Effects of Pre-Diapause Temperature and Body Weight on the Diapause Intensity of the Overwintering Generation of *Bactrocera minax* (Diptera: Tephritidae)

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Abstract

The Chinese citrus fruit fly, *Bactrocera minax* (Enderlein), is an economically important pest of citrus. The fly has an obligatory pupal diapause in soil from November to March. However, techniques for predicting or determining the emergence of the adult have, thus far, not been well documented. In this study, we investigated the effects of different pre-diapause temperatures (8, 12, 16, and 20°C) and pupal body weight (five groups according to pupal weight: G-58, 55.0–61.0 mg; G-68, 65–71 mg; G-78, 75–81 mg; G-88, 85–91 mg; G-95, 92–98 mg) on pupal period (the indicator of diapause intensity). The pupal period of *B. minax* larvae pupated at 8°C was 193.41 d, which was significantly shorter than that of larvae incubated at higher temperatures, suggesting that there was a lower diapause intensity for larvae pupated at lower pre-diapause temperatures. There were also significant differences in the pupal periods at different pupal body weights. The pupal period of G-58 was significantly shorter than that of the heavier groups (G-88 and G-95), and the pupal period increased with increasing pupal body weight in the five groups. Moreover, the pupal period of *B. minax* significantly and positively correlated to pupal body weight. These findings demonstrate that the pre-diapause temperature and pupal body weight are suitable indicators for predicting the pupal period of overwintering individuals, and the results of this study will contribute to the development of new and effective strategies for predicting the occurrence and population dynamics of *B. minax* adult.

Key words: *Bactrocera minax*, diapause intensity, pre-diapause temperature, body weight

The Chinese citrus fruit fly, *Bactrocera minax* (Enderlein), is an economically important pest of citrus in China, Bhutan, Nepal, and India (Wang and Luo 1995, Dorji et al. 2006, Drew et al. 2006). It is a univoltine species with an extended obligatory diapause during its soil-inhabiting pupal stage, which normally occurs from November to March to combat the harsh environmental conditions (Wang and Luo 1995). To date, the only available method for predicting or determining the emergence of adult flies in spring is monitoring by field observation. Considering the current lack of an effective strategy for predicting the emergence of adults in the upcoming growing seasons, it was deemed necessary to develop a new and effective strategy for predicting the timing of future adult emergence. As yet, the possible relationship between the number of overwintering individuals and pre-overwintering environmental temperatures have not been factored into models for predicting field dynamics (Zhou et al. 2012). Therefore, enhancing our understanding of the overwintering ecology of *B. minax* might be helpful in predicting the emergence of adults.

Diapause intensity is measured as the relative duration of developmental arrest (diapause) at a given moment and under particular environmental conditions (Vinogradova 1974). The initiation and maximum levels of diapause intensity between the same and different populations of a given species often differ because of the distinct conditions experienced during the diapause period (including pre-diapause, diapause, and post-diapause) (Danks 1987, Xu et al. 2011, Posledovich et al. 2015). Accordingly, the impact that various environmental conditions during the diapause induction phase may have on the diapause intensity of insects with an obligate diapause needs further investigation. Temperature and photoperiod are the most important factors affecting the diapause intensity of insects, and these have varying degrees of influence depending on the species or a population’s geographic location (Hodek and Hodkova 1988). Exposure to different environmental conditions during diapause induction can enhance or reverse various diapause states, leading to induction via different pathways and thus affecting the duration of the diapause (Fujiyama and Takahashi 1973, 1977; Bodnaryk...
The diapausing insects generally either abstain from feeding or feed only rarely for an extended period of time, which is the major survival challenge for most insects during this period (Hahn and Denlinger 2007, 2011). The body weight of diapausing individuals greatly depends on the degree of food intake before entering diapause. Thus, individuals of different body weights are challenged differently during diapause. Previous studies have suggested that the diapause duration is closely related to body size (Saunders 1997, Danforth 1999, Menu and Desouhant 2002, Xu 2011). In China, the body weight of B. minax pupae varies within 50–100 mg in nature. This kind of overwintering pupal weight variation, and the implied variations in pupal energy reserves, may be related to the amount of food consumed before diapause. Thus, changes in body weight and the accumulation of energy reserves are among the most conspicuous alterations that occur during the pre-diapause period (Tauber et al. 1986, Danks 1987, Denlinger et al. 2005). Therefore, we hypothesize that the diapause intensities of B. minax pupae are related to their pupal body weight.

In the suburbs of Jingzhou, Hubei Province, P.R. China, temperatures in the areas in which B. minax larval pupate vary greatly. In this study, the effects of pre-diapause temperatures and the body weights of fruit fly pupae in relation to the pupal period were investigated. We aimed to provide a better understanding of the factors affecting adult emergence and population dynamics following overwintering in this species.

Materials and Methods

Insect Rearing

Bactrocera minax larvae were collected from infested fallen fruit in a citrus orchard located in Songzi, Jingzhou Country (E 111°39′41.43″, N 30°17′04.57″), Hubei Province, P.R. China, in October 2015. All collected larvae were placed into plastic containers (20 cm diameter, 16 cm height) containing pre-moistened sand (approximately 15–20% absolute humidity), and 300 larvae were placed into each container for use in the following experimental treatments.

Pre-Diapause Temperature Treatment

The range of daily air temperatures in the citrus orchard in October for the 3 yr previous to 2015 is shown in Fig. 1. We selected experimental pupation temperatures according to this range. Then, 2,400 B. minax larvae were segregated into eight individual containers (20 cm diameter, 16 cm height), and two containers were placed in each of the four tested temperatures (8, 12, 16, and 20°C) (RH: 70 ± 5%; 14:10 (L:D) h) for continuous rearing, and the larvae were allowed to pupate. The stages of the individual larvae were observed for the 3 yr previous to 2015 is shown in Fig. 1. We selected experimental pupation temperatures according to this range. Then, 2,400 B. minax larvae were segregated into eight individual containers (20 cm diameter, 16 cm height) containing pre-moistened sand (approximately 15–20% absolute humidity), and 300 larvae were placed into each container for use in the following experimental treatments.

Body Weight Group Treatment

A further 1,000 B. minax larvae were allowed to pupate at a constant temperature (18°C). Afterward, pupae were weighted and divided into five groups, 55–61 mg (G-58), 65–71 mg (G-68), 75–81 mg (G-78), 85–91 mg (G-88), and 92–98 mg (G-95), to investigate the effects of body weights on diapause intensity.

Overwintering Treatment

After pupation, every B. minax pupa from the above experiments was placed individually into separate plastic cups (6.5 cm diameter, 7.5 cm height) filled with sand (approximately 15–20% absolute humidity) and overwintered at 15°C, ca. 75 ± 5% relative humidity, and a photoperiod of 14:10 (L:D) h in climate cabinets (GZX-400BS-III, Shanghai CIMO Medical Instrument Manufacturing Co., LTD, Shanghai). We observed emergence of the adults from March of the following year, and the time, date, and sex of each emergence fly were recorded. The experiment was run until all of the adults had undergone eclosion or the pupae had died.

Diapause Intensity Analysis

We defined the length of time between pupation and emergence as the pupal period. In this study, the intensity of diapause was defined as the average number of days required to complete eclosion after the pupated B. minax larvae had been transferred to the plastic cups (Xiao et al. 2010).

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistical Version 18 (SPSS Inc., Chicago, IL). Differences in diapause intensity were compared using a one-way analysis of variance (ANOVA), followed by Tukey’s test for multiple comparisons with P < 0.05 and t-test for two samples with P < 0.05. Curve fit was also used to detect the relationship between the body weight of B. minax pupae and the diapause intensity.

Results

Effects of Pre-Diapause Temperature on Pupal Period of B. minax

All pupae were incubated at 15°C for overwintering after they have pupated under the different temperature regimes. The pupal period of each pupa is shown in Fig. 2. The pre-diapause temperature was shown to have a significant effect on the pupal period of B. minax (F = 238.017, df = 3, 459, P < 0.001) (Fig. 3A). The mean pupal period extended from approximately 193 d at 8°C to approximately 230 d at 20°C, revealing that a low pre-diapause temperature significantly shortened the length of the pupal period.

There were also significant differences between the pupal period of females and males (Female: F = 122.566, df = 3, 220, P < 0.001, Male: F = 133.37, df = 3, 235, P < 0.001) (Fig. 3B), with the average pupal period increasing in proportion to the increase in pre-diapause temperature. The shortest pupal periods occurred in female and male adults emerging from B. minax pupae that had been induced to diapause at 8°C, while the longest pupal periods occurred in individuals induced to diapause at 20°C (Fig. 3B). No significant differences were found between females and males within the different pre-diapause temperature groups except for those pupating at 16°C (8°C: F = 0.573, df = 1, 109, P = 0.4507, 12°C: F = 3.196, df = 1, 57, P = 0.0791, 16°C: F = 9.83, df = 1, 171, P = 0.002 < 0.05, 20°C: F = 3.553, df = 1, 118, P = 0.0619) (Fig. 3C). The results showed that larvae pupated at lower pre-diapause temperature had shorter pupal period.

Diapause Intensity Analysis

We defined the length of time between pupation and emergence as the pupal period. In this study, the intensity of diapause was defined as the average number of days required to complete eclosion after the pupated B. minax larvae had been transferred to the plastic cups (Xiao et al. 2010).
Effect of Body Weight on Diapause Intensity of *B. minax*

The mean pupal period of every group is shown in Fig. 4A, significant differences occurred between several of the five body weight groups (*F* = 1216.378, df = 4, 116, *P* < 0.001), so five groups pupae were used as the basis for investigating the effect of body weight on pupal period. Separate pupae from each of the five weight groups were maintained at 15°C for overwintering. Significant differences were found among the pupal period of several groups (*F* = 4.395, df = 4, 116, *P* = 0.0024 < 0.05). G-58 had a shorter pupal period than G-88 and G-95. No significant differences, however, were found among G-58, G-68, and G-78, and no significant differences...
were noted among G-68, G-78, G-88, and G-95 groups (Fig. 4B).
These results represented that smaller individuals have less intense diapause intensity, and significantly weaker than those of larger individuals. In addition, there was a strong ($R^2 = 0.1261$, $P < 0.001$) association between pupal period and pupal weight (Fig. 4C). The disparity of individual pupal periods in the G-58 group was more pronounced than in other groups, with only a few individuals in this group successfully emerging into adults.

There were no significant differences in the pupal periods within or between females and males (Female, $F = 1.932$, df $= 4$, 71, $P = 0.1145$; Male, $F = 1.234$, df $= 3$, 39, $P = 0.3105$; G-58, $F = 0.205$, df $= 1$, 10, $P = 0.6607$; G-68, $F = 2.196$, df $= 1$, 36, $P = 0.1471$; G-78, $F = 0.043$, df $= 1$, 37, $P = 0.8362$) (Table 1). Because of an insufficient sample size, only males were analyzed for differences in pupal period for G-58, G-68, and G-78. Although the body weight of $B. minax$ had no significant effect on the pupal period of females and males, the pupal period of $B. minax$ gradually increased with body weight.

Moreover, Braune (1973) pointed out that only the duration and time of termination of the obligatory diapause periods of arrested growth are controlled and influenced by temperature. In addition, Xue et al. (2001) noted that a lower temperature at the pre-diapause stage could play an important role in the normal termination of insect obligatory diapause. In the present study, the pupal period of $B. minax$ decreased in response to lower pre-diapause temperatures (Fig. 3A), indicating that the diapause intensity of $B. minax$ weakened as the pre-diapause temperature decreased. Similar patterns have been reported previously in two other diverse taxa, $Mamestra configurata$ (Bodnaryk 1978) and $Mallada flavifrons$ (Principi et al. 1990). In addition, these results are similar to previous studies that found that with low temperatures were requirement for diapause termination, especially for $B. minax$ (Dong et al. 2013), and other tephritids (Vankirk and Aliniazee 1982; Teixeira and Polavarapu 2005a, b, c, d; Dambroski and Feder 2007). Therefore, pupation at a lower temperature might induce overwintering pupa to enter the diapause stage prematurely or to begin entering post-diapause development earlier.

Most studies have shown that the amount of energy accumulated prior to diapause is reflected in the intensity of diapause. In general, smaller diapausing individuals tend to terminate diapause earlier and the diapause intensity is obviously weaker (Matsuo 2006). This has been demonstrated in a variety of insects, including the moths $Laspeyresia strobiella$ (Bakke 1971, Nesin 1985), Prodoxus
Table 1. Female and male pupal periods of different body weight groups of *Bactrocera minax*

| Group | Female | Male | Female vs Male |
|-------|--------|------|----------------|
|       | ±     | ±    | df  | F   | P   |
| G-58  | 215.5| 210.6| 1   | 0.205| 0.6607|
| G-68  | 221.9| 216.5| 1   | 2.196| 0.1471|
| G-78  | 221.7| 220.8| 1   | 0.043| 0.8362|
| G-88  | 226.3| 220.8| -   | -    | -    |
| G-95  | 228.9| 220.8| -   | -    | -    |

All pupae were divided into five groups according to pupal weight (G-58: 55–61 mg; G-68: 65–71 mg; G-78: 75–81 mg; G-88: 85–91 mg; G-95: 92–98 mg). Values (mean ± SE) labeled with different letters in each group are significantly different by Tukey’s test with *P* < 0.05, and *t*-test for two samples with *P* < 0.05. Sample numbers are given in parenthesis and ‘-’ represents data absence for insufficient samples.

In conclusion, our results provide an overview of the relationships among *B. minax* pre-diapause temperature, diapause intensity, and individual body weight. Pre-diapause environmental temperatures and overwintering individuals’ body weights are important considerations when modeling the diapause duration. The period of *B. minax* adult emergence is a significant time for the effective control of this pest. Therefore, our study may be useful for more accurate estimations of adult emergence times in the field and in developing efficient pest control management for subsequent years.

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