Behavioral Interactions Between Coffee Berry Borers and Ants in Chiang Mai, Thailand

ปฏิสัมพันธ์เชิงพฤติกรรมของมอดเจาะผลกาแฟและมดในจังหวัดเชียงใหม่ ประเทศไทย

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(Received: 1 June 2016; Accepted: 26 August 2016)

บทคัดย่อ: การศึกษาเกี่ยวกับพฤติกรรมของมดที่ตอบสนองต่อตัวเต็มวัยมอดเจาะผลกาแฟ ได้ดำเนินการในสภาพห้องปฏิบัติการ มดที่ใช้ศึกษาจำนวน 7 ชนิดเป็นมดชนิดเด่นที่รวบรวมได้จากแปลงปลูกกาแฟในพื้นที่ภาคเหนือของประเทศไทย ซึ่งได้แก่ *Anoplolepis gracilipes*, *Camponotus nicobarensis*, *Crematogaster* sp. PM2, *Dolichoderus* sp. PM1, *Tapinoma* sp. PM1, *Technomyrmex modiglianii* และ *Technomyrmex yamanei* ในสภาพห้องปฏิบัติการ ทำการบันทึกลักษณะพฤติกรรมของมดที่มีต่อมอดเจาะผลกาแฟ ดังนี้ 1) การใช้หนวดสัมผัส 2) การจู่โจม และ 3) การขนย้าย พบว่ามดทั้งเจ็ดชนิดมีพฤติกรรมการเข้าจู่โจมมอดเจาะผลกาแฟ และบางชนิดทำการคาบขนย้าย ไม่พบมดกัดกินมอดเจาะผลกาแฟ แต่พบความเสียหายของมอดเจาะผลกาแฟหากมดเข้าจู่โจมอย่างรุนแรงและมีการคาบขนย้าย มด *Dolichoderus* sp. PM1 และ *Tapinoma* sp. PM1 พบว่ามีศักยภาพเป็นแมลงตัวห้ำของมอดเจาะผลกาแฟ สำหรับมด *A. gracilipes* และ *C. nicobarensis* มีการจู่โจมมอดเจาะผลกาแฟน้อย

คำสำคัญ: มด มอดเจาะผลกาแฟ *Hypothenemus hampei* กาแฟอะราบิกา การควบคุมโดยชีววิธี

Abstract: The behavioral response of ants to the adult of coffee berry borer (CBB) was studied in laboratory condition. The observed ants were seven dominant ant species collected from coffee plantations in northern Thailand i.e. *Anoplolepis gracilipes*, *Camponotus nicobarensis*, *Crematogaster* sp. PM2, *Dolichoderus* sp. PM1, *Tapinoma* sp. PM1, *Technomyrmex modiglianii* and *Technomyrmex yamanei*. The following ant behaviors against CBB were recorded in the laboratory: 1) Antennal contacting, 2) Attacking, and 3) Carrying. All species attacked CBB and some the species also carried the CBB. However, no ant species fed on the CBB. The CBB got damaged if ants intensively attacked or if they were grasped for carrying. *A. gracilipes* and *C. nicobarensis* were less aggressive against CBB, while *Dolichoderus* sp. PM1 and *Tapinoma* sp. PM1 seemed to be the more effective biological control agents for CBB.

Keywords: Ants, coffee berry borer, *Hypothenemus hampei*, arabica coffee, biological control

Introduction

Arabica coffee is the main cash crop for the hill tribes in the mountainous regions in northern Thailand after it was promoted by the government approximately 30 years ago (Riwthong *et al*., 2015). However, a major pest problem has been caused in some regions by the coffee berry borer (CBB, *Hypothenemus hampei*). Although the origin of CBB is not known, it is now found in all coffee producing areas worldwide, including Thailand (Vega *et al*., 2009; Jaramillo *et al*., 2011; Suttiprapan and Chanbang, 2014; Buranapanichpan and Chanbang, 2014; Thayaping and Suttiprapan, 2015). The fecundity of CBB is high, sometimes producing seven generations during one year (Le Pelley, 1968). Although insecticides have been widely used to control this pest, it is not always effective because the CBB spends most of its life inside the coffee fruit. In addition, CBB has developed a resistance to insecticides (Gonthier *et al*., 2013). Therefore, in recent years, the Thai government and institutions have recommended growers to apply female attractant traps using ethanol and methanol, the entomopathogenic fungus *Beauveria bassiana*, as well as cultural control. Moreover, in other coffee growing countries, the parasitic wasp *Cephalonomia stephanoderis* and predators such as birds, thrips and ants have been used for the biological control of CBB (Vega *et al*., 2009). In South American countries such as Mexico and Colombia, there are several reports on the effects of ants on the CBB. For example, *Pheidole synanthropica* was confirmed to carry the CBB to their nests, and *Azteca instabilis* that have a symbiotic relationship with scale insects on coffee trees expels the CBB (Jiménez-Soto *et al*., 2013). Besides these two ant species, the usefulness for the CBB control is expected for the following species; *Brachymyrmex* sp., *Camponotus* sp., *Crematogaster* sp., *Dorymyrmex* spp., *Gnamptogenys sulcate*, *Pseudomyrmex* sp., *Solenopsis* spp. and *Tetramorium* sp. (Jiménez-Soto *et al*., 2013; Philpott and Armbrecht, 2006). In Thailand, however, ants have not yet been considered as a biological control agent for the CBB. In this study, the ant behavior toward adult CBB was observed in order to know the potential of ants as biological control of the CBB.

Materials and Methods

The behavioral response of ants to adult CBB was observed in the laboratory. Colonies of ants were collected at the coffee plantation of Pa Miang village, Doi Saket district, Chiang Mai province (910m alt., 18°59'N, 99°20'E) on 17 June, 2015. Each ant colony that nested in rotten coffee branches on the ground were put into a plastic bag and brought back to the laboratory. Then, each branch was opened and all ant individuals were collected by an aspirator and moved to a plastic breeding case (20 x 15 x 7 cm). A small plastic case (8– x 5 x 1.5 cm or 6 x 3 x 1 cm) lined with plaster was put inside the breeding case as a nest chamber. Colonies containing only workers without brood were not used because they may not perform normal foraging behavior. Queenless colonies were used for this experiment. Colonies of the following ant species were collected in the coffee plantation; *Camponotus nicobarensis*, *Crematogaster* sp. PM2, *Dolichoderus* sp. PM1, *Tapinoma* sp. PM1, *Technomyrmex modiglianii* and *Technomyrmex yamanei*. All studied ant species are common in this plantation (Onishi *et al*., 2016). In addition, a colony of *Anoplolepis gracilipes* was collected from a coffee plantation of Teentok Royal Project Development Center (18°87'N, 99°32'E). This species was most dominant in that coffee region. The thorax length (mm) and head width (mm) of the ants used in this study were measured for comparing the body size of the CBB. The coffee berries containing adult CBB were collected and kept through the experiment.

After a CBB was introduced in the foraging arena of the breeding case, the behavioral response of the ants was recorded. Figure 1 shows the behavioral process of ants against the CBB. The following ant behaviors, adapted from De la Mora *et al.* (2008) were recorded: (1) antennal contacting: the ant contacted the CBB with its antennae before attacking it, (2) attacking: the ant attacked the CBB with its mandibles or abdominal tip, (3) carrying: the ant grasped the CBB with its mandibles and carried it. Furthermore, 'attacking' was divided into the following two types: (2a) short attacking: the ant attacked the CBB for less than one second, (2b) intensive attacking: the ant continuously attacked for more than one second. Once a worker touched a CBB, we observed its subsequent behaviors. The observation was repeated 20 times for each ant

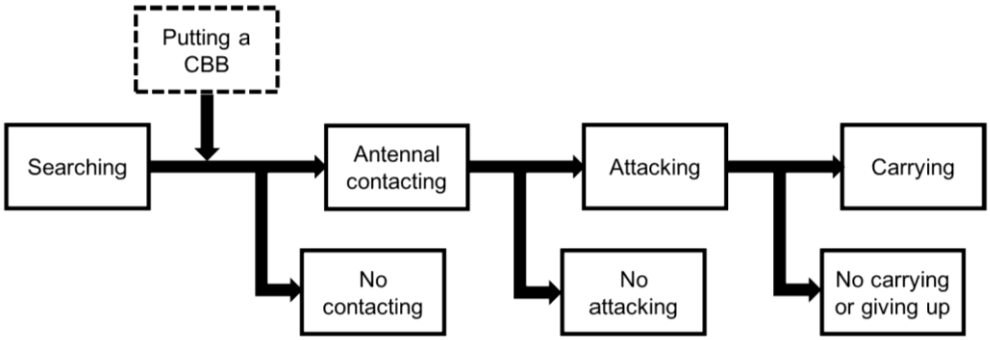

Figure 1. The behavioral process of ants against the CBB. Searching: the ant walked randomly at a moderate speed outside the nest case; Antennal contacting: the ant did contact the CBB with its antennae before the attack; No contacting: the ant did not show any interest for the CBB and did not contact it; Attacking: the ant grasped the CBB with its mandibles or attacked it with the abdominal tip; Carrying: the ant carried the CBB; Giving up: the ant could not lift the CBB and walked away

species. If ants did not recognize a CBB for 10 min, the observation was stopped. For 'attacking' and 'carrying', the duration of these behaviors was also recorded. The differences of behavioral response among species (the ratio of workers performing a given behavior) was examined by pair wise comparison using Fisher test. Multiple comparison of the average duration of the 'Attacking' and 'Carrying' behaviors among ant species was analyzed by Ryan-test using the statistical software R (R Core Team, 2013).

Results

The thorax length (mm) and head width (mm) of the ants used in this study were shown with body size (head width and total body length) of CBB (Figure 2). Among seven ant species, *Camponotus nicobarensis* was the largest species while *Tapinoma* sp. PM1 was the smallest ant.

Most foraging workers touched the CBB with their antennae when they encountered them in the foraging arena. The only exceptions were five workers of *C. nicobarensis* and one worker of *Anoplolepis gracilipes* that ignored the CBB for ten minutes until the observation finished. Figure 3 showed the frequency of each behavior after the ant contacted the CBB. Two to six workers of each species walked away without showing any aggressive behavior toward the CBB. The ratio of such indifferent workers was not different among species (pairwise comparisons using Fisher test). The remaining workers showed short time aggression or intensive aggression. The comparison among the seven ant species indicated that a significant difference in the ratio of workers showing intensive aggression was only found between *Dolichoderus* sp. PM1 and *T. yamanei* (P = 0.023). Figure 4 showed average duration of intensive attacking and carrying performed by each ant species. The duration of intensive attack varied from a few to 150 seconds, however, no significant difference in average duration was detected among ant species (Ryan-test). When *Tapinoma* sp. PM1 workers showed intensive attack, this was usually performed by multiple workers (Figure 5), while in the other species, attacking was always done by single workers.

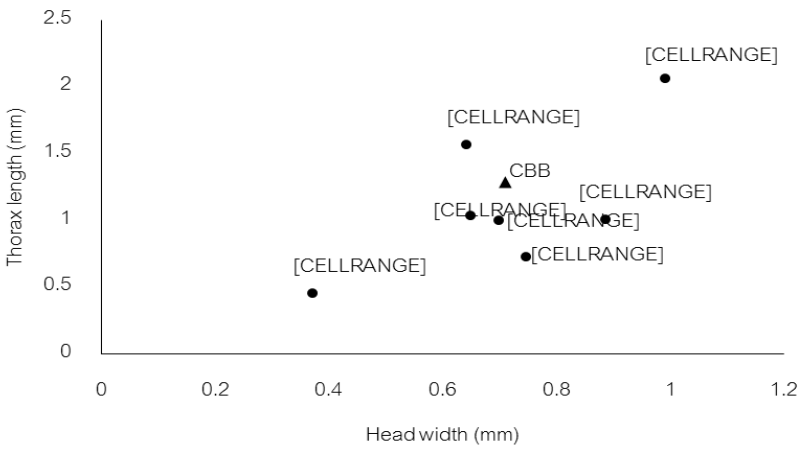

Figure 2. The thorax length and head width (mm) of each ant species. Ta: *Tapinoma* sp. PM1; Tem: *Technomyrmex modiglianii*; Do: *Dolichoderus* sp. PM1; Tey: *Technomyrmex yamanei*; Cr: *Crematogaster* sp. PM2; Ag: *Anoplolepis gracilipes*; Cn: *Camponotus nicobarensis.* The body size of CBB were shown by head width (mm) and body length (mm)

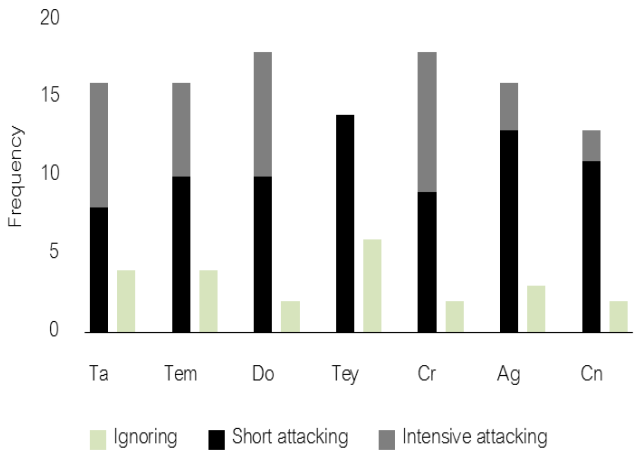

Figure 3. Frequency of each attacking behavior displayed by the seven ant species. Ta: *Tapinoma* sp. PM1; Tem: *Technomyrmex modiglianii*; Do: *Dolichoderus* sp. PM1; Tey: *Technomyrmex yamanei*; Cr: *Crematogaster* sp. PM2; Ag: *Anoplolepis gracilipes*; Cn: *Camponotus nicobarensis*

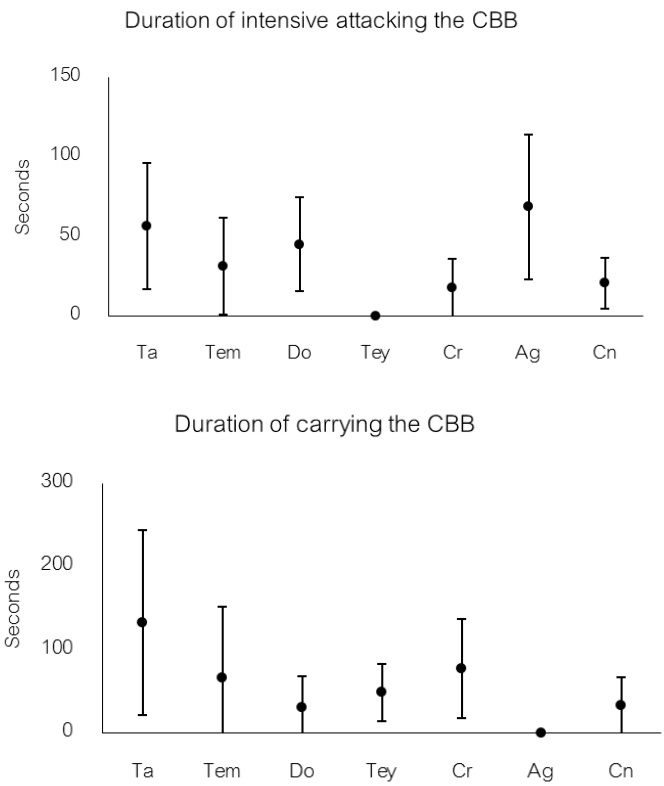

Figure 4. Average duration of each behavior performed by each ant species. Ta: *Tapinoma* sp. PM1; Tem: *Technomyrmex modiglianii*; Do: *Dolichoderus* sp. PM1; Tey: *Technomyrmex yamanei*; Cr: *Crematogaster* sp. PM2; Ag: *Anoplolepis gracilipes*; Cn: *Camponotus nicobarensis*

Figure 5. The workers of *Tapinoma* sp. PM1 gathered around a CBB

After the short or intensive attacks, all workers of *A. gracilipes* (n = 17) walked away but some workers of the other ant species picked up the CBB with their mandibles and carried it to other places in the foraging arena. The ratio of workers that carried the CBB after attacking in *A. gracilipes* was significantly lower than that of *Dolichoderus* sp. PM1 ($P = 0.023$) and *T. yamanei* ($P = 0.023$). The duration of carrying was not significantly different among species, however, the efficiency of carrying CBB seemed to be different among species. Workers of *C. nicobarensis* and *Dolichoderus* sp. PM1 readily picked up the CBB and carried it, while workers of the other species often failed to manipulate the CBB. No workers of any species carried the CBB to their nest chambers, and they never fed on the CBB.

If "intensive attacking" and "carrying" were regarded as "strong interference behavior", the ratio showing the strong interference behavior in *A. gracilipes* was significantly smaller than that of *Dolichoderus* sp. PM1 ($P<0.001$).

Discussion

All ant species studied in this research never fed on the adult CBB, however, they showed attacking and carrying behavior against the CBB. These behaviors may result in expelling the CBB from coffee trees and thus reducing the infestation of coffee berries (Jiménez-Soto *et al*., 2013). The behavioral response against the CBB varied among the seven ant species, although a statistically significant difference was not found in the most species. A possible reason for the absence of statistical differences may be the small sample size. However, our results may give an important insight into the usefulness of ants for biological control of CBB in Thailand. Two relatively large sized ants, *A. gracilipes* and *C. nicobarensis* (see Figure 2), seem to be less effective among the seven ant species we tested. *A. gracilipes* workers never showed carrying behavior, and intensive attacks were observed only three times. *C. nicobarensis* workers often ignored the CBB, and also intensive attacks were rare. In ants, worker body size affects prey size selection as shown in some seed harvesting ants (Kaspari, 1996). For larger sized ants, the adults of CBB may be too small to be hunted as prey. Among the five other ant

species, *Dolichoderus* sp. PM1 seems to be the most effective biological control agent, because of the frequent interference behavior against the CBB. Furthermore, the workers can manipulate the adult CBB very well. Although the frequency is rather low, approx. 50% of encounters resulted in strong interference behavior in the other four species. Especially in *Tapinoma* sp. PM1, the duration of both intensive attacks and carrying was relatively long, although there was no statistical difference when compared to the other ants. The most dominant ant species in the coffee plantation in Pa Miang (Onishi *et al*., 2016), *T. yamanei*, showed a unique behavioral response: they rarely performed intensive attacks, however, they frequently carried the CBB. Thus, *T. yamanei* may have a negative effect on the CBB.

As in former studies in Central and South America (Gonthier *et al*., 2013), relatively smaller ants seem to be effective for controlling the adult CBB in Thailand. Because they can enter the galleries made by the CBB (Gonthier *et al.,* 2013), they can also attack immatures of the CBB inside the berries. The effects of such small ants on the survival of CBB immatures should be investigated for evaluating the usefulness of ants for biological control of the CBB in Thailand.

Acknowledgement

We would like to thank the staff and the students of Division of Entomology, Department of Entomology and Plant Pathology, Chiang Mai University for their support, encouragements, and arranging this study; all staff and the students in the laboratory of Entomology, Faculty of Agriculture, Kagawa University for their help, support and encouragements, and Prof. Johan Billen for comments on the manuscript.

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