Oviposition preference and age-specific life table of the butterfly \textit{Graphium agamemnon} (Lepidoptera: Papilionidae) on four host plants species

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Abstract. Oviposition preference of the butterfly \textit{Graphium agamemnon} (Lepidoptera: Papilionidae) on four host plants species, \textit{Annona muricata} L., \textit{Polyalthia longifolia} L., \textit{Cananga odorata} (Lam) Hook.f. & Thomson and \textit{Michelia alba} L, were observed on 15 females and 10 males in field cage measuring 4 x 6 x 6 m. The females of \textit{G. agamemnon} butterfly deposited its eggs on three species of host plants but no egg deposition on \textit{C. odorata}. The number of eggs laid per plant on \textit{A. muricata} was significantly higher ($p < 0.05$) than those on \textit{P. longifolia} and \textit{M. alba}. However, no oviposition preference was detected between \textit{P. longifolia} and \textit{M. alba} host plants. The age-specific life table analysis was conducted on the more-preferred host plants on oviposition preference test, \textit{A. muricata}. During the immature stages, the mortality ($q_x$) of the egg stage \textit{G. agamemnon} was higher than in the other stages. Furthermore, the fifth instar larvae had the highest mortality ($q_x$) in the larval stage. Predators were the most important factor of mortality in immature stages of \textit{G. agamemnon} and. The spiders family of Salticidae and Oxyopidae prey in all of the larval instars, meanwhile family of Thomisidae preferred in the young larvae.

1. Introduction

Interactions between insects and plants are considered to be largely responsible for the high abundance and diversification of both groups [1]. In insect-plant interactions, the phytophagous insects, as well as butterflies, choose one or several particular plant species which are very closely as a place of food and life [2]. The Papilionidae larval dominated fed on five families of host plant that are Aristolochiaceae, Anonaceae, Lauraceae, Apiaceae, and Rutaceae [3].

Butterflies preferred the host plants for oviposition and feeding depend on the chemicals compound that contained in those plants. Oviposition in butterflies goes through a series of more or less distinct stages corresponding to host orientation, host finding, host assessment and oviposition that mediated by chemical cues associated with the potential host plant [4]. Various oviposition studies have been done for many kinds of treatments such as preferred on host plants species [5], acceptability of host plants [6, 7, 8], suitability of host plants [9, 10, 11], and host plant quality [12, 13, 14], by using the extract of host plants leaves [6, 7] or the whole plants [9, 10, 11, 12, 13].

The oviposition preferences of host plants correlated to feed preference, immature stage period and...
survivorship. The *Papilio polytes* butterfly preferred the *Citrus reticulata* for oviposition and feeding than other *Citrus* and *Murraya koenigii*. Furthermore, life cycle of *P. polytes* was shorter when its larval reared on *C. reticulata* [15]. The host plants species also determined the survivorship of *Papilio polyxenes*, as well as environmental conditions [16].

The swallowtail butterfly *Graphium agamemnon* is a predominantly green and black, both sexes black with bright green spots and streaks on wings. Hind wing tails short and stumpy in both male and female, however, tail in male is shorter than female. This butterfly is commonly known as Green Spotted Triangle, Tailed Green Jay or Green Triangle [17]. The average of wingspan of this butterfly was varies ranging from 83 mm to 100 mm, whereas, immature stage duration and survivorship, depend on host plants that larvae fed [17, 18, 19]. The larval host plants of *G. agamemnon* are the some species that belong to the family of Annonaceae, Magnoliaceae and Lauraceae [20, 21]. This butterfly could be a pest in Aceh if its population is out of control, particularly on *Annona muricata* fruit plant.

Host preferences and the effects of host species on development, survival, caterpillar behavioral ecology and natural enemies occurring mortality agents of *G. agamemnon* will enhance the abilities toward effective pest management strategies for this butterfly. The present study focuses on host plant preference for oviposition by the female of *G. agamemnon* on four species that belong to Annonaceae, Magnoliaceae, and Lauraceae, and constructs the age-specific life table on more-preferred oviposition host plants. All of the plants commonly grow in the towns, home gardens and suburban areas in Aceh, Indonesia.

2. Materials and methods

2.1 Host plants and food plants

Amount of ten seedlings of each host plant *Annona muricata* L., *Polyalthia longifolia* L., *Cananga odorata* (Lam) Hook.f. & Thomson and *Michelia alba* L. were used in the oviposition preferences experiment, and other thirty seedlings of *A. muricata* for age-specific life table experiment. Subsequently ten seedling of *Annona squamosa* L. and *Annona reticulata* L. were used for rearing of the larvae. Meanwhile, the *Ixora javanica* (Blume) DC. and *Lantana camara* L., 20 seedlings each, were used as the food plants for adult butterflies in the field cage. The seedlings (75-100 cm tall) of all host plants and food plants were obtained from a local nursery in Banda Aceh, Indonesia and then the seedlings were planted individually in a plastic bag (35 cm in diameter and 50 cm in height). In each plastic bag, amount 4/5 of plastic bag volume was filled with the mixture media for host plants which containing a ratio 1:1:1 of podsolics: manure: compost and added 10 g of artificial fertilizer. All of the host plants and food plants were planted in the Biological Research Garden, Department Biology Faculty of Mathematic and Natural Sciences, Syiah Kuala University, Banda Aceh, Indonesia.

2.2 Preparation of *Graphium agamemnon* butterflies

Fifty healthy fifth instar larvae of *G. agamemnon* were collected from various host plants in the campus of Syiah Kuala University, Banda Aceh. The larvae were reared on the *Annona squamosa* L. and *A. reticulata* L. in screen cage. In the each screen cage measuring 50 x 50 x 50 cm, consist of 10 larvae that reared until the adult butterflies emerged under the laboratory condition (maintained at temperature 24-27°C; 65-85% RH and 10L:14D of photoperiod). Healthy male and female of adult butterflies were subsequently transferred to the field cage, (4 x 6 x 6 m of length, width and height, respectively and cover by plastic net with 0.5 x 0.5 cm of mess), for mating and oviposition. The flowering plants of *Ixora* sp. and *Lantana camara* were supplied in the field cage as a food source for adults and those food plants were replaced when the flowers fell down.

2.3 Oviposition preference

The oviposition preference experiment was conducted using the completely randomized design with
four treatments of host plant (A. muricata, P. longifolia, C. odorata and M. alba) and five replications. All of 20 seedlings (75-100 cm tall) of four species host plants were provided to the butterflies oviposition for 25 days in a field cage (4 x 6 x 6 m). The host plant seedlings were placed randomly 1 m apart in the middle of the cage and 10 flowering food plants were placed in the nearest wall of the cage. The food plants were replaced when the flowers began falling down. Fifteen females and ten males of newly emerged G. agamemnon were released in a field cage [11]. The number of eggs laid on all of parts of each host plant was recorded daily, at 5.00-6.00 pm. The eggs laid, particularly on a leaf were further categorized into under and upper site. The oviposition preference was observed for 21 days, starting from the third day of eggs laid by females.

2.4 Life table data collection, construction and analysis
The study of an age-specific life table for a generation of G. agamemnon was conducted in a semi-natural in the field cage, measuring 4 x 6 x 6 m in the Biological Research Garden, Department Biology, Faculty of Mathematic and Natural Sciences, Syiah Kuala University, Banda Aceh, Indonesia during two months, April – May 2017. Ten pairs of newly emerged of G. agamemnon were released in to the field cage with fifteen seedling of Annona muricata as the host pants and I. javanica and L. camara, five seedling each. The number of eggs laid, all of surviving immature stages, and their mortality factors were recorded every day. The eggs and pupae with signs of parasitism (dark upper surface of eggs and black spots on the pupae) were collected and reared in the laboratory until the natural enemies emerged [22, 23]. The data from continuous observations on all stages of immature were pooled at the end of observations into respective stages. The life table calculation followed the method of Southwood and Henderson [24]. Identification of the mortality factors of immature stages was followed the method of Suwarmono et al. [11], Furthermore, identification of the parasitoids and predators were using some keys [25, 26, 27], and the specimens that available in the Zoology Laboratory, Biology Dept. Faculty of Mathematic and Natural Sciences, Syiah Kuala University, Banda Aceh, Indonesia.

2.5 Statistical analyses
Based on the females of G. agamemnon laid their eggs only on three of host plants, the statistical analyses were done only on three choosen host plants. The number of eggs oviposited by the female of G. agamemnon on three preferred host plants was analyzed by using the one-way analysis of variance (ANOVA) and followed by the Tukey’s Multiple Mean Comparison. The data were analyzed using the the SPSS software version 21. Meanwhile, the data of age-specific life table was analyzed descriptively.

3. Results and discussion

3.1 Oviposition preference
The study revealed that females of G. agamemnon laid their eggs solitary on three host plants (A. muricata, P. longifolia and M. alba) but no eggs were laid on C. odorata. These results were different that reported in previous study that the larvae of G. agamemnon that distributed in West Java were found in the C. odorata [28]. Distribution and host plants that available in the environment probably affected on the preference for oviposited their eggs. Papilio polytes larvae that were found in Kerinci Seblat National Park, Jambi feed on the Citrus spp. and Clausena excavata, but in Aceh Besar and Sabang, Province of Aceh were never found in those host plant, C. excavata [20].

The females of G. agamemnon shown a different response in oviposition on three of host plants. The number of eggs that laid on the A. muricata was the highest and significantly different ($F = 4.772; P < 0.05; df = 2,12$) from M. alba but no different from P. longifolia. Furthermore, the number of eggs laid on P. longifolia and M. alba was no significantly different (Table 1).

| Host plant species | N | The mean number of eggs laid | Oviposition site of leaves (%) | Leaves aging (%) |
|--------------------|---|-----------------------------|--------------------------------|-----------------|

Table 1. The oviposition and site preferences of Graphium agamemnon on three species of host plants
The similar results were found in Papilio polytes [7, 11] P. demoleus [29], Polygonia c-album [9], Ascia monuste orseis [12], and Pieris brassica [14], that the females preferred the suitable host plant. The A. muricata and P. longifolia were more suitable host plants for the G. agamemnon than M. alba. The number of eggs laid on the young leaves and under the site of leaves was higher than those which were laid on the mature leaves and upper-site of leaves, respectively (Table 1). These results were relevant with P. polytes in the Rutaceous host plants [11]. Female butterflies spread their eggs on suitable host plants [30] to enhance the survival of larvae [31]. Many factors affect the oviposition their preferences including nutrition [12], morphology and texture of leaves [29, 32], color and chemical compound of leaves [33]. Oviposition preference under the surface of leaves is meant to have greater protection for the eggs from the rainfall and natural enemies, meanwhile the young leaves have the higher nitrogen and water content and smooth in texture [15].

3.2 Age-specific life table
The life table experiment was conducted on the A. muricata host plant, based on the females of G. agamemnon which was the most preferred on oviposition test. During the immature stages, the mortality (qx) on the egg stage was the highest than in other stages. Predator and parasitoid were the most important factor mortality in this stage. Some eggs were failure to hatch and broken or fell onto the ground caused by human error (Table 2).

Table 2. Age-specific life table of Graphium agamemnon on Annona muricata host plant

| X   | l   | d   | dF | D'x | q'x | qx | s(lq) | k-value | k-value-ln(s) |
|-----|-----|-----|----|-----|-----|----|-------|---------|---------------|
| Egg | 1120| 756 | Failure to hatch | 18 | 0.016 | 0.675 | 0.325 | 1.124 |               |
|     | 75  |     | Rain                          | 75 | 0.067 |       |       |         |               |
|     | 404 |     | Predator                        | 404 | 0.361 |       |       |         |               |
|     | 248 |     | Parasitoid                      | 248 | 0.221 |       |       |         |               |
|     | 11  |     | Human error                     | 11  | 0.010 |       |       |         |               |

| L1  | 364 | 75  | Rain                          | 6  | 0.016 | 0.206 | 0.794 | 0.231 |               |
|     | 64  |     | Predator                        | 64 | 0.176 |       |       |       |               |
|     | 4   |     | Parasitoid                      | 4  | 0.011 |       |       |       |               |
|     | 1   |     | Human error                     | 1  | 0.003 |       |       |       |               |

| L2  | 289 | 74  | Predator                        | 72 | 0.249 | 0.256 | 0.744 | 0.296 |               |
|     | 2   |     | Missing                          | 2  | 0.007 |       |       |       |               |

| L3  | 215 | 63  | Predator                        | 61 | 0.284 | 0.293 | 0.707 | 0.347 |               |
|     | 2   |     | Missing                          | 2  | 0.009 |       |       |       |               |

| L4  | 152 | 50  | Predator                        | 49 | 0.322 | 0.329 | 0.671 | 0.399 |               |
|     | 1   |     | Missing                          | 1  | 0.007 |       |       |       |               |

| L5  | 102 | 55  | Predator                        | 50 | 0.490 | 0.539 | 0.461 | 0.960 |               |
|     | 3   |     | Parasitoid                      | 3  | 0.029 |       |       |       |               |
|     | 2   |     | Missing                          | 2  | 0.020 |       |       |       |               |

| Pupa | 47  | 22  | Predator                        | 9  | 0.191 | 0.468 | 0.532 | 0.492 |               |
|      | 13  |     | Parasitoid                      | 13 | 0.277 |       |       |       |               |

Adult | 25  | The total killing power (∑k-value) | 3.801
Out of the total mortality, predator was the main agent. In the immature stage of *G. agamemnon*, the mortality rate due to predators was 2.23% and higher than *P. demoleus* in the citrus orchard in Penang, Malaysia [11] and *P. polyxenes* [34]. Two species of predators, *Oxyopes* sp. (spider) and *Seleonopsis* sp. (ant) were the main mortality agents on the eggs. Meanwhile, the parasitoid, *Encarsia sp.* was the only infecting parasitoid that was observed on this stage. The rain wasn’t as an important mortality factor that was reported on *P. demoleus* and *P. polytes* [11], probably caused this study was conducted in the dry season (April - May).

Predator was the mainly mortality agent throughout the larval stage. Mortality during the larval stage was increase correspond to larval instars development. Mortality on the young larvae (L1-L3) was lower than the adult larvae (L4-L5). Higher mortality on the adult larval corresponds to time of larval period. The adult larvae period was longer than the young larvae, consequently, the chance of predator attack the adult larvae increased. The mortality agents observed during the study were Thomicidae, Salticidae, Oxyopidae (*Oxyopes* sp.), *Seleonopsis* sp., and *Sycanus dichotomus*. The spiders attacked the larvae correlated to their body size; the young larvae were more attacked by the small body of spiders, Thomicidae and Oxyopidae. Salticidae was the common spider that found in the field cage during the study. The young nymphs *Sycanus dichotomus* (assassin bug) were attacked the young larvae meanwhile the older nymphs could attack the older larvae. This bug was the very active predator and could find out the larvae which were any sites. Meanwhile, *Seleonopsis* sp. could attack all of larvae instar. These ants attacked the larvae gregariously and they could find out the caterpillars easily. The kinds of predator in this study were limited, probably caused by the study was conducted in the cage. Variation in the number of predators was very much related to the environment. There were two vertebrate predators; birds and lizard were observed attack the caterpillar in the citrus orchard [11].

The parasitoids were attacked on the eggs, fifth instar larvae and pupae. The black spots on the body of larvae or pupae were the sign that they were attacked by parasitoids. The parasitoids growth and develop inside the body of larvae or pupae and emerge several days later. *Pteromalus puparum* was the only one parasitoid that attacked the fifth instar larvae and pupal stages in this study. Earlier studies also reported that *P. puparum* injected their eggs in fresh pupa of *Papilio* spp. [11, 22]

In this study, some of larvae were missing probably the larvae were moving outside the cage to find the food. The contributions of other two mortality agents were less than 1%, its mean they were insignificant effect on population of immature stage of *G. agamemnon*.

The survivorship of *G. agamemnon* was 2.23% and higher than *P. demoleus* in the citrus orchard during the dry season [11]. The survivorship was affected by several factors such as food, climate, and mortality agents. The survivorship of *P. demoleus* in secondary and primary wet seasons was higher than the dry seasons [11]. The total killing power (k-value) of *G. agamemnon* in this study was 3.801 (Table 2). Based on the k-values, the egg stage was the weakness stage (1.124) among the immature stage of *G. agamemnon*, followed by fifth instar larvae stage (0.960).

4. Conclusions
The females of *G. agamemnon* more preferred the *A. muricata* for laid their eggs, and no eggs laid on the *C. odorata*. The females also preferred the underside and young leaves of host plants than the upperside and mature leaves, respectively. Predator was the main factor of mortality during the immature stage of *G. agamemnon*, followed by the parasitoid. The spiders (Thomicidae, Salticidae, and Oxyopidae), *Seleonopsis* sp., and *Sycanus dichotomus* was the mortality agents of the immature stage. *Sycanus dichotomus* both of nymph and adult stages was the very active predator. Parasitoid more contributed on mortality of egg stage. The survivorship of *G. agamemnon* was 2.23% and the egg stage was the weakness stage.
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