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# Honeybee Communication and Pollination

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## 1. Introduction

Honeybees play an important ecological role as pollinators of many plant species, and their products are the basis for a multi-million dollar commercial industry around the world. They are major agricultural pollinators around the world and are keystone pollinators in tropical ecosystems. Pollination has been considered a keystone process to ecosystem function through the facilitation of both plant and animal diversity. The diversity of pollinators and pollination systems is striking. Current understanding of the pollination process shows that, while interesting specialized relationships exist between plants and their pollinators, healthy pollination services are best ensured by an abundance and diversity of pollinators. This is because the flowering plant species only produce seeds if animal pollinators move pollen from the anthers to the stigmas of their flowers. Without this service, delicate interconnected species and processes functioning within an ecosystem would collapse. Many of the most important pollinating insect species are social, adding more complexity between pollination systems and ecosystem dynamics, as it has been well documented that such species have highly evolved methods of methods communication.

For some floral species, pollination may not occur without an evolved communication system some pollinators possess. Communication among insects is extremely important for their survival, especially for social insects that live in complex colonies. Many social insects depend on chemical signals, or pheromones to communicate with each other for guiding nestmates to food source, warn them of danger, and marking territory. Pheromones are chemical signals secreted by an individual that are transmitted and understood only within a single species. In contrast, other types of chemical signals can be perceived by and elicit a response in another species, as in interspecific mutualisms or interceptive eavesdropping (Nieh, 1998). Eavesdroppers may detect the chemical signals of another species and compete for a limited resource. Bees especially use pheromones and chemical signals to locate or detect resources. Honeybees, for example, mark nectar-depleted flowers using mandibular gland pheromones. Honeybees foragers that detect this pheromone are able to recognize which flowers to avoid, thereby increasing colony foraging efficiency. Honeybees are also famous for their remarkable dance language, which is used by workers to recruit nestmates

to resources such as food, water, resin and nest cavities. The waggle dance informs nestmates of the direction and distance of a newly discovered food source. Olfactory communication also plays an important role in recruitment in addition to many other aspects of colony life (Frisch, 1967). For example, honeybees can navigate to food sources by detection of nectar scent and may deposit cuticular hydrocarbon footprints or mandibular gland pheromone to mark nectar-depleted flowers and thereby increase colony foraging efficiency. Honeybee complex social behavior is an important and rich source for behavioral genetics, such as forage marking pheromone releasing by foragers while they are foraging, and including the genetics of aggression. Marking by forage marking pheromone produced by the mandibular gland of honeybee workers is important in basic research studies of bee learning and memory as well in applied studies examining how honeybee foraging behavior can be mitigated.

## 2. The importance of honeybees as pollinators

Honeybees play an important role for cross-pollination, or the transfer of pollen from one plant to the stigma of another plant leading to the process of fertilization. After fertilization, the fruit and seeds develop and mature. Pollination by insects, including honeybees, is important for both monoecious and dioecious plant species, those with an individual plant that bears both male and female reproductive structures and those where the individual only has one set of reproductive structures, respectively. Without this assistance, fruit and/or seeds would not be formed for most flowering species. Many agricultural crops such as *Aeschynomene americana* L., *Ageratum conyzoides* L., *Amomum xanthioides* Wall., *Anacardium occidentale* L., *Antigonon leptopus* Hook., *Balakara baccata* Roxb., *Castanopsis acuminatissima* Rehd., *Cinnamomum kerrii* Kosten., *Coccinia grandis* CL.Voigt, *Cocos nucifera* L., *Coffea Arabica* L., *Conyza sumatrensis* Retz are pollinated by honeybees (Suwannapong *et al.*, 2011). Part of the reason honeybees are so important as pollinators is that they actively seek out flowers with pollen and not only for nectar, unlike other pollinators such as bats and hummingbirds that primarily visit flowers for nectar. Some plants will not produce fruit without honeybee pollination. This floral fidelity of bees is due to their preference for nectars having sugar content and pollen with higher nutritive values.

In recent years, interest in tropical bees has increased. This is appropriate because honeybees likely originated in Tropical Africa and spread from South Africa to Northern Europe and East into India and China (Otis, 1991). The first bees appear in the fossil record in deposits dating from about 40 million years ago in the Eocene. The oldest bee fossil is preserved in a piece of amber found from a mine in northern Burma. It is believed to date back as far as 100 million years when bees and wasps split into two different lineages. The fossilized insect appears to share features both common to the bee and wasp, but is more similar to bees than wasps (Danforth *et al.*, 2006). The earliest known honeybee fossil (genus *Apis*) was found in Europe dating back 35 million years. About 30 million years ago, honeybees appear morphologically very similar to modern species (Koning, 1994). The genus *Apis* is evidently tropical in origin since it is native to Asia, Africa and Europe including such continental islands as Japan, Taiwan and the Philippines (Seeley, 1985). Honeybees did not appear in the Americas, Australia or New Zealand until European settlers introduced them in the 17th century (Zander and Weiss, 1964). Honeybees of the genus *Apis* are the most studied because of their fascinating and complex lifestyle,

communication systems (Nieh, 1998; Nieh and Roubik, 1995), role as keystone pollinators of native plants, pollination of agricultural crops, and the valuable hive products that they produce, such as honey, royal jelly, bee wax, bee pollen, propolis and even bee venom. Honeybees belong to the order Hymenoptera, superorder Apocrita, infraorder Acuelata, superfamily Apoidea, family Apidae, subfamily Apinae, tribe Apini. There are more than 11 extant species of *Apis* worldwide (Michener, 2000) that are classified into two groups, based upon nesting structures and activities. The first group builds single comb, open-air nests: *A. andreniformis*, *A. florea*, *A. dorsata*, *A. breviligula*, *A. binghami* and *A. laboriosa*. These bees are restricted to the Asian tropics and subtropics. The second group consists of species that nest inside cavities where they build multiple combs: *A. cerana*, *A. koschevnikovi*, *A. nigrocincta*, *A. nuluensis*, and *A. mellifera* (Hepburn and Radloff, 2011; Michener, 2000).

Asia has a rich diversity of honeybee species. These include *Apis cerana*, *A. dorsata*, *A. florea*, *A. laboriosa*, *A. breviligula*, *A. binghami* and *A. andreniformis* that are indigenous to the region, whereas the European honeybee, *A. mellifera* was introduced to the region and promoted for beekeeping. The indigenous honeybees make a significant contribution to the livelihoods of the rural poor and protection of the environment through a variety of products and services (Partap, 1992).

In the honey bee colony there are three castes: the queen, several thousand workers, and a few hundred drones. Among the members of the colony, there is division of labor and specialization in the performance of biological functions (Winston, 1987). Workers can flexibly shift among different tasks, depending upon colony need (Ferguson and Winston, 1988; Smith *et al.*, 2008). The tasks performed are primarily age related (Lindauer, 1961; Wang and Moeller, 1970). There is also a strong genetic component to division of labor with workers from different strains, races within the colony showing differences in task ontogeny (Winston and Katz, 1982). Both genetics and environment are important. Workers can perform a subset of multiple tasks at all ages (Lindauer, 1952; Winston, 1992). In general, young workers work inside the nest and older workers work outside (foraging) or at the nest entrance (guarding) (Winston and Ferguson, 1985). The youngest bees perform house cleaning and capping. Brood and queen rearing occupy slightly older workers, nurse bees. Comb building and food processing are handled by middle-aged workers (who serve as a general reservoir of labor that be channeled into performing different tasks inside the nest, as needed). Finally, nest temperature regulation and ventilation, defense, and foraging occupy the oldest bees (Winston, 1992). The caste structure in honeybees is closely linked with the development of brood food glands (hypopharyngeal glands), mandibular glands, and wax glands (King, 1993; Simpson, 1960, 1966; Simpson *et al.*, 1966; Wang and Moeller, 1969).

The honeybee's morphological structure that has co-evolved with the shape and features of flowers make them highly efficient pollinators. Their body is covered with hairs and setae, which pollen sticks to. Some of this pollen rubs off on the next flower they visit, fertilizing the flower. Their mouthparts include a long proboscis that is an appropriate length suitable for floral structures containing nectar. Also, pollen baskets on their legs allow pollen to be carried back to the hive through static electrical charge. This helps pollen (and other small particles) stick to them, while pollinating subsequent flowers they forage upon. Some plants will not produce fruit without honeybee pollination. This floral fidelity of bees is due to

their preference for nectars having sugar content and pollen with higher nutritive values. They enhance agricultural productivity and help maintain biodiversity by providing valuable pollination services. The benefit of honeybees as providers of pollination services for enhancing crop yields and maintaining biodiversity is thought to be much higher than their hive products. (McGreger, 1976; Crane, 1991; Free 1993; Partap and Verma, 1994; Suwannapong *et al.*, 2011). The availability of natural insect pollinators around the world is decreasing rapidly as a result of increased and continued use of pesticides. There is timely need for better management of hive honeybees such as *A. cerana* and *A. mellifera* in rare pollinator areas to increase fruit production. Information on the role of honeybees in pollination leads to increased quality and yield of crops worldwide (McGreger, 1976; Crane, 1991; Free 1993; Partap and Verma, 1994; Suwannapong *et al.*, 2011).

The benefit of honeybees as providers of pollination services for enhancing crop yields and maintaining biodiversity is thought to be much higher than their hive products. Keeping bees for pollination has been shown to increase the crop yield and quality and reduce fruit drop. Honeybee pollination has been reported to increase the fruit juice and sugar content in citrus fruits; reduce the percentage of misshapen fruits in strawberry; and increase the oil contents in rape seed and sunflower (Partap, 1992).

### 3. Honeybee flora

Bee flora, or bee plants, are the plants from which bees collect pollen and nectar. Honeybees forage on a variety of plant species to collect nectar and pollen (McGregor, 1976), including agricultural crops and native plants. They are particularly efficient pollinators for native plants due to the morphological structure of their organs and external features, such as hairs that cover their body to help carry nectar and pollen (Suwannapong *et al.*, 2011). Different species of honeybees that have different morphology may affect their foraging preferences. However, not all plant species are bee flora. Many plant species, including agricultural crops and native plants, are pollinated by honeybees which are good pollinators particularly for native plants. They are such good native plant flora pollinators because they have morphological structures that facilitate pollen attachment, transfer and deposition. For instances, they have a proboscis with the appropriate length and shape to match specific morphology of certain flowers. They also have a body covered with hairs and setae that adhere pollen and pollen baskets that are adaptations for carrying pollen by carrying a static electrical charge. This helps pollen (and other small particles) stick to them (Suwannapong *et al.*, 2011). However, a plant that produces nectar and pollen prolifically in one geographic region may not yield the same amount of nectar and pollen in another region (Erdtman, 1966, 1969; Latif *et al.*, 1960; Singh, 1981). There are three types of bee flora: plants that only supply nectar, plants that only supply pollen, and plants that provide both (Allen *et al.*, 1998; Baker, 1971; Baker and Baker, 1983; Bhattacharya, 2004; Crane *et al.*, 1989; Partap, 1997). Some plants provide only resin, but these are less common. Floral nectar provides energy for flight activity, foraging activity and other activity in the colony. Honeybees also convert the nectar into honey and store it in the honey storage area of the comb. Pollen provides protein, lipids, minerals, and vitamins (Gary, 1975; 1992). Pollen from different plant species differs in nutritive value and attractiveness to honeybees (Baker, 1971; Baker and Baker, 1983; Erdtman, 1966, 1969; Shuel, 1992; Suwannapong *et al.*, 2011).



There are more than 30 species of plants visited by *A. andreniformis* in Thailand such as *Anacardium occidentale* L., *Antigonon leptopus* Hook., *Balakara baccata* Roxb., *Brassica chinensis* Just var., *Castanopsis acuminatissima* Rehd., *Chrysal*, *Cocos nucifera* L., *Coriandrum sativum* L., *Conyza sumatrensis* Retz., *Cucurbita citrillus* L. *Cucumis sativus* Linn, *Cuphea hyssopifolia* H.B.K., *Dimocarpus longan* Lour., *Eugenia javanica* and *Mimosa pigra* (Suwannapong et al., 2011).

The plants visited by *A. florea* include more than 40 species such as *M. pigra*, *Callistemon viminalis*, *Vetchia merrillii* (Becc.) H.E. Mosse, *Cocos nucifera* L., *Melampodium divaricatum*, *Zea mays* L., *C. hyssopifolia* H.B.K., *D. longan* Lour., *Durio zibethinus* L., *E. javanica*, *Eupatorium odoratum* L., *Euphoria longana* Lamk., *Fragaria ananassa* Guedes, *Hopea odorata* Roxb (Maksong, 2008 Suwannapong et al., 2011). Therefore, *A. dorsata* reportedly uses fewer food plants than *A. florea*. Only 38 species are reportedly used by *A. dorsata*: *Ageratum conyzoides* L., *Amomum xanthioides* Wall., *Anacardium occidentale* L., *Blumea balsamifera* L. DC., *Bidens biternata* Merr. and Sherff., *Celosia argentea*, *Cinnamomum kerrii* Kosten, *Citrus aurantifolia* Swing., *C. maxima* (J. Burman ) Merr., *Cocos nucifera* L. (Maksong 2008; Suwannapong et al. 2011). However, few hundred plant species visited by *A. cerana*. There are more than 68 species are *A. cerana* bee plant in Thailand. These include *Aeschynomene americana* L., *Ageratum conyzoides* L., *Amomum xanthioides* Wall., *Anacardium occidentale* L., *Antigonon leptopus* Hook. *Balakara baccata* Roxb., *Bidens biternata* Merr. & Sher, *Brachiaria ruziziensis* Germain & Evrard, *Castanopsis acuminatissima* Rehd., *Cinnamomum kerrii* Kosten, *Coccinia grandis* CL.Voigt, *Cocos nucifera* L., *Coffea Arabica* L., *Conyza sumatrensis* Retz. The number of bee flora of the introduced honeybee species in Thailand are more than 54 species such as *Ageratum conyzoides* L. , *Durio zibethinus* L., *Euphoria longana* Lamk., *Fragaria ananassa* Guedes, *Leersia hexandra* Sw., *Macadamia integrifolia* maiden & Betche, *Mikania cordata* Roxb., *Mimosa pigra*, *Musa acuminata* Colla., *Nephelium lappaccum* L., *Ocimum basilicum* L., *Oryza sativa* L., *Oxalis acetosella* L., *Prunus mume* Sieb., *Psidium guajava* L., *Sesamum indicum* L., *Schoenoplectus juncoideus* (Roxb.) Palla, *Raphanus sativus* L. (Maksong, 2008; Suwannapong et al. 2011). However, there are more than 100 crops in the united states are bee plants for *Apis mellifera* such as *Abelmoschus esculentus*, *Actinidia deliciosa*, *Allium cepa*, *Anacardium occidentale*, *Apium graveolens*, *Arbutus unedo*, *Averrhoa carambola*, *Brassica alba*, *B. hirta*, *B. nigra*, *B. napus*, *B. oleracea* cultivar, *B. rapa*, *Cajanus cajan*, *Carica papaya*, *Carthamus tinctorius*, *Carum carvi*, *Castanea sativa*, *Citrullus lanatus*, *Citrus reticulate*, *Cocos nucifera*, *Coffea* spp., *Coriandrum sativum*, *Coronilla varia* L., *Cucumis melo* L., *Cucumis sativus*, *Cyamopsis tetragonoloba*, *Cydonia oblonga* Mill, *Daucus carota*, *Dolichos* spp. *Dimocarpus longan*, *Diospyros kaki*, *D. virginiana*, *Elettaria cardamomum*, *Eriobotrya japonica*, *Fagopyrum esculentum*, *Feijoa sellowiana*, *Foeniculum vulgare*, *Fragaria* spp., *Glycine max*, *G. soja*, *Helianthus annuus*, *Juglans* spp., *Linum usitatissimum*, *Lichi chinensis*, *Lupinus angustifolius* L., *Macadania ternifolia*, *Malpighia glabra*, *Malus domestica*, *Mangifera indica*, *medicago sativa*, *Nephelium lappaceum*, *Onobrychis* spp., *Persea Americana*, *Phaseolus* spp., *P. coccineus* L., *Pimenta dioica*, *Prunus armeniaca*, *P. avium* spp., *P. cerasus*, *P. domestica*, *P. spinosa*, *P. dulcis*, *P. amygdalus*, *P. persica*, *Psidium guajava*, *Punica granatum*, *Pyrus communis*, *Ribes nigrum*, *R. rubrum*, *Rosa* spp., *R. idaeus*, *R. fruticosus*, *Sambucus nigra*, *Sesamum indicum*, *Solanum melongena*, *Spondias* spp., *Tamarindus indica*, *Trifolium alba*, *T. hybridum* L., *T. incarnatum*, *T. pretense*, *T. vesiculosum*, *Vaccinium* spp., *V. oxycoccus*, *V. macrocarpon*, *Vercia faba*, *Vigna unguiculata*, *Vitellaria paradoxa* ([http://en.wikipedia.org/wiki/list\\_list\\_of\\_crop\\_plants\\_pollinated\\_by\\_bees](http://en.wikipedia.org/wiki/list_list_of_crop_plants_pollinated_by_bees)).

Number	Plant species	Nectar source	Pollen source
1	<i>Ageratum conyzoides</i> L.	+	+
2	<i>Amomum xanthioides</i> Wall.	+	+
3	<i>Balakara baccata</i> Roxb.	+	+
4	<i>Blumea balsamifera</i> (L.) DC.	+	+
5	<i>Bidens biternata</i> Merr. & Sherff.	+	+
6	<i>Brachiaria ruziziensis</i> Germain&Evrard	-	+
7	<i>Brassica chinensis</i> Jusl var.	+	+
8	<i>Castanopsis acuminatissima</i> Rehd.	-	+
9	<i>Ceiba pentandra</i> (L.)	+	+
10	<i>Cinnamomum kerrii</i> Kosten	+	+
11	<i>Citrus aurantifolia</i> Swing.	+	+
12	<i>Citrus maxima</i> (J. Burman ) Merr.	+	+
13	<i>Coccinia grandis</i> CL.Voigt	+	+
14	<i>Cocos nucifera</i> L.	+	+
15	<i>Coffea Arabica</i> L.	+	+
16	<i>Coriandrum sativum</i> L.	+	+
17	<i>Conyza sumatrensis</i> Retz.	+	+
18	<i>Crataeva magna</i> Lour.	+	+
19	<i>Croton oblongifolius</i> Roxb.	+	+
20	<i>Cuphea hyssopifolia</i> H.B.K.	+	-
21	<i>Dalbergia oliveri</i> Gamble ex Prain	+	+
22	<i>Datura metel</i> L.	+	+
23	<i>Dillenia ovata</i> Wall.	+	+
24	<i>Dimocarpus longan</i> Lour.	+	+
25	<i>Diospyros glandulosa</i> Lacc.	+	+
26	<i>Diospyros areolata</i> King & Gamble	+	+
27	<i>Duabanga grandiflora</i> Walp.	+	+
28	<i>Elaeagnus latifolia</i> L.	+	+
29	<i>Erythrina suvumbrans</i> Merr.	+	+
30	<i>Eucalyptus camaldulensis</i>	+	+
31	<i>Eugenia javanica</i>	+	+
32	<i>Eupatorium odoratum</i> L.	+	+
33	<i>Euphoria longana</i> Lamk.	+	+
34	<i>Fragaria ananassa</i> Guedes	+	+

Number	Plant species	Nectar source	Pollen source
35	<i>Gmelina arborea</i> Roxb.	+	+
36	<i>Hopea odorata</i> Roxb.	+	+
37	<i>Jacaranda filicifolia</i> D.Don	+	+
38	<i>Leersia hexandra</i> Sw.	-	+
39	<i>Leucaena leucocephala</i> Wit.	-	+
40	<i>Litchi chinensis</i> Sonn	+	+
41	<i>Macadamia integrifolia</i> maiden & Bette	+	+
42	<i>Mangifera indica</i> L.	+	+
43	<i>Mikania cordata</i> Roxb.	+	+
44	<i>Mimosa diplotricha</i> C. Wright.	+	+
45	<i>M. pigra</i>	+	+
46	<i>M. pudica</i> L.	-	+
47	<i>Muntingia calabura</i> L.	+	+
48	<i>Musa acuminata</i> Colla.	+	+
49	<i>M. sapientum</i> L.	+	+
50	<i>Ocimum sanctum</i> L.	+	+
51	<i>Oryza sativa</i> L.	-	+
52	<i>Oxalis acetosella</i> L.	+	+
53	<i>Passiflora laurifolia</i> L.	+	+
54	<i>Prunus cerasoides</i> D.Don	+	+
55	<i>P. mume</i> Sieb.	+	+
56	<i>Psidium guajava</i> L.	+	+
57	<i>Raphanus sativus</i> L.	+	+
58	<i>Schoenoplectus juncooides</i> (Roxb.) Palla.	-	+
59	<i>Shorea siamensis</i> Miq.	+	-
60	<i>Solanum torvum</i> SW.	+	+
61	<i>Spilanthus paniculata</i> Wall. Ex DC.	+	+
62	<i>Synedrella nodiflora</i> (L.) Gaerth.	+	+
63	<i>Wedelia trilobata</i> (L.) Hiteh.	-	+
64	<i>Wrightia arborea</i> (Dennst.) Mabb.	+	+
65	<i>Zea mays</i> L	-	+
66	<i>Zizyphus mauritiana</i> Lamk.	+	-

Table 1. Nectar, pollen and Nectar and pollen source plants of Thai honeybees (Suwannapong *et al.*, 2011)



The quality of fruits and seed of any plant species are dependent upon the intensity of pollination that involves the transfer of the male gamete, pollen, from the anther to the stigma, the receptive female structure of the flower. If the pollen is viable and compatible with the female tissue, it will produce a pollen tube that grows down into the ovary where fertilization of the ovule occurs, leading to the formation of a seed. Honeybees are a good facilitator for moving pollen from one part of a flower to another, or from one flower to another. Furthermore, insufficient pollen transfer can lead to poor fertilization of ovules, non-symmetrical fruit, and high rates of fruit drop. Many of these problems can be avoided by placing honeybee colonies in the orchard during the bloom period. The proper use and placement of honeybee colonies will help insure maximum benefits of pollination (Partap, 1992; Suwannapong *et al.*, 2011).

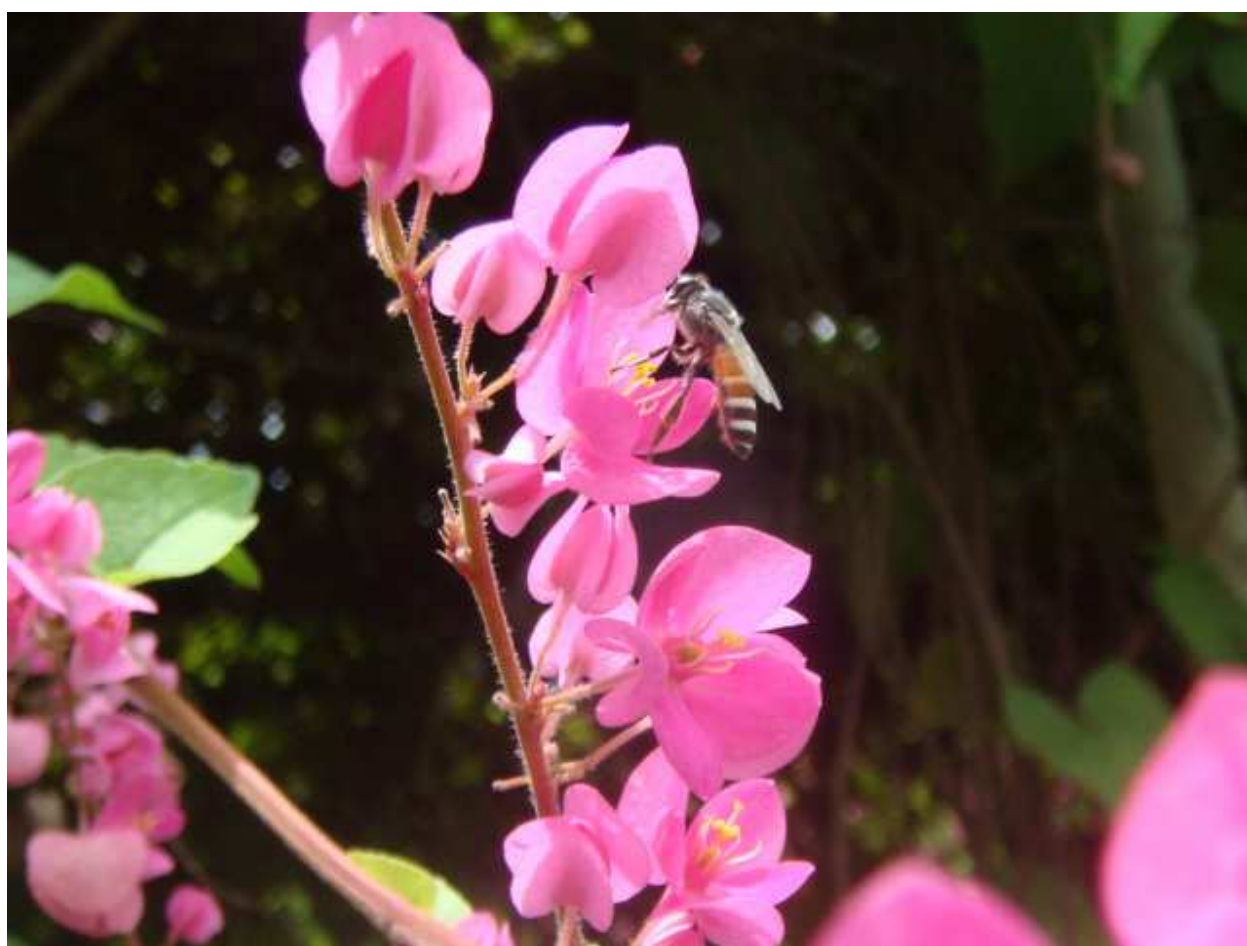


Fig. 1. *Apis florea* is foraging on flower.

Honeybees in the genus *Apis* have been used for crop pollination worldwide. A good system of pollination management is very important for the protection of the abundance and diversity of pollinators, especially in relation to the increasing monoculture systems worldwide that are reducing the diversity of forage resources such as almond and apple orchards, and blueberry crops which require honeybee pollination. Therefore, good management of natural pollination by insects such as honeybees results in increasing quality and quantity of agricultural products and gives rise to new financial opportunities.

### 3.1 Honeybee foraging behavior

A forager may prefer the nectar of one flower species. It is to her advantage to visit flowers producing greater quantities of nectar with a higher sugar concentration. The sugar concentration in the nectar of a given plant species may vary depending upon its location, time of day, and genotype. If nectar with a high sugar concentration is available, a forager of *A. mellifera* can carry as much as 70- 80 mg of nectar per load (Akratanakul, 1976; Partap, 1992; Partap and Partap, 1997). There are differences among flowering plant species with respect to nectar and pollen production. Not all plant species possess nectaries (glands secreting nectar) or have nectar that bees can reach with their proboscis (tongue) (Partap, 1992). Nectaries can be located in various areas of the flower and some species have extrafloral nectaries that may be visited by bees. In addition, some bees may perform nectar robbing, making a small hole at the base of a flower in order to obtain the nectar. In this case, the bee does not perform any pollination service for the “robbed” plant.

Honeybees are unlikely to make many repeat visits if a plant provides little reward. A single forager will visit different flowers in the morning and, if there is sufficient attraction and reward in a particular kind of flower, she will make visits to that type of flower for most of the day, unless the plants stop producing reward or she detects forage-marking pheromones left by other bees to avoid revisiting the nectar-depleted flower.

Workers of all honeybee species carry nectar internally. Part of their alimentary canal is modified to form a “honey sac” or “honey stomach”. After returning to the hive, the forager regurgitates the nectar to one or more house bees, which then dehydrate the nectar and convert it into honey. They use the enzyme invertase, which splits sucrose in the nectar into fructose and glucose, the sugars predominant in honey. To dehydrate the nectar, house bees regurgitate a part of the nectar and hold the droplet in their mouthparts (Partap, 1992; Partap and Partap, 1997).

Honeybee body are covered with abundant setae which pollen grains are attached while she forage both for nectar and pollen. They make pollen to be pellets with nectar and carry them by pollen baskets on the hind tibiae and storage in the pollen storage area (Partap, 1992).

In addition to collecting nectar and pollen, foragers can collect plant gum (propolis) and water (Fanesi *et al.*, 2009; Marcucci, 1995; Bankova *et al.*, 1983, 2000).

Propolis is a resinous hive product collected from various plant materials by honeybee workers. In some countries, and especially in Eastern Europe, propolis has been used in folk medicine for centuries. Its chemical composition includes flavonoids, aromatic acids, esters, aldehydes, ketones, fatty acids, terpenes, steroids, amino acids, polysaccharides, hydrocarbons, alcohols, hydroxybenzene, and several other compounds (Marcucci, 1995; Bankova *et al.*, 1983, 2000) which varies according to the plants in a specific region. The flavonoids (mainly pinocembrin) are considered to be responsible for its inhibitory effect on bacterial and fungus, but only traces of these compounds have been found in propolis of South American origin (Tomás-Barberán *et al.*, 1993). In addition, it works against bacteria in several ways, such as preventing bacterial cell division, and breaking down bacterial walls and cytoplasm. Cinnamic acid extracts of propolis prevented viruses from reproducing, but they worked best when used during the entire infection (Challem, 1995).



Fig. 2. Pollen source plant of *Apis dorsata*

### 3.2 Honeybee communication

Foragers communicate their floral findings in order to recruit other worker bees of the hive to forage in the same area. The factors that determine recruiting success are not completely known but probably include evaluations of the quality of nectar and/or pollen brought in to the hive. Honeybees communicate to each other by two ways: the physical communication by the dance language and the chemical communication by means of pheromone and/or odor that transmit important information to members of the honeybee colony. Pheromones play an important role in recruitment communication (Free, 1987). They use pheromones to guide nestmates for food sources, warn them of danger signal, mark territory area (Leal, 2010). Honeybee can smell or detect odor or chemical signal such as pheromone, flower odor, nectar by sensory receptor located on the flagellum of their antennae (Suwannapong *et al*, 2010).

### 3.3 Foraging behavior using forage – Marking pheromone

Honeybees have sophisticated foraging coordination and communication (von Frisch, 1971; Suwannapong, 2000). This activity is only performed by workers, known as foragers or foraging bees. Some foragers specialize on pollen foraging and some on nectar foraging. Between these extremes, there are a large number of generalists who collect both (Fewell and Page, 1993). The range for the onset of foraging ranges from 18.3 days (Sakagami, 1953)



to 37.9 days of age (Winston and Ferguson, 1985). This food consists of carbohydrates and proteins (nectar and pollen, Seeley, 1985). Under normal conditions, worker bees begin to forage when they are about 2 to 3 weeks old. Foraging is the last chore in the life of a worker. Part of the colony's stored honey is consumed by foraging bees who need fuel and therefore consume a certain amount of honey to ensure that she will have a sufficient energy supply for her round-trip journey (Akcratanakul, 1976; Seeley, 1985). To obtain a full load of nectar and pollen (or both) in a single trip, she may have to visit several hundred flowers (Akcratanakul, 1976). The amount of energy she expends, related to the amount of food she collects, is determined largely by factors such as the amount of nectar obtained per flower, floral density per unit area, the distance from the hive, and weather conditions (Akcratanakul, 1976; Partap, 1992; Partap and Partap, 1997).

It has been also reported that *A. mellifera* foragers use 2-heptanone to mark previously visited flowers, thereby signaling nectar depletion to other bees (Engels *et al.*, 1997; Giurfa, 1991). However, the four native Thai *Apis* species do not appear to use aversive pheromone marking during foraging (Suwannapong, 2000; Suwannapong *et al.*, 2010c). For example, they may revisit the same flower briefly after the first visit and continue to forage on the same flower simultaneously with several bees of their own species or other species. Suwannapong (2000) observed *A. florea*, two to three bees of *A. cerana*, one to two bees of *A. dorsata* and one to two bees of *A. andreniformis* visiting the same flower (Suwannapong, 2000). It is also possible that honeybees, like bumblebees, can learn to associate floral depletion or floral reward using olfactory cues like cuticular hydrocarbon "footprints," which are deposited while walking on the food source (Leadbeater and Chittka, 2007). However, this remains to be investigated.

The mandibular gland of *A. mellifera*, the source of this putative food-marking pheromone is primarily 2-heptanone. However, the primary component of mandibular gland secretions in Thai honeybees is (Z)-11-eicosanol. In general, the ten most abundant components in the mandibular glands of all these species are 80% similar (Suwannapong, 2000).

### 3.4 Forage marking pheromones

Most honeybee pheromones are produced by exocrine glands, which are ectodermal glands of the epidermis that secrete to the outside of the body. Each pheromone consists of odorants with a mixture of low molecular weight that move through the air and are perceived by bee antennae. Some pheromones and semiochemicals are perceived through direct contact with the antenna (Haynes & Millar 1998). Honeybee mandibular glands are pheromone-producing exocrine glands whose secretions may function as alarm pheromone, which is an important component of colony defense (Blum, 1969). The mandibular glands are largest relative to body size in queens, large and well-developed in workers, and very small in drones. The secretory product of workers mandibular glands has an oily appearance, and its major component is 2-heptanone, a volatile substance that accumulates in the central reservoir (Engels *et al.*, 1997).

The function of worker mandibular gland pheromone is unclear. At high concentrations, this pheromone may be repellent (Balderrama *et al.*, 1996). Shearer and Boch (1965) reported that 2-heptanone is the main compound of worker mandibular gland and acts as a secondary alarm pheromone in *A. mellifera* guards. Maschwitz (1964) suggested that

mandibular glands produce alerting pheromones, although a less effective one than the sting apparatus pheromones. Shearer and Boch (1965) identified 2-heptanone from mandibular gland secretions. Guard bees were alerted by, and attacked, filter paper carrying 2-heptanone placed at the hive entrance. Boch and Shearer (1971) therefore suggested that 2-heptanone has two functions: alarm (with lower efficacy than the sting gland) and repelling workers when deposited on exhausted floral resources. However, Nieh (2010) reported that foragers collecting food exhibited no alarm behavior in response to mandibular gland extracts, although they were clearly alarmed by sting gland extract. The response of other *Apis* species to worker mandibular gland pheromone is similarly unclear. Guards and foragers of *A. florea* and *A. cerana* showed diverse responses to Z-11-eicosen-1-ol, the main component of mandibular gland pheromone in these species (Suwannapong *et al.*, 2010). Moreover, the flower-marking hypothesis is not consistent with the finding that 2-heptanone can attract foragers at low concentrations (Shearer and Boch, 1965; Boch and Shearer, 1971; Kerr *et al.*, 1974; Vallet *et al.*, 1991), as would occur on flowers shortly after pheromone deposition.

Worker *Apis* mandibular gland pheromone may play a key role in two important aspects of colony life, defense and foraging, but its function in honeybees remains unclear. Understanding the function of this pheromone is significant because of the importance of studying honeybee forage marking pheromone in terms of practical apiculture; for understanding how honeybees use pheromones to mark nectar depleted flowers to save energy; for describing the role of pheromone as an attractant when a new and rich food source is discovered; and for application to increase honeybee pollination. For example, if the pheromone (like queen mandibular pheromone) is an attractant it can be applied in the field to attract bees to pollinate certain plant crops, which there are now commercial examples that are used to increase crop yields (Currie *et al.*, 1992a; Currie *et al.*, 1992b). On the other hand, if worker mandibular gland pheromone is a repellent, it may be useful in repelling bees where they are not wanted.

#### 4. Physical communication through the waggle dance

Honeybees also communicate for the food sources by physical communication known as bee dances or dancing behavior. When a forager finds a food source, it must communicate the location of the discovered food source to her nestmates. Extensive research shows that honeybees dance inside the nest after a successful foraging trip and communicates to her nestmates with information about the resource. In some social insects, pheromone trails are used to communicate similar messages. What is remarkable about honeybees is that foragers do not follow the scout (the scout may remain in the hive for hours). It conveys to its fellows the direction and distance. Shortly after its return, many foragers leave the hive and fly directly to the food (Wenner, 1964). The remarkable thing about this is that the foragers do not follow the scouts back (the scouts may remain in the hive for hours). It turns out that the scouts can convey to the foragers information about the food of odor, the distance and the direction from the hive. So the scout bees have communicated to the foragers the necessary information for them to find the food on their own. Honeybees guide their nest mate for the distance and direction. The dance essentially encodes the information her nestmates need to know in order to successfully revisit the same resource patch.



Variations of the dance exist, depending on the distance of the communicated source from the colony. The round dance is performed when the resource is within 50 meters from the hive. This dance consists of a scout bee, or returning forager, performing a series of narrow circular movements that may be repeated. Resources that are perceived as rewarding will have higher dance circuits performed. Similar behaviors occur for resources that are greater than 100 meters from the hive. These dances are more commonly known as the "waggle dance," and encodes the direction and distance of the food source to her nestmates. Scout bees fly from the colony in search of pollen and nectar. If successful in finding good supplies of food, the scouts return to the hive and "dances" on the honeycomb. When the dance occurring, the honeybee first walks straight ahead, vigorously shaking its abdomen and producing a buzzing sound with the beat of its wings. The distance and speed of this movement communicates the distance of the foraging site to the others. Communicating direction becomes more complex, as the dancing bee aligns her body in the direction of the food, relative to the sun. The entire dance pattern is a figure-eight, with the bee repeating the straight portion of the movement each time it circles to the center again. Honeybees also use two variations of the waggle dance to direct others to food sources closer to home. The round dance, a series of narrow circular movements, alerts colony members to the presence of food within 50 meters of the hive. This dance only communicates the direction of the supply, not the distance. The sickle dance, a crescent-shaped pattern of moves, alerts workers to food supplies within 50-150 meters from the hive. However, such dances should be thought as a continuum of one type of dance - the waggle dance and not multiple types of dances (Kirchner *et al.*, 1988). There is no evidence that this form of communication depends on individual learning.

The orientation of the dance correlates to the relative position of the sun to the food source, and the length of the waggle portion of the run is correlated to the distance from the hive. Also, the more vigorous the display, the better the food. There is no evidence that this form of communication depends on individual learning. Von Frisch performed a series of experiments to validate his theory. Other honeybee species have a similar method of communicating resources to their nestmates. For example, in *A. florea* and *A. andreniformis* (the "dwarf honeybees"), the dance is performed on the dorsal, horizontal portion of an exposed nest. The runs and dances point directly toward the resource in these species, rather than relative to the sun. Although different species of honeybees have waggle dances, the duration of the waggle dance and the distance being communicated to her nestmates are unique. Such species-specific behavior suggests that this form of communication does not depend on learning but is rather determined genetically. Each honeybee species has a characteristically different correlation of "wagging" to distance, as well. Such species-specific behavior suggests that this form of communication does not depend on learning but is rather determined genetically. Honeybees might use both dance and pheromone to guide the nestmates to find the food source. Various experimental results demonstrate that the dance does convey information, but the use of this information may be context-dependent, and this may explain why the results of earlier studies were inconsistent (Nieh, 1998). In essence, both sides of the "controversy" agree that odor is used in recruitment to resources, but they differ strongly in opinion as to the information content of the dance. Honeybees also have ability on a cognitive map of visible landmarks for their food sources.

In addition to the waggle dance, honeybees use odor cues from food sources to transmit information to other bees. Some researchers believe the scout bees carry the unique smells of flowers they visit on their bodies, and that these odors must be present for the waggle dance to work. Using a robotic honeybee programmed to perform the waggle dance, scientists noticed the followers could fly the proper distance and direction, but were unable to identify the specific food source present there. When the floral odor was added to the robotic honeybee, other workers could locate the flowers. After performing the waggle dance, the scout bees may share some of the foraged food with the following workers, to communicate the quality of the food supply available at the location. Honeybees might use both dance and pheromones to guide their nestmates to find a food source. Various experimental results demonstrate that the dance does convey information, but the use of this information may be context-dependent, and this may explain why the results of earlier studies were inconsistent. In essence, both sides of the "controversy" agree that odor is used in recruitment to resources, but they differ strongly in opinion as to the information content of the dance. Honeybees also have the cognitive ability to visible recognize and learn landmarks for their food sources (Vladusich *et al.*, 2005).

## 5. Color, shape and odor learning in honeybees

Honeybees can distinguish color, shape and symmetry. They have good photoreceptor organs -- compound eyes located beside both side of the lateral head. Each compound eye consists of few thousand of ommatidia (Snodgrass, 1925; Suwannapong and Wongsiri, 1999). Each ommatidium is composed a crystal line lens. Within each ommatidium, light is focused onto eight light sensing cells (retinal cells) arranged in a radial pattern like sections of an orange (Giurfa, 1991; Giurfa *et al.*, 1995). The pigment cells ensure that only light entering the ommatidium -- roughly parallel to its long axis -- reaches the visual cells and triggers nerve impulses. The brain then takes the image from each tiny lens and creates one large mosaic-like picture. Workers of *A. mellifera* have about 4,000-6,000 ommatidia (Giurfa, 1991; Giurfa *et al.*, 1995; Giurfa *et al.*, 1996a; Giurfa *et al.*, 1996b; Suwannapong and Wongsiri, 1999).

Honeybees have trichromatic color vision. Each ommatidium consists of four cells that respond best to yellow-green light (544 nm), two that respond maximally to blue light (436 nm) and two that respond best to ultraviolet light (344 nm). This system enables the honeybee to distinguish colors, and this has been amply demonstrated in behavioral discrimination experiments (Chapman, 1998; Giurfa, 1991; Giurfa *et al.*, 1996a, 1997). Although honeybees perceive a fairly broad color range, they strongly differentiate six major categories of color: yellow, blue-green, blue, violet, ultraviolet, and also a color known as "bee purple", a mixture of yellow and ultraviolet (Chapman, 1998; Giurfa, 1991; Giurfa *et al.*, 1996a). However, bees see red poorly. Differentiation is not equally good at all wavelengths and is best in the blue-green, violet, and bee purple colors.

In addition, honeybees can discriminate various shapes and patterns, inability useful in recognizing different flowers and in local landmark orientation (Giurfa *et al.*, 1995, 1996b). Honeybees can easily differentiate between solid and broken patterns, but show a preference for broken figures (Giurfa *et al.*, 1995). Honeybees also have three smaller eyes in addition to their two compound eyes. These simple eyes that are called ocelli (singular: ocellus), are located near the top of a bee's head. The ocelli only provide

information about light intensity; they cannot resolve images (Chapman, 1998; Giurfa, 1991; Giurfa *et al.*, 1996a, 1996b).

Honeybees collect the nectar and pollen of flowers as food for their colony. When performing this task, honeybees show flower constancy (Gohlke, 1951-1961); individual honeybees exclusively visit flowers of the same species as long as nectar is provided. However, honeybees are not explicitly specialized to forage on specific flower species. They are generalists in their ability to learn the color, odor and shape of all kind of flowers (Backhaus, 1993). Honeybees have ability to learn and remember the color, shape, and fragrance of flowers that are bountiful in these nutrients, and also how to get to them. A bee can learn a new color in about half an hour (after it has made about five visits to collect a food reward), a new pattern in about half a day (after 20–30 rewarded visits), and a new route to a food source in about 3 to 4 visits. It can learn to visit different species of flowers at different locations, at different times of the day (Srinivasan, 2010).

## 6. Factors impacting honeybee population leading the pollination decline

A significant decreasing colony population in many countries evokes concerns about pollination of crops and wild plants. For four consecutive years, the U.S. has experienced combined colony losses far greater than the level considered normal by beekeepers (vanEngelsdorp *et al.*, 2010). European countries have also reported elevated rates of colony mortality and isolated cases of colony abandonment. The continued collapse of honeybee populations may threaten the success of pollination services honeybees provide. While the cause or causes for these losses are not yet completely understood, most researchers agree that it is not due to a single factor (Oldroyd 2007), involving global warming, honeybee pests and diseases, pesticides, and colony collapse disorder.

### 6.1 Global warming

Global warming and climate change may affect the relationship between plants and their pollinators. Numerous studies have already shown advanced flowering times (Abu-Asab *et al* 2001), and other pollinators, such as butterflies, are also peaking earlier in the season (Roy and Sparks, 2000).

Honeybees forage in extreme weather conditions, however the level of pollination has recently dropped as a result of honeybee population decline (often associated with CCD).. Observed losses would have significant economic impacts. Possible explanations for pollinator decline include habitat destruction, extensive use of pesticides, pathogens, parasites, and changing environmental conditions. These factors affect indigenous bee populations in their natural habitat by reducing the availability of both foraging area and nesting locations. In some cases, the flowering period of a plant may not correspond to the peak or emergence of its corresponding pollinator (Jump and Peñuelas *et al* 2002). How honeybees might be affected by such changes remains unknown. Although, honeybees are generalists, and forage on many plant species, and display remarkable plasticity to various environmental conditions. For example, honeybees found in the oases of the Sahara are able to thrive in hot conditions (Ruttner, 1988) by collecting water for evaporative cooling to thermoregulate the colony. Under cold conditions, honeybees will form tight clusters inside the hive and create heat by isometrically contracting their thoracic muscles (Seeley, 1996).

Not all plant-pollinator mutualisms are at risk due to climate change (Rafferty and Ives 2011). It also affects populations isolated by habitat fragmentation, possibly limiting the expansion of bee-dependent plant species that may shift, especially for native bees (Opdam and Wascher 2004). Thus, encouraging the diversity and yield of native plants may foster the growth and sustainability of native bee populations, which are currently in decline and are also important pollinators (Luck *et al* 2003; Kremen *et al.* 2002).

To protect bees farmers should avoid using pesticides or other chemicals, as honeybees often suffer ancillary mortality. Beekeepers should be encouraged to use native plant species instead of exotics. They should also increase the diversity of bee flora in order to increase foraging and crop yields. Most honeybee species are generalists, feeding on a range of plants through their life cycle. By having several plant species flowering at once, and a sequence of plants flowering through spring, summer, and fall, it is possible to support a range of honeybee species that pollinate throughout the season.

## 6.2 Pests and diseases

There are numerous pests and diseases afflicting honeybees. Among them, Varroa mites and Nosemosis are of most concern to honeybee researchers and beekeepers (Fries, 2010; Genersch 2010).

Varroa mites (*Varroa destructor*) were introduced in the U.S. during the 1980s and have contributed to the decline of healthy honeybee colonies (Committee on the Status of Pollinators in North America, 2006). These mites are external parasites that attach themselves and feed on the developing pupae inside the colony, eventually reproducing. Once the adult honeybee emerges, mature Varroa will spread to other bees and developing brood. Parasitized bees that emerge develop with reduced fitness, including reduced lifespan (DeJong and DeJong, 1983), improper development of hypopharyngeal glands (Schneider and Drescher, 1987), and possess deformed wings (Akratanakul and Burgett, 1975). Drones that are parasitized are known to have reduced sperm quality (Collins and Pettis, 2001).

While methods to control populations of Varroa are available (i.e., acaricides), few options are available to beekeepers that completely eliminate the parasite, making their colonies susceptible to future infections (Afssa, 2009).

*Nosema* is considered one of the most widespread adult honeybee diseases, and although *Nosema apis* has been known to beekeepers for more than one hundred years, the emergence of a new species, *Nosema ceranae*, and its dissimilarity from pathology and epidemiology than *N. apis* (Fries, 2010), has renewed interest in how this emergent disease contributes to the recent global decline of honeybees (Paxton, 2010).

*Nosema* primarily affects the digestive system of adult honeybees, resulting in malnutrition, and ultimately, host death (Malone *et al.*, 1995). It also disrupts the proper development of the host's hypopharyngeal glands (Suwannapong *et al.*, 2011), which function to produce proteinaceous food for developing brood and other adult bees, and assist in converting nectar to honey in forager bees and food storers (Seeley, 1996). Colonies that are known to be strong by a beekeeper may experience a sudden collapse of the colony due to the inability of the queen to replace the loss of infected bees (Higes, 2008). The use of fumagillin to



control *Nosemosis*, when used and stored properly, can be effective, and the lack of finding chemicals of the active ingredient in collected honey is also promising for continued and commercial use (Higes, 2011).

### 6.3 Pesticides and chemical spraying

Pesticides are substances used to eliminate unwanted pests. Unfortunately, honeybees are insects and are greatly affected by insecticides. Pesticides have received much attention by beekeepers as the primary cause of colony declines (Doucet-Personeni *et al.*, 2003; vanEngelsdorp *et al.*, 2011). There are several ways honeybees can be killed by insecticides. One is direct contact of the insecticide on the bee while it is foraging in the field. The bee immediately dies and does not return to the hive. In this case the queen, brood and nurse bees are not contaminated and the colony survives. The second more deadly way is when the bee comes in contact with an insecticide and transports it back to the colony, either as contaminated pollen or nectar, or on its body. The main symptom of honeybee pesticide kill is large numbers of dead bees in front of the hives. Another symptom is a sudden loss of the colony's field force. After a honeybee pesticide loss the colony may suffer additionally from brood diseases and chilled brood. In this case the queen, brood and nurse bees are not contaminated and the colony survives. The second, more deadly way is when the bee comes in contact with an insecticide and transports it back to the colony, either as contaminated pollen or nectar or on its body. Honeybee colonies that are exposed to pesticides typically have a large number of dead bees in close proximity to the hive. Another symptom is a sudden loss of the colony's field force. Exposure to pesticides may make them more susceptible to other diseases, such as *Nosema* (Alaux *et al.*, 2010; Vidau *et al.*, 2011).

Colonies that have been exposed to pesticides may recover if proper steps are taken. If a colony has lost a majority of its field force but has abundant honey and pollen it will usually recover without assistance from the beekeeper. If brood and nurse bees continue dying, the pesticide is present in the hive, probably in the pollen supplies. The colony will continue to die as long as the poison remains in the hive. In these cases the combs must be cleaned or removed and soaked in water for 24 hours. The pollen should be washed from the cells and the combs allowed to dry. Another method is to remove the wax comb and replace it with new foundation. To help colonies recover from bee poisoning, the colonies should be fed sugar syrup, pollen, and water, and then a package of bees should be added or weak colonies combined. The bees should be protected from heat or cold, and then moved to a pesticide-free area with natural nectar and pollen sources.

Bees poisoned with a pesticide will contaminate nectar and pollen back at the hive, especially when bee farmers use the wrong condition or formulations. This includes spraying flowers at inappropriate times when honeybees are actively pollinating. In addition, honeybees also have an abnormal communication dance on the horizontal landing board outside the hive after being exposed to pesticides (Johansen, 1977). Moreover, application of sublethal doses of parathion causes mistakes in communicating time sense, distance and direction of feeding sites (Johansen, 1977).

With significant and increasing colony population declines in many countries there is concern about crop and wild plant pollination. The inappropriate use of pesticides has negative effects on honeybee colony populations. Fortunately, pesticides are very well



regulated to control specific host or target species that would otherwise consume the plants and if used appropriately should not have effects on non target species. Pesticides should be applied in the evening hours because honeybees forage during the daytime. The appropriate choice of formulation and less toxic pesticide is also important for reducing associated honeybee mortality.

Most recent research related to pesticides and honeybees has been focused on how pesticide exposure affects their behavior. Neonicotinoids, found systemically throughout all tissues of treated crops and target the nicotinic acetylcholine receptors (nAChRs) in agricultural pests, are potentially the most harmful insecticides to honeybees (Decourtye *et al.*, 2010). Particularly, imidacloprid decreases foraging rates (Yang *et al.*, 2008), olfactory learning (Decourtye *et al.*, 2003; Decourtye *et al.*, 2004), and recruitment to food sources (Kirchner, 1999). Thiacloprid, another neonicotinoid, is toxic to honeybees (Decourtye *et al.*, 2010) and is also extensively used in honeybee pollinated crops (Moritz *et al.*, 2010). Residues of these insecticides are commonly found inside the hives of managed colonies (Mullin *et al.*, 2010; Chauzat *et al.*, 2009), yet no clear relationship has been made between pesticides and the increased loss of managed honeybees.

#### 6.4 Colony collapse disorder (CCD)

Colony collapse disorder is a syndrome that is characterized by the sudden loss of adult bees from the hive. Many possible explanations for CCD have been proposed, but no one primary cause has been found. The number of managed honeybee colonies has dropped caused by combination of many factors such as pathogens, pests, diseases, pesticides and genetic modified plants, this means pollinator declines in general, have become serious environmental concerns.

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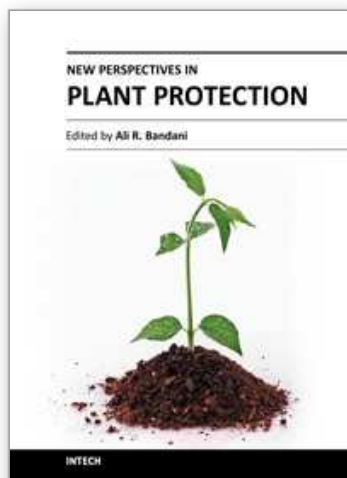


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## **New Perspectives in Plant Protection**

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Crop losses by pests (insects, diseases and weeds) are as old as plant themselves but as agriculture are intensified and cropping patterns including the cultivation of high yielding varieties and hybrids are changing over time the impact of the pests becoming increasingly important. Approximately less than 1000 insect species (roughly 600-800 species), 1500 -2000 plant species, numerous fungal, bacterial and nematode species as well as viruses are considered serious pests in agriculture. If these pests were not properly controlled, crop yields and their quality would drop, considerably. In addition production costs as well as food and fiber prices are increased. The current book is going to put Plant Protection approaches in perspective.

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