RATTAN SPINY MORPHOLOGY AND LITTER COLLECTING STRUCTURES IN ASSOCIATION WITH ANT COLONIES

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RATTAN SPINY MORPHOLOGY AND LITTER COLLECTING STRUCTURES IN ASSOCIATION WITH ANT COLONIES

by

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ABSTRAK

Rotan merupakan pokok palma yang biasa dijumpai di hutan Malaysia tetapi tumbuhan ini jarang diketahui ramai selain dari nilai ekonomiknya dalam pembuatan perabot dan tikar. Banyak spesies rotan mempunyai duri yang banyak dan aturan yang berlainan. Tetapi, kurang ada kajian dilakukan mengenai aspek struktur dan keunikan fungsi duri. Kajian ini memfokuskan stuktur duri rotan yang terdapat pada lima spesies rotan yang biasa dijumpai di bahagian utara Semenanjung Malaysia, iaitu *Daemonorops lewisiana, Daemonorops geniculata, Calamus castaneus, Plectomia griffithii* dan *Korthalsia scortechinii*. Panjang, lebar, sudut condong, kepadatan dan kekuatan duri rotan telah diukur serta dibanding sesama lain untuk mengetahui spesies yang mana mempunyai keupayaan yang paling bagus dalam melindungi rotan tersebut. Ciri-ciri rambut daun pada *D. geniculata*, *D. lewisiana,* dan *C. castaneus* telah direkod. Tidak ada spesies rotan mempunyai struktur pertahanan yang jauh lebih baik berbanding spesies lain dan hal ini disebabkan setiap spesies mempunyai kelebihan tersendiri. *D. geniculata* mempunyai duri yang paling panjang; *D. lewisiana* mempunyai duri yang paling kuat; duri pada *C. castaneus* mempunyai kepadatan yang paling tinggi dan duri yang berarah ke bawah pada *P. griffithii* mungkin berkesan dalam menghalang binatang mamalia pemanjat kecil. Tiada ciri yang unik terdapat pada struktur duri *K. scortechinii* tetapi duri spesies ini masih dapat mempertahankan dirinya dengan koloni semut yang mendiami pada stuktur okrea duri spesies ini. Rotan bergantung pada pelbagai jenis strategi pertahanan dan struktur duri

merupakan salah satu strategi pertahanan. Dalam kajian ini, banyak koloni semut telah dijumpai pada spesies rotan tertentu. Kajian seterusnya memfokuskan hubungan antara rotan dengan semut, spesies semut yang terdapat pada empat spesies (*D. geniculata, D. lewisiana, C. castaneus,* and *K. scortechinii*). Tiada bukti yang menunjukkan bahawa terdapat hubungan obligat antara rotan dan spesies semut. Spesies semut yang berlainan telah dijumpai pada rotan dan semut-semut tersebut mempunyai fungsi yang berbeza. Tetapi, tiada koloni semut didapati pada spesies *P. griffithii*. Kecuali *K. scortechinii* yang menyediakan domatia (okrea yang berkembang) kepada koloni semut, tiga spesies yang lain tidak menyediakan stuktur yang sama kepada koloni semut tetapi koloni semut dapat dijumpai dalam sampah dedaun yang jatuh pada stuktur duri dan daun. Terdapat lebih banyak koloni semut pada rotan yang menyimpan sampah dedaun (*D. lewisiana* dan *C. castaneus*) berbanding dengan jenis rotan yang tidak menyimpan sampah dedaun (*P. griffithii*). Bab yang selanjutnya memberi fokus pada perbezaan struktur duri and anak daun yang terpadat pada dua jenis rotan. Keputusan menunjukkan bahawa *P. griffithii* tidak memiliki struktur yang dapat menyimpan sampah dedaun (duri yang tunjuk ke atas dan daun yang berupa corong), jadi spesies ini tidak dapat menyimpan sampah daun dari kanopi. Duri pada *D. lewisiana* dan *C. castaneus* mempunyai fungsi alternatif dalam pengumpulan sampah dedaun dan struktur tersebut dapat mengalakkan kolonisasi semut. Kesimpulannya, suatu adaptasi yang kompleks dan baru telah (mengumpul sampah dedaun dan provisi bahan membuat sarang) didapati pada rotan dan adaptasi tersebut mengalakkan interaksi antara rotan dan semut melalui susunan daun, anak daun dan duri. Di sebaliknya, rotan memperoleh faedah seperti pertahanan, peningkatan nutrien, serta pendebungaan daripada semut.

RATTAN SPINY MORPHOLOGY AND LITTER COLLECTING STRUCTURES IN ASSOCIATION WITH ANT COLONIES

ABSTRACT

Rattan is a common palm in Malaysian forests but rarely known except for their economic values in furniture or matting products. Many rattan species possess a great number of spines arrangement in various patterns. However, few studies have looked into the different aspects of those spiny structures and their unique functions. This study focused on rattan spine structures in five different species which are common in the northern part of Peninsular Malaysia; they are *Daemonorops lewisiana, Daemonorops geniculata, Calamus castaneus, Plectomia griffithii* and *Korthalsia scortechinii.* Spine length, width, inclination, density, and strength were measured, and comparison from every aspect was taken to find out which rattan species possess the greatest defensive abilities to protect themselves. The leaf hairs characteristics on leaflets of *D. geniculata*, *D. lewisiana,* and *C. castaneus* were also measured. The results showed that none of the species has an outstanding defensive weapon since every species have their advantages. *D. geniculata* has the longest spines; *D. lewisiana* has the strongest spines; *C. castaneus* has the greatest the number in density and *P. griffithii*'s down-pointing spines may effectively deter small climbing mammals. *K. scortechinii* has nothing special in its spiny structures but was still well defended by ant partners colonizing their ocrea structures. Therefore, a rattan plant may rely on multiple defensive strategies and spiny structures only contribute part of its defensive role. During the study, many ant colonies were found on certain species of rattan plants. Ants were founded on four rattan species (*D. geniculata, D. lewisiana, C. castaneus,*

and *K. scortechinii*) and the relationship between ant and rattan were studied. There was no evidence of an obligate relationship existed among the rattan and ant species. Different ant species colonized on those rattan plants and they may serve different services to the plants. However, no ant colonies were found on rattan *P. griffithii*. Except for *K. scortechinii* that directly provide domatia (swollen ocreas) to ant colonies, the other three rattan species have no prepared structure for ant colonies. Instead, ant colonies were found inside the leaf litter trapped inside their spiny and leaflet structures. The presence of ant colonies in litter-collecting rattan (*D. lewisiana* and *C. castaneus*) was significantly higher compared to non-litter-collecting rattans (*P. griffithii*). The differences in spine and leaflet structures between the two types of rattan were studied. Results showed that *P. griffithii* do not possess litter collecting structures (upward-pointing spines and funnel-shaped leaves) so the plant could not collect much leaf litter from the canopy. Hence, the spines of *D. lewisiana* and *C. castaneus* have an alternative function in collecting leaf litter and encourage ants to build colonies on them. It can be concluded that a complex and novel type of adaptation (litter-collection and provision of nesting materials) for rattan which promotes interactions between the rattan and ants through the arrangements of leaves, leaflets, and spines. In return, the rattan may benefit from ants' services, such as protection, nutrient enhancement, and pollination.

CHAPTER 1 GENERAL INTRODUCTION

1.1 Background study

Plants face numerous threats such as herbivores, pathogens, parasitic plants or competitors after germination. Among all the threats, herbivore may be the most hazardous to plants. Herbivores vary in size, from tiny invertebrates like homoptera which suck sap from plant vascular tissue to megaherbivores which can consume a large amount of plant tissue or even uproot the whole plant from soil (Herrera & Pellmyr, 2009). Herbivores can learn and choose different types of plant or different parts of plants as their optimal diets so that they can balance their nutrients and maximize their fitness while avoiding plant's defensive mechanism (Waldbauer & Friedman, 1991; Karban & Agrawal, 2002).

Herbivores also possess countermeasures against plants' defensive weapons. For example, mixed function oxidases are a group of enzymes which can detoxify foreign chemicals from plant materials (Feyereisen, 1999). Some herbivores can even sequester the chemical weapons from the plants and use it in their own tissues or organs to deter predators (Duffey, 1980). If herbivores cannot consume a plant or cannot digest the plant's tissue, the role could be carried out by their symbiotic partners. For example, leaf-cutting ants can cultivate fungus to breakdown cellulose, starch and xylan from plant tissues (Herrera & Pellmyr, 2009).

For plants, herbivory attacks are an inevitable hindrance in the growth process. To protect from tricky and crafty herbivores, plants have two choices to deter herbivores. One is using avoidance strategies and the other one is using tolerance strategies. Avoidance strategies can be further divided into escaping strategies and defensive strategies (Rosenthal& Kotanen, 1994). Plants can escape herbivores by

camouflaging themselves into background or mimicry of an unpalatable items from the surrounding environment. Plants can also alter their growth periods or fruiting time to avoid herbivores during peak periods.

For those plants which cannot hide from herbivores, chemical and/or physical defensive weapons are always armed and may accompanied with warning signals. Chemical mechanisms refer to plants' secondary compounds which may decrease the palatability of plant tissues by increase bitterness or nasty smells, lower the digestible nutrients, increase indigestible substances and also produce toxic compounds which harmful to herbivores. Physical defensive structures deter herbivores by direct contact with herbivores. Spinescence (spines, thorns and prickles), pubescence (trichomes and leaf hairs), sclerophylly (hardened leaves) and minerals (silica) are common physical weapons against a variety of herbivores (Hanley et al., 2007). Other plants which can neither decrease their attraction to herbivore nor find powerful defensive weapons to deter herbivore can only tolerate herbivore's attack. Increasing growth rate, photosynthesis efficiency or increasing tillering etc. are common strategies in tolerance (Strauss & Agrawal, 1999). Plant can also seek for assistance from their mutualistic animal partners to chase herbivores away (Herrera & Pellmyr, 2009). For example, *Macaranga* plants provide food bodies and nest sites for ants and in return ants protect the plant against herbivores (Itino et al., 2001; Itioka, 2005).

The armament race between plants and herbivores has been continuing for millions of years and herbivores are the main evolutionary pressure in shaping plants' defensive traits. Herbivores are also an important selective force to determine which plants' traits can exhibit greater fitness. However, the defensive strategies of an important plant in Malaysia tropical rainforests are rarely studied. Rattan (Subfamily: Calamoideae) is a well-known for the commercial value in matting, furniture mating

and medicines (Dransfield, 1979). Previous studies were mainly focused on how to cultivate rattan plants with more economic value and how to increase their yields (Dransfield, 1992; Xu et al., 2000). Although many rattan species have conspicuous spines, few studies explored on their interaction with other animals. What are the features that spine possesses, and what are the potential alternative functions of those spines? What kind of spine-animal interaction that occurs? Such questions remain unknown.

Several aspects in rattan spinescence were studied. This study investigated methods in measuring the characteristics of spiny structures, looking into the alternative function of their unique spine and leaves arrangements and also the plant-animal interaction. This study would potentially help us to gain more knowledge in exploiting the rattans' potential usages and promote better rattan harvest by reducing herbivores' damages. It is also crucial for us to save several endangered rattan species from extinction. The defensive traits on the rattan could be an insight into mutualistic or antagonistic relationship with animals.

1.2 General Objectives

To describe the spinescence of five different rattan species and to estimate their physical defensive abilities.

To study the ant-rattan relationships

To study the alternative function of spiny structures and to examine the leaf litter collecting structure in some rattan species.

1.3 Thesis structure

This thesis is the first investigation of rattan spiny structures based on protocols of plant traits measurements and close observation of rattan-ant relationships. My

research questions are: what is the general pattern of rattan spiny structureand how to measure it? Are there differences among different species which rattan has the most effective structural defensive ability based on their spiny structures?

Several aspects of spinescence were measured and compared among different species. It could be used as a standardised method to study other plants' spiny structures. After I observed several different rattan spiny structures, my research questions are: why they are arranged differently and are there other functions rather than defence? Spiny structures can be multi-functional and rattan plants may not only rely on spines for defence. After I observed many cases that rattan plants were bearing ant colonies. the relationship between rattan and ants were studied. What are those ants that colonized on rattan plants? Are there any mutualistic relationships between ant and rattan and what benefits they can get from each other? I noticed that some rattan plants always accommodate ant colonies while some do not. My research question is: why ant species prefer certain rattan plants only? Is it likely that ants preferred to build their colonies in rattan plants which collect leaf litter? Why do certain rattans can collect leaf litter but others cannot? I studied the unique characteristics of litter-collecting leaf and spiny structures of certain rattan species and proposed a new adaptation between ant species and litter-collecting rattan plants.

The second chapter is the literature review in theories and previous studies about plant defensive mechanism, ant-plant interactions and researches on rattan plants. The third chapter is the general methodology about my study locations, periods and the statistics I used to analyse field data. The fourth chapter is the first working chapter talking about the spiny structures of five different rattan species. The fifth chapter is the ant species I found on different rattan plants and possible services that ant could provide for rattan plants. The sixth chapter is to study the litter collecting structures of

certain rattan species and figure out whether ants are adapted to build their nests in litter-collecting rattans. The seventh chapter is a general discussion and the last chapter is a conclusion. Each working chapter was written as an independent manuscript and repetitive information may appear in certain parts of each chapter.

CHAPTER 2 LITERATURE REVIEW

2.1 Plant defensive mechanisms

Plant plays the most important role in our planet as producers that absorb energy from the sun and consumed by herbivores. The earliest evidence of terrestrial herbivorous activities was recorded in Early Devonian, when plant tissues were consumed by arthropod herbivores (Labandeira, 2007). Due to the lack of escaping abilities, stationary plants have evolved a range of defence mechanisms against herbivores to increase their survival and reproductive rates. On the other hand, herbivores also evolved numerous countermeasures and a great variety of interaction between animals and plants has contributed to the macroevolution of adaptive traits in our ecosystem.

However, not all defensive strategies are always ready to deter herbivores. Certain types of defence strategy only emerged after plants encountered an attack, which is classified as inducible defence. Some other types of defence are constantly present in plant, which I classified as constitutive defence. Spines, thorns and certain secondary products are induced defence (Herrera & Pellmyr, 2009). Inducible defence has an advantage that is they can save energy and resources when the plant face little pressure from herbivores. Therefore, the defensive traits exhibit in plants should match with the distribution of herbivores or ancient herbivores that exist once upon a time (Burns, 2013).

Plants' defence can also be categorized into physical defence (mechanical defence) and chemical defence. Chemical defence are well studied as researchers see a great variety of compounds in plant which has no specific role in plants' daily routine, i.e. growth, development and reproduction (Fraenkel, 1959). Secondary compounds

are by-products which are derived from primary metabolic pathways (Whittaker, 1970). They can be grouped as toxins if they are interfering with herbivores' important metabolic process or they can be grouped as deterrence if they can stop herbivores from eating the plant (Herrera & Pellmyr, 2009). The deterrence compounds are always associated with features such as offensive-smelling, bitter-tasting or hard-to-digest. Some compound can possess both features, for example, cucurbitin are both toxic and awful-tasting to a large group of beetles (Metcalf et al., 1980).

There are three common groups of plant secondary substances in defence, nitrogen compounds (Alkaloids), terpenoids (monoterpenes and saponins) and phenolics (tannins and flavonoids) (Harborne, 1991). Certain compounds may serve multiple functions in plant, for example, silica may have structural functions in grasses and also act as a defensive weapon that abrades herbivores diet and lowers the digestibility (Van Soest & Jones, 1968; Herrera, 1982). Plants are not guarded by chemical compounds may also be toxic with the help of other organisms. Grasses can produce toxic compounds (alkaloids) after they harbour fungal endophytes (Clay, 1989).

Compared to the mainstream theories of plant defence strategies in chemical defence, plant structural defence are lacking in research. The structural trait that plant display can not only be obvious protuberances but also small or microscopic modification in cell wall structures (Hanley et al., 2007). Therefore, any mechanical and anatomical traits that deter herbivores directly can be considered as structural defence and plant can gain evolutional advantages by possessing structural traits. Karban, & Baldwin (1997) emphasized that the defensive trait that confers a fitness to plant is beneficial under the presence of herbivores. Traits with no physical contact with herbivores cannot be considered as structural traits. Several types of traits that

belong to structural traits can be categorized as spinescence (spines, thorns and prickles), pubescence (hairs and trichomes), sclerophylly (hardened leaves) and granular minerals inside plant tissues (Hanley et al., 2007).

However, the morphological structures are not necessarily for the herbivory pressure adaptation. For example, evidence showed that three Berberis displayed longer spines after fire (Gowda & Raffaele, 2004). Certain morphological changes maybe due to the responses to environmental stimuli. Nevertheless, Strauss & Agrawal (1999) argue about defensive trait as "a trait can be view as defensive even though defence is not its primary function." Many structural characteristics can also provide multiple functions. Leaves are densely covered by a layer of fine hairs (trichomes) which are vital in protecting leaves from herbivores (Werker, 2000). Grey willow (Salix cinerea) increase the trichomes density on their new leaves to stop leaf beetle (Phratora vulgatissima) from browsing (Dalin & Björkman, 2003). Pubescence may also prevent herbivore oviposition as female *Papilio troilus* prefer to lay eggs on the leaves without pubescence (Haddad & Hicks, 2000). Trichomes on some plants of *Datura wrightii* are glandular which deter herbivores by excreting a sticky exudate (Van Dam & Hare, 1998). Although researches also demonstrated that leaves with abundant trichomes will also deter predators' movement and decrease their searching efficiency (Krips et al., 1999), trichomes in many plants are also important in other functions to increase their physiological benefits. For example, trichomes play a role in water balance (Levin, 1973), gas exchange and temperature maintaining (Gutschick, 1999). Hairs also help leaves reduce UV radiation impact (Manetas, 2003). In arid environment, pubescence helps lower temperatures and water loss in daytime during the hot period while maintaining a relatively high leaf temperature during cold weather at night (Press, 1999). Sclerophylly also helps leaves resist wilt and increase leaf life

span (Chabot & Hicks, 1982), maintain water (Lamont et al., 2002) and nutrients level (Chapin et al., 1993), this is in addition to their protective roles in reducing the palatability and digestibility of leaves or shoots tissues (Grubb, 1986) as well as decreasing the chewing effectiveness of herbivores (Perez-Barberia & Gordon, 1998).

A trait is an adaptation evolved under certain pressure or induced by certain stimuli in an environment which may come from biotic effects or abiotic effects. The defensive trait may not be the direct response from herbivores but a 'natural resistance' (Edwards, 1989) and be cautious about the alternative function of a defensive trait (Hanley et al., 2007). Spinescence is a term that describes the characteristic of plant structural trait, spines, thorns and prickles. Spines, specifically, are modified leaves, thorns originated from modified branches or twigs, while prickles come from cortical or epidermal tissue (Cornelissen et al., 2003). Grubb (1992) used 'spine' to describe any projection with a stiff sharply point, which is a big set of term including thorns and prickles.

Although there are various spiny structures in different plant species and they originated from different plant tissue, Cornelissen et al., (2003) argued that spinescence have an obvious function in plant's defence and Hanley et al., (2007) claimed that spinescence play an evolutionary role in deterring herbivores. If spinescence is a weapon against herbivores, there should be an increase in number of plants with spinescence in areas with relatively higher herbivore pressures. In Africa, spiny structures are a common feature in areas where browsing pressure is high due to the megaherbivores (Brown, 1960). In arid places of southern Africa, plants in moist environments tend to be more spinescent since herbivores also prefer to assemble in sites of similar condition (Milton, 1991). When megaherbivores face pressure from

large carnivores, their pressure exert on plant are weaker and plant species are less thorny in areas with the presence of herbivores' predators (Ford et al., 2014).

Even though megaherbivores have gone extinct for many years, pressures from human-introduced livestock still exert onto the spinescence as palm species possess longer and denser leaf spines in areas with cattle compare to areas without cattle (Goldel et al., 2016). In Australia and New Zealand, structural defence disappeared on plants in offshore islands where herbivores have never set foot on (Burns, 2013; Burns, 2016). *Damnacanthus indicus*, has smaller leaves in regions with deer compared to regions free of deer (Takada et al., 2001). Presence of spinescence does not only match with regional distribution of herbivores, it also matches the ontogeny of plant species. For example, juvenile trees of *Acacia tortilis* and *A. nilotica* are physically well-defended compared to the adult trees because the adult trees are too high for the consumption by mammalian herbivores (Brooks & Owen-Smith, 1994). In Western Australia, shrubs vertically increase their structural defence since megaherbivores are capable to reach and feed on adult plant while shrubs in Eastern Australia, shrubs vertically decrease their structural defence since adult plant are unreachable for herbivores (Burns, 2013). Similar patterns show in New Zealand plant *Pseudopanax crassifolius* where leaves are no longer spiny after they reach adult stage above 3 meters, which is the highest point an avian herbivore can reach (Fadzly et al., 2009; Burns, 2016). The behaviour of spinescence due to the presence of herbivores indicates that plant structural traits are induced by herbivores' pressure and several studies found a significant increase in plant spinescence after the plants was consumed by herbivores. Leaf spines of European holly, *Ilex aquifolium*, exhibited more branching structures and smaller leaves with higher spinescence after herbivory attack, but undisturbed plants showed a decrease in leaf spinescence (Obeso, 1997). Spines on the leaves of

American holly, Ilex opaca, were initially thought to help cool the plants, but they were found in more abundance on shadowed leaves rather than the leaves under higher sun exposure. American holly deployed more spines in southern areas where the number of herbivory was higher than the northern areas and herbivores preferred leaves with fewer spines (Supnick, 1983). *Acacia depranolobium* have longer thorns after browsing by domestic goats compared to trees that were never browsed by domestic goats (Young, 1987). *Solanum lycocarpum* trees showed a significant increase in spine abundance and spine length after they were attack by moths and moths preferred leaves with shorter and fewer spines (Alves-Silva & Del-Claro, 2016). The abundance of palm stem spine remained the same, but leaf spines increased significantly with the presence of livestock (Goldel et al., 2016)

Even though evidence showed that spinescence could be induced by abiotic factors (Gowda & Raffaele, 2004), majority of the spines are inducible weapon to deter herbivores and several studies proved the effectiveness of spinescence in deterring different herbivores. Cooper & Owen-Smith (1986) studied the impact of plant spinescence on megaherbivores and found that spiny structures can reduce the effectiveness of browsing by restricting the bite size, retarding biting rate, eventually reduce the tissue and foliage loss every time when a plant is fed by a megaherbivores. Belovsky et al., (1991) found that spinescence have no effect on feeding rates, but a reduction in biomass ingested among five herbivore species existed, ranging from small mammal rabbits to large herbivores kangaroos. An increase in spine density of *Acacia tortilis* can reduce the pruning rate of goats and spines can also protect twigs (axillary meristems) which grow new leaves (Gowda, 1996). Midgley et al., (2001) suggested that thorns in African *Acacia* are mainly functions as protective weapons for stems but plays minor role in protecting leaves. Researches also showed that plants

suffer more damage after the removal of spinescence, which further proved the importance of plant defensive function. Wilson & Kerley (2003) found that plant spinescence can limit the intake rate of herbivores and removal of spinescence can remove the restrictions of feeding style and herbivores can enlarge their bite and eventually achieve higher intake rate. A unique experiment designed by Cooper & Ginnett (1998) indicated that spines on stems can decrease the foraging efficiency of small climbing mammals and woodrats, where these animals can get access to more branches of shrub after the thorns were removed from the plant. Removal of thorns on branches of Acacia seyal plant suffer significantly greater damage from browsers (Milewski et al., 1991). Deer were feeding more on seedlings of two *Acacia* species after their thorns were removed (Cash & Fulbright, 2005).

Many studies above have proven that spines are defensive weapons. The effectiveness of spinescence in deterring herbivores, however, has been questioned in several cases. The marginal leaf spines of American holly, *Ilex opaca*, are not the key factor to deter caterpillars but the glabrous cuticle and tough margins of leaf take a greater part in deterring invertebrate herbivores. Rabbits and deer showed little discrimination between foliage with or without spinescence (Potter and Kimmerer, 1988). Considering the relative size of spines and distance between each spine, it can be assumed that spines are evolved under vertebrate herbivores' pressure and they have little effects in deterring invertebrate herbivores.

However, consideration should be given that plants may not solely rely on spine for defence and plants may have multiple defensive traits, which were described as 'plant defence syndrome' by Agrawal & Fishbein (2006). Theoretically, spines may have other functions but they play an ambiguous role in defending herbivores. Other than a protective role, spines on cactus may benefit the plant in other ways, such as

helping zoochorous dispersal (Bobich & Nobel, 2001), conducting water toward roots (Benson et al., 1982) protecting stem from freezing temperature (Loik & Nobel, 1993), extreme hot temperature (Nobel et al., 1986) and maintaining rate of photosynthesis in extreme circumstance (Loik, 2008). Recurved spines help rattan plants or climbing palms to hold onto other plants and scramble up to higher level of canopy level of the forests (Dransfield, 1979; Putz, 1990). Spines of acacia trees are swollen, and they provide nest sites to their mutualistic ants (Young et al., 1996). The presence of multi-functioning spines could explain that spines may not be induced by one stimulus, such as pressure from herbivores, since protective role may not be the primary role of spinescence. An additional physical barrier other than spinescence is 'divaricate branching'. Divaricate plants' branches deploy wide angles and they interweave each other with very small leave (Burns, 2016). Divaricate branching is a common feature in plants found in New Zealand and it is considered as a defensive trait against avian herbivores, extinct moa. (Greenwood & Atkinson, 1977; Lee et al., 2010). Bond et al., (2014) proved that avian herbivores (emus and ostriches) faced difficulty when feeding on divaricate plants and plants with divaricate branching experienced less damage compared to non-divaricate plants. Researches also showed that the divaricate branching can tolerate wind (Darrow et al., 2001), avoid photoinhibition (Howell et al., 2002) and build microclimate to prevent water-loss and damage from frost (McGlone & Webb, 1981). Therefore, structural traits are not always induced by herbivores and plant may keep such structural features regardless the presence of herbivores since they have alternative roles.

To avoid herbivores' attack, the structural traits and chemical traits described above are all belong to defensive traits. However, stationary plants can also rely on other traits, such as escaping strategies, to avoid herbivores. Changing of the

phenology of leaf production is an escaping strategy to avoid the consumption by herbivores on young leaves (Aide, 1988). Plant produce young leaves during the time when herbivores are rare (Aide, 1992; Coley & Barone, 1996). Young leaves can also flush synchronously in order to saturate the need of herbivores and some leaves may escape from damage (Aide, 1993). Yong leaves which delay greening can also avoid herbivores attention (Coley & Barone, 1996).

Another escaping strategy is certain plant can mimic other coloration to be invisible. In other words, plants try to avoid herbivores' attention. Many herbivores rely on their vision to seek for food. Hence, plants which are visually obvious are found to be heavily defended by chemical weapons (Yamamura & Tsuji, 1995). Conversely, other plants choose to be inconspicuous in an environment so that visually dependent herbivores may only notice conspicuous plants. Camouflage strategy is the trait that helps an organism blend in with its surrounding environment. This strategy is common in the animal kingdom but plants' camouflage strategies can be divided into two categories, which are mimicry and crypsis (Wainwright, 2017). Crypsis is described as the situation where the appearance of plant resembles its background image in order to reduce its herbivores' detection. This strategy is normally the first strategy of plants' ontogeny as seedling plants out of the ground can perfectly blend in background coloration. Burns (2010) concluded 4 hypothesis for the crypsis strategy to be viable, (1)plant's predators must be visually orientated and foraging in daytime; (2)plants need grow in a special habitat but not widely spread in every common habitat which background colour may be different; (3) height of the plant must be lower, the closer to the ground the harder to be found; (4) the background appearance must be unpalatable so that the background itself will not attract any attention from herbivores. In New Zealand, the juvenile plants of *Elaeocarpus hookerianus* possess various

shaped leaves with mottled brown in colour, which is similar to the colour of background leaf litter (Fadzly & Burns, 2010). Another plant in New Zealand is *Pseudopanax crassifolius*, which seedling stage appearance also similar to the background litter colours (Fadzly et al., 2009, Burns, 2010). In tropical forests, cryptic coloration of seedling plants which resemble the background colouration can be found in *Macaranga bancana* and (Fadzly et al., 2016) and *Amorphophallus bufo* (Liu et al., 2017). The plants changed their colour and appearance after they grow higher since the strategy is no longer effective, but plants can deploy alternative defence strategies throughout their ontogeny. The dry vegetative bracts covering *Monotropsis odorata* have a similar coloration to ambient litter. Klooster et al., (2009) removed the bracts of *Monotropsis odorata* and found the plant suffered more herbivores' attack. Therefore, the cryptic coverage helps the plant to avoid herbivores. Leaf colour of *Corydalis benecincta* are dimorphic, which presents two different colours (grey and green). Niu et al., (2014) found that herbivores can hardly distinguish the grey leaves from background grey rocks colour so grey morphs suffer less herbivory and have higher survivor rate.

Another type of camouflage strategy is that plant mimic an unpalatable object from surrounding environment, which are called masquerade (Wainwright, 2017). Herbivores are able to spot the presence of the plant, however, will not see it as an edible object (non-plant-mimicking defensive masquerade) or the animals deem it as an edible plant parts, but the parts are not appealing at all (plant-mimicking defensive masquerade) (Lev-Yadun, 2014). Australian mistletoes resemble their non-palatable hosts are considered as a protective strategy against herbivores (Barlow & Wiens, 1977). Although studies showed that herbivores do not distinguish between host-mimicry plant and non-host-mimicry plant, it is found that the plant with no visually

mimic strategy were lower in nutrient contents (Canyon & Hill, 1997). Another mimic strategy used by epiphytic woody vein, *Boquila trifoliolata*, mimics the leaves of their host plants' foliage and suffer less damage compared to unsupported veins and veins climbing on leafless trunks (Gianoli & Carrasco-Urra, 2014).

Defensive masquerade strategy is not necessary to be less conspicuous. In contrast, plants may use bright colour to mimic unappealing patterns (i.e. insects damaged tunnels) to lower the attraction of herbivores or even warn herbivores with aposematic coloration (Lev-Yadun, 2014; Lev-Yadun & Niemela, 2017). Dark spots in plant *Xanthium trumarium* mimics numerous ants crawling on the plants; conspicuous reddish spots on the pods of three annual legumes which resemble caterpillars and the aphid-like dark anthers on *Paspalum paspaloides* may have potential visual deterrence against herbivores (Lev-Yadun & Inbar, 2002). Plants can also pretend to be attacked by fungal or herbivores to reduce herbivore's tendency to feed on the plants. The white coloration on leaves of some coastal and sand-dune plants may be the mimicry of fungal attack to deter herbivores and insects form ovipositing on the leaves (Lev-Yadun, 2006). Leaves deploy fake appearance that they were attacked or damaged by insects, especially tunnelling damaged, signalling defensive information to later attackers (Yamazaki, 2010). If herbivores consume plants tissues which are formerly infested by other insects or pathogens, they may face certain risks, such as higher competition, cannibalism or predation, induced chemical or physical defences by damaged plants (Yamazaki, 2010; Lev-Yadun & Niemela, 2017). Hence, herbivores may avoid eating plants that are occupied, damaged or infested and plants which fake those signals (pseudo-variegation) may gain an advantage in evolutionary selection.

The visual signals of plant targeted for herbivores are not necessary to mimic images or patterns but they can also be conspicuous warning colours. The physical defensive weapons (spines, thorns and prickles) are always associated with aposematic coloration (Lev-Yadun, 2001; Lev-Yadun, 2009a). The spiny plants in genus *Launaea* are white variegated, which are conspicuous structural signals providing indirect protection to another four non-thorny plants with white variegated leaves (Lev-Yadun 2009b). Leaves of *Silybum marianum* in Israel resemble green zebras and the special pattern serves as an aposematic coloration against herbivores (Lev-Yadun, 2003). In the animal kingdom, predators will avoid colourful preys as they associate colourful signals with unpalatabilities and non-toxic animals mimic those warning signal to avoid predators (Cott, 1940; Harvey et al., 1982). Hence, herbivores may also acquire the sense to avoid plants with aposematic colorations.

The intention of acquiring these escaping and defensive traits is to avoid herbivores' attack. Some other plants do not develop such strategies to avoid herbivory, but they choose to tolerate attack from herbivores or environments. Tolerance is defined by Strauss & Agrawal (1999) as "the degree to which plant fitness is affected by herbivore damage relative to fitness in the undamaged state." To say that a plant can tolerate herbivory means that it can regrowth and reproduce after certain parts are consumed by herbivores. Tolerance is always interchanged with the term 'compensation' and compensation can be used to indicate the degree of tolerance which a plant possesses. The mechanisms behind plants tolerance against herbivores may involve several following compensatory responses, such as increasing their leaf photosynthetic rate (Houle & Simard, 1996), increasing the shoot regrowth rate (Danckwerts, 1993), increasing number of tillers/branches and leaves (Rosenthal & Welter, 19995) or higher reproductive efficiency and percent fruit set, relocation of

biomass from roots to shoot, decrease leaf longevity etc. (Mabry & Wayne, 1997). Considering the cost that plant needed to regrowth after herbivory, the allocation of resource to one strategy (tolerance) may suppress the resource to another strategy (defence), which is evident by the existence of trade-off between tolerance and defence (Fineblum & Rausher, 1995). Nevertheless, positive relation between tolerance and defence also proved in plants Arabidopsis thaliana (Mauricio et al., 1997) and *Salix planifolia* (Rosenthal & Welter, 19995). The mechanism of the trade-off between tolerance and resistance are still unknown but tolerance may influence the evolutionary path of plants' resistant traits and the diverse composition of plant communities (Rosenthal & Kotanen, 1994; Strauss &Agrawal 1999).

Since defensive and escaping traits are costly, plants' avoidance strategies directing resources to secondary compounds or structural weapons may eventually reduce those resources for growth or reproduce. Therefore, several hypotheses predict that there is a negative association between plant growth and defence (Stamp, 2003). Optimal Defence (OD) hypothesis predicts that there is a trade-off between a high-level of defence and a great impact on plant fitness from herbivores' damage (McKey, 1974; Rhoades, 1979). The Growth-Differentiation balance (GDB) hypothesis (Lorio, 1986; Herms & Mattson; 1992) and The Carbon-Nutrient balance (CNB) hypothesis (Bryant et al., 1983) have a similar prediction that plant will balance the allocation between growth and differentiation-related process (defence) and the resource directed to defence will result in resource diversion from other needs. The optimal defence theory will consider the value of plant tissues or organs and the probability of being attack, then allocate more resource to defend the most precious plant tissues or organs and the parts which has the highest chance to be consumed (McKey, 1974; Rhoades, 1979; Stamp, 2003). This hypothesis assumes that herbivores are the primary selective

force in shaping plants' defensive traits and when herbivores are absent, the less-defended plants have higher fitness than well-defended plants since they have more resource to be allocated in growth and reproduction (Stamp, 2003).Similarly, Feeny (1976) argued the relationship between 'apparent' plant and degree of defensive traits by predicting that the 'apparent' plant occupying enormous landscape or persisting for a long time should invest more in defence, while the 'unapparent' plant live in specific spots transiently with low possibility to be found by herbivores should invest relatively less in defence. Coley et al., (1985) considered the impact of nutrient availability on plant defence, which predicts that the plants in nutrient-poor sites are expected to invest more resource in defence as they have no fast-recovering ability while plants in nutrients rich areas are expected to invest lesser nutrients in defence since they have stronger ability to tolerate attack from herbivores. Nevertheless, Edwards (1989) pointed out that the primary selective force may not come from herbivores but 'neutral resistance'. Apart from defending herbivores, a defensive trait may also help resist harsh environment and the trait may be evolved before a plant experience the herbivory pressure. Therefore, a defensive trait may display alternative functions and the growth/reproductive abilities are not mutually exclusive with defence.

The resistant behaviours that plant deploy are a direct defence against herbivores, whereas plant can interact with animals in more diverse ways. Certain plants can protect themselves indirectly by producing a mixture of volatiles to attract herbivores' predators, parasites or other natural enemies (Aljbory & Chen, 2018). Plants can also build mutualistic allies with certain animal species and their animal partners may protect or help them in various ways. (Herrera & Pellmyr, 2009).

2.2 Ant-Plant interactions

The consumption from herbivores and the deterrence from plants are just one way in animal-plant interaction. In many ecosystems, no other animals can interact with plants as diverse as ants

Ants are distributed in extraordinary geographic range of ecosystem and they make up more than 10% of biomass in various ecosystems. They can also alter the local habitat dramatically as leading predators in many habitats and help direct energy/nutrients cycles (Herrera & Pellmyr, 2009). Ants belong to Family Formicidae under the order Hymenoptera. There are more than 10 000 species under 296 genera has been described around the world (Bolton, 1994). Ants existed approximately 100 million years ago and the oldest ant fossil (*Sphecomyrma freyi*) was found in the amber of Upper Cretaceous age (Wilson et al., 1967). Ants are highly socialized animals that live in organized colonies. Ants in their colonies can be divided as sterile workers and soldiers and fertile drones and queens based on their reproductive ability. Each individual of ants can work independently and cooperate other ants to show group behaviours so that an increase in overall success in duties can be achieved. The great teamwork as well as the diverse ways interacting with plants have contributed to ants' success in today's ecosystems (Herrera & Pellmyr, 2009).

Certain ant species are antagonistic to plants. The leaf-cutter ants are the herbivores of many plant species in tropical forests and savannahs of South America. The leaf-cutting ants (Attini) mainly cut grass or some dicots, some even collect flowers and fruits (Herrera & Pellmyr, 2009). Leaf-cutting ants cannot digest those leaves directly but rely on their mutualistic partner, a fungus (*leucoagaricus gongylophorus*) to produce enzymes to breakdown the plant tissues (cellulose, starch and xylan). They also detoxify insecticides and ant repellent produced by plants (North

et al., 1997). In return, ants remove other fungus competitors such as yeast, bacteria or alien fungus spores by antibiotic compounds produced by ants and the constant licking behaviours (North et al., 1997, Herrera & Pellmyr, 2009). Ants will also reject harvesting plant materials with fungicide which can damage their mutualistic fungus (Ridley et al., 1996). Currie et al., (1999) found that bacteria (*Streptomyces sp.*) on the cuticle of ants can produce specific antibiotics to supress the growth of parasite fungus (*Escovopsis*) and promote the development of mutualistic fungi as well. By the help of cultivated fungi, leaf-cutting ants are the leading herbivores in many habitats as garden fungi help ants digest nutrient from the plants and ants help maintain the fungi garden from infection and invasion from other organisms.

Nevertheless, the relationship between ant and plants are not always antagonistic. In contrast, myriads of ant species build mutualistic relationships with plants in various ways. Ants are dominant predators which forage a range of prey. The ancient Chinese take a good advantage of this feature of weaver ants. Colonies of weaver ants were placed on branches of orchard trees and prevented from leaving the tree. After weaver ants build colonies on the tree, a considerable number of invertebrate herbivores were removed and trees with ants' protection produce more fruits than trees without protection (Beattie, 1985). Experiments demonstrated that predation of wood-ants on trees significantly suporessed the population of non-tended aphid species and the trees which were protected by ants had significant lower number of Lepidoptera larvae and significant lower rate of defoliation than trees without ants' protection (Skinner & Whittaker, 1981).

As plant species enjoy the protection from ants, certain plant species offer rewards to their symbiotic ant species. Certain plants provide nest sites and domatia so that ants can live inside the plant and defend their colonies along with the plants.

Nest sites can be cavities, cracks or excavated tunnels by other animals and ants can modify those natural cavities into other shape or size by usage of soil particles, debris or carton (Beattie, 1985). The domatia of plants is a specific adaptation for ant colonies and ants were encouraged to occupy the interval cavities of the domatia on the particular part of plants (Herrera & Pellmyr, 2009). Plant species bearing domatia are known as myrmecophytes. The domatia structure evolved by plants can always be found on stems, leaves or spines. A famous example is the domatia of some *Acacia* species in America with expanded and hollow thorns (Janzen, 1966). Ants (*Psedomyrmex*) gnaw a small entrance out at the tip of the hollow thorns and moved in. *Acacia* plants also secrete nectar and Beltian bodies (food bodies containing proteins, lipids and carbohydrates) to ants and in return ants deter not only invertebrate herbivores but also mammalian herbivores, gnaw off invading veins or other plant competitors (Janzen, 1966; Janzen, 1967).

The mutualistic relationship between ants (*Pheidole*) and their host plant, piper (Piperaceae), showed positive results in removing stem borers, fungal spores and suppressed fungal activities after ants built colonies from the hollow cavity of sheathing leaf base to the stem pith tissue of the entire plant (Letourneau, 1998). myrmecophytes thrived in many tropical habitats, which takes up 380 individuals (16 plant species) per hectare associating with 25 species of ants (Fonseca & Ganade, 1996). Certain ant species even provide extra nutrients to the plants that offer them domatia. Stem cavities of *Hydnophytum formicarum* receive animal wastes from cohabiting ants and broke down those wastes for nutrient (Rickson, 1979). *Philidris* ants can also 'feed' on their mutualistic epiphyte *Dischidia major* by placing organic debris into their modified leaves (pitchers) where tissue of leaf wall could use those nutrients (Peeters & Wiwatwitaya, 2014).

Food bodies are a common reward from plants that provide lodging with ants. Food bodies are a great variety of small epidermal structures containing various nutrient such as protein and lipids to attract ant foragers (Beattie, 1985). Food bodies in which predominant metabolite substance is lipid are known as Pearl bodies while the vascularized food bodies with protein and lipid are known as Beltian bodies (Herrera & Pellmyr, 2009). Rickson (1969) observed the food bodies from the tip of each rachis and pinnule of *Acacia cornigera* as food source to their symbiotic ant *Pseudomyrmex ferruginea.* Tropical plant species from genus *Cecropia* even produce food bodies containing a great amount of animal glycogen, which is extremely rare in plants and ants were found collecting those Mullerian bodies avidly in order to build a mutualistic relationship with the plant and serve as protectors (Rickson, 1971; Rickson, 1973). Plant *Piper cenocladum* will not produce any food bodies if their specialist ant *Pheidole bicornis* are not present and the production of food bodies will be rebooted after their symbiotic ant species reinvade the plants (Risch & Rickson, 1981). Another famous example of plants defended by mutualistic ant partner is the *Macaranga* plants in Southeast Asian tropical forests. Heil et al., (1997) found that *Macaranga* plants invested up to 9% of above-ground biomass costs into their Beccarian bodies (type of Pearl bodies) and ant-inhabited plants produced much more food bodies than ant-free plants. Although the investment on food bodies is expensive to the plants, *Macaranga* can still benefit from the effective protection from ants' anti-herbivores strategies. The plant has a regulatory system to ensure that food bodies' investments were kept in high rates when ant partners exited (Heil et al., 1997).

Extra-floral nectaries (EFNs) are another common reward provided by plants. EFNs are secreted from leaves, twigs or flowers' external surface so they are not involved in the plants' pollination systems. They are not the rewards to pollinators

such as bees or wasps but as an attractant to ants for seeking their protection as a number of studies demonstrated that the exclusion of ant partner on the plants bearing EFNs resulted in an increased damage from herbivores and seed predators (Beattie, 1985). However, the effect of protection may not be consistence as Barton (1986) revealed that the different level of ant protection due to the densities of the ants and the type of herbivores among three separate population of *Cassia fascicul.* EFNs are a mixture of nutrients of sugars, amino acids and lipids that not only attract ants but also various other predatory animals such as wasps and spiders. Studies have shown that they can also benefit the plant offering EFNs by attacking herbivores (Beattie, 1985; Herrera & Pellmyr, 2009).

Plants that benefit from ants' protection may not only from direct protection, but also from indirect protection. The indirect ant-guard system is always complicated by involving herbivores in a three-way relationship (ants, plants and herbivores). The herbivores are Homoptera (Beattie, 1985) or lepidopteran larvae (Herrera & Pellmyr, 2009). Homoptera are sap-sucking herbivores such as aphids, leafhoppers, scale insects, mealybugs and coccids. Homoptera possess slender mouthparts (proboscis) penetrating vascular tissue and sucking sap from phloem directly. After passing through the gut of homoptera, sap will come out from the anus and become honeydew droplets for ants (Beattie, 1985). This honeydew contains a variety of nutrients such as different kinds of sugar, amino acids, alcohols, plant hormones, salts, amides and vitamins (Brian, 1977). Ants harvest honeydew from homopterans and in return they will tend and protect the insects from predators and parasites. Even though lepidopteran larvae are not plant-suckers, they will also produce honeydew for ants after chewing plant tissues (Herrera & Pellmyr, 2009). In addition to protection, ants also keep colonies of homoptera in good hygiene, which is crucial for their survival