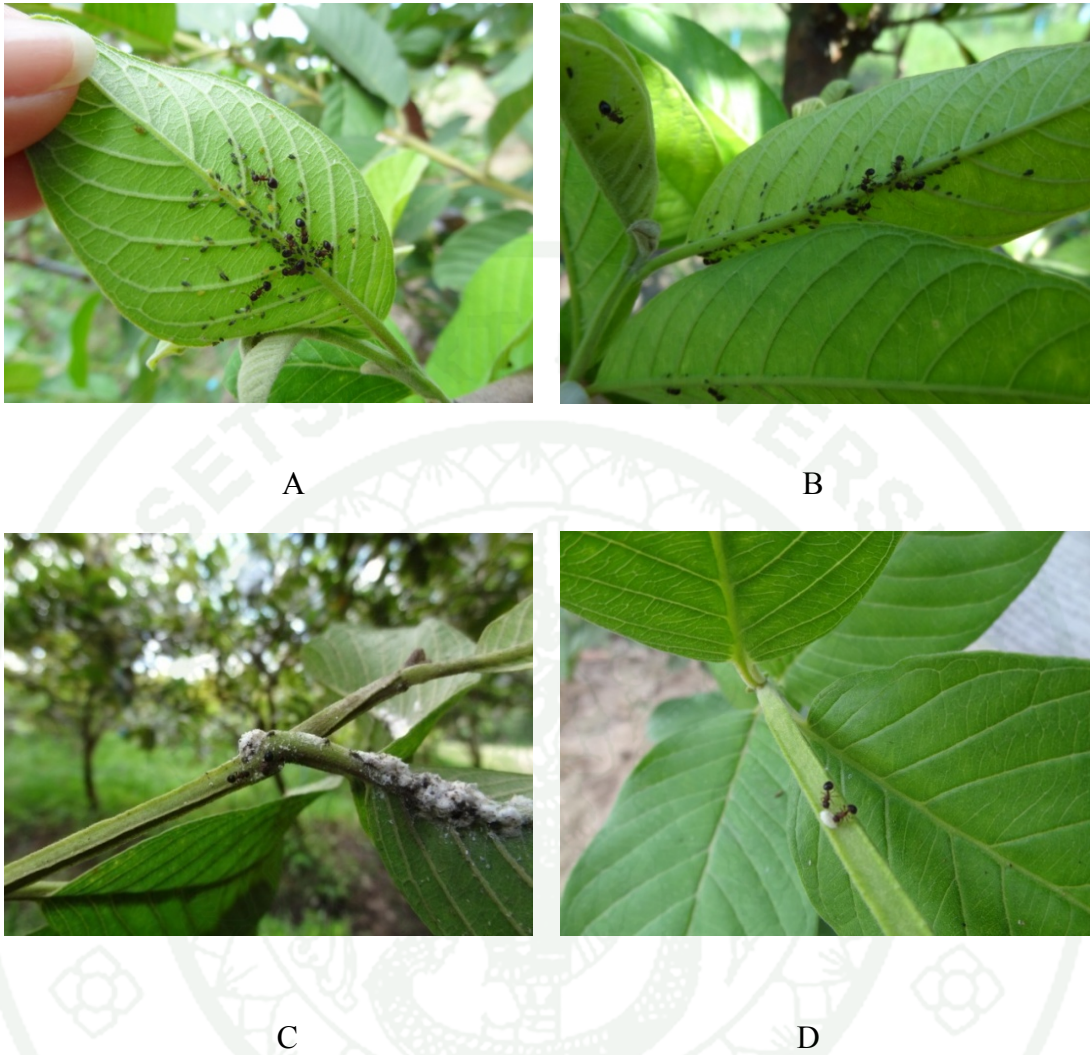
with protective covering or resembling coccids in appearance are able to gain access to the coccid colonies, and are not attacked by ants.

*Mutualism between M. bicolor and honeydew producing hemipterans*

Kumari and Gautam (2009) reported that *M. bicolor* together with other seven ant species were found on the green apple tree in Himachal Pradesh, India. *M. bicolor* were tending the green apple aphid, *Aphis pomi* De Geer. The other ant species were *Camponotus dichorous (basalis)* Forel, *Camponotus* nr. *compressus* (F.), *Camponotus* sp., *Lepisiota frauenfeldi* Mayr, *Pheidole indica* Mayr, *Polyrhachis lactipennis* Smith and *Tapinoma melanocephalum* (Fabricius). All ants stimulated green apple aphid to produce more honeydew. On the other hand green apple aphid were protected from natural predators by these ants.

Jesudasan *et al.* (2004) mentioned that *Meranoplus bicolor*, *Camponotus paria*, *C. rufoglaucus*, *C. taylori*, *Crematogaster* sp., *Crematogaster dalyi*, *Monomorium indicum*, *Myrmicaria brunnea*, *Oecophylla smaragdina*, *Paratrechina longicornis*, *Polyrhachis exercita*, *P. rastellata*, *P. scissa*, *Tapinoma melanocephalum*, *Tetraponera rufonigra* and *T.aitkenii* were attracted to the honeydew of the 2nd, 3rd and 4th nymphal instars of whitefly. They also reported that the number of ants tending whitefly was positively related to the colony of whitefly on that leaf. Ants took more time for antennal grooming on the whitefly compared with palpating the honeydew by its mandibles. Large ants attended more number of whitefly nymphs and spent longer time for feeding while smaller ones shared the exudation with its own members and also with other species. The ant attended whiteflies were attacked by parasite less than non-ant attended whiteflies.

At Kamphaeng Saen, Nakhon Pathom, *M. bicolor* were commonly associated with *A. gossypii* (Figure 3 A and B) and *F. virgata* (Figure 3 C and D) in guava plantation.



**Figure 3** *Meranoplus bicolor* associated with *Aphis gossypii* (A) and (B), and *Ferisia virgata* (C) and (D).

## Management of ant in Agricultural System

### *Insecticide spraying*

In earlier days, Hamilton and Seagrave-Smith (1959) report on ant control in Hawaii, U.S.A. Ants frequently become a pest on guava plants, not because of their direct effects, but because they are associated with the spread of aphids and scale insects. Ants were controlled by spraying with either chlordane or DDT on the trunk and main branches, as well as on the ground underneath and around the base of the guava tree.

In general, ant control in crop production and urban pest management has been done by spraying contact insecticides as barrier treatments (Klotz *et al.*, 2002). Highly toxic insecticides with long residue have been used as surface sprays to treat ant infestations (Stanley, 2004). However, spraying of chemicals kill or repel foraging workers, but have no effect on queens and their members in the colonies (Daane *et al.*, 2006). Chemical residues kill portion of ants foraging on the ground surface and the colony themselves may not be eliminated (Stanley, 2004). Williams and Vail (1993) showed that broadcast application of toxic bait is generally considered to be the most effective and efficient method to control multiple ant colonies over a large area. Foragers of ants collect and take baits back to their colonies. Insecticide-impregnated food materials eaten by foraging workers are transferred to other individual workers, the brood and the queen (Daane *et al.*, 2006). Ant toxic baits usually kill queen, brood and other workers which eliminates the entire colony (Williams and Vail, 1993). Nelson and Daane (2007) pointed out that grape growers in California are interested in alternative method to exclude the association between Argentine ant and mealybugs instead of using insecticidal sprays. The reasons were sprayings could contact only worker ants that were actively foraging but not the nest mate in the colony. They had to reapply the pesticides because their effectiveness declined over time, and finally, the insecticides killed beneficial insects and contaminated the environment.

### *Solid ant bait*

In agriculture, granular treatments for ant control are commercially available for use especially in plant nurseries, the granular baits or solid baits usually containing hydramethylnon. Solid ant bait, typically targeting protein-feeding ants, has been somewhat successful (Daane *et al.*, 2006). Hooper-Bui and Rust (2000) reported that 0.1% hydramethylnon at caused 100% mortality in workers at the end of 24 hours and at even much lower rates, 0.001 and 0.05% with continuous exposures for 2 weeks, the mortality of workers was 60 and 85%.

Despite from the above mentioned, hydramethylnon had positive result for solid bait. Ulloa-Chacón and Jaramillo (2003) used granular baits containing hydramethylnon against the ghost ant, *Tapinoma melanocephalum*. They found that hydramethylnon at 2.0% was ineffective in order to eradicate the nest mate of the ghost ant, workers, queens, and brood. The baits killed only 50% of the colonies at the end of the second week and did not reach 100% at the end of the experiment. Klotz *et al.* (1996) tested a solid formulation bait of 0.9% hydramethylnon in silkworm pupae. They found that no ghost ant colonies had been eliminated after 10 weeks of treatment. In addition, they observed that the use of hydramethylnon was ineffective against native ants from the subfamily Dolichoderinae. Hansen (2002) showed that the structure of granular or solid bait was not suitable to control carpenter ants, *Camponotus* spp. The foragers transported the bait but dropped it before reaching the nest.

### *Liquid ant bait*

Most ants in agricultural system prefer to forage for sugars (Klotz *et al.*, 2002; and Daane *et al.*, 2006). This preference has led to the development of liquid baits (Rust *et al.*, 2004; Daane *et al.*, 2006). Klotz *et al.* (2002) proposed the important components of ant toxic bait e.g. the attractant should be made from ant food or pheromone, the toxin in bait should be non-repellent to ant and delay in action for at

least allowing the forager to return to the nest. The toxin in the bait should be 10-fold of the lethal effective dose because the toxin would have to spread and dilute between colony members. Ant baits should include some additives of formulation such as emulsifiers, dyes, preservatives, etc. Toxic baits are better than chemical sprays in many ways. They are easy to apply with no influence from soil types on baits' efficacies. Only one or two treatments are usually sufficient for long-term control. The toxicant of bait is spread to all members of the colony, therefore baits could target on certain species. The liquid ant bait requires a very small amount of toxicant compared with insecticidal spray which reducing contamination to the environment (Collins and Callcott 1998).

Chemicals that have been certified as safe and widely used in commercial baits to eliminate ants are boric acid, fipronil, indoxacarb, abamectin, and insect growth regulators. Liquid ant baits containing boric acid mixed with sugar and water generally exploit the natural feeding habit of ants that collect honeydew or nectar (Stanley, 2004). Boric acid was first registered in the United States as an insecticide in 1948 for control of cockroaches, termites, fire ants, fleas, silverfish, and many other insects (United State Environmental Protection Agency [U.S. EPA], 1993). Boric acid acts as a stomach poison affecting the insects' metabolism, and the dry powder is abrasive to the insects' exoskeleton. The sugar solution of boric acid ant bait can also disrupt water regulation causing ants to ingest more of the bait to counterbalance dehydration (Stanley, 2004). In comparison to spray insecticides, liquid ant bait do not come in to contact with crop plants. They result in less exposure to non-target organisms, and less input to the environment (Stanley, 2004).

Boric acid ant bait is generally considered to be safe to use in household kitchens in order to control cockroaches and ants. Boric acid in liquid baits usually used to eradicate ants around the house and in industrial buildings. (Klotz *et al.*, 1998). Stanley (2004) reported that 5.3% boric acid, and 4.3% sodium borate was effective in eliminating *Monomorium pharaonis* colonies in buildings. At low concentrations of 1% boric acid in 10% sugar solution is effective in killing laboratory colonies of *M. pharaonis*, *Tapinoma melanocephalum*, and *Solenopsis*

*invicta* (Stanley, 2004). The high concentration of boric acid in liquid baits at 5.4% in Terro Ant Killer®; kills ants rapidly and prevents recruitment; repels some ant species (Stanley, 2004). In California, the wine growers used the liquid ant bait to control Argentine ants that associated with the grape mealybug, *Pseudococcus maritimus* in the vineyard (Daane *et al.*, 2006).

Liquid ant baits containing thiamethoxam, imidacloprid, and boric acid was used to reduce Argentine ant densities (Daane *et al.* 2006). The insecticidal ant baits are important tool for ant control in agriculture systems. They not only are effective in order to control ant but also are safe for people, non-target animals, and benefits insects (Klotz *et al.* 2002). Liquid ant bait has been using for alternative control methods in agricultural, urban, and natural habitats for Argentine ant control (Daane *et al.*, 2006).

#### *Sticky barrier*

Applying a sticky barrier or horticultural glue generally use to prevent the ants and some pests from climbing the tree in orchard or plantation. Almost all hemipterans, aphids, scale insects that cause sooty mold fungus may not develop into serious problems on orchard if the ants are effectively controlled (Hamilton and Seagrave-Smith, 1959). Daane *et al.* (2006) reported that using sticky barriers to remove Argentine ants on vine trunks reduced both mealybug densities and crop damages. In South African vineyards, using sticky barrier affected natural enemy densities (Nyamukondiwa and Addison, 2011). Conversely, sticky barriers are labor intensive to apply. They are not practical for use in young plant nurseries. They are not effective for controlling arboreal ants like *Crematogaster peringueyi* (Nyamukondiwa and Addison, 2011). Stewart-Jones *et al.*, (2008) reported that the excluding ants by sticky barriers is a laborious method that can only realistically be used in gardens or in very small orchards.

## MATERIALS AND METHODS

### Mutualism between *M. bicolor* and honeydew-producing hemipterans

#### *Study species*

Start-up colony of the shield ant, *M. bicolor*, were obtained from field collected queens, workers, and broods from a guava (*Psidium guajava*) plantation at Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand (14.0358 °N, 99.9826 °E). The colony of *M. bicolor* was reared in plastic boxes filled with soil, and was maintained in the Laboratory of Entomology Department, Kasetsart University, Kamphaeng Saen Campus. The colony and their brood were fed with dilute honey and nymph of hemipterans.

*A. gossypii*, the cotton aphid culture used in the experiment was collected from the guava plantation, Horticulture Department, Kasetsart University, Kamphaeng Saen, Nakhon Pathom. The aphid populations was maintained on young guavas (40 cm in height). The host plants and the cotton aphids in this experiment were kept in the nursery.

*F. virgata*, the striped mealybug were collected from the guava plantation of Horticulture Department, Kasetsart University, Kamphaeng Saen, Nakhon Pathom. The mealybugs were stocked on young guava trees (40 cm in height) like the cotton aphids. Their colonies were maintained on guava trees in the nursery.

The study was conducted during April–December 2012 in the unsprayed varietal collection plots (varieties: Phant Si Thong, Kim Ju, and Vhan Pi Roon), consisting of 336 guava trees, *Psidium guajava*, of Horticulture Department, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Thailand (14.0358 °N, 99.9826 °E). The predominant ground-nesting ant species in the study area was the native *M. bicolor*, with only a few colonies of the invasive ant species the tropical

fire-ant, *Solenopsis geminata* (Fabricius) near the perimeter of the plantation. The honeydew-producing hemipterans were *A. gossypii* and *F. virgata*. The natural enemies, mainly predators, were two species of coccinellid beetles, *Menochilus sexmaculatus* (Fabricius) and *Coccinella transversalis* Fabricius (Coleoptera: Coccinellidae), and one species of syrphid fly, *Pseudodorus clavatus* (Fabricius) (Diptera: Syrphidae).

#### *Ant benefit and honeydew preference*

The direct benefit of *M. bicolor* was obtained from weighing a certain number of foraging ants, and then calculating the difference of weight gain between foragers descending and ascending the guava branches. Weight gains of *M. bicolor* were measured after visiting hemipteran colonies as honeydew receiving. Foraging ants were randomly chosen from the field to weigh for honeydew loading. There were selected 50 ant foragers ascending the guava branch before reaching hemipteran colonies, and the other 50 individuals descending the branch with full load of honeydew. Honeydew loads were measured from the weight differences of ants filled with honeydew and ants ascending the guava branch. Individual worker of *M. bicolor* was captured in an empty hard gelatin capsule (size 0; outer diameter 7.65 mm, height 21.7 mm, and volume 0.68 ml of Torpac Inc., NJ), and shortly after, the capsule containing the arrested ant was weighed on a digital balance. The actual ant weight was obtained from the subtraction of the capsule weight. There were 2 sets ( $n = 200$ ) foraging ants visiting *A. gossypii*, and one set ( $n = 100$ ) of ants visiting *F. virgata*. The honeydew loads were confirmed with the laboratory set up by feeding of *M. bicolor* workers with honeydew. A set of field collected workers ( $n = 100$ ) leaving their nests for foraging were randomly chosen, holding in captivity for 24 hours without food, and subsequently captured inside the gelatin capsule for weighing. After weighing, half of the 24-hour arrested *M. bicolor* was offered with guava leaves occupied by honeydew exudates of *A. gossypii*, and the other half of ants with honeydew from *F. virgata*. All *M. bicolor* workers were weighed for the second time after they filled up their stomachs. This was indicated by they refused to feed on the

provided honeydew. Afterward, the ant workers were returned back to their former habitats.

The analysis of covariance (ANCOVA) of IBM SPSS Statistics (Verma, 2013; Meyer *et al.*, 2013) was used in order to answer the question on the difference of honeydew preference between *A. gossypii* honeydew compared with those of *F. virgata*. Body weight of *M. bicolor* workers with empty stomach (24-hour unfed workers) or weight before receiving honeydew was treated as covariate, and the criterion variable or dependent variable was ant weight after eating honeydew from each hemipteran species. This analysis of covariance approach was used in order to adjust the initial variations of *M. bicolor* worker size.

#### *Hemipteran benefits*

The honeydew-producing hemipteran benefits were demonstrated by interference of ants, predominantly *M. bicolor*, with sticky barrier applying around the base of the main brace in order to exclude the ant. The abundances of hemipterans and natural enemies were compared between presence and absence of *M. bicolor*. There should be more hemipterans and less natural enemies in the ant-attended guava branches.

Thirty guava trees, age 6 years old, approximately 1.65 m in height and 2.5–2.75 m in diameter from the pesticide-free guava plot as our study units. One of the two similar branches was chosen at random from each selected tree to perform ant-exclusion treatment, using sticky barrier around the base of the branch covering 20 cm in length. The target branch was first wrapped around with plastic wrap, and then applied with generic horticultural glue (colorless and odorless). The objective of the gluey barrier is to prevent ants and other crawling insects from reaching hemipteran colonies at the guava shoots, allowing only the entering of air-borne insects, including winged aphids, mealybug crawlers, ladybugs, and syrphid flies. The barriers were examined periodically, and reapplied the glue as required, in order to maintain the effectiveness as ant barriers throughout the experimental period. The other branch

was left unmanipulated as the ant–presence treatment. There was the total of 60 experimental units. This ant–exclusion/ presence experiment was started in May, beginning with equal numbers of both *A. gossypii* and *F. virgata* between the two treatments on the same guava tree. Insect observations were made during peak seasonal activities of both hemipterans and their natural enemies in June–August 2012.

On each chosen guava tree, we randomly selected one branch tip from the total of 3–5 tips of each experimental unit, in order to make observations. All shoots tips belonging to each experimental unit had an equal chance to be picked on each data collection day. The number of hemipterans: *A. gossypii*, *F. virgata*; and larvae of predators: *M. sexmaculatus*, *C. transversalis*, and *P. clavatus*, occupying the branch tips of 30 cm in length of both presence and absence of *M. bicolor* were counted at various intervals throughout the duration of the experiment from April–December 2012. We counted the insects at interval of 3–5 days, with the total of 7 times per month during June–August, coincided with the peak activities of both hemipterans and their predators, and every 15 days in other months. However, the observation data or multivariate responses of the five dependent variables were derived from the average of 7 times  $\times$  3 months = 21 field observations during peak activities of the insects in June–August 2012. We recorded the number of *M. bicolor* moving up or down (bidirectional) passed a fixed point on the treatment branch with no gluey barrier for 3–min period, to ascertain ant activity throughout the overall experimental period from April–December 2012. All observations in the field were done during 08:30–11:30 hours.

Most studies of ant-hemipteran interaction included either ant or hemipteran removals from study plants, and made comparisons with the unmanipulated partners. The conclusions, in general, relied on statistical analysis by the uses of univariate analysis of variance (ANOVA), which concentrated on one dependent variable, with attempts to make findings from multiple analysis of ANOVA (Flatt and Weisser, 2000; Billick *et al.*, 2007; Daane *et al.*, 2007; Mgocheki and Addison, 2009; Styrsky and Eubanks, 2010). Herein I used multivariate analysis of variance (MANOVA) of

IBM SPSS Statistics (Meyer *et al.*, 2013; Rencher and Christensen, 2012), in order to draw one solid conclusion of ant-hemipteran mutualism based on the comparison of five dependent variables from two groups, presence and absence of *M. bicolor* on guava branches. These five dependent variables or multivariate responses were number of insects: i.e. nymphs and adults of *A. gossypii*; nymphs and adults of *F. virgata*; larvae of *M. sexmaculatus*; larvae of *C. transversalis*; and larvae of *P. clavatus*. All insect counts were transformed into  $\log(y + 1)$  format; where  $y =$  number of insect, in order to agree with statistical assumptions.

Several outputs were requested from the MANOVA analysis of IBM SPSS. Box' s Test of Equality of Covariance Matrices expected to see if the dependent variable covariance matrices are equal across the levels of the presence-absence of *M. bicolor*. Bartlett' s Test of Sphericity was demanded to ascertain sufficient correlation between dependent measures in order to proceed with the analysis. The core MANOVA output was inquired for the multivariate null hypothesis evaluation of no differences between presence and absence of *M. bicolor* on the composite dependent (number of insects) variate. When the multivariate test is statistically significant, we can proceed with some assessments of each dependent variable. I performed not only Leven' s Test of Equality of Error Variances testing homogeneity of variance violations for each dependent measure, but also the Tests of Between-Subjects Effects to evaluate the statistical significance of each dependent variable separately. Bonferroni-corrected alpha level was applied to avoid alpha inflation in order to evaluate these presence and absence of *M. bicolor* effects. We divided .05 by the number of ANOVAs and obtained .05/5 or a Bonferroni-corrected alpha level of .01.

### **Effectiveness of liquid ant baits**

The experiment was done on 52 guava trees (*Psidium guajava*) variety Phant Si Thong of Horticulture Department, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Thailand (14.036056 °N, 99.982834 °E), covering 200 m<sup>2</sup> with 2 x 3 plant spacing. These guava trees were part of the unsprayed varietal collection

plots within the total of 336 trees in 0.32 hectares. These seven years old guava trees were pruning regularly to maintain the height at approximate 1.6 m with the diameter ranging from 2.5-2.75 m for convenience in order to tie up with shakes-supported system. . The observations of *M. bicolor* visitation rate at sugary liquid bait, and hemipteran densities, *A. gossypii* and *F. virgata* were made during in April 2012-March 2013.

The bait trial experiment was done during April 2012-March 2013. The bait test was performed on randomly selected 44 out of 52 guava trees as our study units. The liquid bait toxicants and application rate used were boric acid powder 0.3% (RECL Limited [RANKEM], New Delhi, India; 99.7% [A.I.]; analytical reagent), imidacloprid 0.0001% (Bayer Crop Science, Samut Prakan, Thailand; Provado®) and fipronil [ 0.0001% (BASF Thailand, Samut Prakan, Thailand; Ascend®). The selection of toxicant was judged from common use with liquid bait, availability in local market, and solubility in sugar water. The concentration of 3% boric acid was median value of concentrations that have been used in ant baiting from 0.5–5.6%, and .0001% of either imidacloprid or fipronil was derived from application rates that have been tested with liquid ant baits elsewhere (Klotz *et al.*, 2003; Daane *et al.*, 2006, 2008; Cooper *et al.*, 2008; Nyamukondiwa and Addison, 2011). All toxicants were dissolved in 25% sugar syrup, filled in an empty 600-ml plastic drinking water, and distributed in bait stations. The bait stations or dispensers, in general, were similar to those of UC-designed PVC station (Cooper *et al.*, 2008). We used part of PVC pipe 22 cm (80 mm diameter, PVC Class 5) to make the bait dispenser. The 22-cm section was capped on both openings with PVC end caps (PVC Class 13.5), the bottom end cap was sealed with pipe glue in order to prevent leakage of liquid bait. Eight holes (3 mm diameter) were drilled around the 22-cm pipe in eight directions at the distance of 4.5 cm from both openings, with the total of sixteen holes. These holes provided ant entries and air ventilation including outlets of the smell of sugary liquid bait for attraction of foraging ants. In sequence, an empty 600 ml drinking water with 500 ml liquid bait, drilled a hole (1 mm diameter) in the middle of the cap, capped the bottle, and then put it upside down inside the dispenser. The liquid was slowly coming out of the bottle and maintaining at the level of approximate 3 cm inside the dispenser for

foraging ants to collect. The cylindrical dispensers were placed on the ground and fastened with the main stem of the guava plants. I used 44 bait dispensers in this experiment. There were four treatments: three treatments belonged to three types of toxicants i.e. boric acid, imidacloprid, and fipronil, dissolving in 25% sugary liquid; including one treatment with only 25% sugary liquid as check or control. Each treatment was replicated eleven times. The 44 dispensers were cleaned and refreshed at 2-week time in order to remove ant remains and dirt materials, as well as preserving the quantity and quality of the liquid baits.

One branch terminal was selected from guava tree with bait dispenser attached, in order to make observations. I counted nymphs and adults of hemipterans: *A. gossypii*, *F. virgata*; and larvae of predators: *M. sexmaculatus*, *C. transversalis*, and *P. clavatus*, occupying the branch terminal of 30 cm in length at various intervals throughout the duration of the experiment from April 2012-March 2013. In addition, the number of *M. bicolor* entered the bait dispensers for 5-min period, and *M. bicolor* associated with *A. gossypii* and *F. virgata* were recorded as ant visitation. I observed the insects at interval of 3-5 days, with the total of 8 times per month during June-August, coincided with the peak activities of both hemipterans and their predators, and every 15 days in other months. Nevertheless, the observation data or multivariate responses of the eight dependent variables mentioned earlier were derived exclusively from the average of 24 field observations (8 time x 3 months) during peak seasonal activities of the insects in June-August. All observations in the field were done during 8:30-11.30 hours.

A one-way between-subjects multivariate analysis of variance (MANOVA) was used to compare eight dependent variables or mean vectors from four groups or treatments, i.e. 25% sugary liquid + 3% boric acid; 25% sugary liquid + 0.0001% imidacloprid; 25% sugary liquid + 0.0001% fipronil; and only 25% sugary liquid, by using IBM SPSS Statistics (Meyer *et al.*, 2013; Rencher and Christensen, 2012). The observed multivariate responses of this 4-group MANOVA were no. of *A. gossypii*; no. of *F. virgata*; no. of *M. sexmaculatus* larvae preying on *A. gossypii*; no. of *M. sexmaculatus* larvae preying on *F. virgata*; no. of *C. transversalis* larvae preying

on *A. gossypii*; no. of *C. transversalis* larvae preying on *F. virgata*; no. of *P. clavatus* larvae preying on *A. gossypii*; and visitation rates of *M. bicolor* at bait dispensers or the number of ant entering the bait dispenser in 5 min. There were no *P. clavatus* found on *F. virgata* colonies, and thus resulting in eight instead of nine dependent measures. All insect counts were transformed into  $\log(y + 1)$  format; where  $y$  = number of insect, in order to agree with statistical assumptions. Additional one-way between-subjects MANOVA was done on the three dependent measures concerning number of *M. bicolor*, i.e. ant foragers entered bait dispenser, and visual counts of ants associated with either of the two hemipterans.

Various outputs were requested from the IBM SPSS of IBM SPSS similar to the previous session. The Post Hoc Paired Comparisons were performed to determine particularly which treatment groups differed significantly on each dependent measure. The Ryan, Einot, Gabriel, and Welsch Studentized Range test was used as recommended by Gamst *et al.* (2008) for these pairwise comparisons. Bonferroni-corrected alpha levels were applied to avoid alpha inflation in order to evaluate these liquid bait effects. The alpha level of 0.5 was divided by the number of ANOVAs or number of dependent measures involved and obtained .05/8, and .05/3 or Bonferroni-corrected alpha levels of .006, and .016, respectively. The Tamhane's T2 post hoc test from the IBM SPSS was requested in order to anticipate homogeneity of variance violation of some dependent variables.

## RESULTS AND DISCUSSIONS

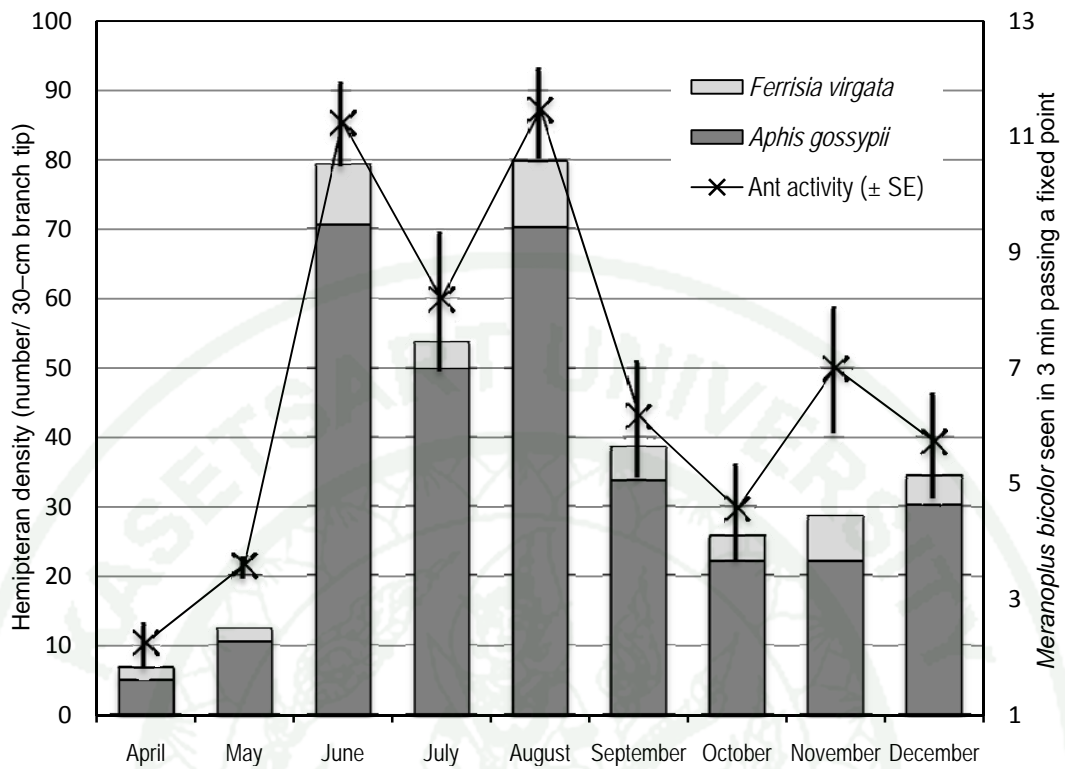
### Mutualism between *M. bicolor* and hemipterans

*M. bicolor* generally foraged on honeydew of hemipterans as carbohydrate source throughout the year in guava plantation at Kamphaeng Sean. Monthly averages ( $\pm SE$ ) of *M. bicolor* activity from April–December 2012 were presented in Figure 4. *M. bicolor* activity coincided with population fluctuations of both hemipterans (*A. gossypii*, and *F. virgata*), with peaks seasonal activities in June–August (Figure 4). There were very high correlation coefficient ( $r^2$ s) between ant activity and either *A. gossypii* or *F. virgata* density at  $r = .97$  ( $p$ -value  $< .0005$ ;  $n = 9$ ).

*M. bicolor* dominated the other ground-nesting ant species, *Selenopsis geminata*, in the studied guava plot, although *S. geminata* has been considered as one of the most invasive ant species worldwide (Wetterer, 2011), but not in this guava ecosystem with history of pesticide application. There were no *S. geminata* workers observed on the experimental guava trees. The tolerance to pesticides of *M. bicolor* was probably due to the protection of long fine hair covering the entire body Schödlh (1998), together with the defensive behavior of *Meranoplus* by curling up the body and feigned dead when disturbed (Hölldobler, 1988).

### Ant benefit and honeydew preference

In the studied guava plantation, foragers of *M. bicolor* leaving their nests weighed approximately 2.48 mg ( $SE = .08$ ;  $n = 150$ ). After visiting hemipteran colonies, *M. bicolor* with honeydew loaded, descending the branch back to their nests weighed on average 8.69 mg ( $SE = 0.1$ ;  $n = 150$ ). The honeydew loading is about 6.21 mg ( $8.69 - 2.48$ ) or roughly estimate around 2.5–fold ( $6.21 \div 2.48$ ) of the mean forager weight departure from their nests. The weighting capacity of *M. bicolor* workers was reconfirmed in a confined study of laboratory feeding of ant workers to different kinds of honeydew from both hemipteran species.



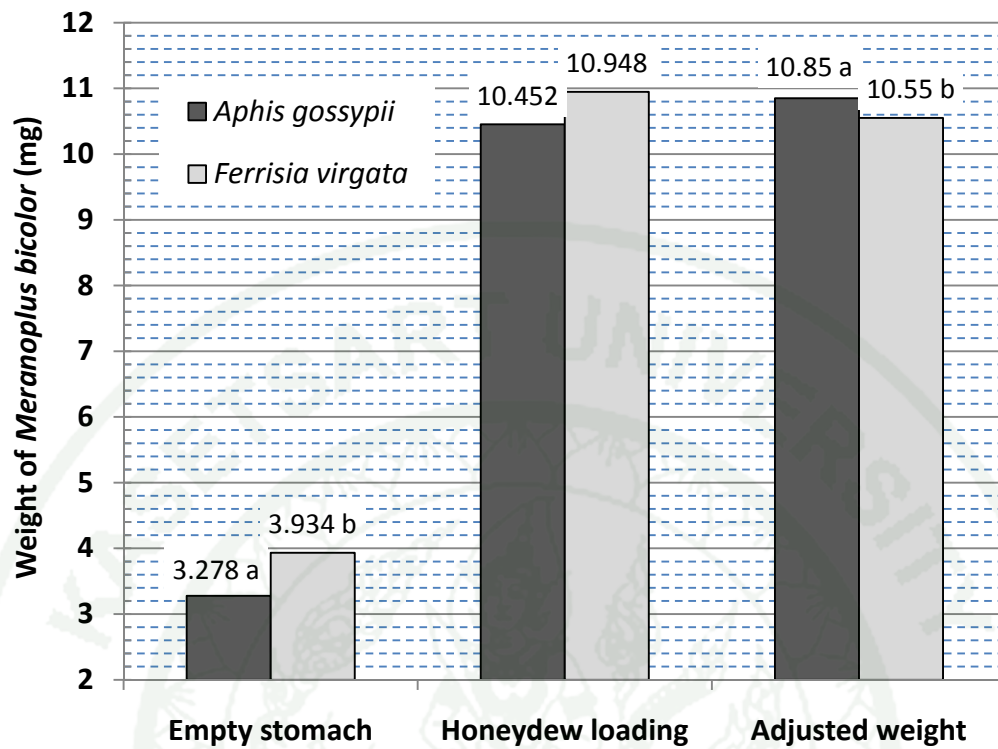
**Figure 4** Monthly average of *Meranoplus bicolor* activity ( $\pm$  SE, vertical line), and hemipteran density (*Aphis gossypii* and *Ferrisia virgata*) from 30 ant-tended guava branches at Kamphaeng Saen, Nakhon Pathom, Thailand in year 2012.

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After 24 hours in captivity, *M. bicolor* workers weighed 3.61 mg (SE = 0.12; n = 100) on average. Larger workers seemed to be more tolerance and easier for seizing were selected in order to withstand the 24-hour starvation before obtaining honeydew. These workers were fully fed with honeydew from different hemipteran species, and later weighed approximately 10.70 mg (SE = 0.15; n = 100). The overall expected value of honeydew loading is 7.09 mg (10.70 – 3.61), with an estimate of 2.96-fold (7.09 – 3.61) of the average worker weight after 24 hour in caging. The former 2.5-fold honeydew loading from field foragers was slightly lesser; this was probably due to the offering of honeydew by trophallaxis among workers before returning to their nests (Pfeiffer and Linsenmair, 2007).

A one-way between-subject ANCOVA assessing the difference of honeydew loadings from two hemipteran species of *M. bicolor* workers showed that the covariate effect or weight of 24-hour captured *M. bicolor* before honeydew feeding was statistically significant,  $F(1, 97) = 786.297$ ,  $p\text{-value} < .001$ . Moreover, a statistically significant effect of honeydew source, from either *A. gossypii* or *F. virgata* honeydew, was obtained,  $F(1, 97) = 8.387$ ,  $p\text{-value} = .005$ .

Mean weight of ants before eating honeydew of *F. virgata* group was higher than that of *A. gossypii* group, leading to higher full up honeydew loading from *F. virgata* compared with that from *A. gossypii* (Figure 5). However, the use of ANCOVA approach removed the covariate effect and unveiled the reversal outcome. Mean weight of *M. bicolor* plus honeydew from *A. gossypii* was significant higher when corrected for weight prior to receiving honeydew (adjusted mean = 10.85; SE = .072; 95% CI = 10.707–10.992) than mean weight of worker ant with honeydew loaded from *F. virgata* (adjusted mean = 10.55; SE = .072; 95% CI = 10.408–10.693) Figure 6. This could indicate that *M. bicolor* workers prefer *A. gossypii* honeydew to that from *F. virgata*.



**Figure 5** Mean weight of *Meranoplus bicolor* workers (mg) with empty stomach (24-hour without food), mean weight with honeydew loading, and adjusted mean weight from different hemipteran species, *Aphis gossypii* and *Ferrisia virgata*. Value on top of column chart indicates data label; different letters followed mean values represent statistically significant differences ( $p$ -values  $\leq .005$ ).

Ants are expected to concentrate their honeydew collection activities on hemipteran species offering higher reward in terms of both quantitative and qualitative effects. Hemipteran species that produce larger amount of honeydew, or having honeydew with the presence of preferred sugars or amino acids should be more attractive to certain ant species (Cushman, 1991; Völkl *et al.*, 1999; Yao, 2014). Ant preference for particular sugars in hemipteran honeydew can be species specific (Blüthgen and Fiedler, 2004). Several ant species react strongly to honeydew that holds large amounts of melezitose (Völkl *et al.*, 1999), while others prefer sucrose to melezitose (Blüthgen and Fiedler, 2004). On the other hand, *A. gossypii* honeydew consisted of mainly sucrose, fructose, and erlose (Lawo *et al.*, 2009), with no appearance of melezitose. Honeydew composition of *F. virgata* is unknown; however, some studies of mealy bugs' honeydew show composition of fructose, glucose, sucrose, and small amounts of melezitose and raffinose, together with a variety of amino acids (Gray, 1952; Salama and Rizk, 1969).

Another difference in honeydew quality beside the composition of sugars and amino acids is a physical property specifically honeydew viscosity. In this study, honeydew excreted by *F. virgata* was more viscous than that by *A. gossypii*. A study in Argentine ant showed that workers fed eightfold longer on gel sucrose composition, and removed fivefold less sucrose than workers feeding in liquid sucrose (Silverman and Roulston, 2001). The later would agree with lesser amounts of honeydew loading of *M. bicolor* from *F. virgata* than that from *A. gossypii* in this study.

#### *Hemipteran benefits*

A two-group between-subject MANOVA was done on logarithmic transformed data [ $\log(y + 1)$ ;  $y$  = observation data] of five dependent variables: no. of *A. gossypii*; no. of *F. virgata*; no. of *M. sexmaculatus* larvae; no. of *C. transversalis* larvae; and no. of *P. clavatus* larvae. The independent variable or treatment was the presence-absence of ant, particularly *M. bicolor*, in guava plantation. There were two treatments, i.e. ant-tended and ant-excluded. In general, the ant-excluded treatment

with sticky barrier was quite effective against *M. bicolor*, the slow-moving ant species. Even though some ants could accidentally reach the colonies of hemipterans on the exclusion treatment from adjacent branches due to the contact with nearby branches via wind blowing, however, these ants could not return back to their nests or could not be able to recruit additional ant foragers.

The sample consisted of 60 guava branches divided into equal amounts of presence and absence of *M. bicolor*. The output of Box's Test of Equality of Covariance Matrices was statistically significant (Box's  $M = 69.998$ ;  $p$ -value  $< .001$ ), showing that the dependent variable covariance matrices were not equal across the levels of the presence-absence of *M. bicolor*. Therefore, Pillai's trace was used to evaluate all multivariate effects (Meyers *et al.*, 2013). Bartlett's Test of Sphericity was statistically significant (approximate chi-square = 99.838;  $p$ -value  $< .001$ ), indicating sufficient correlation between the dependent variables to proceed with the MANOVA. Using Pillai's trace as the criteria, the combined dependent variable was significantly affected by the presence-absence of *M. bicolor*, Pillai's trace = .807,  $F(5, 54) = 45.244$ ,  $p$ -value  $< .001$ . There were reliable multivariate differences between ant-tended and ant-excluded treatments on the combined dependent variate. The partial eta squared = .807 (partial  $\eta^2$ ), equivalent to the full eta squared ( $\eta^2$ ) in this two-group design (Levine and Hullett, 2002), indicating that we had a very high proportion of the total variance (.807, or about 81%) explained by the activity of *M. bicolor*.

Each dependent measure or each observed insect density was assessed individually in order to determine the strength of the statistically significant multivariate effect. The result of the tests of the univariate effects is shown in Table 1. We had statistically significance univariate effects on all dependent variables (Table 1;  $p$ -values  $< .001$ ). Of all insect species under investigation, *A. gossypii* provided the highest effect size ( $\eta^2 = .62$ ), while *M. sexmaculatus* had highest effect size in terms of natural enemies ( $\eta^2 = .55$ ) (Table 1).

**Table 1** Tests of univariate effects of *Meranoplus bicolor* activity on five dependent variables<sup>a</sup>: *Aphis gossypii*; *Ferrisia virgata*; *Menochilus sexmaculatus*; *Coccinella transversalis*; and *Pseudodorus clavatus*. Data are visual counts of insects occupying 30–cm length of branch terminal ( $n = 60$ )

Source	Dependent var.	Type III SS	df	MS	F	p-value	Eta squared ( $\eta^2$ )
Ant	<i>A. gossypii</i>	.466	1	.466	96.188	.000 <sup>b</sup>	.624
	<i>F. virgata</i>	1.681	1	1.681	61.859	.000	.516
	<i>M. sexmaculatus</i>	1.744	1	1.744	72.151	.000	.554
	<i>C. transversalis</i>	.977	1	.977	27.876	.000	.325
	<i>P. clavatus</i>	.925	1	.925	37.525	.000	.393
Error	<i>A. gossypii</i>	.281	58	.005			
	<i>F. virgata</i>	1.576	58	.027			
	<i>M. sexmaculatus</i>	1.402	58	.024			
	<i>C. transversalis</i>	2.034	58	.035			
	<i>P. clavatus</i>	1.430	58	.025			
Corrected total	<i>A. gossypii</i>	.746	59				
	<i>F. virgata</i>	3.257	59				
	<i>M. sexmaculatus</i>	3.146	59				
	<i>C. transversalis</i>	3.011	59				
	<i>P. clavatus</i>	2.355	59				

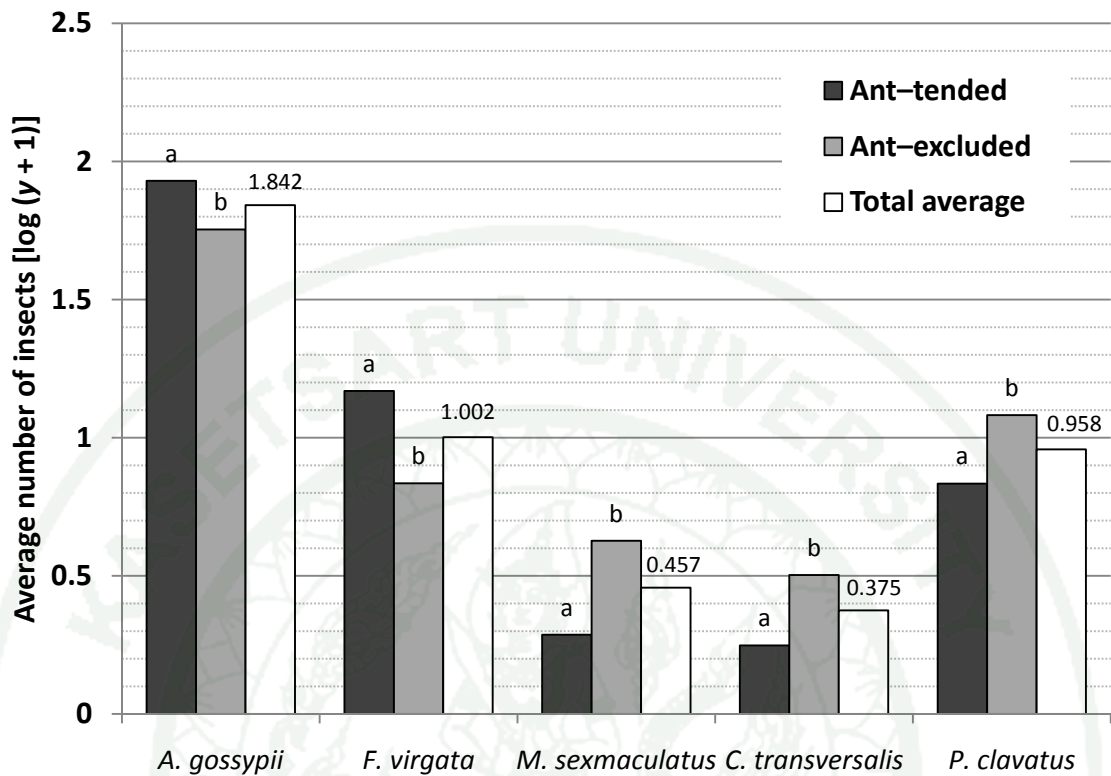
<sup>a</sup> The multivariate test of combined dependent measure was statistically significant; Pillai's trace = .807,  $F(5, 54) = 45.244$ ,  $p$ -value < .001.

<sup>b</sup> .000 indicates < .001.

In general, *M. bicolor* preferred to associated with *A. gossypii* more than *F. virgata*, in this meaning the preference of honeydew collecting, as indicated by higher value of the strength of effect or effect size (Levine & Hullett, 2002; Meyers *et al.*, 2013), i.e.  $\eta^2 = .62$  and  $\eta^2 = .52$ , respectively (Table 1). This could be the second evidence in supporting the previous study of honeydew preference of 24-hour captured *M. bicolor*. Among the three natural enemies or predators, *M. sexmaculatus* had more strength of effect ( $\eta^2 = .55$ ), i.e. would be more effective predator, than the other two competitors ( $\eta^2$ s = .39, and .32) in this *M. bicolor*–hemipteran association (Table 1). Even though the surphid fly, *P. clavatus*, was more abundant than the other two coccinellid predators, but its appearance in the guava plot was restrict to June till August. In addition, there were no *P. clavatus* larvae found preying on the striped mealybug, *F. virgata*, in this study.

The graphic information for the univariate analysis is presented in Figure 6; providing each dependent measure's observed means, and total averages obtaining from 30 guava trees in the study. The presence of *M. bicolor*–tended hemipterans had a considerable impact on insect populations not only hemipteran themselves, but also their natural enemies. On ant–tended treatment, there were higher densities of both hemipteran species, together with lesser amounts of all natural enemies compared with the ant–excluded treatment (Figure 6). There was more abundant in density of roughly 7.6–fold [ $(\text{antilog}(1.824) - 1) \div (\text{antilog}(1.002) - 1)$  or  $68.50 \div 9.05 = 7.57$ ] of *A. gossypii* than *F. virgata* from 30 guava trees in the study (Figure 6).

The mutualistic relationships or trophobiotic interactions between *A. gossypii* and *F. virgata* with ants have been classified as facultative and very common phenomenon by Delabie (2001). There are two main reasons from this study in supporting the above mentioned. Firstly, both hemipteran species are polyphagous and cosmopolitan species (Blackman and Eastop, 2000; da Silva-Torres *et al.*, 2013).



**Figure 6** Effect of *Meranoplus bicolor*-exclusion on hemipterans (*Aphis gossypii* and *Ferrisia virgata*) and their natural enemies (*Menochilus sexmaculatus*, *Coccinella transversalis* and *Pseudodorus clavatus*). Different letters on top of dark columns represent statistically significant differences ( $p$ -values  $< .001$ ); numbers on top of clear columns indicate data labels of total average or grand mean from 30 guava trees.

Any mutualistic relationship with ants should be opportunistic or facultative rather than obligatory. Secondly, *M. bicolor* is the most common species of the genus *Meranoplus* in the Oriental Region (Schödlh, 1998), and is widely distributed as ground nesting species in disturbed habitats of agricultural ecosystem, therefore the acquiring for food in the vicinity should be by selection of most abundant resources.

This study showed that ant attending had a considerable effect on hemipterous pest densities in guava plantation. There were more individuals of hemipterans in the ant tending guava branches, together with lesser amounts of natural enemies mainly predators because of ant guarding activities. In return the benefit, ants received carbohydrate sources in terms of honeydew from both hemipterans. In general, the results of this ant exclusion experiment using gluey barrier are agreed with previous studies done in fruit orchards; e.g. cherry (Stutz and Schmidt-Entling, 2011), apple (Stewart-Jones *et al.*, 2008; Miñarro *et al.*, 2010; Nagy *et al.*, 2013); and in vineyards (Mgocheki and Addison, 2009).

#### **Effectiveness of liquid ant baits**

There were more than 70 well established medium to large-sized ground nesting colonies (200-350 individuals) of *M. bicolor* existed in the guava plot under study. Only ant colonies near the experimental guava trees were measured about distances from guava trees. The average distance of nearest ant colonies to guava trees was 30.33 cm ( $SE = 0.97$ ;  $n = 44$ ), ranging from 15-48 cm.

The effectiveness of three insecticidal materials dissolved in 25% sugar solution was assessed in the field using a multivariate analysis of variance (MANOVA) procedure. A four-group between-subjects MANOVA was performed on logarithmic transformed data [ $\log(y + 1)$ ;  $y =$  observation data] of eight dependent variables. Box's Test of Equality of Covariance Matrices was statistically significant (Box's  $M = 228.01$ ;  $p$ -value = .019), indicating unequal variance-covariance matrices across levels of sugary liquid ant bait and thus required the use of Pillai's trace in assessing the multivariate effect. Bartlett's Test of Sphericity was statistically

significant (approximate chi-square = 193.19;  $p$ -value < .001), providing sufficient correlation between the dependent variables to proceed with further analysis.

Using Pillai's trace as the judgment, the composite dependent variate, i.e. the number of *M. bicolor* seen in 5 min at bait dispensers, and seven sets of numbers of insects observed on 30-cm of terminal guava branch, was significantly affected by liquid ant bait attached to the basal of the main stem, Pillai's trace = 1.876,  $F(24, 105) = 7.302$ ,  $p$ -value < .001. There were dependable multivariate differences among liquid bait toxicants and control treatment on the weighted composite dependent variates. The amount of explained variance (eta square or  $\eta^2$ ) obtaining by subtracting the value of Wilk's lambda (.004) from 1.00 was .996 or 99.6%, indicating that there was a very high proportion of total variance explained by the treatment effects. In this study, two of the eight dependent variables (no. of *C. transversalis* on either *A. gossypii* or *F. virgata*) had statistically significant Levene's test, indicating heterogeneity of variances among treatments on the two dependent measures. Thus, in evaluating the effect of *C. transversalis* populations, there was an increase in the stringency of the alpha level to .001 in order to assess statistical significance. Those dependent variables not violating the homogeneity of variance assumption were assessed against a Bonferroni-adjusted alpha level of .006 (.05  $\div$  8).

Tests of Between-Subjects Effects, equivalent to univariate ANOVAs, were done on each dependent variable separately in order to determine the strength of the statistically significant multivariate effect. Liquid ant bait attached to the basal of guava tree significantly affected all dependent measures, considering of both stringent alpha level of .001 and Bonferroni-adjusted alpha level of .006, with all  $p$ -values < .001 (Table 2).

**Table 2** Tests of univariate effects of liquid ant bait on eight dependent variables<sup>a</sup>.

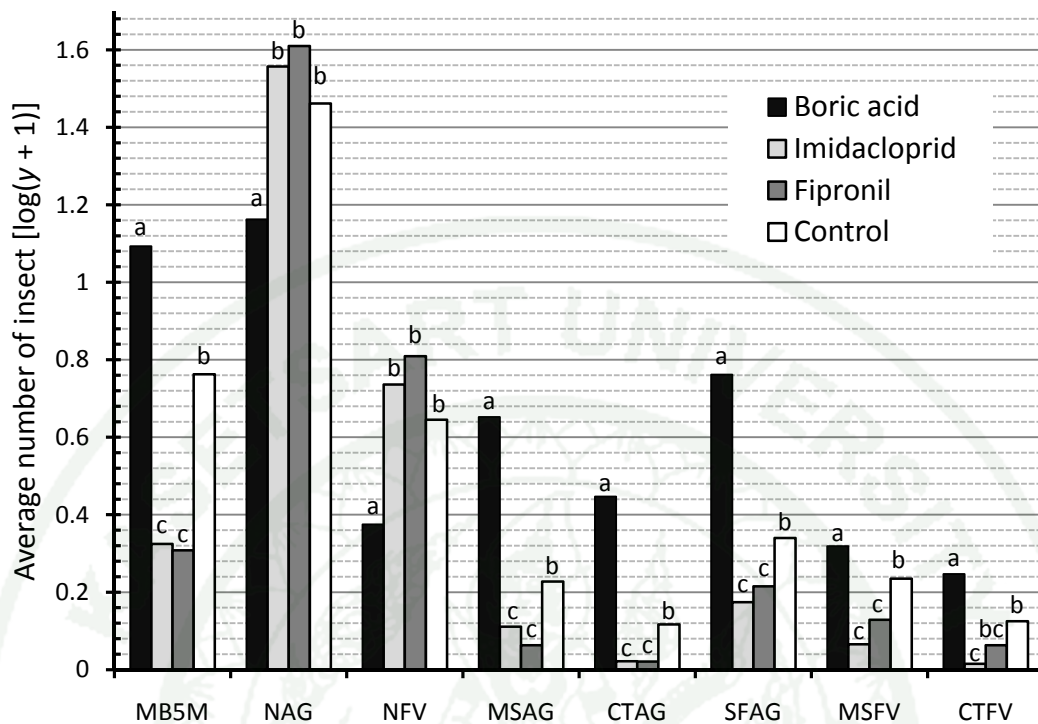
Source	Dependent var. <sup>a</sup>	Type III SS	df	MS	<i>F</i>	<i>p</i> -value	$\eta^2$
Liquid ant bait	MB5M	4.712	3	1.571	273.69	.000 <sup>b</sup>	.954
	NAG	1.318	3	.439	11.67	.000	.467
	NFV	1.191	3	.397	11.23	.000	.457
	MSAG	2.375	3	.792	103.44	.000	.886
	CTAG	1.342	3	.447	95.02	.000	.877
	SFAG	2.383	3	.794	138.23	.000	.912
	MSFV	.415	3	.138	27.134	.000	.671
	CTFV	.332	3	.111	30.49	.000	.696
Error	MB5M	.230	40	.006			
	NAG	1.506	40	.038			
	NFV	1.414	40	.035			
	MSAG	.306	40	.008			
	CTAG	.188	40	.005			
	SFAG	.230	40	.006			
	MSFV	.204	40	.005			
	CTFV	.145	40	.004			
Corrected total	MB5M	4.942	43				
	NAG	2.824	43				
	NFV	2.604	43				
	MSAG	2.681	43				
	CTAG	1.531	43				
	SFAG	2.613	43				
	MSFV	.619	43				
	CTFV	.478	43				

<sup>a</sup> The multivariate test of combined dependent measure was statistically significant; Pillai's trace = 1.876,  $F(24, 105) = 7.302$ ,  $p$ -value < .001., Visitation rate: ( $n = 44$ )

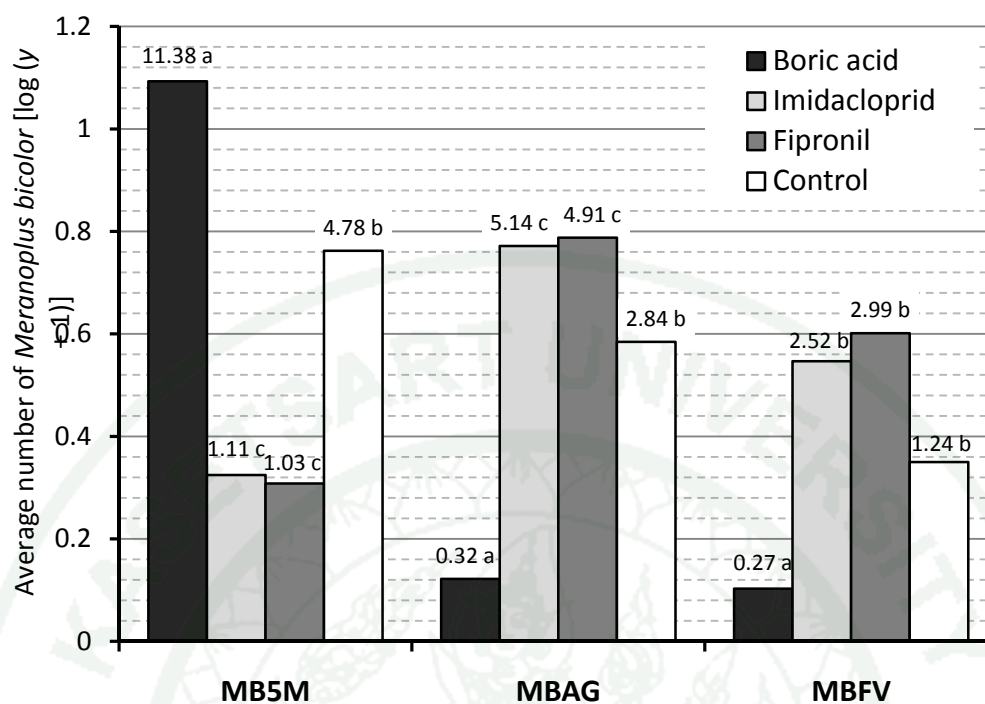
<sup>b</sup> .000 indicates < .001.

The Ryan, Einot, Gabriel, and Welsch Studentized Range (REGWQ) tests were used for dependent variables showed homogeneity of variance, and the Tamhane post hoc tests were applied for those measures with heterogeneity of variance. Liquid bait dispenser with boric acid had statistically significant difference concerning efficiency by having greater ant visitation rate (more attractive to ant foragers), higher pressures of predators preying on hemipterans (less protective from ants because most ant foragers were deviated to bait dispenser), and as a result lower hemipteran densities on guava branch compared with other treatment effects (Figure 7). On average, there were many *M. bicolor* entered bait dispenser with boric acid of 2.4–fold  $[(\text{antilog}(1.093)) - 1] \div [\text{antilog}(.762) - 1]$  or  $11.38 \div 4.78 = 2.38$ , 10.2–fold, and 11–fold compared with bait dispensers of control, imidacloprid, and fipronil, respectively (Figures 7 and 8: MB5M). This visitation rate of *M. bicolor* at bait dispenser contributed the highest value of the strength of effect or effect size (Levine and Hullett, 2002; Meyers *et al.*, 2013), i.e.  $\eta^2 = .954$  (Table 2). There were significantly fewer ants associated with colonies of either *A. gossypii* or *F. virgata* on terminal guava branch in treatment with boric acid bait compared with others (Figure 8: MBAG and MBFV).

There were no statistically significant differences between imidacloprid and fipronil on all dependent measures (Figures 7 and 8). The two toxicants imidacloprid and fipronil exhibited relatively low effectiveness comparing with control treatment in many assessment categories. These were significantly lower ant visitation rates at bait dispensers (Figures 7 and 8: MB5M), and higher ant foragers associated with *A. gossypii* colonies (Figure 8: MBAG), but there was no treatment effect in number of ant–tended *F. virgata* on guava branches (Figure 8: MBFV). In terms of observed natural enemies, imidacloprid and fipronil treatments exhibited lesser amounts of natural enemies probably due to frequent ant protection (Figure 7: MSAG, CTAG, SFAG, and MSFV) compared with untreated control. Moreover, there were no statistically differences not only among fipronil, imidacloprid, and control treatments on both hemipteran densities (Figure 7: NAG and NFV), but also between fipronil and control on CTFV as well (Figure 7).



**Figure 7** Comparison of sugary liquid ant baits with different toxicants and untreated control in guava plantation at Kamphaeng Saen. MB5M – *Meranoplus bicolor* entered bait dispenser in 5 min; NAG – no. of *Aphis gossypii*; NFV – no. of *Ferrisia virgata*; MSAG – no. of *Menochilus sexmaculatus* on *A. gossypii*; CTAG – no. of *Coccinella transversalis* on *A. gossypii*; SFAG – no. of *Pseudodorus clavatus* on *A. gossypii*; MSFV – *M. sexmaculatus* on *F. virgata*; and CTFV – no. of *C. transversalis* on *F. virgata*. Observed means sharing the same letter represent statistically non-significant difference (REGWQ test for dependent measures with homogeneity of variance; Tamhane post hoc test for dependent variables violated homogeneity of variance assumption; alpha level = .05).



**Figure 8** Comparison of liquid bait toxicants on *Meranoplus bicolor* activities: MB5M – *M. bicolor* entered bait dispenser in 5 min; MBAG – *M. bicolor* associated with *Aphis gossypii* on terminal branch; and MBFV – *M. bicolor* associated with *Ferrisia virgata* on terminal branch. Observed means on top of columns followed by the same letter represent statistically non-significant difference (REGWQ test for MB5M and MBAG; Tamhane post hoc test for MBFV; alpha level = .05). MANOVA of composite dependent measure (MA5M, MBAG, and MBFV) was statistically significant; Pillai's trace = 1.11,  $F(9, 120) = 7.833$ ,  $p$ -value < .001.

Many ant species including *M. bicolor* primarily forage on honeydew of hemipterans as carbohydrate source like Argentine ants (Rust *et al.*, 2000; Klotz *et al.*, 2002), and therefore are suitable for the application of liquid baits (Silverman and Roulston, 2001; Rust *et al.*, 2004). This specialization in liquid consumption is indicated by the adaptation of worker crops that allow eating large amount of honeydew equivalent to the body weight of ant foragers (Markin, 1970), or up to 2.5–fold of forager weight as appeared in *M. bicolor* (Burikam and Kantha, 2014). The preference in liquid consumption of *M. bicolor* was mentioned in earlier study (Burikam and Kantha, 2014). The shield ant frequently collected more honeydew from the cotton aphid than from the striped mealybug on guava branches. This was due to not only the abundance of the cotton aphid honeydew, but also its physical property of more watery than that of the striped mealybug.

There were many *M. bicolor* foragers visited bait dispensers with boric acid compared with other testing bait toxicants including control which consisted of only 25% sugar water (Figures 7 and 8: MB5M). This was probably due to two reasons. Firstly, boric acid gave no smell in addition to the smell of sugary liquid in bait dispenser, while both imidacloprid and fipronil treatments used in this study created some smell of chemicals that could mask the smell of sugar at the beginning, and not only that, both formulations gave out sour-smelling later within 3–4 days. These unwanted attributes could deter ant foragers coming to bait dispensers. Secondly, at the average field temperature of 30 °C during June–August 2012 (range 26–32 °C) at Kamphaeng Saen, the 25% sugary liquid could slowly ferment within a few days and thus gradually reduced the performance of smell in order to attract ant foragers. Unlike sugar water with boric acid that could remain its freshness over longer period of time because of the antibacterial and antifungal properties of dissolved boric acid.

Cooper *et al.* (2008) studied liquid baits to control Argentine ants associated with mealybugs of commercial vineyards in California pointed out that poor results with imidacloprid in their report were probably due to rapid photodegradation of the material and to ultra low concentration of active ingredient at .0001%. Our study of *M. bicolor* associated with hemipterans in guava revealed the inferior performance of

imidacloprid bait as well, but the cause was initially due to unpleasant smell, and consequently failure to draw ant foragers away from hemipterans (Figure 8: MBAG and MBFV). This substandard situation is similar to that of sugary liquid bait with fipronil as well. Cooper *et al.* (2008) pointed out lessons learned from their works in coastal California vineyards during 2000–2001 that bait stations left in the field for longer than 3 weeks, without preservative added, decomposed as the sugar fermented. In this study, the dispenser was cleaned and renewed all bait materials every 2 weeks. These were not enough to help maintaining the performance of all baits as before throughout the experiment except those with boric acid. The shortening time of bait material renewal at less than 2 weeks could be tedious and time consuming if baits were attached to every tree. However, it should be ideal if we could expand the time for renewing the bait material up to one month.

## CONCLUSION

In conclusion, mutualistic relationships between *M. bicolor* and honeydew-producing hemipterans were revealed. *M. bicolor* preferably collected honeydew of *A. gossypii* more than that of *F. virgata* in guava plantation. This was probably not only easier to find, i.e. more abundant of *A. gossypii* honeydew, but also more ingestible, due to the physical property of more watery liquid compared with *F. virgata*. Other alluring properties of honeydew to ants could be honeydew composition in terms of sugars and amino acids, which needed further investigations. The ant-exclusion promoted an increment in predator densities, and thus leading to a tentatively conservation biological control of hemipterous pests in guava ecosystem. *M. bicolor* workers to die following after insecticide exposure.

Study on sugary liquid ant baits showed evidence in supporting the effectiveness of bait dispenser with 3% boric acid dissolved in 25% sugar water in deviating *M. bicolor* foragers away from mutually benefit honeydew-producing hemipterans, therefore enhancing natural enemy pressures, and finally leading to reduction of hemipterous pest densities. The future commercial development of liquid ant bait in guava orchards needs additional knowledge including the density and timing of bait arrangement, suitable time for bait material renewal or alternatively adding of preservatives, and more importantly the bait efficacy in order to reduce ant density in the target area.

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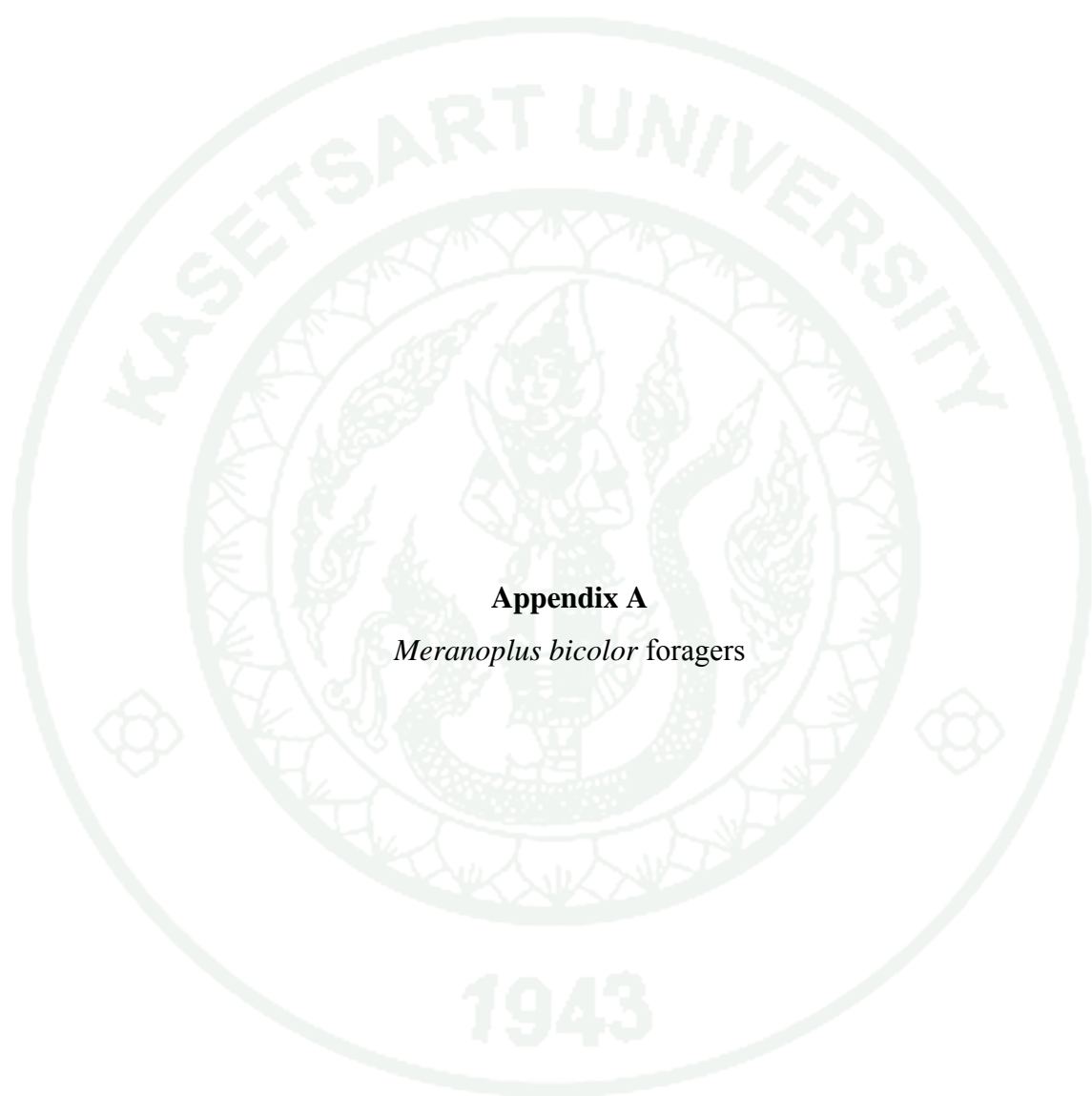
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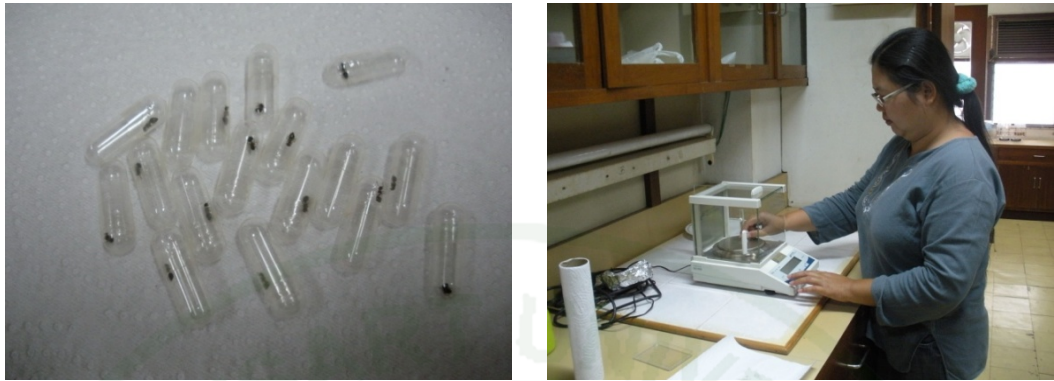
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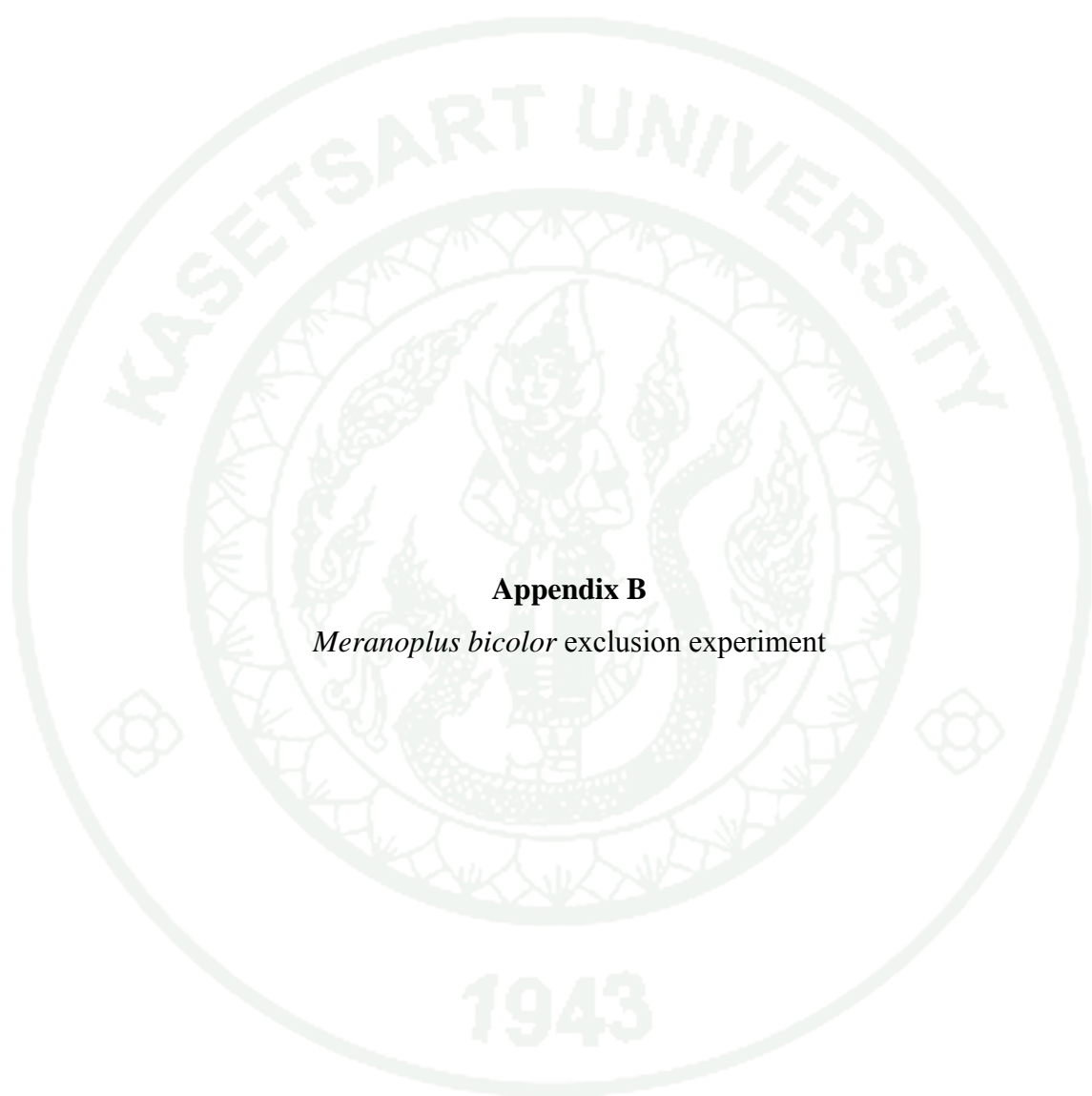




**Appendix A**  
*Meranoplus bicolor* foragers



**Appendix Figure A** Individual worker of *M. bicolor* was captured in an empty hard gelatin capsule (size 0; outer diameter 7.65 mm, height 21.7 mm, and volume 0.68 ml of Torpac Inc., NJ) (left); and weighed on a digital balance (right).



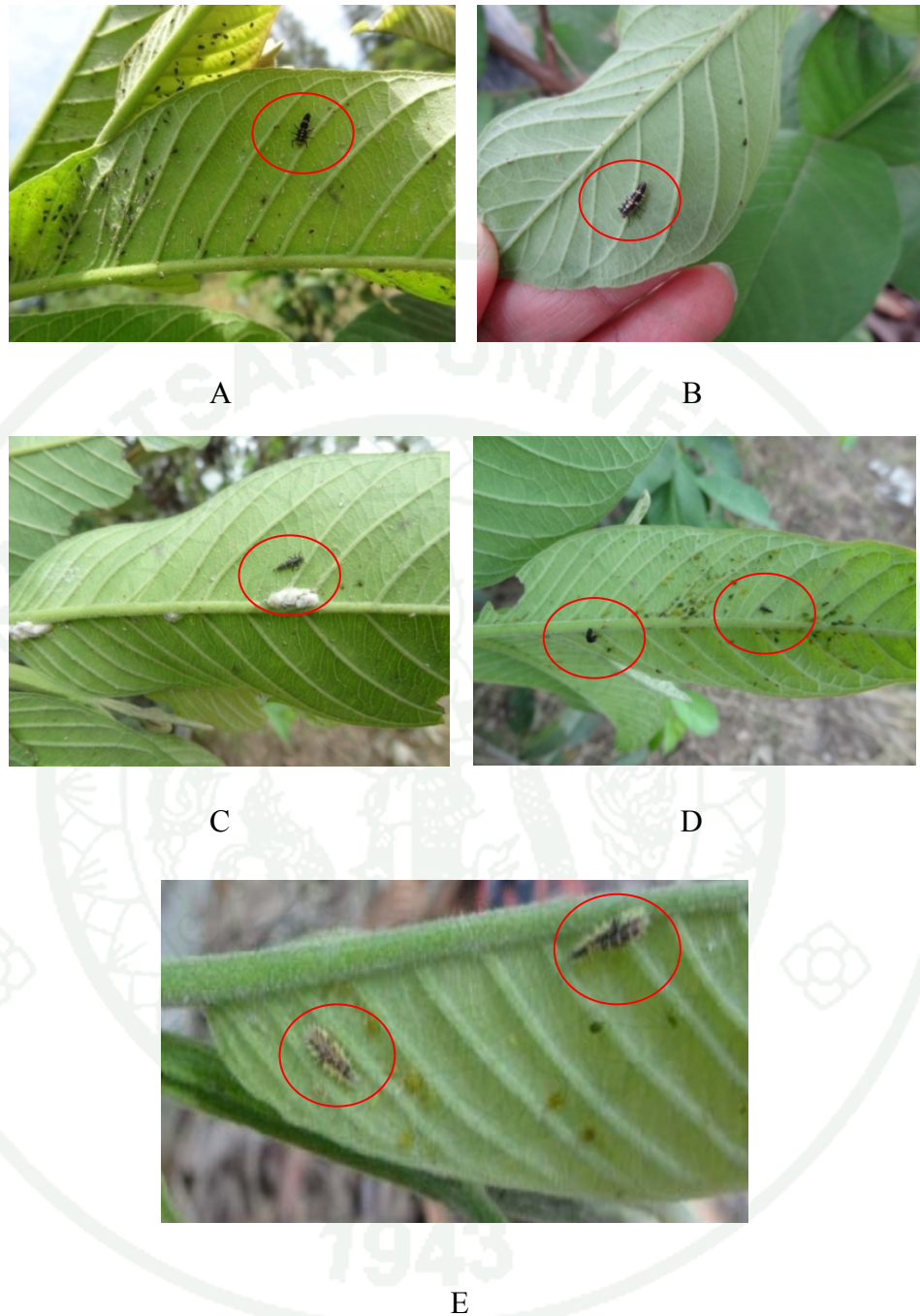
**Appendix B**

*Meranoplus bicolor* exclusion experiment

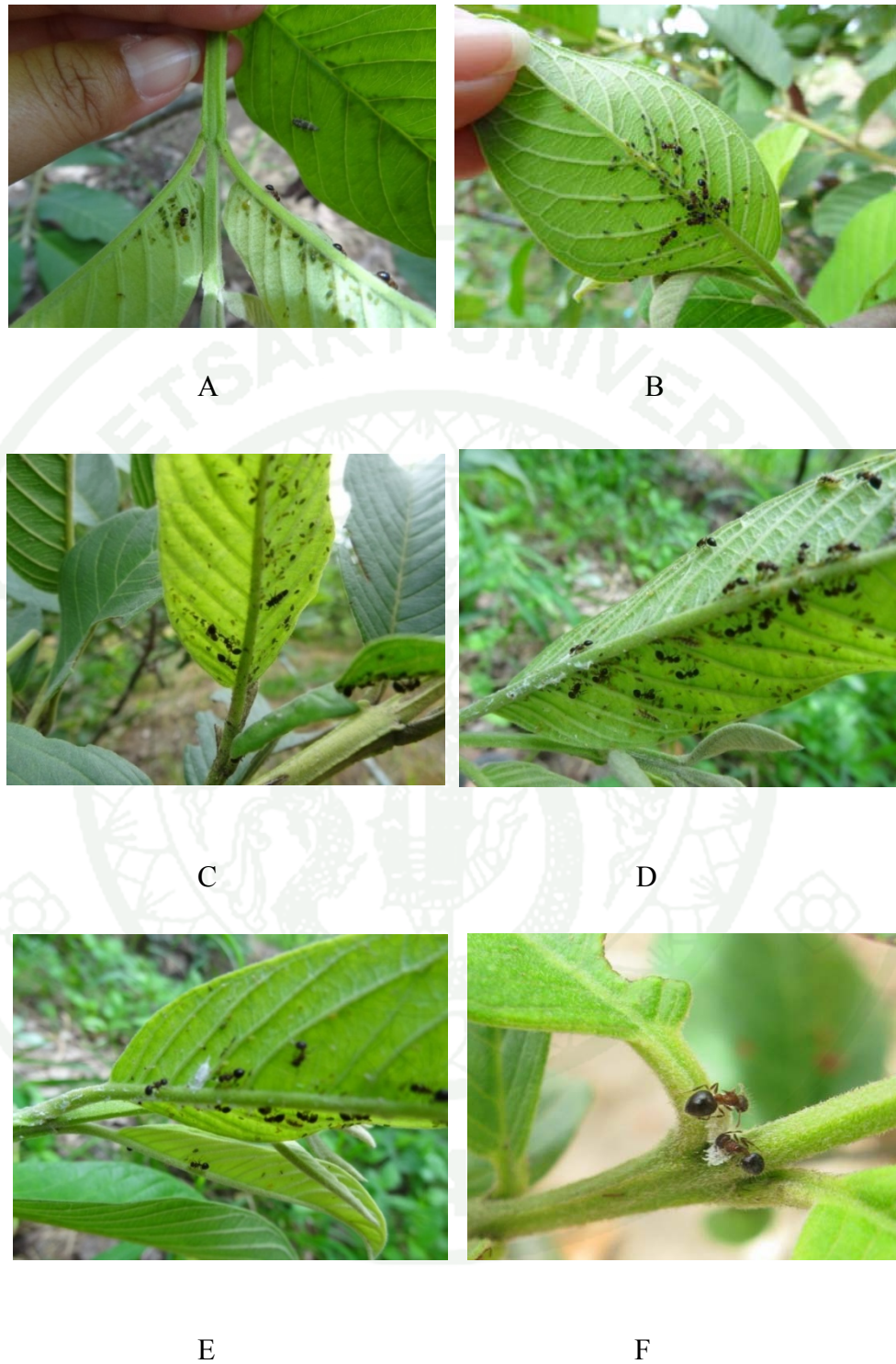


**Appendix Figure B1** Ant-excluded treatment with sticky barrier of 20 cm length around the base of guava branch.

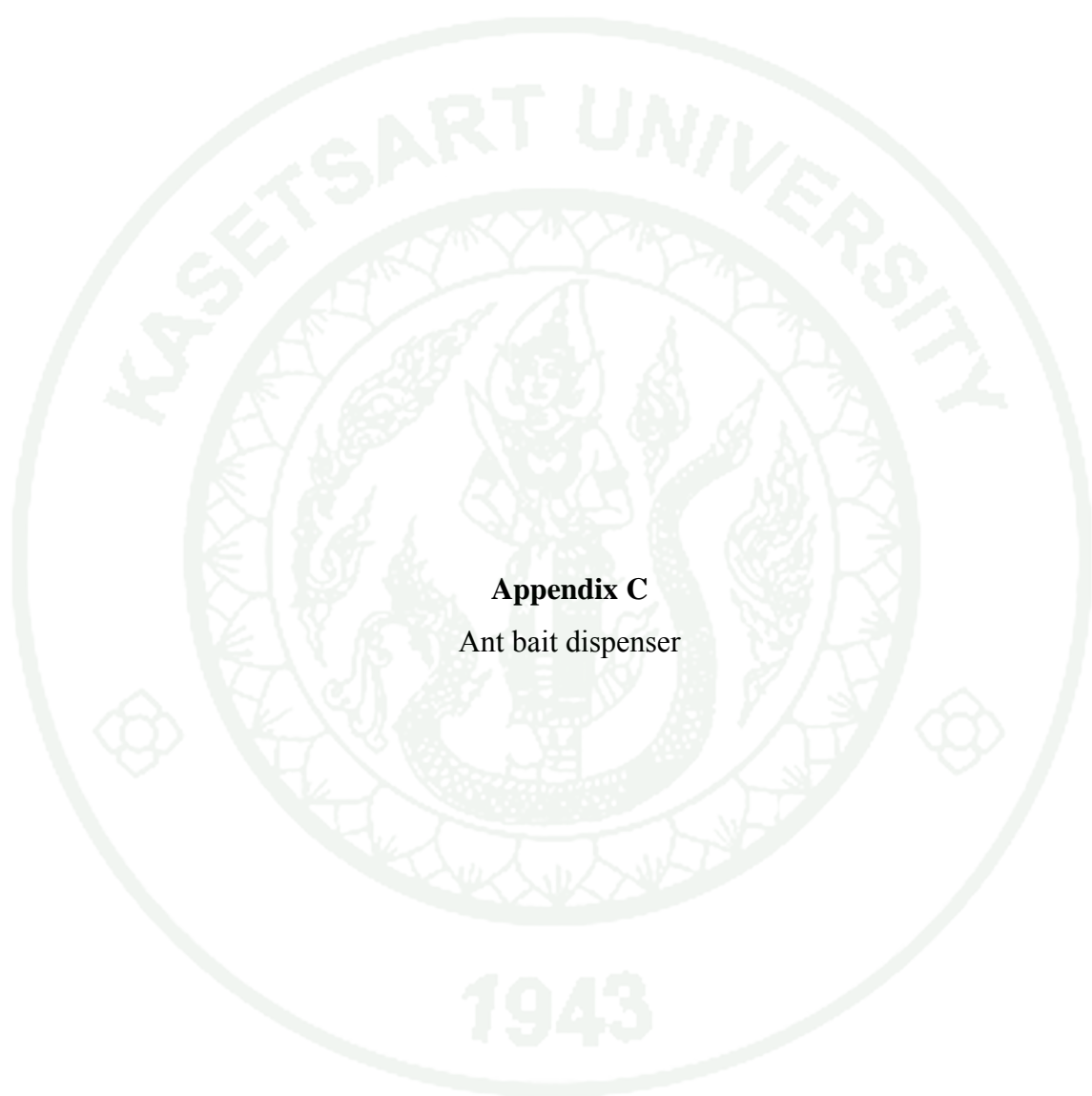
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**Appendix Figure B2** Larvae of natural enemies of *Meranoplus bicolor*; *Menochilus sexmaculatus* (A, B), *Coccinella transversalis* (C, D), and *Pseudodorus clavatus* (E).



**Appendix Figure B3** Relatively high densities of *Aphis gossypii* (A, B, C, D), and *Ferrisia virgata* (E, F) on ant-tended guava branches.



**Appendix C**  
Ant bait dispenser

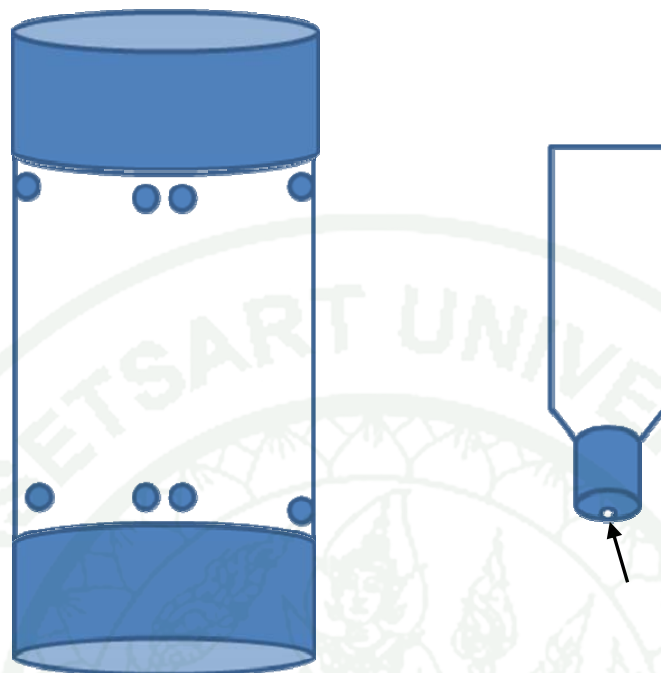


A



B

**Appendix Figure C1** Bait dispenser attached to the base of guava. (A), an upside down 600-cc plastic bottle filled with 25% sugar water and toxicant (B).



**Appendix Figure C2** Side view diagram of ant bait dispenser showing eight entry holes (3 mm in diameter) (left); and a 600-cc plastic bottle with one hole (1 mm in diameter) on the cap (right).

## CURRICULUM VITAE

NAME : Ms. Duangthip Kantha

BIRTH DATE : January 9, 1976

BIRTH PLACE : Chachoengsao, Thailand

EDUCATION : 

<u>YEAR</u>	<u>INSTITUTE</u>	<u>DEGREE/DIPLOMA</u>
1999	Kasetsart Univ.	B.Sc.(Agriculture)
2006	Kasetsart Univ.	M.S. (Entomology)

POSITION/TITLE : Entomologist

WORK PLACE : Entomology Department, Faculty of Agriculture,  
Kasetsart University

SCHOLARSHIP :

1. Academic Scholarship from Kasetsart University Foundation, Thailand (2011-2014)
2. Ph.D. International Publication Grant, Graduate School, Kasetsart University (2012)
3. Ph.D. Support Thesis Grant, Graduate School, Kasetsart University (2012)

AWARDS :

1. Excellence Academic Student Prize (Master Degree), Graduate School, Kasetsart University (2006)
2. The 3<sup>rd</sup> Award in the Invention " Ant Bait for Agriculture System" (University and Collage Level), Inventors Day, Bangkok, Thailand (2013)