



E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(3): 1658-1661
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Received: 16-03-2017
Accepted: 17-04-2017

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Morphological changes in *Chilo partellus* (Swinhoe) undergoing diapause

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Abstract

The purpose of this work was to provide a practical pictorial guide for identification of diapausing larvae of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) based on morphological and morphometric characteristics in the laboratory of Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India during February, 2014. On the basis of pictorial guide, the larvae of *C. partellus* which completely lack cuticular pigmentations, markings, spots and asetose tubercle are the distinctive diapause larvae. Transitional morphs are less conspicuously pigmented and considered as intermediate of non-diapausing (spotted morphs) and diapausing larvae (immaculate morphs). There was significant reduction in body weight, size and head capsule width in diapause than the non-diapause larvae. The pupal and adult sizes of diapause experienced population were smaller than the non-diapausing counterparts. Adults emerged from diapausing larvae were darker in color than those emerged from non-diapausing larvae. This pictorial key to identify diapausing larvae will be highly useful for the entomologists working on diapause, and the agriculture practitioners to find and kill diapausing larvae of *C. partellus* in their resting sites to disrupt further multiplication.

Keywords: *Chilo partellus*, diapause, diagnostic key, identification

1. Introduction

Diapause is hormonally mediated developmental response that occurs at a specific stage in insect species [1]. Diapausing insect passes through a series of physiological events such as induction, maintenance, termination, and post-diapause development [2]. During diapause, not only the physiological (suppression of metabolic activity), but several morphological (weight, size, body colour, pigmentation and sclerotization) and behavioral changes (cessation of feeding, exceeding normal developmental time and construction of resting sites) may also be encountered in insects [3].

Spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is one of the most serious constraints and causing enormous losses of about 18 to 25% in maize and sorghum under different agro-climatic conditions in Asia [4]. Host plant maturity coupled with warm and cold season enforces *C. partellus* to enter aestivation (summer diapause) [3, 5] and hibernation (winter diapause) [6], respectively. *Chilo partellus* undergoes hibernation under North Indian conditions [6] and aestivation under South Indian conditions [7]. During diapause, *C. partellus* larvae moult several times designated as supernumerary moults [3]. Genetic determination of locally adapted life-history traits such as diapause and polyphenism in response to feeding on host plants and climate change are important to understand their bio-ecology and population dynamics in insects [8]. In many insect species, diapause state is very rarely accompanied by morphological characteristics that permit their easy identification. Therefore, purpose of the present study was to establish a detailed diagnostic key based on photographs to differentiate diapausing and non-diapausing larvae of *C. partellus*. This paper will help not only to scientists engaged in diapause related research in *C. partellus* but also to untrained farmers to find and kill diapausing larvae of *C. partellus* in their resting sites to disrupt further multiplication.

2. Materials and Methods

Larvae of *C. partellus* were collected during February, 2014 from experimental field of Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India (Latitude; 28°38'23" N and Longitude; 77° 09'27"E, height above mean sea level is 228.61 meters), and the larval culture was maintained on artificial diet [9] under laboratory conditions

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at 27 ± 1 °C, $65 \pm 5\%$ relative humidity, and 12:12 (L:D) photoperiod. Adults emerged from the culture were released in oviposition cages and provided with cotton swab soaked in water. The cages were covered with wax-paper from outside to serve as oviposition substrate. The wax-papers were changed daily and kept at 27 ± 1 °C and photoperiod 12:12 (L:D) for hatching. From this culture late 5th instar (~25 days old) were exposed to earlier established aestivation ($32^\circ\text{C} + 13\text{L}:11\text{D}$) [5] and hibernation ($22^\circ\text{C} + 11.5\text{L}:12.5\text{D}$) [6] inducing conditions together with dry diet, considering natural climatic conditions prevailing during winter and summer in India. To terminate diapause, 45 days old diapausing larvae were exposed to $27^\circ\text{C} + 12\text{L}:12\text{D}$ together with fresh diet from their respective diapause inducing conditions. The diapausing and non-diapausing larvae were examined and photographed for body colour, pigmentation, and sclerotization to capture prominent visible differences among them. Furthermore, the differences in colour and size of pupae and adults emerged from diapausing and non-diapausing larvae were also established. The larval morphometrics (weight, length and head capsule width) of *C. partellus* larvae undergoing hibernation and aestivation along with their non-diapausing counterparts were also recorded. The larval weights were recorded using electronic balance (Precision balance, CB-Series, Contech). Head capsule width and length were recorded using Leica StereoZoom microscope (Leica Microsystems Ltd, Heerbrugg, Switzerland). There were five replications for each larval state in a Completely Randomized Design.

2.1 Statistical analysis

The data on weight, length and head capsule width of aestivating, hibernating and non-diapausing larvae were compared using one-way analysis of variance. The significance of differences between treatments were judged by F-test, and treatment means were compared using least significant difference at $P = 0.05$.

3. Results and Discussion

Chilo partellus undergoes facultative diapause as mature larvae inside the mature stems and/or stubbles of host plants [10] (Fig. 1). Present studies clearly showed polymorphism in mature non-diapausing and diapausing (aestivation/hibernation) larvae of *C. partellus* (Fig. 2). When mature spotted (no-diapause) larvae were exposed to diapause inducing conditions (aestivation/hibernation), they first transformed into transitional morphs (diapause and non-diapause intermediates) within 2 days of transitional period. In transitional morphs, the cervical shield and pinaculæ were less pigmented than in spotted morphs. In one to two days, the transitional morphs transform into immaculate larvae. These immaculate morphs were without pigmentation around the setae, having uniform milky white colour, and considered diapausing larvae in *C. partellus*. Chippendale and Reddy [11] have also shown that diapause larvae of *Diatraea grandiosella* Dyar lack cuticular pigmentation and designated as immaculate larvae. Immaculate diapause larvae of some borer species can be easily distinguished from non- or pre-diapause spotted ones which have segmental arrangement of sclerotized pinaculæ [11, 12]. This kind of polymorphism is of special physiological interest because the transitional morph from the spotted to immaculate larva positively identifies the onset of diapause. Furthermore, the presence of a transitional period makes it possible to obtain metabolic information about pre-diapause mature larvae.

There were no differences in body color and pigmentation in hibernating and aestivating larvae, and collectively designated as diapause larvae to demonstrate the detailed diagnostic features of diapausing and non-diapausing larvae. The size of diapause larvae was found much reduced than the non-diapause larvae (Fig. 3A₁ & 3A₂). The cuticular pigmentation disappear in diapause larvae and body color turns creamy to milky white, while the body of non-diapausing larvae remain light brown with dark brown to black spots (Fig. 3B₁ & 3B₂). The absence of tanned areas in the cuticle, head capsule and pro-thoracic shield of diapause larvae of *C. partellus* could be due to low titer of ecdysone in haemolymph. As this enzyme is involved in formation of the tanning agent N-acetyldopamine in non-diapause larvae, might be absent from the haemolymph- of newly-ecdysed diapause larvae resulting in disappear of cuticular pigmentation [13]. The aetose tubercles disappear in diapause larvae, while present in non-diapausing counterparts (Fig. 3C₁ & 3C₂). Aetose tubercle is a useful character for distinguishing the larvae of *C. partellus* from other species of *Chilo*. They are present on each of meso and meta-thorax in between theta and Pi group and one aetose tubercle behind the spiracle in each of the 1-7 abdominal segments [14]. The head capsule colour in diapause larvae turns reddish brown with blur epicranial suture, being dark brown to black with distinct epicranial suture in non-diapausing larvae (Fig. 3D₁ & 3D₂). The colour of pro-thoracic shield was dark brown in non-diapause larvae, while it turned to light brown to creamish in diapausing larvae (Fig. 3E₁ & 3E₂). The diapause larvae failed to pupate and construct diapause chamber (Fig. F), and exceeded normal development time by exhibiting at least >2 supernumerary moults (Fig. 3G). Termination of diapause is followed by pupation, wherein pupal size was found reduced in diapause larvae than the non-diapause counterparts (Fig. 3H₁ & 3H₂). Similarly, adults emerged from diapause larvae were comparatively smaller in size and darker in color than those emerged from non-diapause larvae (Fig. 3I₁ & 3I₂).

The morphometric parameters viz., larval weight, length and head capsule width of *C. partellus* larvae significantly varied across physiological states, i.e. non-diapause, hibernation and aestivation (Table 2). The diapause (hibernation and aestivation) larvae of *C. partellus* were significantly lighter than the non-diapause larvae ($F = 11.33$; $df = 2, 8$; $P < 0.001$). The aestivation larvae were significantly heavier than the hibernation counterparts. The length ($F = 8.004$; $df = 2, 8$; $P < 0.037$) and head capsule width ($F = 4.86$; $df = 2, 8$; $P < 0.02$) of non-diapause larvae were significantly more than the non-diapause larvae. Among diapause, head capsule width of aestivation larvae was significantly more than the hibernation larvae, while the differences for larval length were non-significant (Table 2). This loss of body weight and size in diapause larvae could be due to reduced feeding, diet deficient in water and protein contents and/or due to loss of energy while constructing diapause chamber [3]. It is generally assumed that the number of larval ecdysis is genetically fixed [15]. However, we have observed occurrence of >2 supernumerary moults (additional moults) in diapause larvae of *C. partellus*. The existence of supernumerary moults in *C. partellus* suggests that the corpora allata produces active forms of juvenile hormone during the entire course of diapause [16-18].



Fig 1: Photograph showing the diapause larvae of *Chilo partellus* inside the old stubbles of sorghum plant from the experimental field of Division of Entomology, ICAR-IARI, New Delhi, India.

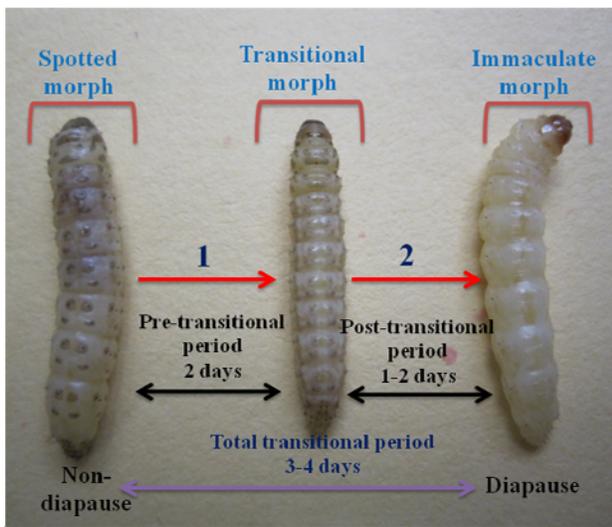


Fig 2: Late 5th instar *C. partellus* larvae on exposure to diapause inducing conditions, normal spotted larvae (non-diapause) passes through transitional morph (intermediate of non-diapause and diapause) before assuming the typical immaculate diapause morph.

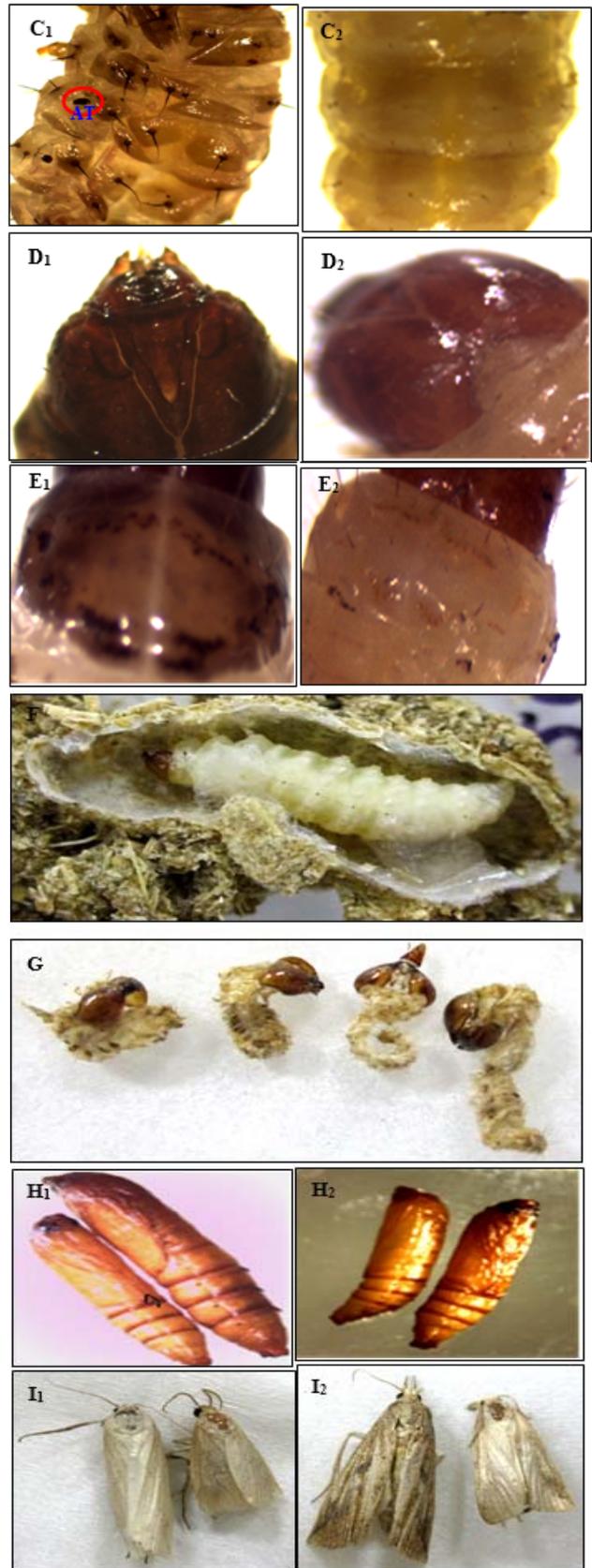
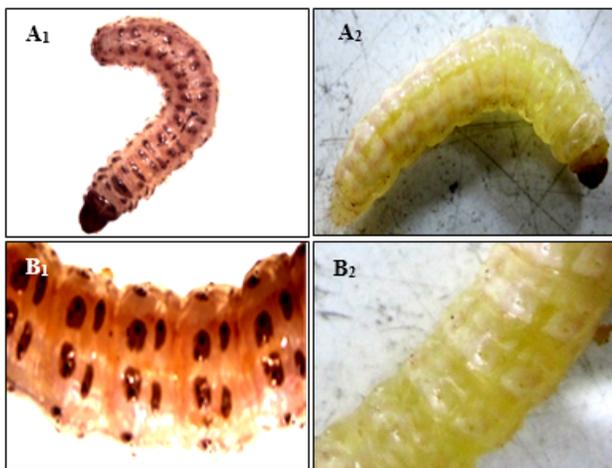


Fig 3: Pictorial diagnostic key for identification of larval diapause in *Chilo partellus* alongwith some important post-diapause pupal and adult characteristics.

Table 1: Diagnostic key for identification of diapausing and non-diapausing larvae of spotted stem borer, *Chilo partellus*.

Characteristics	Non-diapause	Diapause (Aestivation/Hibernation)*
Size of larvae	Large (Fig. 3. A ₁)	Reduced (Fig. 3. A ₂)
Body colour and pigmentation	Light brown with dark brown pigmentation (Fig. 3. B ₁)	Creamy to milky white with reduced or no pigmentation (Fig. 3. B ₂)
Asetose tubercles (AT)	Present (Fig. 3. C ₁)	Absent (Fig. 3. C ₂)
Head capsule colour	Dark brown to black with clear epicranial suture (Fig. 3. D ₁)	Reddish brown sometime with distinct epicranial suture (Fig. 3. D ₂)
Color of prothoracic shield	Dark brown (Fig. 3. E ₁)	Light brown to creamy (Fig. 3. E ₂)
Symptoms to enter diapause (diapause chamber)	No-diapause chamber	Fail to pupate and construct diapause chamber (Fig. 3. F)
Supernumerary moults	No supernumerary moults	>2 supernumerary moults (Fig. 3. G)
Pupal size	Larger (Fig. 3. H ₁)	Smaller (Fig. 3. H ₂)
Adult colour and size	Light brown and larger in size (Fig. 3. I ₁)	Dark brown and smaller in size (Fig. 3. I ₂)

Table 2: Variation in morphometrics of diapausing and non-diapausing larvae of spotted stem borer, *Chilo partellus*.

Larval state	Weight (mg/larva)	Length (mm)	Head capsule width (mm)
Normal	122.2 ± 1.20a	16.06 ± 0.24a	1.52 ± 0.002a
Aestivation	96.06 ± 0.75b	14.42 ± 0.24b	1.45 ± 0.009b
Hibernation	85.87 ± 0.62c	13.64 ± 0.32b	1.36 ± 0.005c

Within column values followed by different letters are significantly different from each other (Tukey's HSD post-hoc test at $P \leq 0.05$)

4. Conclusion

The present study concluded that the diapause in *C. partellus* is linked to a decrease in weight and size of larvae, pupae and adults, and further it suggests that activity of stationary moults do not appear to provide the insect any adaptive value. Moreover, present studies will be helpful in distinguishing diapausing and non-diapausing larvae, aid-in diapause-based physiological experimentation, immature stage identification, and management of *C. partellus* under field conditions.

5. Acknowledgements

The financial support from Department of Science and Technology, Ministry of Science and Technology, Govt. of India, New Delhi (SERB No:SB/SO/AS-020/2013), India is gratefully acknowledged.

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