FOOD PREFERENCE, FORAGING, COMPETITION ~~ACTIVITY~~ AND CONTROL OF THE LONG-LEGGED ANT, *Anoplolepis gracilipes* (FR. SMITH) (HYMENOPTERA: FORMICIDAE)

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FOOD PREFERENCE, FORAGING, COMPETITION ~~ACTIVITY~~ AND CONTROL OF THE LONG-LEGGED ANT, *Anoplolepis gracilipes* (FR. SMITH) (HYMENOPTERA: FORMICIDAE)

by

CHONG KIM FUNG

Thesis submitted in fulfillment of the requirements for the degree of Master of Science

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To

My most beloved

Father, Mother, Brothers and Sisters

who have given me endless of love,

greatest encouragement and support

all this while

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TABLE OF CONTENTS

Acknowledgements		ii
Table of Contents		iii
List of Tables		vi
List of Figures		viii
List of Appendices		x
List of Presentations		xi
Abstrak		xii
Abstract		xiv
CHAPTER 1 - GENERAL INTRODUCTION		1
CHAPTER 2 - LITERATURE REVIEW		
2.1	Ants	4
2.2	Basic biology of ants	4
2.3	Feeding behaviour of ants	7
2.4	Foraging behaviour of ants	10
2.5	Aggression behaviour of ants	13
2.6	General biology and behaviour of *A. gracilipes*	16
2.7	Control of ants	20
CHAPTER 3 - FOOD PREFERENCES OF *A. gracilipes*		
3.1	Introduction	26
3.2	Materials and methods	
	3.2.1 Insects	27
	3.2.2 Effects of nutritional content of various foods	27
	3.2.3 Effects of various food qualities, food consistencies and food forms	

3.2.3.1 Various food qualities (food with different concentration) 29
3.2.3.2 Various food consistencies 29
3.2.3.3 Various food forms 30
3.2.4 Effects of different combination of food class 30
3.2.5 Effects of various food particle sizes 32
3.2.6 Periodical c~~C~~hanges of f~~F~~ood p~~P~~references 32
3.3 Results and discussion
3.3.1 Effects of nutritional content of various foods 33
3.3.2 Effects of various food qualities, food consistencies and food forms 38
3.3.3 Effects of different combination of food class, food particle sizes and periodical changes of food preferences 43
3.4 Conclusion 48

CHAPTER 4 - FORAGING BEHAVIOUR OF *A. gracilipes*

4.1 Introduction 49
4.2 Materials and methods
4.2.1 Insects 50
4.2.2 Foraging activity of *A. gracilipes* 50
4.2.3 Effects of food type on the foraging group size of *A. gracilipes* 50
4.3 Results and discussion 51
4.4 Conclusion 60

CHAPTER 5 - INTRA- AND INTERSPECIFIC~~IES~~ AGGRESSION AND COMPETITION IN *A. gracilipes*

5.1 Introduction 61
5.2 Materials and methods
5.2.1 Insects 62

5.2.2 One to one interaction test
5.2.2.1 Interspecific aggression 64
5.2.2.2 Intraspecific aggression 65
5.2.3 Mortality test
5.2.3.1 Interspecific aggression 65
5.2.3.2 Intraspecific aggression 66
5.2.4 Food competition 66
5.3 Results and discussion
5.3.1 One to one interaction test 67
5.3.2 Mortality test 78
5.3.3 Food competition 87
5.4 Conclusion 93

CHAPTER 6 - CONTROL OF *A. gracilipes* BY BAITING

6.1 Introduction 95
6.2 Materials and methods 96
6.3 Results and discussion 98
6.4 Conclusion 105

SUMMARY AND CONCLUSION 106

BIBLIOGRAPHY 108

APPENDICES

VITA

LIST OF TABLES

		Page
Table 3.1	Different combinations of carbohydrate, protein and lipid food	**31**
Table 3.2	Preference of *A. gracilipes* for various carbohydrate foods (all prepared in 30 % w/w)	**34**
Table 3.3	Comparative preference of *A. gracilipes* for human-edible and laboratory grade carbohydrate sources (all prepared in 30% w/w)	**35**
Table 3.4	Preference of *A. gracilipes* for various protein foods	**36**
Table 3.5	Preference of *A. gracilipes* for various lipid foods	**37**
Table 3.6	Preference of *A. gracilipes* for various concentrations of brown sugar (carbohydrate), prawn meat (protein) & egg yolk (lipid)	**39**
Table 3.7	Preference of *A. gracilipes* for various consistencies of brown sugar (carbohydrate), prawn meat (protein) & egg yolk (lipid)	**41**
Table 3.8	Preference of *A. gracilipes* for various forms of brown sugar (carbohydrate), prawn meat (protein) & egg yolk (lipid)	**42**
Table 5.1	Measurements of body size (tip of mandible to tip of gaster), head width and mandible width of the studied worker ant	**63**
Table 5.2	Mean behavioural indices of *A. gracilipes* when interact with the opponent species and the outcome of the interaction in a~~the~~ period~~od~~ of 15 minutes	**69**
Table 5.3	Intraspecific aggressive behaviour of *A. gracilipes* from different colonies in a~~the~~ period of 15 minutes	**77**
Table 5.4	Mortality of both *A. gracilipes* and opponent species when interacting with each other within 24 hours	**79**
Table 5.5	Mortality of *A. gracilipes* from different colonies when interacting with each other within 24 hours	**86**
Table 5.6	Mean time of first ant encounters food source and mortality of both *A. gracilipes* and opponent species during 3 hours interaction	**92**

Table 6.1	Study sites of *A. gracilipes* in Universiti Sains Malaysia Minden campus	**97**
Table 6.2	Mean percentage of reduction of workers after treatment	**100**
Table 6.3	Occurrence of other ant species during treatment	**104**

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LIST OF FIGURES **Page**

Figure 2.1	Structure of boric acid	**22**
Figure 2.2	Structure of fipronil	**23**
Figure 2.3	Structure of indoxacarb	**24**
Figure 2.4	Structure of chlorantraniliprole	**25**
Figure 3.1	Preference of *A. gracilipes* for various combination of food	**44**
Figure 3.2	Preference of *A. gracilipes* for various particle sizes of solid prawn meat	**45**
Figure 3.3	Periodical changes of food preference of *A. gracilipes*	**47**
Figure 4.1	Foraging activity of *A. gracilipes*.	**52**
Figure 4.2	Relationship between foraging acitivity of *A. gracilipes* and ambient temperature	**54**
Figure 4.3	Relationship between foraging acitivity of *A. gracilipes* and ambient relative humidity	**56**
Figure 4.4	Relationship between foraging acitivity of *A. gracilipes* and ambient light intensity	**57**
Figure 4.5	Foraging group size of *A. gracilipes* to carbohydrate, protein and lipid food	**58**
Figure 5.1	Set-up~~Test arena~~ for food competition experiment	**68**
Figure 5.2	Mean behavioural indices of *A. gracilipes* when interacting ~~with different size of~~ opponent species of different sizes	**74**
Figure 5.3	Mean behavioural indices of *A. gracilipes* versus mean head width of opponent species	**75**
Figure 5.4	Mean behavioural indices of *A. gracilipes* versus mean mandible width of opponent species	**76**
Figure 5.5	Mortality of *A. gracilipes* when interacting with ~~with different size of~~ opponent species of different sizes	**82**
Figure 5.6	Mortality of *A. gracilipes* versus different head width of opponent species	**83**

Figure 5.7	Mortality of *A. gracilipes* versus different mandible~~head~~ width of opponent species	**84**
Figure 5.8	Mean mortality of opponent species versus mean behavioral indices of *A. gracilipes*	**85**
Figure 5.9	Mean numbers of *M. pharaonis* and *A. gracilipes* that attracted to sucrose solution 30% during 3 hours interaction	**88**
Figure 5.10	Mean numbers of *M. floricola* and *A. gracilipes* that attracted to sucrose solution 30% during 3 hours interaction	**89**
Figure 5.11	Mean numbers of *T. indicum* and *A. gracilipes* that attracted to sucrose solution 30% during 3 hours interaction	**91**
Figure 6.1	Self-design bait station	**99**

LIST OF APPENDICES

Appendix A	A worker ant of *A. gracilipes*
Appendix B	A queen ant of *A. gracilipes*
Appendix C	A female alate of *A. gracilipes*
Appendix D	A male alate of *A. gracilipes*
Appendix E	Eggs of *A. gracilipes*
Appendix F	Immature stages of *A. gracilipes*

LIST OF PRESENTATIONS

1. **Chong, K. F.** & Lee, C. Y. Control of the long-legged Ant, *Anoplolepis gracilipes* in Universiti Sains Malaysia's Minden Campus (Hymenoptera: Formicidae). The 12th Biological Sciences Graduate Congress (BSGC). Universiti Malaya, Malaysia. 17-19 Dec 2007.
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KEGEMARAN MAKANAN, PENCARIAN~~AKTIVITI MENCARI~~ MAKANAN, PERSAINGAN

DAN KAWALAN SEMUT *Anoplolepis gracilipes* (FR. SMITH)

(HYMENOPTERA: FORMICIDAE)

ABSTRAK

Tesis ini menumpu kepada kajian kegemaran makanan, ~~perilaku m~~pencarian makanan, ~~inter- and intraspesifik~~ ke~~per~~lakuan agresif inter- dan intraspesifik serta kawalan terhadap *Anoplolepis gracilipes* (Fr. Smith). Beberapa faktor yang mempengaruhi kegemaran makanan *A. gracilipes* telah dikaji~~jalankan~~. *A~~.~~noplolepis gracilipes* paling mengemari gula hitam (brown sugar) dan D (+) sakarosa~~ccharose~~ sebagai makanan karbohidrat, udang sebagai makanan protein dan kuning telur sebagai makanan lipid. Makanan yang lebih berkualiti dan makanan yang lebih lembut (consistency) dapat menarik perhatian lebih banyak semut ($p<0.05$). Mereka lebih suka makanan cecair dan separa-pepejal berbanding dengan makanan pepejal. Kombinasi makanan yang berbeza mempengaruhi kegemaran makanan species ini ($p<0.05$). Penambahan makanan karbohidrat atau makanan protein ke dalam makanan lipid menarik lebih banyak semut jika dibandingkan dengan makanan lipid sahaja ($p<0.05$). Walau bagaimanapun, saiz butiran makanan tidak mempengaruhi kegemaran makanan *A. gracilipes* ($p>0.05$). Kajian perubahan kegemaran makanan menunjukkan *A. gracilipes* lebih gemar makanan karbohidrat diikuti dengan makanan protein dan lipid. Aktiviti pencarian makanan *A. gracilipes* dipengaruhi oleh suhu dan kelembapan persekitaran. Aktiviti pencarian makanannya berkorelasi

secara positif dengan kelembapan persekitaran tetapi berkorelasi secara negatif dengan suhu persekitaran. Aktiviti pencarian makanan *A. gracilipes* adalah paling tinggi pada pukul 10 pagi~~am~~. Keamatan cahaya tidak memainkan peranan dalam aktiviti pencarian makanan *A. gracilipes.* Selain itu, makanan yang berlainan jenis tidak mempengaruhi saiz kumpulan semut yang mencari makanan. Dalam kajian kelakuan agresif interspesifik~~ agresif~~, *A. gracilipes* menunjukkan ke~~per~~lakuan agresif terhadap kebanyakan species lawan~~semut yang lain~~ (opponent species). Kelakuan agresif *A. gracilipes* dipengaruhi oleh saiz badan, kelebaran kepala dan mandibel species lain. Mortaliti *A. gracilipes* dalam kajian secara berkumpulan dengan species lain juga dipengaruhi oleh saiz badan, kelebaran kepala dan mandibel species lain. Dalam u~~ka~~jian kelakuan agresif intraspesifik~~ agresif~~, *A. gracilipes* dari koloni yang berlainan menunjukkan ke~~per~~lakuan agresif terhadap satu sama lain. Dalam u~~ka~~jian persaingan makanan, *Monomorium pharaonis* (L.) tidak berupaya bersaing dengan *A. gracilipes.* Walaupun begitu, *A. gracilipes* membenarkan *Monomorium floricola* (Jerdon) dan *Tapinoma indicum* (Forel) bersama-sama mencari makanan dengan mereka. Keberkesanan 4 jenis insektisid iaitu fipronil, indoxacarb, chlorantraniliprole dan boric acid telah di~~gunakan dalam kajian kawalan~~uji terhadap *A. gracilipes.* Fipronil (0.01% w/w), indoxacarb (0.05% w/w) dan boric acid (2% w/w) berkesan terhadap *A. gracilipes* menghasilkan~~dengan lebih daripada~~ 90% pengurangan semut pekerja pada sehari selepas rawatan. Walau bagaimanapun, chlorantraniliprole (0.05% w/w) memerlukan lebih daripada dua bulan untuk mengurangkan populasi *A. gracilipes.*

FOOD PREFERENCE, FORAGING, COMPETITON ~~ACTIVITY~~ AND CONTROL OF THE LONG-LEGGED ANT, *Anoplolepis gracilipes* (FR. SMITH)

(HYMENOPTERA: FORMICIDAE)

ABSTRACT

This dissertation focuses on food preference, foraging behaviour, inter- and intraspecific aggression behaviour as well as control of *Anoplolepis gracilipes* (Fr. Smith). Various factors that influence the feeding preferences of *A. gracilipes* were investigated. *Anoplolepis~~.~~ gracilipes* preferred brown sugar and D (+) saccharose as carbohydrate foods, prawn meat as proteinaceous food and egg yolk as lipid source. Higher qualities and lower consistencies of food significantly attracted more ants ($p<0.05$). Liquid and semi-solid food were ~~were more~~ preferred by the ants when compared to solid food. Different combinations of food affected their feeding preferences ($p<0.05$). Adding carbohydrate food or proteinaceous food into lipid food ~~would~~ significantly attracted more ants than lipid food alone ($p<0.05$). However, food particle sizes did not affect their feeding preference ($p>0.05$). Periodical changes of food preference study showed that they preferred carbohydrate food followed by proteinaceous and lipid foods. Foraging activity of *A. gracilipes* was significantly influenced by both ambient temperature and relative humidity. The activity rhythm of this species was positively correlated with ambient relative humidity, but negatively correlated with ambient temperature. The peak foraging

time~~ of the foraging schedule~~ of *A. gracilipes* was at 10 am. Light intensity did not play any role in affecting the foraging activity of *A. gracilipes*. D~~On the other hand,~~ ~~d~~ifferent types of food also did not affect the foraging group size of *A. gracilipes*. In interspecific aggression test, *A. gracilipes* showed aggression behaviour towards most of the opponent species. Aggression behaviour of *A. gracilipes* was significantly ($p<0.05$) influenced by the body size, head width and mandible width of opponent species. Mortality of *A. gracilipes* during group tests was also significantly related to body size, head width and mandible width of opponent species. In intraspecific aggression test, different colonies of *A. gracilipes* showed aggressive~~on~~ behaviour when interacting with each other. In food competition test, *Monomorium pharaonis* (L.) was unable to compete with *A. gracilipes.* However, *A. gracilipes* allowed *Monomorium floricola* (Jerdon) and *Tapinoma indicum* (Forel) to forage together. The effectiveness of four insecticides namely fipronil, indoxacarb, chlorantraniliprole and boric acid was tested against *A. gracilipes*. Fipronil (0.01% w/w), indoxacarb (0.05% w/w) and boric acid (2% w/w) were effective against *A. gracilipes* with more than 90% reduction of worker a day after post-treatment. On the other hand, chlorantraniliprole (0.05% w/w) required more than 2 months to cause a reduction in *A. gracilipes* population.

CHAPTER ONE

GENERAL INTRODUCTION

Ants are eusocial insects that have existed since the Cretaceous Period (Hölldobler & Wilson 1990) and spread throughout the world. There are about 10,000 species of ants that have been identified but less than 0.5% of them are considered as pest in the human environment (Lee & Robinson 2001).

In Malaysia, ants areis the important pest group after termites and cockroaches (Lee & Yap 2003). Currently, ant management accounts fored about 10% (US$18-20 million) of the total business turnover of the pest management industry (Lee & Yap 2003; Lee 2007). Ants are also the top pest in the United States since 1998 (Robinson 1999) and pest ants status has risen in many other developed countries like Singapore and South Korea (Lee 2007). According to Lee (2007), the increase in importance of ants as pests may due to the introduction of baits in the control of cockroaches. The decreased usage of residual sprays in cockroaches control strategy may result in the overmore abundancet or survival of other crawling pests likes ants. Gooch (1999) reported that ants areis the most difficult pests to control.

Ants are usually considered as nuisance pest as they forage in large group ton our foods and drinks. However, they also have potential to act as mechanical vectors for various pathogenic organisms (Lee & Tan 2004). Pharaoh ants have been reported to contaminate sterile surgical instruments as well as transmit bacteria to wounds of patient (Lee & Yap 2003). Fire ants' sting can cause respiratory tract obstruction, worsening of pre-existing medical condition or frank anaphylaxis (deShazo *et al.* 2004; Klotz *et al.* 2005). Other species like carpenter ants are economically important as they cause damage to wooden structures (Lee & Tan 2004).

The important species of household ants in Malaysia are *Pheidole megacephala* (Fabricius), *Monomorium pharaonis* (Linn.), *Tapinoma melanocephalum* (Fabricius), *Monomorium destructor* (Jerdon), *Paratrechina longicornis* (Latreille) and *Solenopsis geminata* (Fabricius) (Lee & Yap 2003). Recently, the long-legged ant, *Anoplolepis gracilipes* (Fr. Smith) (formerly known as *Anoplolepis longipes* (Jerdon)) is becoming increasingly important due to its negative impact on urban, agricultural as well as the native ecosystem. This species has been recognized by The World Conservation Union (IUCN) and the Global Invasive Species Programme as one of the world's 100 worst invaders (ISSG 2001).

The most significant impact was the deadly attack on nesting birds and native invertebrates in the Seychelles (Feare 1999,~~ as~~ cited by Wetterer 2005) and endemic crabs in Christmas Island, Australia (O'Dowd *et al.* 2003;~~,~~ Abbott 2006). They also attacked hatching birds and ~~-~~reptiles (Feare 1999,~~ as~~ cited by Wetterer 2005) as well as newborn pigs, dogs, cats, rabbits and rats (Haines *et al.* 1994). The association between *A. gracilipes* and honeydew-secreting scale insects may lead to canopy dieback because of the growth of sooty moulds (Haines *et al.* 1994;~~,~~ O'Dowd *et al.* 2003). *Anoplolepis*~~.~~ *gracilipes* also displaced the 'keystone' species as they were able to form multi-queen supercolonies in the rainforest (O'Dowd *et al.* 2003). Lester & Tavite (2004) reported that there was significant reduction in ant species diversity with increasing *A. gracilipes* density in newly invaded areas in Tokelau, New Zealand. In addition, because of its rapid population growth, *A. gracilipes* beca~~o~~me an important agricultural and household pest in Tokelau, New Zealand. In the urban environment, they always cause nuisance especially when they forage in large numbers. Formic acid sprayed by the ants can cause skin burns and eye irritation.

Common methods to control ants are by using residual treatment and baiting (Lee & Tan 2004). Recently, baiting became a popular approach~~mean~~ due to its minimal usage of insecticide and more target-specific characteristics. This method could work against the ant colony without the need to locate the nest (Lee & Tan 2004). However, the major challenge in controlling ants is the varying biology and behaviour among the different ant species. This makes control of ants difficult, as there is no single bait that is able to control all species. Baiting is only effective if the correct bait is applied against correct species. Therefore, it is very important to understand the biology and behaviour of the targeted species before we can use the correct bait or strategy to manage them.

Anoplolepis~~.~~ gracilipes is difficult to control because of its large population in nature, rapid spread, wide foraging range and general feeding habits. To date, there is limited information and knowledge available on this species. The objectives of this study are:

a. To determine the feeding preferences of *A. gracilipes* namely the effects of food nourishment; food qualities, consistencies and forms; food particle sizes as well as the periodical changes of food preferences.

b. To study the foraging activity of *A. gracilipes* and to examine the effects of food type on foraging group size of *A. gracilipes*.

c. To study the aggressive~~on~~ behaviour of *A. gracilipes* when interacting with other ant species as well as in the presence of food.

d. To determine the effectiveness of several novel insecticide compounds against *A. gracilipes*.

Field Code Changed

Field Code Changed

CHAPTER TWO

LITERATURE REVIEW

2.1 Ants

Ants are eusocial insects (Hölldobler & Wilson 1990) and are the members of the order Hymenoptera. They are classified under the family Formicidae. They are among the most abundant and species-rich taxa on the planet (Hölldobler & Wilson 1990). Besides that, they are also the most diverse and abundant social insects compared to other social insects such as termites and bees (Lee & Chong 2003).

Ants live in an organized colony where daily activities include food gathering, defense against enemy, colony moving and breeding. These are carried out as a unit ~~that~~ made up of hundreds or thousands of individuals (Lee & Chong 2003). Each species has its own peculiarities of structure and behaviour which make it distinguishable from other species (Sudd 1967). Different species of ants vary greatly in their nesting habits, distribution, physical characteristics, food preferences, methods of dispersal and etc. (Haack & Granovsky 1990).

2.2 Basic biology of ants

Ant has three body regions: head, thorax and abdomen or gaster. It has a pair of elbowed antennae and three pairs of legs. Swarmer or alate has two pairs of wings. The forewings are larger than the hind wings. All legs and wings are attached to the thorax (Lee & Chong 2003). Ants can be distinguished from other insects by the narrow pedicel (one or two nodes) which are located between the thorax and abdomen (Haack & Granovsky 1990). Ant heads vary in shape (eg. circular, triangular, rectangular or elliptical) (Lee & Chong 2003). The mandibles on the head are important organs. These mandibles play a role in biting, pricking, piercing, cutting, building, sawing, carrying, leaping and bounding but never for eating (Haack & Granovsky 1990). The antennae have many touch and smell sensory cells. Ants have lateral compound eyes. However, queens, males and workers of some species have additional three simple eyes or ocelli. Their eyes are not fully developed. They are only able to detect movement rather than seeing objects distinctly. The ocelli are adapted for detecting light and darkness (Haack & Granovsky 1990). Some of the species have a sting at the tip of the gaster, for example fire ants (Lee & Tan 2004).

Ants undergo complete metamorphosis (holometabolous). Their life cycle consists of four distinct stages, egg, larva, pupa and adult stage (Lee & Chong 2003). The eggs are white in colour and microscopic in size. The eggs will hatch into larvae. The larvae are fragile, legless and need nursing from the workers. These larvae will then develop into pupae which resembles adults. They are immobile, soft and white in colour. The pupae will then develop into adult and the newly hatched adults are known as callow because of their fair body colour. They will undergo coloration as they grow older (Lee & Chong 2003).

Ants perform caste system in the colony. This division of labour is important for the survival of the colony (Lee & Chong 2003). Ant colonies consist of two castes, the reproductive individuals and the non-reproductive individuals. Reproductive individuals are queens, male and female alates while non-reproductive individuals are workers and immature stages or brood (Lee & Chong 2003). The queen is the largest individual in the colony. Most ant species have multiple queens per colony (polygynous) while some other species have only one queen per colony (monogynous). The queens function as 'egg-laying machines' except that they have to rear their first batch of brood (Haack & Granovsky 1990). Male alates have large eyes and huge thorax that harbors great wing muscles. Male and female alates will mate in the nest or on the ground. After mating, the female alates will shed their wings, lay eggs and become the queen of the colony while the male alate will usually die within two weeks (Haack & Granovsky 1990). The female alate only has to mate once to reproduce continuously throughout her life (Lee 2004). The workers are sterile females and are the most abundant individual in the colony. They perform a great majority of tasks in the colony for example gathering food, nursing the brood, defending and constructing the nest. The size of the workers may vary depending on species. Most of the species have one size worker (monomorphic) and their role in the colony may change according to their age. Younger workers normally work within the nest (eg. tending to the queens and brood) while the older workers forage for food and defend the colony (Haack & Granovsky 1990). Some species have two size workers (dimorphic) or multiple sizes (polymorphic). Dimorphic workers consist of major and minor workers while polymorphic workers consist of minim, minor and major workers. The minims usually perform lighter tasks such as tending to the queens and brood. The majors need to forage for food, construct nest and defend against enemies. On the other hand, the minors are more flexible as they either tend the nest or forage for food (Lee & Chong 2003). The immature stages consist of eggs, larvae and pupae. They have to be fed and groomed by the workers. (Haack & Granovsky 1990).

Ants establish a new colony through two methods namely swarming and budding (Haack & Granovsky 1990; Lee & Chong 2003). A mature colony will produce male and female alates. The male and female alates will leave the nest and mate on the ground or in the air (swarming). After mating, the male will die while the female will find a suitable place to lay eggs. The first batch of brood will be tended by the queen. After the first batch of workers emerges, the queen will function as an ‘egg-laying machine’ and all the other tasks will be carried out by the workers. Then the colony will slowly develop. Budding involves movement of several mated queens with a group of workers that carries the brood and establish a new colony at a new site (Lee & Chong 2003). In some cases, queens are not necessarily needed to start a new colony as the workers are able to produce new queens through special feeding of the larvae (Lee & Chong 2003).

2.3 Feeding behaviour of ants

Ants feed on a wide range of food including sweet, greasy, starchy, plant and animal materials. They usually obtain carbohydrates from nectaries on leaves and in flowers as well as ‘honeydew’ from aphids, scale insects, mealybugs and whiteflies (Nash 1969). ‘Honeydew’ is a rich food that contains amino-acids, carbohydrates and some lipids (Sudd 1967). On the other hand, ants obtain proteinaceous and lipid foods from dead insects or their preys. Some species such as *Solenopsis fugax* Latreille feed on stolen brood from other ants’ nests. Abbott (1978) stated that the difference in food distribution in ants is due to the different functions played by the different food types. Carbohydrate serves as energy sources for the workers as the workers need to carry out multiple tasks. Protein and lipid are the two most crucial nutrients to ensure continuous production of brood in an ant colony. Queens need proteinaceous food to produce eggs while larvae need protein to grow or develop. Lipid is usually shared among all members in the colony (Haack & Granovsky 1990).

When workers find liquid food, they will ingest the food until their abdomen becomes swollen. After that, they return to the nest and share the food with other members of the colony (Lee & Chong 2003). When the workers find solid food, they will carry the food back to the nest because they cannot digest the solid food as they lack the~~of~~ necessary endopeptidase in their foregut (Stradling 1978). If the food is too big or too heavy to be carried back to the nest, they will cut the food into pieces (Haack & Granovsky 1990). The solid food are given to the 4th instar~~s~~ larvae (Lim *et al.* 2005) to be digested. The larvae will then regurgitate the digested food back to the workers. Then, the workers will feed the other workers and queens. This process of exchanging food is called trophallaxis (Lee & Chong 2003).

The f~~F~~eeding behaviour of ants are influenced by caste composition, weather, satiation, food qualities, food forms, food abundance, colony age, stage and presence of brood as well as long-term feeding history (Stein *et al.* 1990). When the colony is in a productive stage where there are more brood in the colony, the foragers tend to forage more towards proteinaceous and lipid foods (Stradling 1978). Alternation of food is necessary to make sure the colony obtain both varied and balanced diet (Edwards & Abraham 1990). The ~~L~~larvae play a very important role in regulating the colony's nutrient distribution (Lee 2007). The ~~L~~larval protein storage ~~protein~~ profiles change~~s~~ with the stages of larvae and dietary protein levels (Lim *et al.* 2005). In temperate countries, ants will forage for proteinaceous food during spring and summer due to maximum brood development (Stein *et al.* 1990; Sims 2006). From early to late autumn, the food preference ~~is equal~~ towards carbohydrate, protein and lipid foods is equal (Sims 2006).

When the colony is satiated with a nutrient, they will forage for other nutrients although the previous nutrient is still available or abundant (Lee & Tan 2004). Ants are able to distinguish between poor and rich resources (Lenoir 2002) and they will usually go for richer or more rewarding resources (Crawford & Rissing 1983; Fewell 1990; Nonacs & Dill 1990; Venna & Ganeshaiah 1991). More profitable or higher quality of food allows the ants to maximize the net rate of energy delivery to the nest (Davidson 1978). Food choice and recruitment are related to quantity, quality and location of food source (Nonacs & Dill 1990,; Nonacs & Soriano 1998). The foragers will collect more food especially food that are only produceds at certain period. They will also go for foods that are situated nearer to the nest. According to Hölldobler (1976), the definition of food quality is different to an ant colony and is defined according to the size and concentration of source, distance to the source as well as the degree of colony starvation.

Most species prefer liquid food. According to Howard and Tschinkel (1981), liquid comprises a major portion and form an important part of the diet in many social insects. Ants usually get carbohydrate sources in liquid form such as nectar and honeydew from Homoptera. Stradling (1978) reported that 81% of the food intake in *Formica rufa* (Linnaeus) was in liquid form and the balance of 19% was solid food. Haack and Vinson (1990) reported that solid baits requires more time to handle and needs longer visits by *M. pharaonis* than liquid food. Some species prefer bigger food to maximize the net rate of energy intake to the nest (Davidson 1978) but others prefer smaller food as they do not need to cut the food into pieces and can readily feed it to the larvae.

Some species such as *Myrmica rubra* (L.), the fire ant, *Plagiolepis pygmaea* Latreille produce two types of eggs; reproductive eggs and trophic eggs (Abbott 1978). Trophic eggs are non-viable and function as food as the trophic eggs are rich in protein. There are several species that cannibalize larvae even though the food is available (Sorensen *et al.* 1983). For example, workers of *Camponotus floridanus* (Buckley) cannibalize larvae when the ratio of larvae to worker are too high (Sorensen *et al.* 1983).

2.4 Foraging behaviour of ants

Ants forage for foods and water. However, only about 15% of the workers in the colony will take part in the foraging activity (Petal 1978). The job of the workers will shift from nursing when~~during~~ young ~~age~~ to foraging as they grow older. They will forage either individually or in groups. According to Bernstein (1975), there are three types of foraging strategies in ants. First, food exploitation and collection are done by an individual ant. Second, food exploitation is done by an individual ant but food collection is done in group (mass recruitment). Lastly, groups of ants will forage on a specific trail but food collection is done by an individual ant. Foraging strategies of ants differs from species to species. Most ants use the second strategy as it is efficient and~~, save~~ energy and time saving. The amount of food a single ant can transport back to its nest depends on the speed of discovery and the time spent capturing and carrying back to its nest (Sudd 1967).

Ants communicate with each other by using pheromone. Trail pheromone is laid by foragers from food sources to the nest and this will recruit other foragers to go to the food sources and carry the food back to the nest (Lee & Chong 2003). Food recruitment is directly related to food source quality (Wilson 1962; Chadab & Rettenmeyer 1975). Roces (1993) reported that the evaluation of resource quality depends on the motivational state (modulated by the information received during recruitment) and intensity of the recruitment signals. Foraging strategies of ant is influenced by the demand of the ant colony (Sudd & Sudd 1985; Traniello 1989), abundance of food (Traniello 1989), weather (Fellers 1989) as well as inter- and intraspecific competition (Carrol & Janzen 1973).

Insects like many other organisms show daily, seasonal and annual cycles of activity and development. They may be nocturnal, diurnal or crepuscular. According to Saunders (1982), ants will display rhythm in their foraging activity. Activity rhythm of ants is controlled by both endogenous and exogenous factors.

Endogenous rhythm is a self-sustained or 'free-run' periodic system in the absence of temporal cues such as daily cycles of light and temperature. If ants are imprisoned in the dark at a constant temperature, they will show peaks of activity at the same time of day (Sudd 1967). This phenomenon is cause by the internal 'biological clock' of the ants. The 'biological clock' comes from their experience and time sense. For example, if ants are fed regularly at the same time each day for a period of time, they will search for the food at that particular time on succeeding days. It is also reported that *M. rubra*, *Lasius niger* (L.), *Formica fusca* (Linnaeus) and *Camponotus ligniperda* (Latreille) can be trained to forage at intervals of 3, 5, 21, 22, 24, 26 and 27 hours (Grabensberger 1933, cited by Sudd 1967).

Exogenous rhythm is a rhythm of activity which is a direct response to environmental cues such as temperature and cycle of light (Saunders 1982). Since ants are poikilothermic, they are very sensitive to climatic fluctuations (Fellers 1989). Their foraging activities are strictly controlled by environmental factors such as ambient temperature or soil temperature (Bernstein 1974; Peakin & Josens 1978; Whitford *et al.* 1980; Traniello 1989; Porter & Tschinkel 1993), water stress (Traniello 1989), moisture, radiation and wind (Pol & de Casenave 2004). Temperature plays an important role in the energy balance and metabolism of ant societies (Roces & Núñez 1995). Temperature would directly affect the oxygen consumption, water loss and transport costs of the foraging ants (López *et al.* 1992). Markin (1970a) reported that foraging activity of *Iridomyrmex humilis* (Mayr) is strongly correlated with temperature where the optimum foraging activity was between 15 and 30 °C. According to Peakin and Josens (1978), respiration rates of ant would double with every 10°C increase in temperature. *Camponotus pennsylvanicus* (De Geer) will travel faster as temperature increases and their foraging activity is significantly correlated with temperature, night length and wind speed (Nuss *et al.* 2005).

Foraging activity of ants is affected by season, altitude and weather (Bernstein 1974). For example, *Prenolepis imparis* Emery forages diurnally during cooler parts of the year but changes to nocturnal in~~at~~ late spring and early summer and then ceases entirely during midsummer (Fellers 1989). Rain will directly stop the foraging activity (Sudd 1967). Besides that, competition for food and space between species will also induce changes in activity schedule (Carrol & Janzen 1973). Subordinate species will change their foraging time in order to avoid foraging together with the dominant species. On the other hand, interaction among dominant species can also be avoided by separate~~d~~ peak periods (Fellers 1989).

Foraging responses of ants will change according to the demand of the colony (Sudd & Sudd 1985). When the colony needs a particular nutrient for the growth or development of their colony, the workers would search for that particular food. The workers must be able to modulate their foraging intensity to suit the change in their colony. Foraging responses of ant also changes depending on food types (Detrain & Deneubourg 1997), spatio-temporal distribution of food, food size (Hölldobler 1976) and food quality (Hölldobler & Wilson 1990). Bernstein (1974) found that there was a relationship between seasonal foraging time and food abundance in the desert ants. Bernstein (1975) also showed that the foraging strategies of ants changes in order to response to various food density. *Pogonomyrmex* sp. forage individually when food is abundant but forage in groups when food is scarce.

2.5 Aggression behaviour of ants

Recognition of nestmates and discrimination of intruders are important in eusocial insects especially in maintaining the integrity of insect societies (Hölldobler & Wilson 1990). It is believed that queen dominance (Carlin & Hölldobler 1983), worker-produced gestalt (Lahav *et al.* 1998) and environmentally-derived factor (Crosland 1989; Liang & Silverman 2000) are the three main keys to~~sources of~~ recognition key. Lim *et al.* (2003) found that diet affected the ability of *P. longicornis* workers to recognize their former nestmates after being subjected to different type of food.

Ants communicate through chemical cues or pheromones. Chemical communication and the organization of defense depends on a number of features including different behavioural thresholds to different quantities of a given secretion; pheromones that are multicomponent and multifunctional; the use of different glands in different circumstances and different pheromones in different castes (Howse 1983). African ant when disturb produces a droplet at the tip of its sting. One drop will attract their nestmates to the source while five drops will result in 'alarm' and cause rapid running as well as recruit others from a distance. *Oecophylla longinoda* (Latreille) has a chemical communication system involving at least five separate gl~~r~~ands: sternal gland (short-range recruitment), rectal sac (mark territory), mandibular gland (alert nestmates toward the source and bite), poison gland (formic acid) and dufour gland (alarm and trail pheromone) (Bradshaw *et al.* 1975). Minor workers in some species have different mandibular gland chemicals than the~~from~~ major workers as they are rarely found outside the nest.

Interspecific competition is a near-ubiquitous feature of ant communities (Hölldobler & Wilson 1990). They compete fiercely for the same resources, for example, food, space and mate. This will lead to antagonistic behaviour such as fights, escape and submission (Buschini & Leonardo 2001). Different species of ants will have different level of aggression~~veness~~ (Horvitz & Schemske 1984). Dominance hierarchies may be mediated by morphology such as ~~(e.g.~~ size (~~:~~ Fellers 1987), physiology ~~(e.g.~~ such as defensive compounds~~:~~ (Adam & Traniello 1981) and/or behaviour (Hölldobler & Wilson 1990). According to Dreisig (1988), aggressive or competitive ability correlates with body size. Jutsum (1979) also reported that difference in worker size ~~difference~~ is an important criteria in determining the interspecific aggressive interaction of leaf-cutting ants.

The common weapons for defence and/or attack are sting, toxic smears and repellents (Howse 1983; Hölldobler & Wilson 1990). Sting is a powerful weapon in some members of the subfamily Myrmicinae, eg. *S. geminata*. They sting to paralyse their preys or enemies. The sting is very effective against arthropods because the venom is lipophylic which can spread well on the cuticle. The sting will also attract their nestmates to snap anything that moves as the sting contains alarm pheromones (Brian 1983). In Pseudomyrmecinae, the sting is used for defense instead of predation. The sting can repel both vertebrates and invertebrates (Brian 1983). Howse (1983) reported that members of the subfamily Formicinae do not have sting, they usually bite and spray poisonous secretions. The most commonly used tactic of *A. gracilipes* when interacting with the opponent species is to twist its gaster forward to spray formic acid. According to Brian (1983), formic acid is effective against both arthropods and mammals either directly or after the skin has been in contact~~ed~~ with the acid. *Monomorium minimum* (Buckley) workers appear~~ed~~ to possess a powerful poison which when spread in the air, have a strong repellent effect against *Solenopsis invicta* (Buren) workers. *M~~.~~onomorium minimum* is often successful even in direct competition for food with *S. invicta* (Baroni Urbani & Kannowski 1974). *Oecophylla smaragdina* (Fabricius) will arise~~ up~~ its abdomen and secrete mandibular gland pheromones to alert or attract other nestmates so that they can locate the target and induce biting (Brian 1983).

Smaller ants have more difficulty in defense compared to larger ants that have strong mandibles and stings. They can either be mobile (run very fast to avoid); develop chemical repellents that act at a distance; abandon resistance in the face of attack or immobilize at a distance (Howse 1983). According to Retana & Cerdá (1995), tempo was related to the defense strategy of ants. Low-tempo species usually use immobile strategy to avoid attacks but high-tempo species would use escape strategy when in contact with more aggressive species. Some *Camponotus* sp. workers would response in submission behaviour where they would fold into a pupal position and become motionless.

Competition for food and other resources are common in ant communities. According to Hölldobler & Wilson (1990), ant dominance may derive from the ability to discover, recruit and consume the resources. According to Feller (1987), coexistence in ant communities was mediated by trade-offs competitive abilities (discover ability versus dominate and recruit ability). In ant communities, food competition usually results in direct combat between workers (Levings & Traniello 1981). Group of competing species can be arranged into either linear dominance hierarchies or intransitive competitive networks (Connell 1978). Coexisting species used various competition strategies. According to Wilson (1971), there are three kinds of competitors. Opportunists refer to those that can discover or exploit food quickly and effectively. Extirpators are those that are able to dominate food aggressively while insinuators are those that are able to discrete thieves and insert themselves inconspicuously. Sanders & Gordon (2003) revealed that competitive success differs at individual and colony level. In one to one interaction, factors such as worker size or the use of chemical defense compound may dictate dominance (Sanders & Gordon 2003) whereas in colony level, competitive success may strongly depend on colony size (Holway 1999). Smaller species must have higher number of individual to protect resources (Dreisig 1988). On the other hand, smaller species with large colonies will be able to win in the competition even though the opponent colony have larger workers or more rapid recruitment rates (Palmer 2004).

2.6 General biology and behaviour of *A. gracilipes*

Anoplolepis gracilipes is a member of the subfamily Formicinae. It was formerly known as *Formica gracilipes* Smith and *A. longipes*. The common names of this species are long-legged ant (Wetterer 2005), yellow crazy ant, red crazy ant and crazy ant (Lee & Tan 2004). The workers are long and slender with body size about 4-5 mm (Appendix A). They have long legs and antennae (scapes two times longer than head length). They have yellow-brownish body colour and have small oval heads with large eyes. This species can be distinguished from other species by several characters which includes a clypeus that is produced medially, convex anterior margin, mandible with eight teeth and antennae with 11 segments (Wetterer 2005). This species does not have a sting but kills prey by spraying formic acid.

Anoplolepis~~A.~~ *gracilipes* is primari~~l~~ly a species of the moist tropical lowlands (Wetterer 2005). The native range of *A. gracilipes* is still unknown. It is proposed that they are either from Africa or Asia (Wetterer 2005) but they have successfully spread throughout the world especially the islands. *Anoplolepis*~~A.~~ *gracilipes* spread to new locations (between cities, states, countries and biogeographic regions) through a variety of pathways for example sea cargo that involves timber trading; soil, machinery and road vehicles transporting; horticulture and material packaging.

Anoplolepis~~A.~~ *gracilipes* is a well-known tramp ant species. It is polygyny (multi-queen) and monomorphic (one size worker). The queens are much larger (10 mm) and robust than workers (Appendix B). Most dispersal and colony foundation appears to occur through colony budding. Generally, they lack intraspecific aggression among workers (Passera 1994). They can form diffuse supercolonies over large areas (up to 750 ha) in its introduced regions. Worker production is continuous and sexual stages (Appendix C and D) can be present year round. Colonies readily migrate if disturbed (Passera 1994).

Worker eggs (Appendix E) need 76-84 days to reach maturity at 20-22°C (Fluker & Beardsley 1970). The eggs hatch in 18-20 days and worker larvae needs 16-20 days to develop into pupae (Appendix F). Worker and queen pupae needs around 20 and 30 days respectively for development. Workers live approximately 6 months while queens live for several years. Queens lay about 700 eggs annually throughout their ~~whole~~ life time.

Anoplolepis~~A.~~ *gracilipes* have generalized nesting habits. They may nest in soil, below woody debris, below rocks, at the base of trees, underneath accumulated leaf litter, in animal burrows, tree hollows, at the base of epiphytes (Rao & Veeresh

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1991), bamboo cuts placed on ground (Lee & Tan 2004) as well as artificial structures such as PVC pipes.

Anoplolepis~~A.~~ gracilipes has a broad diet. They are scavengers as well as scavenging predators. It preys on isopods, myriapods, mollusks, earthworms, arachnids, other insects, land crabs, birds, mammals and reptiles (Lewis *et al.* 1976; Haines *et al.* 1994). They obtain carbohydrates and amino acids from plant nectaries and honeydew from Homopterans. They will tend to the Homopterans on stems and leaves of trees and shrubs (Haines *et al.* 1994) and this tend to cause a outbreak in the infestations of a variety of sap-sucking scale insects.

In Christmas Island, *A. gracilipes* forage day and night on the forest floor and in the canopy. They communicate through pheromones. The workers are good exploiters because they walk very fast across every available surface in the foraging areas. They will lay trail pheromone once they find food and this will recruit their nestmates to the food sources. Their foraging activity is limited by rains and high temperatures (Haines *et al.* 1994).

Anoplolepis~~A.~~ gracilipes has been recognized by The World Conservation Union (IUCN) and the Global Invasive Species Programme as one of the world's 100 worst invaders (ISSG 2001). It invades urban, agricultural and the native ecosystems. The impact of this species includes decimation of endemic species, changing habitat structure and resource availability, loss of biodiversity and altering ecosystem processes.

Anoplolepis~~A.~~ gracilipes attack and kill nesting birds and native invertebrates in the Seychelles (Feare 1999~~, as~~ cited by Wetterer 2005) as well as endemic crabs in Christmas Island (O'Dowd *et al.* 2003; Abbott 2006). They also attack hatching birds, reptiles (Feare 1999~~, as~~ cited by Wetterer 2005), newborn animals such as pigs, dogs, cats, rabbits and rats (Haines *et al.* 1994). *AnoplolepisA. gracilipes* directly threatens the conservation effort in Christmas Island. A number of species such as red land crabs, Abbott's Booby, Christmas Island Gecko, Christmas Island Hawk Owl, Christmas Island Thrush, Christmas Island white-eye and blue-tailed skink are at risk either directly through predation or indirectly through habitat alteration or resource depletion.

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AnoplolepisA. gracilipes are able to displace the 'keystone' species as they form multi-queen supercolonies in the rainforest (O'Dowd *et al.* 2003). Lester & Tavite (2004) reported that there was significant reduction in ant species diversity with increasing *A. gracilipes* densitiy in newly invaded areas in Tokelau. On Christmas Island, an estimation of 15-20 million red land crabs have been killed since the supercolonies of *A. gracilipes* were first noted in 1989. Elimination of red land crabs will result in a rapid shift and cause drastic alterations in forest structure and composition. In addition, extirpation of the red land crab from the ecosystem facilitates 'follow-on' invasions of the giant African land snail and a variety of environmental weeds. Besides that, *A. gracilipes* causes the outbreaks of sap-sucking scale insects which will stress trees and lead to the decrease in seed production and high mortality in some canopy species.

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AnoplolepisA. gracilipes is also a pest in agricultureal. They will tend sap-sucking scale insects on crops and irritate livestocks. In the Seychelles, they are a human nuisance, interfering with farm workers and disrupting households (Haines *et al.* 1994). They will spray formic acid when disturbed. The formic acid can cause skin burns and eye irritation. In addition, *A. gracilipes* are severe household pest and a nuisance in public buildings, hotels, hospitals, food and drink processing establishments (Lewis *et al.* 1976).

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In the Seychelles in the 1970s, toxic baits which are formulated with aldrin (chlorinated hydrocarbons) and a carrier of sieved coir waste were developed for the control of *A. gracilipes* and proved more effective than chemical spray treatments (Lewis *et al.* 1976; Haines & Haines 1979a; Haines & Haines 1979b; Haines *et al.* 1994). However, due to safety reasons, many of these products have been deregistered. On Christmas Island, fish-meat bait with fipronil at 0.1 g/kg (Adonis®) was used to control *A. gracilipes* (Abbott *et al.* 2005). The baits were distributed on foot throughout the rainforest. In some inaccessible areas, an aerial baiting programme was carried out and this programme was highly successful. Direct nest treatment was done for small localized incursions. In Tokelau, the government focused on implementing an education, awareness and training programme to engage Tokelauans in the ~~*A. gracilipes*~~ management of *A. gracilipes*.

2.7 Control of ants

A good understanding of ant identification, biology and behaviour is necessary before a control programme can be initiated (Haack & Granovsky 1990). Different species show~~ed~~ marked variations in biology and behaviour. It is impossible to have a universal solution for ants control. The best way to control the ants is by direct treatment or direct removal of nests. However, the nests are usually not accessible and have numerous entries to a single nest (Haack & Granovsky 1990). Therefore, residual treatment, dust treatment and baiting are alternative ways to control the ants (Lee & Chong 2003).

Residual treatment is done using formulated insecticides such as bendiocarb, chlorpyrifos, diazinon, carbaryl, propoxur and some pyrethroids (Lee & Chong 2003). However, it is difficult to achieve total elimination of ant colony using this method.

It is recommended to use non-repellent insecticides for indoor treatment. Repellent insecticides such as pyrethroids may induce budding of ants from their original nest site. On the other hand, perimeter treatment can use both repellent and non-repellent insecticides to prevent the ants from foraging into buildings (Lee & Tan 2004).

Dust treatment can be done against ants that nest inside switchboxes, motor, wood structures, wall voids, cracks and crevices (Lee & Tan 2004). Dust should be applied in light amounts to prevent drifting, staining or residue problems (Haack & Granovsky 1990).

Baiting is an effective method of ant control. Baiting is getting popular because it requires minimal amount of insecticides and can be used in~~at~~ sensitive areas such as zoos, children centres and computer rooms where residual treatment is not suitable (Lee & Yap 2003). The active ingredient in baits should be adequate to kill the ants but yet slow-acting so that the ants can carry the toxic back to the nest and transfer to other nestmates. The baits are generally formulated with neurotoxic insecticides (eg. fipronil), stomach poisons (eg. boric acid), metabolic inhibitors (eg. hydramethylnon) or insect growth inhibitors (eg. methoprene) (Lee 2007). The baits are formulated in liquid, gel, paste and granular form (Lee 2007).

Besides the active ingredient<u>s</u>, another important factor to successful baiting is the food attractant<u>s</u> in bait<u>s</u>. The bait must be palatable and able to compete with other food sources in the infested area. The physical or form of the bait must be used correctly. For example liquid baits are used against ghost ant and crazy ant because they do not response to paste and granular baits (Lee 2007). In addition, sanitation and bait placement will also influence the performance of baits. <u>G</u>~~A g~~ood sanitation such as well-kept food, water resources and clean floors will increase the performance of baits as the ants cannot access other food sources in the infested area

(Lee & Chong 2003). The baits should be placed on the ant trails where the ants can immediately find the baits, for example, under the sinks, wash basins, along skirting boards and places with ample heat and moisture (Lee & Chong 2003).

Boric acid (H_3BO_3 or $B_2O_3{\cdot}3H_2O$) (Figure 2.1) is a popular active ingredient of many pesticide products. Boric acid is derived from boron. Boron is a natural substance found in rocks, soil and water (NPTN 2007). Boric acid also occur naturally in fruits, vegetables and forage crops (BP 2007). It is effective against urban and agricultural pests, for example, insects, spiders, mites, algae, molds, fungi, weeds and etc. (NPTN 2007). In agriculture, boric acid is used as an insecticide, herbicide and fungicide. It will dessicate and interrupt photosynthesis in plants and suppresses the growth of algae. It is also used as wood preservative that controls decay-producing fungi in lumber and timber products. Boric acid acts as a stomach poison for ants, cockroaches, termites and silverfish. According to Knight & Rust (1991), boric acid is an excellent toxicant for ant bait because it is water-soluble, slow-acting, non-repellent and has a low mammalian toxicity. Boric acid disrupts the water balance and digestion of the insect (BP 2007). As a result, the insects will be dead due to dehydration and starvation. Boric acid also abrade the exoskeletons of the insects (BP 2007). Boric acid formulations exist in various forms including aerosols, liquids, wettable powders, granules, dust, pellets/tables and impregnated materials such as stakes and baits (NPTN 2007). Boric acid is generally of moderate acute toxicity and has been classified as Toxicity Category III for most acute effects including oral and dermal toxicity as well as eye and skin irritation (BP 2007).

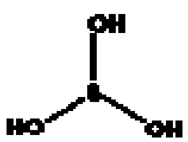

Figure 2.1 Structure of boric acid.

Fipronil (Figure 2.2) with a formula of 5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile has been proven as an effective insecticide against many insect pests including ants, termites and cockroaches. It is a member of the phenyl pyrazole class (Tingle *et al.* 2000). It acts on γ-aminobutyric acid-gated (GABA) receptors and interferes with the passage of chloride ions through chloride channels and thereby disrupting the activity of the central nervous system ~~activity~~ which results in death (Cole *et al.* 1993). It is much sensitive in insects compared to mammals (Hainzl & Casida 1996). It is a slow-acting and non-repellent insecticide which can act as a stomach poison or contact poison (Soeprono & Rust 2004; Klotz *et al.* 2007). Fipronil can be degraded by sunlight to produce various metabolites for example fipronil-desulfinyl (MB 46514) which is more stable and more toxic than the parent compound (Tingle *et al.* 2000).

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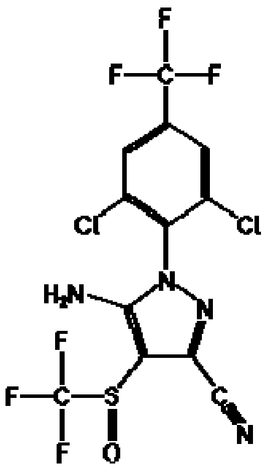

Figure 2.2 Structure of fipronil.

Indoxacarb is from the oxadiazine class of insecticide with a formula of methyl (*S*)-*N*-[7-chloro-2,3,4a,5-tetrahydro-4a-(methoxycarbonyl)indeno[1,2-*e*][1,3,4]oxadiazin-2-ylcarbonyl]-4′-(trifluoromethoxy)carbanilate (Figure 2.3). It is another novel insecticide that is highly toxic to insects but low toxicity to mammals, birds, earthworms and aquatic organisms. Indoxacarb was registered in the U.S.A. for use on apples, pears and other crops in May 2001. It is a broadspectrum insecticide that is effective against codling moth, white apple leafhopper, pandemic leafroller and lacanobia fruitworm (McKinley *et al.* 2002). It acts on the sodium channels, neuronal nicotinic acetylcholine receptors and GABA receptors (Narahashi 2001). It blocks off the insect voltage-gated sodium channel by the N-decarbomethoxyllated metabolite (Wing *et al.* 2000). As a result, the insect will cease feeding, become paralyzed and eventually die (McKinley *et al.* 2002). Indoxacarb must first be metabolized by the insect into an N-decarbomethoxyllated metabolite in order to become acutely toxic (Furman & Gold 2006b). It can act as contact and as a stomach poison (Moncada 2003).

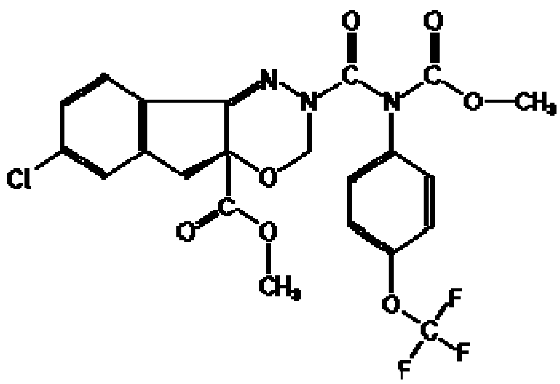

Figure 2.3 Structure of indoxacarb.