

## Larval Food Preference of the Swallowtail Butterfly *Papilio polytes* L. (Lepidoptera: Papilionidae) on Four Species of Rutaceae

### Preferensi Makan Larva Kupu-kupu *Papilio polytes* (Lepidoptera: Papilionidae) Terhadap Empat Spesies Rutaceae

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**ABSTRAK.** Preferensi makanan larva *Papilio polytes* L. pada empat tanaman inang (*Citrus aurantifolia* (Chrism.) Swing, *Citrus reticulata* Blanco, *Citrus hystrix* DC, dan *Murraya koenigii* L.) telah diselidiki di laboratorium. Preferensi makan instar pertama *P. polytes* berbeda nyata ( $p < 0,05$ ) untuk tanaman inang di semua kombinasi daun tanaman inang yang diuji, kecuali kombinasi *C. hystrix* dan *C. reticulata*. Selanjutnya, preferensi makan larva instar ketiga terhadap tiga daun tiga spesies spesies jeruk kecuali *M. koenigii*, sedangkan larva instar ketiga tidak menunjukkan preferensi makan yang signifikan diantara *Citrus spp.* Pada larva instar kelima, tidak menunjukkan adanya preferensi makan pada daun dari semua spesies jeruk, tapi konsisten konsumsi terhadap *M. koenigii* dibandingkan *Citrus spp.* dalam berbagai kombinasi yang diuji. Larva *P. polytes* instar pertama dan instar ketiga lebih selektif dalam hal makan daripada instar kelima. Kandungan nitrogen dalam daun keempat tanaman inang berbeda nyata ( $p < 0,01$ ), dengan *C. reticulata* adalah yang tertinggi (4,52%), sedangkan kadar air daun berbeda nyata antara *M. koenigii* (71,72%) dibandingkan ketiga spesies *Citrus* (76,38-79,12%), tetapi tidak berbeda nyata diantara ketiga spesies jeruk itu sendiri.

**Kata kunci:** preferensi makan, *Papilio polytes*, tanaman inang, nitrogen, kandungan air

**ABSTRACT.** The food preference of the *Papilio polytes* L. larvae on four rutaceous host plants, *Citrus aurantifolia* (Chrism.) Swing, *Citrus reticulata* Blanco, *Citrus hystrix* DC, and *Murraya koenigii* (L.) Sprengle, were investigated in the laboratory. The first instar of *P. polytes* larvae significant preferential feeding ( $P < 0.05$ ) for a host plant in all paired host plant leaves combinations tested, except for the *C. hystrix* and *C. reticulata* combination. Furthermore, the third instar larvae had significant preference for leaves of the three *Citrus* species over *M. koenigii*, while the third instar larvae showed no significant feeding preference among the *Citrus* spp. Indiscriminately feeding shown on the fifth instar larvae on leaves of all *Citrus* species but consistently consumed less *M. koenigii* compared to *Citrus* spp. in the various combinations tested. The first and the third instar larvae of *P. polytes* were more selective in terms of feeding than the fifth instar. The nitrogen content in leaves of all four host plants differed significantly ( $P < 0.01$ ), with *C. reticulata* was the highest (4.52%), while the water content of leaves was significantly the lowest in *M. koenigii* (71.72%), compared to the three *Citrus* species (76.38 – 79.12%) among which the water content did not significantly differ.

**Key words:** Food preference, *Papilio polytes*, host plant, nitrogen and water content

#### INTRODUCTION

Feeding preference is important traits that define the ability of a phytophagous insect to efficiently utilize a particular plant species (Futuyama & Peterson, 1985). The ability of immature insects

to discriminate host for feeding based on the quality of the host plant can be important in crop pests because of the role of non-crop plants as alternate hosts (Portillo *et al.*, 1996).

Plant chemistry, generally, is the most important factor that regulates suitability and acceptance by

herbivore. Host plant nutrient, primarily water and nitrogen are essential universally important determinants of insect herbivore feeding, growth, survival, and population dynamics (Ghumare & Mukherjee, 2003; Kursar *et al.*, 2006).

Herbivores response to variable food quality involves flexible behavioral and physiological processes. This compensatory response typically falls into three different categories: increased rates of food intake, consumption of additional plant tissue that complements the limiting nutrient, and increasing digestive efficiency of limiting nutrient (Slansky, 1993). Compensatory feeding for nutritionally suboptimal food may be a common adaptive response found in many herbivore species (Wheeler & Slansky, 1991).

*Papilio polytes* is known to feed on various genera of Rutaceae, such as *Murraya*, *Triphasia*, *Glycosmis*, *Zanthoxylum*, *Toddalia*, *Evodia*, and *Poncirus* in the forests (Corbet & Pendlebury, 1992). Saljoqi *et al.* (2006) studied the biology of eggs and host preferences of *P. demoleus* on various species of citrus and *P. trifoliata*. In recent years, this butterfly has adapted to the urban/suburban environments and is found to feed on citrus plants (Corbet & Pendlebury, 1992); this feeding adaptation has made *P. polytes* a potentially serious pest in citrus orchards.

Understanding the feeding preference of various host species of *P. polytes* is an important prerequisite for developing pest management strategies for this insect. Various studies have investigated the relationship between adult host preference and larval performance by using this assumption (Berdegue *et al.*, 1998; Forister, 2004). The objective of the present study was to evaluate the feeding preference and selectivity of different larval stages of *P. polytes* on four host plant species.

## MATERIALS AND METHODS

**Host-plants.** Selection of the rutaceous host plants for this study was based on their potential value as commercial plants. Three commercially grown *Citrus* species, [*C. aurantifolia* (Christm.) Swing., *C. hystrix* DC., *C. reticulata* Blanco], and a partially domesticated rutaceous species, *M. koenigii* (L.) Sprengel (Backer & Van Den Brink, 1965), were used in this experiment. Forty young seedlings (50-55 cm tall) of each species were separately planted in plastic bags (each bag 25 cm diameter and 35 cm high) containing a 3:1:1 mixture of podsolic, compost and manure,

respectively. Each plastic bag contained one seedling of a single species. Each seedling was fertilized fortnightly with 10 g of artificial fertilizer and 100 g manure.

**The insect.** Larvae of *P. polytes* were collected from citrus trees at the Universiti Sains Malaysia (USM) main campus in Penang. The collected larvae were reared in the laboratory on *Citrus microcarpa* to avoid any potentially confounding effect of dietary history on host plant preference. The larvae were placed in a screen cage measuring 50 x 50 x 50 cm and were maintained at 24-26 °C, 60 – 85% relative humidity, and a photoperiod of L12:D12, until adult emergence. Ten pairs of the emerged *P. polytes* adults were transferred to a field cage (2 x 2 x 2 m) to facilitate mating and oviposition. Flowers of *Ixora* sp. collected from the plants at USM campus were supplied as food for the caged adult *P. polytes*. The flowers contained in a glass jar filled with water were sprayed with 10% sucrose solution twice daily to ensure sufficient provision of nectar, and placed in the cage; the flower were replaced every three days.

**Feeding preference.** Three larval stages, the first, the third and the fifth instars, were used in this experiment. Preliminary studies were conducted to determine the amount (weight) of leaves consumed in 24 h by each larval stage. Based on the average weight of leaves of each host plant consumed in the preliminary trial by the first, third, and fifth instar larvae (first instar: 0.10-0.11 g, third instar: 0.30-0.32 g and fifth instar 0.55-0.60 g), the weight of leaves for this feeding experiment was determined. All larval instars were fed with leaves from the four host plants using the two-choice and four-choice experiments.

### First instar larvae.

**Two-choice test.** The methodology for this test was modified after DiTommaso and Losey (2003) and Ghumare and Mukherjee (2003) and the test was conducted for a 24 h period. All possible paired combinations for the two-choice test of the host plant leaves (*C. aurantifolia* – *C. hystrix*, *C. aurantifolia* – *C. reticulata*, *C. aurantifolia* – *M. koenigii*, *C. hystrix* – *C. reticulata*, *C. hystrix* – *M. koenigii* and *C. reticulata* – *M. koenigii*) were evaluated using petri dishes (each 10 cm diameter). One of very young leaf of each *Citrus* species (approximately 2 cm long, 1 cm wide, and weighed 0.10-0.11 g), and three young foliages of *M. koenigii* approximately equal in size and weight to the *Citrus* leaves were used for the

first instar larvae experiment. The leaves were collected from the respective plants and the end part of their petiole was wrapped with moistened cotton to prevent dehydration prior to placing them in the petri dish. Thereafter, twenty newly hatched *P. polytes* larvae starved for 4-5 h were introduced in each petri dish containing the respective combination of leaves and allowed to feed on the leaves for 24 h period.

The leaves in each petri dish were weighed at the beginning of the experiment and reweighed 24 h post feeding by the first instar larva. The water loss from the leaves in each petri dish due to evaporation was estimated by placing a piece of young leaf of each *Citrus* spp. (and three young foliages in the case of *M. koenigii*) in each of ten petri dishes (10 cm diameter) prepared as in the two-choice combination. The leaves in each of these petri dishes (without larvae) were weighed twice, at the beginning of the experiment and later at 24 h. Thus, the weight difference indicated water loss due to evaporation (Ojeda-Avila *et al.*, 2003). The larval consumed was recorded from weight of leaf before introduced to larvae subtract the weight of leaf after 24 h and mean of water losses. Both feeding preference of the first instar and leaves water content were replicated 10 times.

**Four-choice test.** The experimental design for the four-choice test was similar to the two-choice test, except that one very young leaf of all three *Citrus* (*C. aurantifolia*, *C. hystrix* and *C. reticulata*) and three young foliages of *M. koenigii* were offered in the same petri dish to the first instar larvae. Each of the ten petri dishes prepared for this experiment contained leaves of all four host plants approximately equal in size and weight. Twenty newly hatched *P. polytes* larvae starved for 4-5 h were introduced into each dish. The leaf host in each dish was weighed before the larval introduction and at 24 h post-introduction of the larvae. The water loss from each petri dish due to evaporation was estimated by simultaneously running additional 10 petri dishes without larvae as described above for the two-choice test.

**Third instar larvae.** The procedures used and the experimental design for evaluating the feeding preference of the third instar larvae for two-choice and four-choice test of four host plants were similar to that employed for the first instar. However, in this experiment, five newly-moulted third instar larvae were introduced per petri dish and larger petri dishes (15 cm diameter and 2.5 cm high) were used. In each dish one

young of leaf each *Citrus* spp. (each approximately 4 cm long, 2 cm wide, and weighing 0.30-0.32 g) was used; *M. koenigii* leaves used in the experiment were similar in size and weight to any one of the leaves of the *Citrus* species. The water loss from each petri dish due to evaporation was estimated by simultaneously running additional petri dishes without larvae as already described above. The replication for two-choice and four-choice test was 10 times each.

**Fifth instar larvae. Two-choice test.** The experimental design for the two-choice test for the fifth instar larva was very similar to the two-choice test conducted for first and third instar larvae except that one newly-moulted fifth instar *P. polytes* larva per petri dish (each 15 cm diameter and 2.5 cm high) was used. Two young leaves (each approximately 5 cm long and 3 cm wide and weighing 0.55-0.60 g) of each *Citrus* species and 5-7 young foliages of *M. koenigii* similar in size and weight to the *Citrus* species were used for all possible paired combinations (six pairs) for the two-choice test. The six combinations of the leaves were replicated 10 times. The leaf in each dish was weighed before the larval introduction and at 24 h post-introduction of the larva. The water loss from each petri dish due to evaporation was estimated by simultaneously running additional 10 petri dishes without larvae as already described.

**Four-choice test.** The experimental design for the four-choice test for the fifth instar *P. polytes* larvae was similar to the four-choice test for the first and third instars, except that one newly-moulted fifth instar *P. polytes* larva per petri dish (each 15 cm diameter and 2.5 cm high) was used. Two young leaves (each approximately 5 cm long and 3 cm wide and weighing 0.55-0.60 g) of each *Citrus* species and 5-7 young leaves of *M. koenigii* similar in size and weight to the *Citrus* species were used. Each of the ten petri dishes prepared for this experiment contained leaves of all four host plants approximately equal in size and weight. The vegetation in each dish was weighed before the larval introduction and at 24 h post-introduction of the larva. The water loss from each petri dish due to evaporation was estimated by simultaneously running additional 10 petri dishes without larvae as described above for the two-choice study.

**Nitrogen content of host plant leaves.** The Kjeldahl method was used to determine the percentage of total nitrogen in the leaf (Eaton *et al.*, 2005). Approximately 0.10 g of dried and

homogenized young leaf materials were used for this test. The percentage of total nitrogen was

calculated using the following formula:

$$\%N (\% \text{ dried material}) = \frac{(Vs - Vb) \times \text{normality HCl} \times 14.007 \times 100}{\text{Weight of sample}}$$

% protein (% dried material) = % N x 6.25

Where: Vs = volume HCl with sample; Vb = volume HCl without sample

**Water content of host plant leaves.** Twenty young leaves of each *Citrus* species and *M. koenigii* were collected from each host plant. All leaves of each species were separately weighed for wet weight (WW) and dried in an incubator at 80°C for 24 h and then weighed again for dry weight (DW). The leaves water content of each species was calculated as a percentage  $(WW - DW/WW) \times 100\%$  (Barros & Zucoloto, 1999).

**Statistical analysis.** The weight of leaves consumed on feeding preference of the *P. polytes* for each combination of the host plant in two-choice test were analyzed using an independent *t*-test. The feeding preference on the four choice test, larval performance parameters and chemical compound of leaves were evaluated by using the one-way analysis of variance (ANOVA). Means associated with each variable of the host plant were further separated using the Tukey's test. All data were analyzed using the SPSS software version 12 (Dytham, 2003; Pallant, 2005).

## RESULTS AND DISCUSSION

**Feeding preference.** Feeding preference of the first instar *P. polytes* larvae showed significant preferential feeding ( $P < 0.05$ ) for a host plant in all paired host plant leaves combinations tested, except for the *C. hystrix* and *C. reticulata* combination (Table 1). It is obvious from the data in Table 1 that *C. reticulata* leaves were generally the most preferred by the first instar larvae. The third instar larvae had significant preference for leaves of the three *Citrus* species over *M. koenigii*, while no significant feeding preference among the *Citrus* spp. was shown by the third instar larvae. The fifth instar larvae fed indiscriminately on leaves of all *Citrus* species but consistently consumed less *M. koenigii* compared to *Citrus* spp. in the various combinations tested.

The trend in the feeding preference of *P. polytes* larvae in the four-choice test was similar to the

two-choice test where first instar larvae were relatively more selective than the third and fifth instar larvae (Table 2). All tested instars of *P. polytes* larvae consumed *C. hystrix* leaves more than the leaves of the other host plants offered; *M. koenigii* was the least preferred.

This study showed that the first and third instar larvae of *P. polytes* were more selective in terms of feeding than the fifth instar. The younger instars preferred and consumed more leaves of the *Citrus* spp. having a higher nitrogen and water content than the leaves of *M. koenigii*. The younger instar larvae generally prefer host plants of higher quality because they are important determinants of their performance (Zalucki *et al.*, 2002; Cizek, 2005). In addition, young larvae are more sensitive to mechanical leaf traits (e.g. toughness) owing to their smaller mouthparts and less developed musculature (Hochuli, 2001). The low preference for *M. koenigii* leaves may have been due to their tougher texture compared to the leaves of *Citrus* spp. The stronger odor of the curry leaves could also be a feeding deterrent for the younger instar of *P. polytes*. The first instar always prefer young leaves which contain less of the secondary metabolites, less fiber (Cizek, 2005), and toughness (Coley & Barone, 1996).

The fifth instar larva was less selective about its food than the earlier instars because at this stage the larva needed a lot of nutrients (volume) to transform into pupa. It has been reported that 80% of food consumed during the larval stage occurs in the fifth instar. Young larvae are generally more sensitive to food quality, including nitrogen and secondary metabolites; also, the older larvae are able to feed on a wider range of host plants (Waldbauer & Friedman, 1991).

Although the pupal stage is non-feeding, there are massive physical transformations that occur during pupation. Therefore, the fifth instar larva consumes a lot of food and consequently becomes less discriminate in terms of the plants it consumed. Early instars often feed on young

leaves, while later instars can also utilize mature leaves, because the young instar need to the higher nutrition for growth (Floater, 1997).

Table 1. Mean consumption (g) by the first, third and fifth instars larvae of *Papilio polytes* during a 24 h period when provided with six different combinations of leaves of host plants, *Citrus aurantifolia* (Ca), *Citrus hystrix* (Ch), *Citrus reticulata* (Cr) and *Murraya koenigii* (Mk) in the laboratory.

Combinations of host plants	Weight of leaves consumed (g)		
	First Instar (g/20 larvae)	Third Instar (g/5 larvae)	Fifth Instar (g/larva)
Ca : Ch	0.05 : 0.06* t = 2.40	0.17 : 0.14 <sup>ns</sup> t = 0.96	0.31 : 0.30 <sup>ns</sup> t = 0.32
Ca : Cr	0.05 : 0.06* t = 2.57	0.14 : 0.16 <sup>ns</sup> t = 0.70	0.29 : 0.30 <sup>ns</sup> t = 0.29
Ca : Mk	0.06 : 0.05* t = 3.50	0.17 : 0.12* t = 2.90	0.32 : 0.25 <sup>ns</sup> t = 1.08
Ch : Cr	0.05 : 0.06 <sup>ns</sup> t = 1.36	0.15 : 0.16 <sup>ns</sup> t = 0.34	0.30 : 0.33 <sup>ns</sup> t = 0.29
Ch : Mk	0.06 : 0.05* t = 2.86	0.19 : 0.12* t = 7.41	0.37 : 0.26* t = 2.86
Cr : Mk	0.06 : 0.04* t = 2.36	0.17 : 0.14* T = 1.55	0.32 : 0.29 <sup>ns</sup> t = 0.98

Means in a combination for each instar marked with an asterisk "\*" are significantly different ( $P < 0.05$ ,  $n = 10$ ,  $t$ -test), ns = not significant.

Table 2. Weight (mean  $\pm$  SE, g) of *Citrus aurantifolia*, *Citrus hystrix*, *Citrus reticulata* and *Murraya koenigii* consumed by the first, third and fifth instar *Papilio polytes* larvae during a 24 h period in the laboratory.

Host plants	Weight of leaves consumed (g)		
	First Instar (g/20 larvae)	Third Instar (g/5 larvae)	Fifth Instar (g/larva)
<i>Citrus aurantifolia</i>	0.030 $\pm$ 0.002 b	0.092 $\pm$ 0.014 ab	0.160 $\pm$ 0.021 a
<i>Citrus hystrix</i>	0.031 $\pm$ 0.003 b	0.103 $\pm$ 0.019 b	0.168 $\pm$ 0.021 a
<i>Citrus reticulata</i>	0.027 $\pm$ 0.002 ab	0.096 $\pm$ 0.017 ab	0.155 $\pm$ 0.019 a
<i>Murraya koenigii</i>	0.019 $\pm$ 0.002 a	0.044 $\pm$ 0.007 a	0.103 $\pm$ 0.019 a

Means in column under each instar followed by the same letter are not significantly different ( $P < 0.05$ , Tukey's test)

Table 3. Nitrogen and water contents (mean  $\pm$  SE) of host plants.

Host-plant	Nitrogen content (%)	Water in leaves (%)
<i>C. aurantifolia</i>	4.37 $\pm$ 0.01 c	78.47 $\pm$ 1.03 b
<i>C. hystrix</i>	4.29 $\pm$ 0.01 b	76.38 $\pm$ 0.79 b
<i>C. reticulata</i>	4.52 $\pm$ 0.01 d	79.12 $\pm$ 1.55 b
<i>M. koenigii</i>	3.73 $\pm$ 0.01 a	71.72 $\pm$ 1.46 a

Means in the same column followed by different letters are significantly different (Tukey's test,  $P < 0.01$ ).  $n$  nitrogen = 3,  $n$  water = 20.

Naturally, the oviposition preference of *P. polytes* is very much related to the placing of off-springs on the most suitable host plant. This is very important especially for the first instar larva because of its fragile body and poor mobility (Zalucki et al., 2002). There are three important factors, which determine the affinity of young larvae on suitable food choice. First is the provision of energy and the size of the larvae because the ability to cut through leaves depends on the size of head capsule, mass of chewing muscles and mandible morphology (Bernays & Janzen, 1988; Hochuli, 2001). Second is the break down of ingested nutrient into fragments. Toughness of food material may affect the end size of the food particles. Finally is the absorption of food across the gut epithelium. The absorption efficiency of the digested nutrients is also positively correlated with the gut or body sizes.

The later instars could move and feed on many parts of the plant. The fifth instar larvae for example are bigger and stronger; therefore, they feed on both young and mature leaves and on young stems. According to Gall (1990), the older larvae of *Cotacola* spp. (Lepidoptera: Noctuidae) could consume all parts of host plants because they could move about easily.

#### **Nitrogen and water contents of host plants.**

The nitrogen as well as water content in the leaves of *M. koenigii* were the lowest and significantly different ( $F_{3,8} = 7.20$ ,  $P < 0.01$ ) from all three *Citrus* spp. analyzed. Nitrogen content in the leaves of *C. reticulata* was the highest and significantly different from the other two citrus species; however, the water content in the leaves of all three citrus species was somewhat similar (Table 3)

The hierarchy of water content of all four-host plants was similar to the nitrogen content (Table 3). Consequently, the weights of leaves consumed by *P. polytes* larvae on *Citrus* species were higher than those consumed on *M. koenigii*.

The higher  $N_2$  level and water content in the host plants increased the developmental rates of herbivorous insects. In contrast low nitrogen and water contents in host plants result in poor performance level (Gonzales & Gianoli, 2003; Chen et al., 2004; Jones & Despland, 2006). Incorporated leaf nitrogen content and leaf water are key indices of plant leaf quality and insect growth performance, and leaf water is physiologically, ecologically and evolutionary important to herbivores insects (Scriber & Slansky, 1981).

#### **CONCLUSION**

This study showed the young larvae were more selective of their food than the older larvae. These larvae would search thoroughly for suitable host plants as their food source. The larvae more consumed on plants with higher nutritive values (*Citrus* spp.) than those consumed on plants with lower nutritive values (*M. koenigii*). Based on the laboratory study, the trend in the feeding preference of *P. polytes* larvae in the four-choice test was similar to the two-choice test.

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