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Factors Inducing and Terminating Larval Diapause in a Stem Borer, Busseola fusca in Western Kenya

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Abstract
Identification of factors regulating diapause induction and termination was attempted with a stem borer, Busseola fusca (Lep.: Noctuidae). Diapause was induced when matured stems (15 week old) of sorghum were given to last instar larvae. It was recognized that the conditions where sorghum stems with a constant age were provided were not favorable to the larvae and as a consequence, however, no diapause incidence was induced. Water played a significant role in the termination of late diapause. During the late diapause period, larvae in the field were exposed to rainfalls in the short rainy season. This precipitation, however, terminated diapause in only a few larvae. This indicates that diapause termination of a stem borer requires sustained exposure to wet conditions, which do not usually tale place during the short rainy season. Drinking water did not stimulate a break of the larval diapause. Diapause larvae transferred to artificial wet conditions pupated without increase in fresh weight. It is thus concluded that primarily significance for diapause termination in B. fusca is association with water.

Discipline: Insect pest
Additional key words: plant phenology, rainfall, sorghum, water contact water uptake

Introduction

An African corn borer, *Busseola fusca*, which is a major insect pest on sorghum and maize in Africa, enters aestivation diapause at the final larval instar[22]. Due to the semi-arid conditions in Western Kenya, particularly around the Mbita Point Field Station (MPFS), a monocropping system is employed for sorghum culture. Under such conditions, *B. fusca* has two generations a year and the second generation diapauses in the dry stalks and stubbles of the host plants during the dry season, which lasts 6 to 8 months from June/July to February/March.

An analysis of relationship between the diapause incidence and plant phenology of maize was carried out in *B. fusca* in Nigeria[23]. It is suggested that the degradation of feed quality in matured maize plants be a main factor for inducing diapause. In this connection, it is still to be identified yet what a relationship exists between fresh sorghum stems of different ages as a feeding stuff for larvae and diapause incidence.

Onset of rainfall frequently coincides with the time of diapause termination[6]. Rainfall or artificial wetting stimulates pupation in diapausing larvae of the stem borers, *Diatraea elineolata*[10] and *Rupela albinella*[24]. Results of various studies indicate that water is associated with breaking of the diapause of *B. fusca*[19,20,22]. It was reported that 10 mm of rainfall per day could terminate the diapause of *B. fusca*[25]. However, other studies suggest that an incidental contact with water be not the cause of abrupt termination of the diapause[9] and that provision of water be necessary only after the completion of the diapause development[1]. Further studies are therefore required to clarify the influence of water on the termination of diapause of *B. fusca*, including artificial wetting in a laboratory and raindrops in nature.

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In various insect species, water is actively absorbed or imbibed by larvae at the end of their diapause. Such water uptake may be needed for the resumption of subsequent morphogenesis[3]. In the larval diapause in the rice borer, *Chilo simplex*[11], the Southwestern corn borer, *Diatraea grandiosella*[18] and the European corn borer, *Ostrina nubilalis*[2,12], it is recognized that water uptake is required for the pupation of the overwintered larvae. In order to identify a function of water pertaining to the diapause termination in *B. fusca*, further studies are also required.

Materials and methods

1) Insect rearing to identify factors for inducing diapause

Eggs of *B. fusca* were collected from adults of the second generation. A fresh sorghum stem (8 cm length) was given to a newly hatched first instar larva which was reared in a glass vial (2.5 cm diameter, 8.5 cm height) with a plastic cover having small holes. The feeding stuff was replaced with a fresh stem every other day.

A series of three experiments were set up. In the first experiment, newly hatched larvae were provided at the start with sorghum stems of 2 weeks old. Thereafter, an older stem was given in accordance with the age of developing larvae: for example, as of day 7, larvae were fed on a 21-day-old sorghum stem. Older stems such as 3, 4, 5 and 6 weeks old were also subjected to investigations. In the second experiment, the same procedure as the first one was employed until the last molting, being followed by a different treatment; i.e., the last instar larvae of 16 days old were given 15-week-old stems, instead of 6-week-old ones. In the third experiment, sorghum stems of only a constant age were provided the developing larvae.

2) Insect rearing to identify factors for terminating diapause

B. fusca larvae under diapause were collected in a field of sorghum near the MPFS in the period August to February when the field was ploughed. For a dry condition, a Whatmann filter paper and 1 g of cotton wool were laid on the bottom of a disposable petri-dish (9 cm). For a wet condition, a filter paper and 2 g of cotton wool in a petri-dish were soaked with 2 m*l* of water. About 0.2 m*l* of water was added every third day to maintain the moist condition. In each of the dry and the wet conditions, two larvae were placed in a petri-dish, taking into account that a crowded condition can always induce high mortality[14]. In feeding water, it was supplied *per os* with a Pasteur pipet to a diapausing larva once a day. The feeding was discontinued when the larva lost an interest in water. Then, the larva was weighed to check the quantity it consumed. All the laboratory experiments were conducted under room temperature; i.e., 26.5 ± 2°C.

Results

1) Factors inducing diapause

In the first experiment, data on the longest period of prepupation and the least occurrence of pupation were taken, having started with 2-week-old sorghum stems (data not shown). It was found that a 4-week-old sorghum stem was the most favorable diet, resulting in the quickest larval development in 21 days, the least mortality and the highest incidence of pupation (Fig. 1–A). In the second experiment (Fig. 1–B), only 2% pupated and 13% went into diapause. The prepupation period was considerably delayed; i.e., 40–42 days. In the third experiment where the larvae were fed on a sorghum stem with a constant age, a high mortality was induced in all feeding conditions; i.e., stems of 2 weeks old (data not shown), 4 weeks old (Fig. 1–C), and 5 weeks old (data not shown). No pupation took place in 14-day-old stems. Only 2% and 6% pupated in 4- and 5-week-old stems, respectively. In summary, it is concluded that the conditions where sorghum stems with a constant age are provided are not favorable to the larvae and as a consequence, however, no diapause incidence is induced.

2) Factors terminating diapause

(1) Rainfall and pupation in nature

In the Mbita region of Kenya, a short rainy season comes irregularly after a long rainy season which starts usually in March or April. In 1986, the short rainy season started in November (Fig. 2). In order to clarify the influence of precipitation during the short rainy season on dispausing larvae, incidences of pupation in nature were investigated. In November, precipitation of more than 10 mm per day took place twice, but it showed little influence on larvae since no pupation was observed after these rainfalls. After the rather intensive rainfalls in early December, pupae represented 10 to 20% of the field samples. By the time of ploughing in February, the rainfalls were sporadic, and the proportion of pupae in nature remained at only 10%. When the long rainy season started, most larvae pupated[21]. This implies that precipitation during the short rainy season does not give a major cue to the complete diapause termination of *B. fusca*.

(2) Artificial dry and wet conditions

Diapausing larvae sampled on November 30 did not pupate under the dry condition (Fig. 3-A).

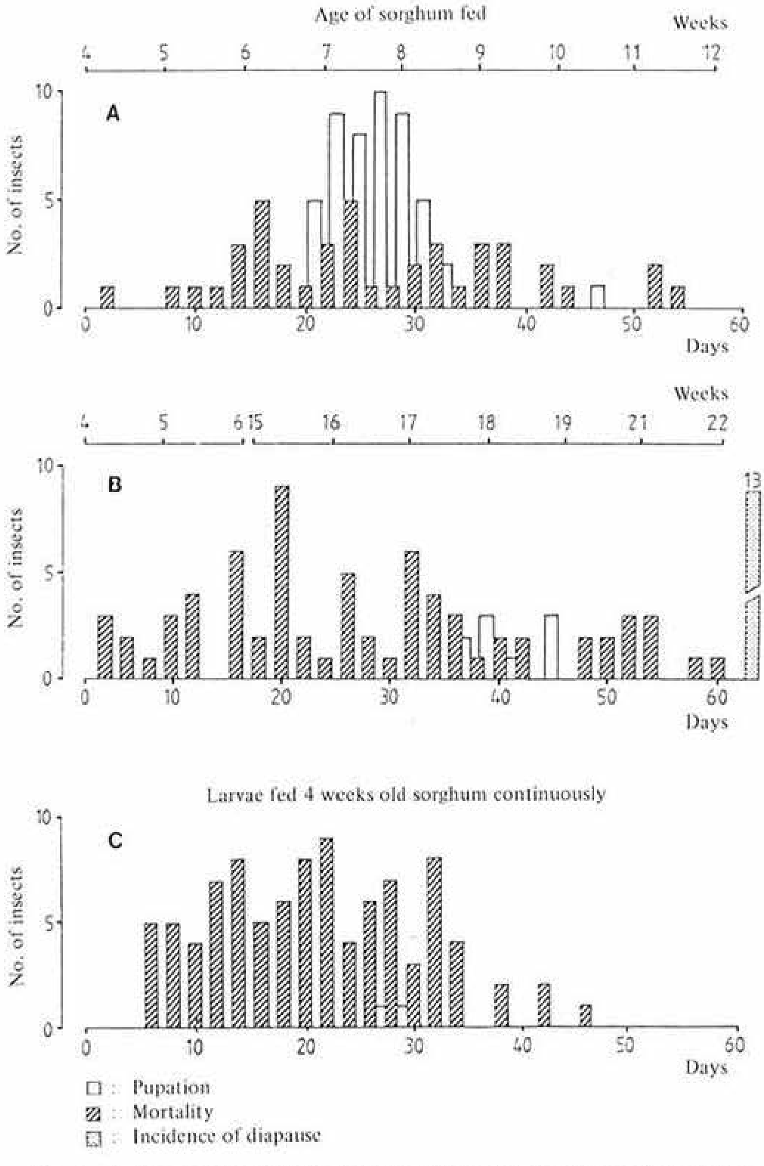

Fig. 1. Incidence of pupation and mortality after provisions of sorghum fresh stems of different stages
A: The abscissa indicates age of larvae (days) and host plant (week), B: Fresh sorghum stems of advanced age (15 weeks) were given for the last instar larvae and beyond, C: Fresh stems of constant age (4 weeks old) were provided.

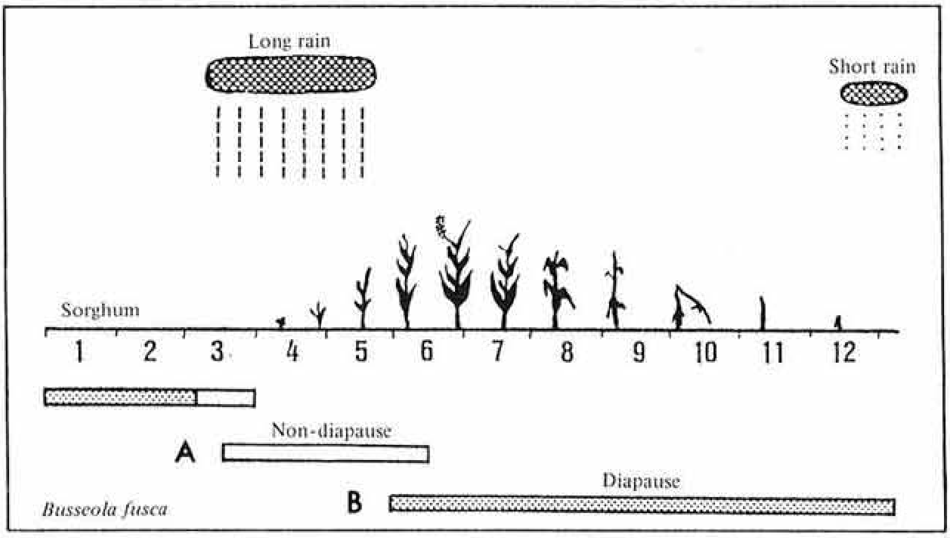

Fig. 2. Life cycle of *Busseola fusca* and the host plant phenology at the Mbita Point in Western Kenya
A: Non-diapause 1st generation, B: Diapause 2nd generation.

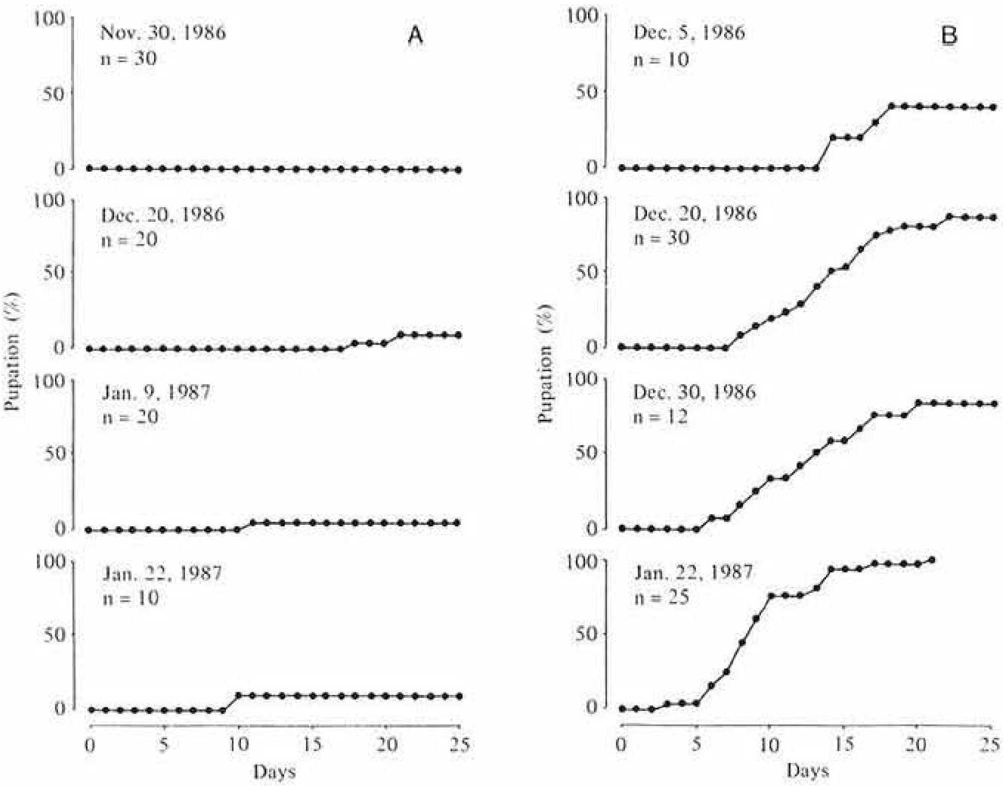

Fig. 3. Incidences of pupation in the dry and wet conditions
Date indicates a sampling time in the field and also a time when the larvae were transferred to a dry condition (A) and a wet condition (B)[13].

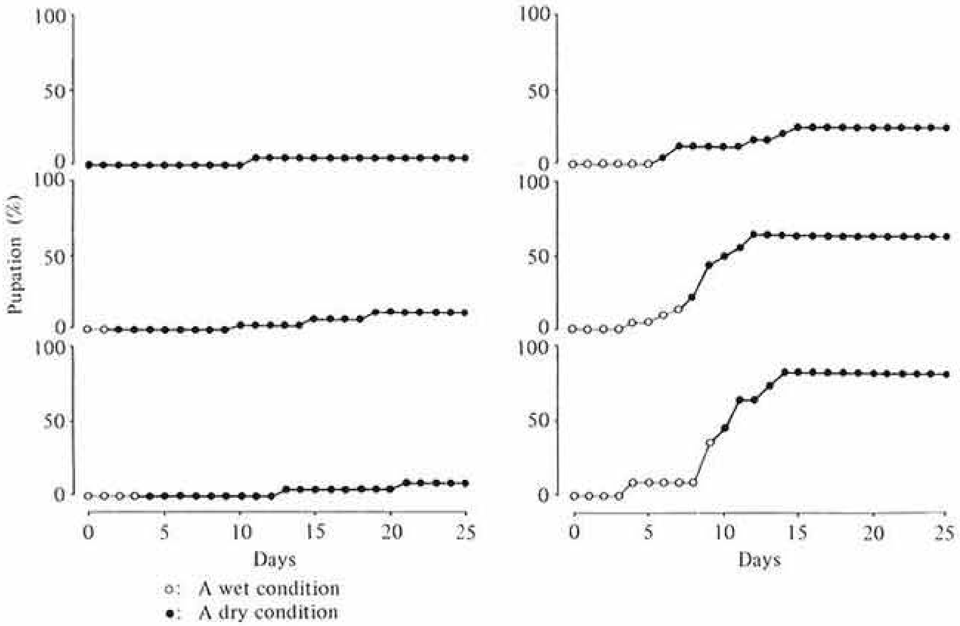

Fig. 4. Incidences of pupation after exposure to a wet condition for different periods
In each group, 20 to 30 larvae were used.
All the larvae were collected on Jan. 9[13].

Pupation took place in diapausing larvae sampled only after that date, but the pupation rates were very small; i.e., 5 to 10%. The dry conditions did not stimulate pupation of the diapausing *Busseola* larvae.

During the initial period of 3 to 4 months after the onset of diapause, artificial wetting did not terminate diapause. Of the larvae sampled on December 5 and transferred to the wet condition in the laboratory, 40% pupated within 25 days (Fig. 3-B). As time proceeded, the larvae showed a greater response to water. For example, in regard to the larvae sampled on January 22, their prepupation period was shortened by about 7 days and 100% of the larvae pupated within 25 days (Fig. 3-B).

Diapausing larvae collected from the field during the short rainy season pupated under the condition of artificial wetting in the laboratory (Fig. 3-B). However, under the field condition, in spite of exposure to precipitations over 10 mm per day, the rate of pupation in the open air was as low as that under the dry condition in the laboratory. In order to interprete the apparently contradictory data in conformity, diapausing larvae were placed under the wet condition for the durations of 0, 3, 5, 7 and 9 days.

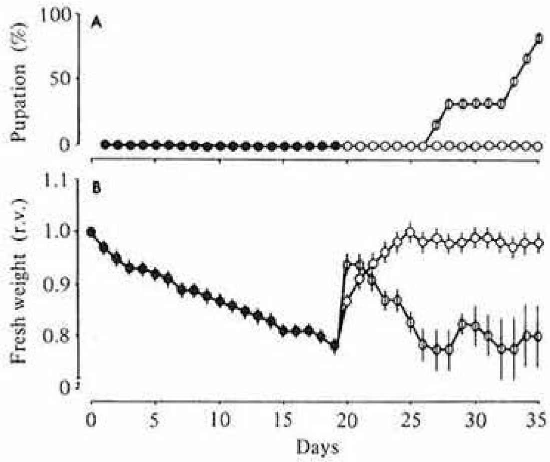

Fig. 5. Effect of water uptake on diapause termination in *Busseola fusca* larvae
A: Incidence of pupation, B: Changes of fresh weight in a relative value (r.v.).
The larvae (n = 28) were sampled on Dec. 3 and transferred to a dry condition on the same day (●). After 19 days, 14 larvae were given water *per os* and kept in a dry condition (○), and the other 14 larvae were transferred to a wet condition (⦶).
Vertical lines indicate standard error[15].

They were then transferred to the dry conditions and the rates of pupation were observed (Fig. 4). Up to 3-day exposure to water, no effect could be seen on the larvae. The rate of pupation was slightly increased after 5-day exposure to the wet condition; 25% of the larvae pupated. The highest level of pupation was achieved after 7 to 9 days of continuous exposure to water.

The diapause larvae sampled on December 3 and continuously placed under the dry condition for 19 days lost their weight (Fig. 5). However, when they were transferred to the wet condition, the dehydrated larvae gained weight and 83% of them pupated within 16 days. Apart from this group, the other half of the dehydrated larvae were given water orally so that water contact could be minimized. Water uptake was confirmed by an increase in fresh larval weight (Fig. 5). However, despite the considerable water uptake, i.e., uptake of the same amount of water as that in the wet condition within 3 days, no pupation took place.

Discussion

1) Diapause induction

It is well known that qualitative and quantitative changes in feeding stuffs affect diapause induction of a stem borer. It is shown in *Leptinotarsa* that feeding on physiologically old leaves can induce diapause[7]. An analysis of relationships between the diapause induction of *B. fusca* and the phenology of maize was carried out in Nigeria[23]. The result obtained indicates that the diapause incidence increases with the age of maize: i.e., 24% diapause on 6- to 9-week-old maize plants and 91% diapause on 12- to 15-week-old maize plants. The general status of such a positive correlation between the diapause incidence and the age of plants fed is consistent between those data demonstrated by Usua and those obtained in the above-stated study. However, a lower diapause incidence took place in the present study with sorghum stem, as compared with maize.

Feeding on young stems with a constant age had negative effects on the larval development, causing a prolonged larval growth duration, a high mortality and a very low rate of pupation. The unfavorable diet condition, however, did not lead to the early start of diapause.

2) Diapause termination

It is generally concluded that a provision of water to insects under diapause leads to its termination only if the insect's diapause development has already completed[3,5]. *Busseola* larvae do not respond to water at all during the first 3- to 4-month period of diapause. This may imply that water is effective for diapause termination only during its late period. It is very likely that in *B. fusca* as well, water is only effective when the diapause development has been completed[15].

The completion of diapause development in *B. fusca* occurred in early December as evidenced by its response to water supply (Fig. 2–B). Commencement of the short rainy season in Mbita Point coincided with the completion of diapause development. The precipitations in this period, however, did not terminate diapause in the majority of larvae in the field. The result of a laboratory experiment revealed that continuous contact to enough moisture lasting for over one week was required to terminate diapause (Fig. 3). One of the characteristics of rainfall patterns in the short rainy season in the Mbita area is that in most cases rain falls for a short period during the night. During this season, under strong sunshine and high temperature in the following day, the atmospheric and soil moisture is not maintained long enough, as it is in the long rainy season. *Busseola* diapause comes to an end with a heavy and continuous rainfall[8,17,20]. It may therefore be concluded that only a long rainy season can provide conditions of continuous and sufficient moisture for *B. fusca* to terminate its diapause in that region. In regard to the water contained in the plant tissues, it is recognized that lack of the tissue water is one of the major limiting factors for the immediate resumption of growth[4,24,26]. The present study indicates that it is likely that water contact is more effective than water uptake for the termination of larval diapause in *B. fusca*. The data obtained showed that the larvae given water *per os* failed to pupate (Fig. 4). In contrast, the larvae placed under the artificial wet condition pupated in a significantly higher rate consuming the same quantity of water.

The above-stated characteristics of *B. fusca* in relation to water contact as a diapause terminating factor might be associated with its high ability of ecological adaptation to the tropical conditions. In the short rainy season, it is very likely that the larvae were holding enough water to resume growth, because it was recognized that the diapausing larvae, transferred to a wet condition from the field, pupated immediately without any increase of the body weight (data not shown). If an ingested water were the only limiting factor, most of the larvae could terminate diapause long before the host plants are made available for feeding. In a temperate region, however, a limiting factor for further growth of post-diapause insects could be related not only to water uptake but also to low temperature. In these two ways, the reproductive period of the insects and the availability of the host plants could be well synchronized, even if rains out of season provide enough water for immediate resumption of growth in that region.

Possibility of photoperiodic regulation of the diapause induction or termination was not included in this study on *B. fusca,* because no significant variation in daylength takes place in the Mbita Point Field Station in Kenya (0°22′S). Instead, *B. fusca* utilizes plant phenology as an incentive for diapause induction and waterdrops of rainfall as a diapause termination signal.

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