PHOTOPERIOD INFLUENCE ON THE BIOLOGY AND PHENOLOGICAL CHARACTERISTICS OF Dichelops melacanthus (DALLAS, 1851) (HETEROPTERA: PENTATOMIDAE)

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(With 6 figures)

ABSTRACT

The influence of the photoperiod on the biology and seasonal morphs of Dichelops melacanthus (Dallas, 1851) was studied in the laboratory. Four different photoperiods (11, 12, 13, and 14 hL = hours of light) were tested, keeping the temperature at 25 ± 1°C and the relative humidity at 65 ± 5%. Nymph developmental time tended to be longer under shorter photophases (11 and 12 hL). The 14-hour photophase (long day) resulted in lower nymph mortality rates. Females maintained at 13 and 14 hL showed greater weight gain (1st-28th day) than females under 12 hL. D. melacanthus showed reproductive diapause induced by short photophases, especially when exposed to 11 hL. The 14-hour photophase (long day) resulted in lower nymph mortality rates. Females maintained at 13 and 14 hL showed greater weight gain (1st-28th day) than females under 12 hL. D. melacanthus showed reproductive diapause induced by short photophases, especially when exposed to 11 hL. Under 13 and 14 hL, 85% and 65% of females oviposited, respectively, in comparison to 10% and 15% of females ovipositing under 11 and 12 hL, respectively. Fecundity (number of egg masses and number of eggs/female) was greater in the longer than under the shorter photophases. Seasonal dimorphism induced by photoperiod was observed in D. melacanthus adults. Under short-day conditions (11 and 12 hL), adults showed short and rounded shoulder spines, grayish brown abdomen (mainly in 11 hL), high lipid contents, and lower percentage of mature reproductive organs. Under long-day conditions (13 and 14 hL), the stink bugs showed greatly developed shoulder spines, green abdomen, low lipid contents, and mature reproductive organs.

Key words: diapause, development, reproductive performance, dimorphism, photophase.

RESUMO

Influência do fotoperíodo na biologia e nas características fenológicas de Dichelops melacanthus (Dallas, 1851) (Heteroptera: Pentatomidae)

A influência do fotoperíodo na biologia e nas formas sazonais de Dichelops melacanthus (Dallas, 1851) foi estudada em laboratório. Foram utilizadas quatro condições fotoperiódicas diferentes (11, 12, 13 e 14 hL = horas de luz), mantendo a temperatura a 25 ± 1°C e a umidade relativa a 65 ± 5%. O tempo de desenvolvimento das ninfas teve um efeito mais prolongado nas fotofases mais curtas (11 e 12 hL). A fotofase de 14 horas (dia longo) foi um melhor condicionamento para o desenvolvimento ninfal, apresentando baixos índices de mortalidade durante esse período. Fêmeas mantidas sob 13 e 14 hL apresentaram maior ganho de peso (1º-28º dia) do que fêmeas sob 12 hL. D. melacanthus apresentou diapausa reprodutiva induzida por fotofases curtas, principalmente sob 11 hL. Em 13 e 14 hL, 85% e 65% de fêmeas ovipositaram, respectivamente, em comparação a 10% e 15% de fêmeas que ovipositaram sob 11 e 12 hL, respectivamente. A fecundidade (número de massas de ovos e número de ovos/fêmea) foi maior nas fotofases longas do que nas curtas. O dimorfismo sazonal induzido pelo fotoperíodo foi observado em adultos de D. melacanthus. Em condições de dias curtos (11 e 12 hL), os adultos apresentaram...
INTRODUCTION

The photoperiod is an abiotic factor that influences insect biology and behavior, and can be considered the main factor regulating diapause (Ali & Ewiess, 1977). Diapause is the reduction or pause of insect metabolic activities during an unfavorable period and involves physiological, epidemiological, biochemical, and behavioral changes (Leather et al., 1993).

Studies that associate photoperiod and diapause have been conducted with several species of pentatomids. Albuquerque (1993) observed in the laboratory that Oebalus poecilus (Dallas) is induced to diapause by short days. Adults of Aelia fieberi (Scott) are induced to diapause under short photoperiods, and long days promote reproduction (Nakamura & Numata, 1997). Mourão & Panizzi (2002) observed that Euschistus heros (Fabr.) presents reproductive diapause, which is induced by a 12-hour (or less) photophase. Usually, diapausant insects have immature reproductive organs and high lipid contents, associated with a feeding pause (Kiritani, 1963).

Another dormancy type, the oligopause, is common among insects living in climates with moderate winters. Insects in oligopause periodically feed during the dormant period, even with lipid accumulation during the preparatory period (Leather et al., 1993).

According to McPherson (1974), photoperiod causes phenological changes in pentatomids, such as different seasonal morphs. For example, E. heros showed short and rounded shoulder spines when maintained under short photophases during nymphal stage (Mourão & Panizzi, 2002).

Stink bugs of the genus Dichelops (Diceraeus) are important soybean pests and have recently been associated with corn and wheat, causing heavy damage to young plants cultivated under the no-tillage system (Panizzi & Chocorosqui, 2000). These bugs, which were collected in southern Brazil during a one-year period, showed different seasonal morphs (Chocorosqui & Panizzi, unpublished). In Rio Grande do Sul State, this pentatomid shows behavior similar to that of E. heros during autumn and winter. The insects were inactive, usually sheltered under crop residues, with abdomens turned upward. The abiotic and biotic factors involved in insect seasonality need to be understood in order to develop better control methods for integrated pest management programs.

This study was carried out to evaluate photoperiod influence on nymph development, and also on adult longevity, weight gain, reproductive performance, and phenological changes of Dichelops melacanthus (Dallas) in the laboratory.

MATERIAL AND METHODS

Nymph biology

Adults of D. melacanthus were collected in 1999 at the Embrapa Soybean Farm, in Londrina, PR (latitude 23°55'46"S) and placed in a gerbox (11.0 x 11.0 x 3.5 cm) to obtain eggs. They were fed green pods and dry soybean seeds [Glycine max (L.) Merrill] variety ‘Paraná’, and maintained at 25 ± 1°C, 65 ± 5% RH, and 14-hour photophase. Egg masses were mixed to avoid a genetic effect. Eggs were conditioned in Petri dishes (9.0 x 1.5 cm) lined with moistened filter paper and maintained in four different photophases: 11 hours of light (11 hL), 12 hL, 13 hL, and 14 hL. Temperature and relative humidity were kept constant (25 ± 1°C and 65 ± 5%, respectively).

During the 1st instar, nymphs were maintained only with distilled water. On the first day of the 2nd instar, 30 nymphs from each photophase were individualized in Petri dishes (9.0 x 1.5 cm) lined with filter paper. They were fed with green pods and dry soybean seeds, and supplied daily with distilled water. The same photoperiod used for the egg stage was maintained throughout nymphal development. Instar changes and mortality were observed daily.

Upon emergence, adults were separated by sex and weight on an electronic scale (Mettler Toledo PB 303). Instar duration, developmental time, and nymph mortality were calculated for nymphs under
each photoperiod. Treatments were set up in a completely randomized design, with the number of nymphs (n = 30) considered replicates. Data were submitted to analysis of variance, and means compared using Tukey’s test (p ≤ 0.05).

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**Adult biology**

Egg masses were obtained as described above. Eggs from different egg masses were mixed and divided into four groups, which were conditioned in Petri dishes (9.0 x 1.5 cm) lined with moistened filter paper, and maintained under four different photophases: 11 hL, 12 hL, 13 hL, and 14 hL. Temperature and relative humidity were kept constant (25 ± 1°C and 65 ± 5%, respectively).

Groups of ten 2nd instar nymphs were placed in a gerbox (11.0 x 11.0 x 3.5 cm) lined with filter paper and fed with green pods and dry soybean seeds. Nymphs were reared under the same photoperiod as that of the egg stage. Food was renewed every other day and humidity was maintained by using moistened cotton in a plastic container (3.0 cm diameter). At adult emergence, 20 couples were formed for each photoperiod. Each couple was conditioned in a gerbox and fed with the same food offered to the nymphs. They were supplied daily with distilled water and food was renewed every other day.

Fresh body weights of females and males were taken on the 1st, 7th, 14th, 21st, and 28th days of adult life. The weight gain between two evaluations was calculated by subtracting the last weighing from the previous one. The total weight gain was obtained by subtracting the weight on day 28 from the weight on day 1. The insects were observed periodically to verify feeding occurrence in each photoperiod. The reproductive activity was evaluated through the following parameters: percentage of ovipositing females, preoviposition period, number of egg masses/female, number of eggs/female, and egg viability.

Longevity and survival of males and females were also evaluated in the several photophases. Treatments were set up in a completely randomized design, and the number of couples (n = 20) considered replicates. Data were submitted to analysis of variance, and means were compared using Tukey’s test (p ≤ 0.05).

**Seasonal morphs and lipid contents**

Egg masses of *D. melacanthus* obtained in the laboratory were mixed to eliminate the genetic effect. Four groups of eggs (n = 50) were conditioned under different photophases: 11 hL, 12 hL, 13 hL, and 14 hL, with constant temperature and relative humidity (25 ± 1°C and 65 ± 5%, respectively). Nymphs were conditioned and fed in the same way as nymphs reared for adult biology. Abdomen coloration of one-day-old adults (n = 20) was evaluated.

Adults were maintained, under the same conditions described above, for 20 days, time enough to reach sexual maturity under ideal conditions. After that, adults were killed by freezing to evaluate the following parameters: right shoulder spine length, shoulder spine shape (short, long, rounded, or pointed) and abdomen coloration. The shape of the shoulder spine was evaluated based on its morphological aspect. Afterwards, the adults were divided into two groups (n = 10 each). In the first one, lipid content was measured using the following method: 1. insects were taken to the oven (60°C) for about 48 hours; 2. dry weight (DW) was obtained using an electronic scale (Mettler Toledo PB 303); 3. insects were identified with a number and individualized in cloth packages (3.0 x 4.0 cm); 4. packages were conditioned in extraction tubes (7.5 x 3.0 cm); 5. 250 ml of the extraction solvent (hexane) was added to a volumetric balloon and placed in the Soxhlet extractor, with circulation water and tubes; 6. equipment was heated up to ca. 120°C. After the first cycle, the extraction process was maintained for three hours; 7. adults were placed again in the oven (60°C) for 24 hours; 8. weight without lipid (“thin weight” = TW) was obtained, and the following formula was applied to obtain percentage (%) of stored lipid:

% Lipid = (DW – TW) x 100/DW

The second group of adults was dissected and the reproductive organs developmental stage evaluated. Alcohol (70%) was used for cleaning the abdominal cavity of the insects to allow the best view of the reproductive organs, which were classified as immature or mature. Mature females presented distended ovaries, usually filled with green eggs. Immature females presented small and empty ovaries, without any differentiation or development. Mature males were characterized by developed testicles occupying most of the abdominal cavity. Immature males showed normal testicles, but smaller than developed testicles. The portion of the abdominal cavity occupied by the testicles was smaller than in mature males.
Treatments were set up in a completely randomized design, with replicates (n) as stated above. Data were submitted to analysis of variance, and means compared using Tukey’s test (p ≤ 0.05).

RESULTS AND DISCUSSION

Nymph biology

Photoperiod influenced mortality of *D. melacanthus* nymphs. During the 2*nd* instar, mortality tended to be greater at 11 hL (46.7%) (Fig. 1). However, it dropped during the 3*rd* instar, and no nymphs died during the 4*th* and 5*th* instars under 11 hL. Total nymph mortality (instars 2-5) in this photophase was 53.4%. In the other photophases, nymph mortality ranged from 10.0 (12 and 13 hL) to 16.7% (14 hL) during the 2*nd* instar. Nymphs reared under 12 hL showed greater mortality during the 5*th* instar, reaching 43.4%. Under 13 hL, mortality was 30.0% and 50.0% during the 3*rd* and 4*th* instar, respectively. Total nymph mortality was the same as for 11 hL of photophase (53.4%). Under 14 hL, only 30.0% of *D. melacanthus* nymphs did not complete development. Mourão & Panizzi (2000b) working with another species of pentatomid, *E. heros*, also observed greater mortality of those under short photoperiod (56.7%), in comparison to nymphs under 14 hL (28.3%).

Nymphs tended to complete development faster under longer photoperiods. The 2*nd* instar lasted longer at 11, 12, and 13 hL, in comparison to 14 hL (Table 1). In the 3*rd* instar, nymphs tended to develop faster at 14 hL. During the 4*th* and 5*th* stadia, the developmental time was longer at 12 hL than at 13 and 14 hL. There was also a significant difference in 5*th* instar duration between nymphs reared at 14 hL (5.9 days) and 11 hL (8.6 days).

For males, total developmental time (instars 2-5) was shorter under 14 hL, in comparison to the other photoperiods. For females, there was a significant difference only between 14 hL (17.4 days) and the shortest photophases (11 and 12 hL). These results are supported by Mourão & Panizzi (2000b), who reported longer developmental time for *E. heros* nymphs under short photoperiod (10 hL), than that under 14 hL. The fresh body weight of males and females of *D. melacanthus* did not differ statistically among treatments.

![Fig. 1 — Mortality (%) of *Dichelops melacanthus* nymphs reared under different photoperiods, feeding on green pods and dry soybean seeds in the laboratory (n = 30).](image-url)
Adult biology

Weight gain of males and females of *D. melacanthus* occurred mostly during the first days of adult life, up to day 14 (Fig. 2). In spite of the tendency to greater weight gain under 13 and 14 hL until day 7, there was no significant difference in weight gain/week among all the photophases, during the 4-week evaluation period. A significant difference was detected in the total weight gain of females (days 1-28). Females maintained in 13 and 14 hL (summer photophases in southern Brazil) showed greater weight gain than females at 12 hL. In the former photophases, nutrient intake may have been greater. However, this tendency to greater food intake in longer photophases did not occur with females at 11 hL, which gained weight similarly to those under 12 hL. Obviously, further studies are needed to clarify this point. Adults in all photophases were observed to feed continuously, showing that this activity was not affected by reduced photoperiod.

Reproductive performance of females also varied with the different photoperiods tested (Table 2): 85% and 65% of the females oviposited at 13 and 14 hL, respectively, but under 11 and 12 hL, only 10% and 15% of females oviposited, respectively. Preoviposition period was longer for females maintained under 11 and 12 hL, in comparison to females at 14 hL. For females under 13 and 14 hL, fecundity (number of egg masses and number of eggs) was greater than that of females under 11 and 12 hL (local winter photophases); egg hatchability, however, only tended to be higher in longer photophases. Mourão & Panizzi (2002) reported that female *E. heros* kept under 14 hL showed better reproductive performance, in comparison to females under 12 hL, which did not oviposit, and in 10 hL, which produced unfertile eggs. For the pentatomid *Nezara viridula* L., Ali & Ewiess (1977) observed no differences in reproductive performance under either 10 hL (short photophase) or 14 hL (long photophase). However, there were significant differences in fecundity and preoviposition period for *Dolycoris baccarum* L. under long-day (266 eggs and 38 days, respectively) and short-day conditions (95 eggs and 54 days, respectively) (Hodek & Hodková, 1993).

Seasonal morphs and lipid contents

Shoulder spine length of *D. melacanthus* was statistically different among treatments. Males maintained under 14 hL presented more pointed and longer shoulder spines than males under 11 and 12 hL (Table 3, Fig. 3). Males at 13 hL also presented longer and sharper shoulder spines, but the spine length did not differ from that under the other treatments. Females under summer photophases (13 and 14 hL) presented pointed and longer shoulder spines compared to females under winter photophases (11 and 12 hL). The latter, as well as the males, had rounded and shorter shoulder spines.

The photoperiod influence on the shoulder spine shape and length has been reported for *O. poecilus* (Albuquerque, 1989) and *E. heros* (Mourão & Panizzi, 2002). According to McPherson (1975), the shoulder spine length and body coloration are the most relevant aspects of seasonal dimorphism controlled by the photoperiod in pentatomids.

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**TABLE 1**

Mean ±SEM development time and fresh body weight of *Dichelops melacanthus* maintained under different photoperiods, and fed with green pods and dry soybean seeds (number of nymphs in parentheses).

<table>
<thead>
<tr>
<th>Photophase (hours)</th>
<th>Development time</th>
<th>Fresh body weight</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2th instar</td>
<td>3th instar</td>
<td>4th instar</td>
</tr>
<tr>
<td>11</td>
<td>6.2 ± 0.54 a</td>
<td>4.6 ± 0.29 a</td>
<td>5.5 ± 0.29 ab</td>
</tr>
<tr>
<td>12</td>
<td>5.4 ± 0.27 a</td>
<td>5.5 ± 0.50 a</td>
<td>7.7 ± 1.00 ab</td>
</tr>
<tr>
<td>13</td>
<td>5.4 ± 0.36 a</td>
<td>5.1 ± 0.64 a</td>
<td>4.3 ± 0.33 b</td>
</tr>
<tr>
<td>14</td>
<td>4.1 ± 0.08 b</td>
<td>3.9 ± 0.19 a</td>
<td>4.1 ± 0.38 b</td>
</tr>
</tbody>
</table>

*Means followed by the same letter in each column do not differ significantly using Tukey’s test (p < 0.05).*
TABLE 2
Reproductive performance of Dichelops melacanthus under different photoperiods, feeding on green pods and dry soybean seed in the laboratory (number of females in parentheses).

<table>
<thead>
<tr>
<th>Photophase (hours)</th>
<th>Females ovipositing (%)</th>
<th>Preoviposition period (days) (X ± SEM)(^2)</th>
<th>Number/female(^1)</th>
<th>Egg hatchability (%) (X ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Egg masses (X ± SEM)</td>
<td>Eggs (X ± SEM)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>10 (2)</td>
<td>29.0 ± 2.00 a</td>
<td>1.5 ± 0.50 b</td>
<td>14.5 ± 6.50 b</td>
</tr>
<tr>
<td>12</td>
<td>15 (3)</td>
<td>23.3 ± 5.36 ab</td>
<td>3.6 ± 1.45 ab</td>
<td>25.3 ± 6.33 ab</td>
</tr>
<tr>
<td>13</td>
<td>85 (17)</td>
<td>17.3 ± 1.59 bc</td>
<td>6.2 ± 1.23 ab</td>
<td>47.7 ± 12.74 a</td>
</tr>
<tr>
<td>14</td>
<td>65 (13)</td>
<td>12.4 ± 0.73 c</td>
<td>7.9 ± 2.68 a</td>
<td>55.8 ± 13.83 a</td>
</tr>
</tbody>
</table>

\(^1\)Data on female ovipositing.

\(^2\)Means followed by the same letter in each column do not differ significantly using Tukey’s test (P ≤ 0.05).

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**Fig. 3** — Shoulder spine morphs of *Dichelops melacanthus* observed under 13 and 14 hL (A) and 11 and 12 hL (B) of photophase.

**TABLE 3**  
Phenological characteristics of *Dichelops melacanthus* adults maintained under different photoperiods, fed with green pods and dry soybean seeds in the laboratory (number of adults in parentheses).

<table>
<thead>
<tr>
<th>Photophase (hours)</th>
<th>Shoulder spine length (mm)$^1$</th>
<th>Shoulder spines$^2$</th>
<th>Abdominal coloration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (X ± SEM)</td>
<td>Female (X ± SEM)</td>
<td>Long (%)</td>
</tr>
<tr>
<td>11</td>
<td>2.1 ± 0.06 b (20)</td>
<td>2.2 ± 0.03 b (20)</td>
<td>0.0</td>
</tr>
<tr>
<td>12</td>
<td>2.1 ± 0.05 b (20)</td>
<td>2.2 ± 0.04 b (20)</td>
<td>0.0</td>
</tr>
<tr>
<td>13</td>
<td>2.2 ± 0.04 ab (20)</td>
<td>2.3 ± 0.05 a (20)</td>
<td>100.0</td>
</tr>
<tr>
<td>14</td>
<td>2.4 ± 0.05 a (20)</td>
<td>2.5 ± 0.04 a (20)</td>
<td>100.0</td>
</tr>
</tbody>
</table>

$^1$Means followed by the same letter in each column do not differ significantly using Tukey’s test ($p \leq 0.05$).  
$^2$Data based only on morphologic aspect.  
$^3$G = green, GB = grayish brown.

Two distinct abdominal colors, green and grayish brown, were observed. Under 12, 13, and 14 hL, 100% of males and females showed green abdomens on the 1st day of adult life (Fig. 4). On the 20th day, green was the predominant color (100%) for females kept under 13 and 14 hL, and males under 14 hL. Only 10% of males kept at 13 hL changed their abdominal color to grayish brown.
after 20 days. At 12 hL, 10% of males and 20% of females changed abdominal color from green to grayish brown after 20 days. Grayish brown was the predominant color of males maintained under 11 hL. On the 1st day, only 30% of females under 11 hL showed grayish brown abdomen. However, after 20 days, 90% of the females showed this coloration.

These results indicate that *D. melacanthus* abdominal coloration is related to two factors: photoperiod and adult age. For field-collected insects, whose age cannot be estimated, abdominal coloration is not a good parameter to indicate hibernation state or seasonal morph. Furthermore, body coloration was not considered a reliable parameter of the physiological state of *E. heros* (Mourão & Panizzi, 2000a). A similar conclusion reached by Seymour & Bowman (1994) working with the pentatomid *N. viridula*, contradicted that of Harris et al. (1984), who referred to body coloration (russet) as the easiest and fastest method to determine diapause and seasonal morph of this bug.

The lipid contents extracted from *D. melacanthus* adults were significantly higher when insects were maintained under short photophases (11 and 12 hL), in comparison to the 14 hL one (Fig. 5). The lipid contents at 13 hL did not differ from those found under the other treatments. These results agree with those of Kiritani (1963), who noted that insects in diapause show immature reproductive organs and great amounts of stored lipid. Immature reproductive organs were observed when insects were maintained under short photophases (Fig. 6). Under 13 and 14 hL, all insects presented mature testicles or ovaries. Under 12 hL, 40% of males and 60% of females had immature reproductive organs. Under 11 hL, the percentage of immature organs was higher (89.9 and 81.8%, for males and females, respectively) than under the other photophases, confirming the occurrence of reproductive oligopause (hibernation with periodic feeding) under this photoperiodic condition. Hibernation induction by short photophases was reported for other pentatomids (Higuchi, 1994; Nakamura et al., 1996; Mourão & Panizzi, 2000a).

Fig. 4 — Abdominal coloration of *Dichelops melacanthus*: A = grayish-brown (adults kept under 11 hL). B = green (observed under all photophases, but mostly under 13 and 14 hL).
Fig. 5 — Lipid content (%) in dry body (X ± SEM) of *Dichelops melacanthus* 20-days-old adults, maintained under different photoperiods, feeding on green pods and dry soybean seeds in the laboratory. Means followed by the same letter do not differ significantly (Tukey's test, $p \leq 0.05$; $n = 20$).

Fig. 6 — Immature reproductive organs (%) of *Dichelops melacanthus* under different photoperiods, feeding on green pods and dry soybean seeds in the laboratory ($n = 10$).
These laboratory studies indicate that a 14 h photophase, corresponding to the longer days of summer in northern Paraná State, was the best photoperiodic condition for the development of D. melacanthus nymphs. This pentatomid presented reproductive oligopause in the laboratory, induced by 11 and 12 hL, and characterized by the occurrence of periodic feeding, even under typical winter photophases. The highest percentage of immature reproductive organs occurred under the shortest photophases. The highest percentage of immature reproductive organs occurred under the shortest photophase. D. melacanthus also showed photoperiod-induced seasonal dimorphism. The abdominal color varied according to both photophase and insect age, and so it was not considered a reliable parameter to evaluate the physiological state or the seasonal form of this pentatomid. The lipid content extracted from D. melacanthus adults was higher under short-day rather than long-day conditions.

In conclusion, these data demonstrate that D. melacanthus presents different morphs, with different physiological traits, that are driven by photoperiodic conditions. These data coupled with field observations show that the brownish, high-lipid-content, and sexually immature types are found in the soil. This makes plowing and/or elimination of crop residues a potential control strategy for managing this pest.

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