

ECOLOGICAL AND PHYSIOLOGICAL ASPECTS OF AESTIVATION-DIAPAUSE IN THE LARVAE  
OF TWO PYRALID STALK BORERS OF MAIZE IN KENYA

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CENTRALE LANDBOUWCATALOGUS



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# Ecological and physiological aspects of aestivation-diapause in the larvae of two Pyralid stalk borers of maize in Kenya

Proefschrift

ter verkrijging van de graad van  
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## STELLINGEN

## I

Door de grote verscheidenheid in de aard van de periodieke rusttoestand van insekten en de mechanismen die hieraan ten grondslag liggen is elke klassifikatie van de rusttoestand, gebaseerd op externe ekologische factoren (Müller) of op interne fysiologische veranderingen binnen het insekt (Mansingh) gebrekkig en daardoor betrekkelijk zinloos.

Müller H.J. (1970) Nova Acta Leopoldina 35, 1-27.

Mansingh A. (1971) Can. Ent. 103, 983-1009.

Dit proefschrift.

## II

Het verdwijnen van de kutikulaire pigmentatie als criterium voor de diapauze dient met voorzichtigheid te worden gehanteerd.

Dit proefschrift.

## III

Konklusies als die van Hirano betreffende de larvale groei van *Chilo suppressalis* en de drooggewicht bestanddelen van het door deze rijststengelboorder genuttigde voedsel zijn aanvechtbaar.

Hirano C. (1964) Bull. Nat. Inst. Agr. Sci., Ser. C., no. 17.

Dit proefschrift.

## IV

De konklusie van Chippendale en Reddy dat de induktie van diapauze in de "southwestern corn borer" *Diatraea grandiosella* een "extremely temperature-dependent process" is, waarbij fotoperiode een ondergeschikte rol speelt, berust op onvoldoende gegevens.

Chippendale G.M. and Reddy A.S. (1973) J. Insect Physiol. 19, 1397-1408.

## V

De bewering van Usua dat er een kausaal verband bestaat tussen het optreden van diapauze in de mais stengelboorder *Busseola fusca* en gelijktijdig voorkomende veranderingen in de samenstelling van diens waardplant is zuiver spekulatief.

Usua E.J. (1973) Ent. exp. & appl. 16, 322-328.

## VI

Het toekennen van een keuringscertificaat aan bepaalde fruitgewassen door de NAK-B<sup>+</sup> is geen enkele garantie voor de levensvatbaarheid van die gewassen indien ze in het najaar in pakketten worden aangeboden en is dus misleidend voor de konsument.

<sup>+</sup> NAK-B Nederlandse Algemene Keuringsdienst voor Boomkwekerijgewassen.

## VII

De diskrepancie tussen de automatische vermelding van de man in het paspoort van zijn echtgenote en de onmogelijkheid voor de man zijn echtgenote in zijn paspoort te vermelden is een symptoom van de opgedrongen onafhankelijke rol van de man in de huidige maatschappij.

Paspoort instructie Nederland 1952, art. 23.

## VIII

Door de sterke invloed van "tribalism" op de maatschappelijke structuur van vele derde wereld landen, worden tal van ontwikkelingsprocessen ernstig bemoeilijkt.

## IX

De bezwaren van Kenyase autoriteiten tegen het dragen van de traditionele kledij door de Masai zijn onjuist en leiden slechts tot onnodige irritatie.

## X

Vele Nederlanders tonen plotseling een beter begrip voor het levensritme van volkeren in tropische gebieden tijdens een hittegolf dan tijdens normaal in Nederland voorkomende temperaturen.

Proefschrift van P. Scheltes

Ecological and physiological aspects of aestivation-diapause in the larvae of two Pyralid stalk borers in Kenya.

Wageningen, 6 september 1978.

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# 1 General introduction

Stem borers are being considered as major pests of many Gramineae all over the world (JEPSON 1954, METCALF and FLINT 1967, HILL 1975). All important graminaceous crops such as maize, millet, rice, sorghum and sugarcane - often the subsistence food crop in developing countries - may seriously be affected. In the temperate regions relatively few important stem borers are known. Very notorious are the southwestern corn borer *Diatraea grandiosella* and the European corn borer *Ostrinia nubilalis*, both Pyralidae. In the subtropics and the tropics stem borers are much more abundant. Well-known destructive examples are found among the Noctuidae e.g. the maize borer *Busseola fusca* (maize, sorghum), the pink stalk borer *Sesamia calamistis* (maize, sorghum, millet, rice, sugarcane), and the purple stem borer *S. inferens* (rice, sugarcane) and among the Pyralidae e.g. the spotted stalk borer *Chilo partellus* (maize, sorghum, millet, sugarcane, rice), the coastal stalk borer *C. orichalcociliella* (maize, sorghum, millet, sugarcane), the rice stem borer *Chilo suppressalis* (rice, maize), the sugarcane stalk borer *Eldana saccharina* (sugarcane, maize, sorghum) and several *Tryporyza* spp. (rice) (HILL 1975).

The life-cycle and damage of all borers is very similar. Normally eggs are laid on the undersurface of young leaves or between leaf sheaths and stems. After hatching the young larvae feed actively on the tender leaves but as soon as their size increases they bore into the stems. In young plants larvae attack the growing point and cause so called 'dead hearts'. In older plants the caterpillars bore into the main stem, which subsequently is hollowed out over a considerable length. Plants thus affected have a poor growth, a reduced yield and are more susceptible to wind damage and secondary infections. Small and weak plants usually suffer more from borer attack than healthy plants. In tropical East Africa where crops are regularly grown under adverse conditions (unpredictable rainfall, poor soils) this has often been demonstrated (COAKER 1956, MATHEZ 1972, HILL 1975). In such cases stem borer damage may be disastrous. When larvae are fully grown, they start preparing for pupation by cutting an exit hole in the stem to enable

the emerging moths to escape. The life cycle may be continuous in areas where suitable conditions for growth of the host plant are permanently present. Usually however the cycle is interrupted by a cold or dry season during which plant growth is impossible. The larvae then enter diapause (called 'hibernation' when occurring during a cold season and 'aestivation' when in the dry season) inside the old stems or stubble. On return of favourable conditions, pupae develop and the emerging moths oviposit on the newly planted host plants.

Chemical control of stem borers has often proved to be rather unsuccessful. Best results are obtained in the early season against the young larvae which have just hatched from the eggs laid by moths which had developed from diapausing larvae. Older larvae have penetrated the stalks in which they are well protected against insecticides. The duration of larval development varies widely within one population of stem borers and after the more or less synchronous development of the 1st generation attacking the crops, all developmental stages of borers can soon be found within one field. From that time chemical control, unless applied very regularly, is little effective (SCHMUTTERER 1969, HILL 1975).

Cultural control of stem borers is most frequently aimed at a destruction of the diapausing larvae from which the next crop infestation originates. Uprooting, burning, ploughing and flooding of the old stems are widely adopted methods, but only effective when applied at a large scale. In developing countries many individual farmers cultivate a large number of small plots and such a common approach is therefore difficult to accomplish. This is especially so when old stems are utilized e.g. in Tanzania maize stems were found to be used as building material (SWAINE 1957) and in Kenya I observed cut maize stems as boundaries of terraces on slopes. It is common practice to abandon maize and sorghum plants in the field after harvest in Kenya. If stems are cut (mainly for fodder) stubble is normally left behind. All these cultural practices increase the chances of survival for diapausing larvae during the dry season.

Aestivation-diapause of stem borers has often been referred to as a crucial link in the life-cycle which enables the insect to overcome the unfavourable hot and dry season (SCHMUTTERER 1969, HILL 1975). Nonetheless, thusfar almost all attention has been focused on the phase of the insect which is directly doing the damage and hardly any to the phase from which this damage initially results. The aim of this research was to fill this omission. Investigations were carried out on the factors which induce, maintain and terminate aestivation-diapause in two tropical stem borers. A better knowledge of the factors determining their seasonal activity may ultimately lead to a better control of these destructive insects.

## 2 The incidence of aestivation-diapause as related to climate

### 2.1 INTRODUCTION

Diapause (hibernation) has been thoroughly studied for a large number of insects in the temperate regions (LEES 1955, 1968, DANILEVSKII 1961, BECK 1968). In contrast very little attention has focused on aestivation-diapause of the tropics. The present research examines our knowledge of the climatological factor(s) that may induce aestivation-diapause in 2 species of Pyralidae in Kenya. The experimental spotted stalk borer *Chilo partellus* (Swinhoe) and the coastal stalk borer *Chilo orichalcociliella* (Strand), are major pests of maize in East Africa. An understanding of this crucial link in the life cycle of these insects may lead to better techniques of control in the future.

At the start of this research in Kenya, little more was known than the existence of a larval resting stage in old mature and often dry stalks or stubble of the host plant left in the field after the growing season (NYE 1960, SCHMUTTERER 1969, MATHEZ 1972). Hardly any information is available on the seasonal incidence of the aestivation-diapause or on the environmental conditions that induces the arrested development. In this paper we will concentrate on the role of climate factors in the induction of diapause. The role of the host plant will be dealt with later (chapter 4).

### 2.2 LITERATURE

*Criterion for diapause* The most frequently used criterion for larval diapause of Lepidoptera is the failure of the mature larva to pupate within an arbitrarily determined length of time after cessation of feeding. The critical length of time significantly exceeds the normal period required for pupation. The diapause of larvae of the European corn borer *Ostrinia nubilalis* (MUTCHMOR and BECKEL 1959, BECK and HANEC 1960), the pink bollworm *Pectinophora gossypiella* (ADKISSON et al. 1963, KHALIFA et al. 1975), the rice stem borer *Chilo suppressalis* (FUKAYA 1967) and the southwestern corn borer *Diatraea grandiosella*

(CHIPPENDALE and REDDY 1973) was determined in this manner. Larvae are usually kept under dry conditions, without food, in vials with moist absorbent filter-paper. Sometimes they are provided with the natural material in which diapause takes place e.g. EL-SAYED and RUSTOM (1960) and HASSANEIN and GALAL (1969) supplied field collected larvae of *P. gossypiella* with fragments of cotton fibre to prevent cannibalism and to facilitate the construction of cocoons. Such fibres obviously can not be considered as adequate food. The use of this method implies that diapausing larvae are capable of surviving starvation and drought (without pupating). The quality of diapausing larvae to resist starvation can be attributed to the usually high content of metabolic reserves and to the very slow expenditure thereof because of the low overall activity. In the tropics resistance to desiccation is of great importance since aestivating larvae are usually exposed to extremely dry conditions. Solid data on this however are rare. USUA (1974) demonstrated that at all relative humidities the percentage weight loss by non-diapausing larvae of the maize stem borer *Busseola fusca* is at least double that of diapausing larvae. His experiments were carried out near thermal death point. But even at a more normal temperature of 23°C CHIPPENDALE and REDDY (1972) found that both field and laboratory collected mature larvae of *D. grandiosella* (originally a tropical insect) when placed in vials with only moist absorbent paper have a high rate of weight loss until they ecdyse to an unspotted form associated with diapause. Thereafter weight loss is about twice as low. The increased drought resistance may well be caused by changes in the respiratory (intermittent opening of the spiracles) as well as the cuticular (reduced permeability) transpiration as has been found for many other insects (LEES 1956, BARTON-BROWNE 1964, CLOUDSLEY - THOMPSON 1975).

Field collected larvae have not always been tested for diapause in the absence of food. KATIYAR and LONG (1961) provided field larvae of the sugarcane borer *Diatraea saccharalis* with pieces of young maize stems during the period of (winter) diapause determination and the same method was adopted by USUA (1970, 1973) for *B. fusca* to investigate the incidence of aestivation. In both cases larvae did actively feed on the maize, although among diapausing larvae the feeding rate was distinctly lower.

Many Lepidoptera larvae inhabiting the tropics or of tropical origin offer - next to the failure to pupate and the drought resistance - a third widely used criterion for diapause: at the beginning of diapause they loose the cuticular pigmentation of their pinnaculi and turn into an unspotted morph. Such a transition has been observed for *Diatraea lineolata* (HYNES 1942, KEVAN 1944), *Scirpophaga* (= *Tryporyza*) *innotata* (VAN DER GOOT 1925, ROTHSCHILD 1971),

*Coniesta ignefusalis* (HARRIS 1962), *B. fusca* (HARRIS 1962, USUA 1970), *Chilo partellus* (GONÇALVES 1970, MATHEZ 1972, DELOBEL 1975b), *Chilo orichalcociliella* (MATHEZ 1972, DELOBEL 1975a), *D. saccharalis* (KATIIYAR and LONG 1960) and *D. grandiosella* (CHIPPENDALE and REDDY 1972).

*Climatic factors inducing diapause* Diapause in the temperate and subtropical climates is primarily induced by short days and low temperatures. Generally the critical daylength is dependent on the latitude where the insect is found and the incidence of diapause is inversely proportional to the prevailing temperature (BECK 1968). Examples of borers are the rice stem borer *Chilo suppressalis* (INOUE and KAMANO 1957, KISHINO 1969) and the European corn borer *Ostrinia nubilalis* (MUTCHMOR and BECKEL 1959, BECK and HANEC 1960, SKOPIK and BOWEN 1976). KATIIYAR and LONG (1961) working with the sugarcane borer *Diatraea saccharalis* in Louisiana (USA) also indirectly suggested that diapause in the larvae is induced by short photoperiods since pupation no longer occurs from October (during which month the temperature is still "well above the threshold for pupation") and since diapausing larvae were collected from stems which were "green and succulent and appeared to constitute a satisfactory food supply". In spite of this the authors did not exclude a food effect altogether. It should be mentioned here that in the tropics *D. saccharalis* was not found to diapause (KEVAN 1942). Another insect in the south of the U.S. of which diapause is controlled photoperiodically is the pink bollworm *Pectinophora gossypiella*. High temperature can largely nullify the short day effects, while also food (a high fat content) has a diapause inducing effect under short days (ADKISSON et al. 1963, MENAKER and GROSS 1965, ANKERSMIT and ADKISSON 1967). Although larvae of the southwestern corn borer *Diatraea grandiosella* - an originally tropical insect that recently migrated into the southern US - enter diapause under the influence of temperatures as high as 23-25°C and a short photoperiod (12L:12D), it was suggested by the authors (CHIPPENDALE and REDDY 1973) that temperature was the more important factor of the two. Their evidence was based on the overriding importance of temperature for the induction of diapause (without respect to photoperiod no diapause at constant temperatures of 27-30°C and 100% diapause at 20°C), and on the observation that diapause in the field is induced when night temperatures start falling below 20°C whereas the daylength is still diapausing averting. No photoperiodic effect could be related to development of the lesser cornstalk borer *Elasmopalpus lignosellus*. Moderately low temperatures significantly prolong the development but this retardation could according to HOLLOWAY and SMITH (1976) not be considered as diapause.

At lower latitudes seasons depend less upon changes in daylength. Temperature and other factors e.g. moisture (rain) and changes in the composition of the host-plant which are of minor importance in the temperate regions tend to play a major role in the tropics (chapter 4). But even at these latitudes photoperiod can be involved. This is best exemplified by *Nomadaeris septemfasciata* which responds to photoperiod in an area (Tanzania) where the difference between the longest and the shortest day is only 1 hr. (NORRIS 1965). However other factors are reported to induce diapause or quiescence more frequently.

Pupal diapause of the Sudan bollworm *Diparopsis watersi* is in Cameroon primarily dependent on temperature; alternating low nocturnal temperatures and high diurnal temperatures during the larval life induce diapause (JACQUEMARD 1976). GALICHET (1964) came to the same conclusion in Chad but he thought that the period of fluctuating temperatures needed to be followed by a period of very high temperatures (38°C during 6 hrs/day) after pupation. He also mentioned that photoperiod is not related to the induction of diapause. The incidence of diapause in larvae of the white rice borer *Rupela albinella* in Surinam is normally very low throughout the year but suddenly rises to 50% of the larval population during a period of relatively high temperatures. Compared to the same period of other years, neither food, dry period nor rainfall could be indicated as the agent causing diapause; ageing of the host plant stimulates diapause (HUMMELEN 1974).

In Egypt, larval diapause of the pink bollworm *Pectinophora gossypiella* seems to be primarily initiated by low temperatures. Again composition of the food (cotton boll) which changes towards the end of the season plays an important additional role (EL-SAYED and RUSTOM 1960, HASSANEIN and GALAL 1969). Results of PREVETT (1971) are very similar: the Indian meal-moth *Plodia interpunctella* collected from Pretoria (South Africa) entered a pre-pupal diapause at temperatures below 20°C. Time required for larval development at 17.5°C is more than 14 x longer compared to time at 30°C but larvae from a strain collected in Kano, Northern Nigeria, develop only 3.5 x slower at the lowest temperature. PREVETT considered the difference in developmental time at the two temperatures as normal for the Nigerian strain. He suggested that the diapause factor is bred out in strains inhabiting warm climates. The influence of photoperiod was not studied for *Plodia* nor for *Pectinophora* although in both cases the latitudes (South-Africa, Egypt) are such that an effect might be expected.

The spotted stalk borer *Chilo partellus* has a distribution in southern Asia from Afghanistan in the west to Thailand in the east (HILL 1975) and in East Africa from Sudan in the north (SCHMUTTERER 1969) to Mozambique (GONÇALVES 1970)

and Madagascar (DELOBEL 1975b) in the south. The environmental stimuli which lead to preparations for diapause of the larvae are however quite different. In North India larvae do not pupate below 13°C (BUTANI 1955). Also observations of other scientists indicate that the diapause of *C. partellus* in South Asia is governed by low temperatures (PANT and KALODE 1964, KHAN and KHAN 1968, MOIZ and QURESHI 1969). In East Africa however we find less agreement. It is not even clear whether diapause exists in all areas. In Mozambique for example, *C. partellus* develops continuously but has "a low activity" from May to September (GONÇALVES 1970). INGRAM (1958) even specifically mentioned the absence of a resting stage in Uganda, but also he stated that "the development is probably slowed down in the dry season". When later NYE (1960) did find a few aestivating larvae this incidence was attributed to the extremely dry host plant in which they were located. Another example of a continuous life-cycle the whole year around was found in Sudan "during the rainy season or under favourable conditions in irrigated areas"; larval diapause occurs in areas with a conspicuous dry or dead season (SCHMUTTERER 1969). The more severe dry season in the Lake District of Tanzania was held responsible for the presence of aestivating larvae (NYE 1960). From these examples it appears that the diapause of *C. partellus* is a facultative one only emerging when the environmental conditions - in which moisture seems to be crucial - deteriorate. This may well also be the case in Kenya where aestivating larvae are found under similar circumstances (SCHELTES 1976). The situation seems to be somewhat different in Madagascar where again relatively low temperatures are thought to be of prime importance in the diapause of *C. orichalcociliella* (DELOBEL 1975a) and *C. partellus* (DELOBEL 1975b). But again next to temperature DELOBEL mentioned impoverishment of food, drought and even daylength as factors which possibly stimulate the retarded larval development.

In Nigeria USUA (1968, 1973) studied the temperature effect on the larvae of the maize stem borer *Busseola fusca*. The rate of larval development was fastest at 28-30°C. Higher temperatures (32°C) were lethal, lower temperatures (23°C) resulted in delayed growth which however was not regarded as diapause by USUA. The onset of diapause was shown to be primarily induced by the state of maturity of the host plant. Earlier SWAINE (1957) and SMITHERS (1959) had suggested a similar relation for the same insect in Tanzania and southern Rhodesia respectively. They, however paid main attention to the moisture content of the plant. Important evidence to do so came from observations that *B. fusca* continues to develop in irrigated maize fields grown out of season (and in tillers of wild grasses and sorghum containing relatively much water) whereas

the development of the larvae in non-irrigated fields during the dry season ceases. Also for other tropical stem borer larvae diapause was thought to be primarily induced by the host plant: e.g. *Tryporyza innotata* (VAN DER GOOT 1925), *T. incertulas* (ROTHSCHILD 1971), *D. lineolata* (HYNES 1942, KEVAN 1944). Further details on the food condition in relation to diapause will be presented in chapters 4 and 5.

## 2.3 MATERIALS AND METHODS

### 2.3.1 Rearing methods

The colony was started with larvae collected from maize fields in the Coastal Province near Kikambala, Kenya (about 4° south of the Equator). Although two different species of *Chilo* (*C. partellus* and *C. orichalcooiliella*) occur in this area, only *C. partellus* was reared in the laboratory. This species is not only the more important one of the two in East Africa but is also a major pest in South Asia where *C. orichalcooiliella* is absent (HILL 1975). Larvae were reared on a wheat-germ diet (after CHATTERJI et al. 1968) which was slightly modified to obtain better microbial control. The composition of the diet is given in table 1.

Within 24 hrs of hatching eggs were transferred to a corked glass tube (diameter 2.5 cm, length 7.5 cm) that on the bottom contained about 15 ml diet which was slightly loosened from the tube wall with a needle. In the field newly hatched

Table 1 Composition of the artificial diet for rearing *Chilo partellus*

component	weight (g)	volume (ml)
wheat germ	15.00	
vitamin-free casein	17.50	
glucose	11.50	
Wessons' salt +	5.00	
cellulose	2.50	
vitamin fortification mixture (Vanderzant) +	7.00	
ascorbic acid	1.70	
aureomycin	0.18	
methyl para hydroxybenzoate	0.75	
agar	12.50	
KOH 4M		2.50
formaldehyde 10%		2.00
water		450.00

+ Obtained from Nutritional Biochemical Corporation, Cleveland, Ohio.



larvae tend to be gregarious while feeding in the humid environment of young rolled leaves. The minute space between the tube wall and the diet proved to be an ideal place for young larvae. To prevent larvae from crawling around in the tube without reaching the diet, the tubes were placed upside down towards a weak light source (the early larval instars are positively phototropic and negatively geotropic). The corks covering the tubes were provided with a hole and were lined with fine stainless steel gauze to prevent escape.

Third instar larvae were transferred with a fine brush to a layer of 1 cm diet in hard-plastic boxes (11.5 x 17.5 x 4 cm) with screened holes, where they stayed till pupation. The diet was changed twice a week. Pupae collected from the diet were put on a tray containing moist cotton wool and placed in a cylindrical cage (diameter 15 cm, length 30 cm) made from a plastic drain-pipe and covered with a muslin cloth. The walls of this cage were lined with creased wax-paper to provide suitable oviposition sites for emerging moths. Egg masses were cut out daily from the wax-paper and transferred into closed plastic boxes containing moist cotton wool to maintain high humidity. All tubes and boxes used for rearing were soaked overnight in a 1% sodium hypochlorite solution before use. The insectary was maintained at a temperature of  $27 \pm 1.5^{\circ}\text{C}$ , a relative humidity between 70 and 85% and a 12L:12D photoperiod. Rearing of *C. partellus* in this way was carried out without interruption during five consecutive years or about 40 generations. Occasionally fresh larvae from the field were added to the insectary-stock, but this was done only to reduce the possibilities of an inadvertent selection that might make comparison with the natural field population unreliable.

### 2.3.2 Criterion for diapause

Experiments were undertaken to investigate the possibilities to use the "failure to pupate criterion" for diapause of *Chilo partellus* (see 2.2). When aestivating larvae are supplied with fresh food (diet or maize) diapause is rapidly terminated (unpublished observations). The use of dry food or no food at all (as done for most insects) may however be equally unreliable. It has often been suggested that dry food induces aestivation-diapause in stem borers (HYNES 1942, KEVAN 1944, SWAINE 1957, SMITHERS 1959, NYE 1960). This hypothesis first needed to be tested. We therefore compared the survival and the loss in body weight under dry conditions of 50 aestivating larvae and 50 non-aestivating larvae. The aestivating larvae were collected from a maize field near Kikambala three days before the experiment was started. Judged from the presence of

exclusively unspotted larvae and no pupae over a period of more than 2 months these larvae could safely be regarded as aestivating. Non-aestivating larvae, obtained from the ICIPE insectary, were 20 days old and in the early 6th instar (normally males have six, females seven larval instars). Since larvae regularly have to be observed to determine their survival the effect of disturbance (handling and removal of cocoon) was included in these experiments. This was done by subdividing the 50 aestivating and non-aestivating larvae into groups of 25 larvae each. The individuals of one group were put into corked glass tubes (2.5 x 7.5 cm). Larvae were taken from their tube twice a week and their eventual cocoon was removed. The larvae which remained undisturbed throughout the experiment were placed in small (1.5 x 0.5 cm) cylinders made from stainless steel small-mesh gauze. These larvae were weighed directly inside their cylinders and survival was checked by observing movements. When a very thick cocoon made this observation impossible, a tiny window was made with a fine needle.

Field collected larvae were routinely supplied with their natural "diapause environment", i.e. pieces of dry maize stems containing 10-20% of water, when the presence of diapause was investigated. In order to find the critical length of time which a larva has to exceed before it can be called diapausing we determined death and pupation of non-diapause larvae of different ages (at least 50 larvae in each age group) in dry maize. Field collected larvae surviving in dry maize for a longer period than 95% non-diapausing larvae do under the same conditions were considered to diapause. This criterion is in fact based on the capacity of an aestivating larva to survive dry conditions. In the following text and figures we will therefore refer to dormant larvae for which the aestivation was established this way as to "larvae resistant to drought". This in contrast to larvae for which the loss of the cuticular pigmentation is used as criterion for aestivation (see 2.2). These diapausing larvae will be called "unspotted larvae".

All experiments mentioned above were carried out in a constant temperature room ( $25.0 \pm 1.0^{\circ}\text{C}$ ) where a relative humidity of 70-80% and a photoperiod of 12L/12D was maintained.

### 2.3.3 Field experiments

*Experimental fields* Field research was carried out at the Coast Agricultural Research Station in Kikambala, Coast Province, Kenya. The experimental fields from which samples were obtained were sized 1000-2000 m<sup>2</sup> ( $\frac{1}{4}$ - $\frac{1}{2}$  acre) and were planted with maize (Coast Composite). Samples were taken from maize grown under two different conditions: a. maize grown during the wet season (April-August/

September); These plots were subject to the natural weather conditions. b. maize grown during the dry season (December/January-March/April); fields were irrigated twice a week as indicated in the experiments concerned.

*Sampling* Samples of larvae and pupae were collected on regular weekly intervals. Samples were collected by uprooting one maize plant after a fixed number of steps (depending on the number of plants present in the field) when going through the rows of plants. Sample size always consisted of at least 100 stems/field. Stems were dissected immediately after collecting and the number of larvae and pupae in each stem was recorded. Usually within the same day larvae (placed inside maize stems from the sampled field) and pupae were sent to Nairobi by taxi-cab.

*Observations* Temperature, relative humidity and rainfall were recorded daily at a weather station situated in an open field next to the experimental fields. On the day of sampling the growth stage and the condition of the maize plant was described. The role of the plant in the induction of diapause will be described later (chapter 4 + 5). The analysis of the larval material (of which in this chapter only the cuticular pigmentation and the resistance to drought are mentioned, but in the next chapter also some physiological characteristics) normally took place at the ICIPE laboratories in Nairobi within 3 days after sample collection. The figures on the seasonal changes in population levels and on the incidence of aestivation were combined for the two species *Chilo partellus* and *Chilo orichalcoiliella*. This had to be done because the larvae can only be distinguished on the base of differences in the pattern of the cuticular spots (MATHEZ 1972) which gradually disappear as the larvae enter diapause. No behavioural or other differences related to diapause could be detected between the two species.

For the determination of aestivation-diapause only larvae were used which had at least reached the fifth instar (determined by the size of the head capsule).

#### 2.3.4 Effects of photoperiod and temperature

The development of larvae obtained from the insectary was checked under different photoperiodic and temperature regimes, comparable to the natural environment in which larvae live.

To determine the effect of photoperiod three groups of 100 1st instar larvae each were subjected to photoperiods of 12½, 12 and 11½ hrs per day respectively at a temperature of 25°C. Food was always changed during the photophase.

Temperature effects on 1st, 3rd and 5th instar larvae were observed under a 6 hr ( $> 25^{\circ}\text{C}$ ): 18 hr ( $25^{\circ}\text{C}$ ) thermoperiod. The tested temperatures during the 6 hr thermoperiod were 25, 30, 33, 35 and  $37^{\circ}\text{C}$ . The high temperatures always coincided with the beginning of the photophase of the photoperiod which in these experiments was kept at 12L:12D. Each experiment was started with 100-150 1st, 50-60 3rd or 35-50 5th instar larvae.

Time required for pupation was recorded for each experiment. Experiments were continued until all surviving larvae had pupated. Larvae dying during the observation period were not taken into account.

## 2.4 RESULTS

### 2.4.1 Drought resistance as criterion for diapause

The suitability of drought resistance as a criterion for aestivation-diapause was investigated. Experiments were carried out to study survival and loss of body weight of aestivating and non-aestivating larvae under dry conditions and to evaluate the effects of disturbance on the larvae.

Fig. 1 shows that 30 days after the experiment was started almost no non-diapausing larvae had survived whereas 60% of the undisturbed diapausing larvae

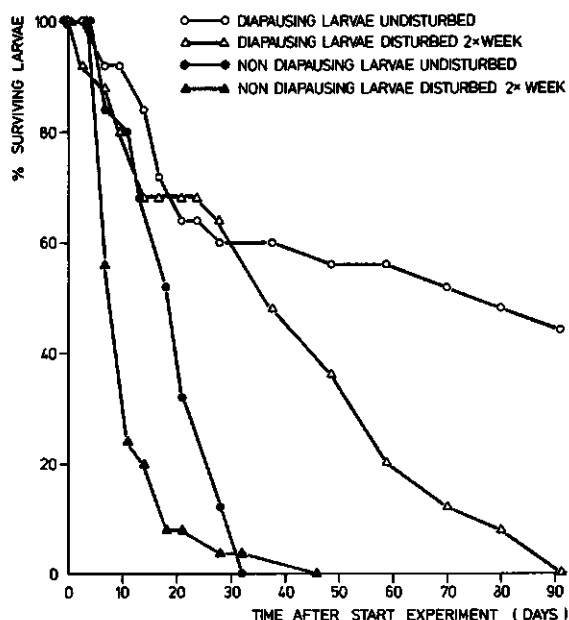


Fig.1. Effects of dryness and disturbance on the survival of diapausing and non-diapausing larvae of *Chilo partellus*. Each group contained 50 larvae when the experiment was started.

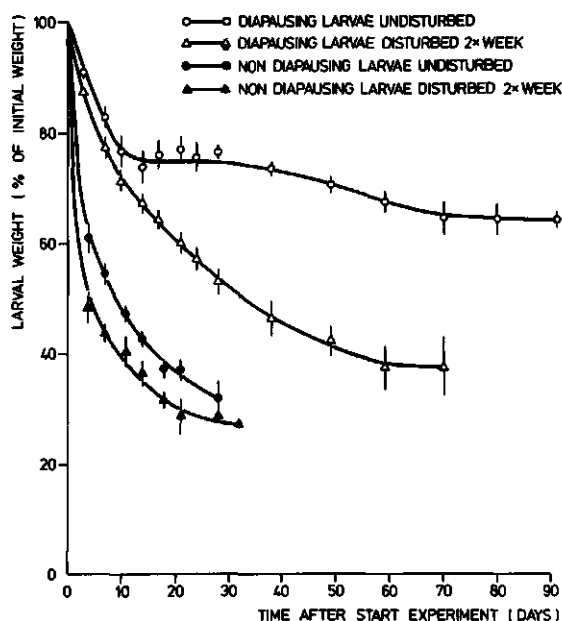


Fig.2. Effects of dryness and disturbance on the loss of body weight (mean  $\pm$  S.E.) of diapausing and non-diapausing larvae of *Chilo partellus*. Each group contained 50 larvae when the experiment was started.

were still alive. Disturbance reduced survival of both types of larvae. The effect was however more obvious in aestivating larvae, especially after 30 days. By 90 days all diapausing larvae that had been disturbed twice a week died. By contrast about 50% of the undisturbed larvae were still alive after 90 days.

Similar results were obtained for the effect of dryness and disturbance on the larval weight of diapausing and non-diapausing larvae (fig.2.). Weight decreased rapidly immediately after larvae were exposed to dryness. This decrease was slower for the diapausing larvae than for non-diapausing ones. After 30 days all surviving non-diapausing larvae were reduced to about 30% of their initial body weight. By that time disturbed diapausing larvae dropped to 50% of their original weight and undisturbed larvae to 75%.

Although the results show that 20 days old non-diapausing larvae were not capable of surviving dry conditions, this is not true for larvae of all ages. Fig. 3 shows the relation between larval age and death or pupation (the opposite of survival) on dry maize. Young larvae less than 14 days old always die when reared on dry maize stems. Thereafter the percentage pupation increases with larval age. Fig. 4 shows for the same experiment the time needed for the larvae to die or pupate. The longest time which a larva could survive (without pupating or dying) was  $7.5 \pm 0.6$  days (mean  $\pm$  S.E.) (fig.4.). This was when larvae were 21 days old. The upper confidence limit ( $p = 0.05$ , student t-test) for this distribu-

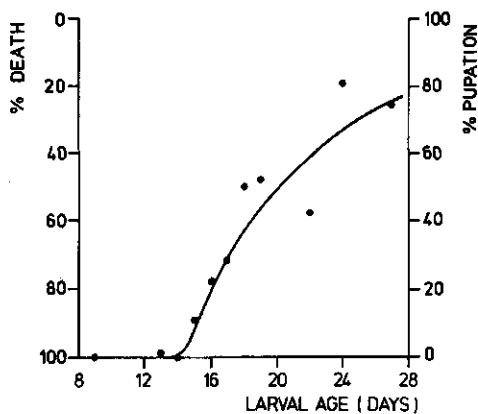


Fig.3. Relation between age and development of larvae of *Chilo partellus* in dry maize stems. Each age group consists of 50-150 larvae.

tion is 15.11 days. Thus, if a larva survived in dry stem and at 25°C (when disturbed biweekly) for more than 15 days it was considered to be in diapause.

#### 2.4.2 Field experiments

##### 2.4.2.1 Climate during the field experiments

The climatic conditions (rainfall, relative humidity and maximum and minimum temperatures) showed very similar fluctuations during 1973, 1974 and 1975 (fig. 5-8). Rains were frequent in June and continued during part of July. After that period dry conditions prevailed but were interrupted occasionally by rain. Relative humidity was somewhat higher during the rains and thereafter than during periods of drought. Decreases in humidity were usually accompanied by increases in temperatures. Normally rains began in early April, but in 1974 the rains only started on the 25th of May.

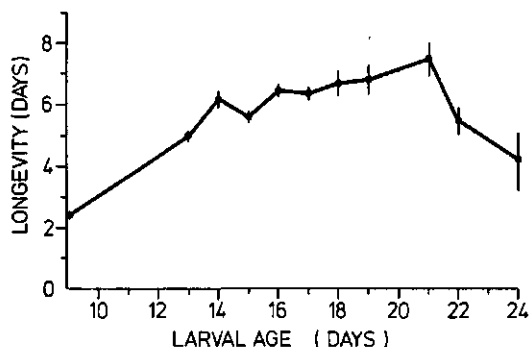


Fig.4. Relation between age and longevity (average number of days + S.E.) of larvae of *Chilo partellus* in dry maize stems. Each age group consists of 50-150 larvae.

#### 2.4.2.2 Seasonal fluctuation in the larval and pupal populations

Fluctuations in the larval and pupal populations of *Chilo* in the experimental maize fields during the growing seasons of 1973, 1974 and 1975 are shown in figures 5A, 6A and 7A. For irrigated fields where maize was grown during the dry seasons of 1974 and 1975 data are given in fig. 8A. Seasonal and yearly variations were considerable. The populations showed some similarity during the wet seasons of 1973 and 1974 (fig. 5A and 6A) after an initial peak in the number of larvae early in the growing season. 1½-2 months after sowing, there was a rather rapid decrease in the larval population to levels of about half the previous size or even less. Thereafter the number of larvae remained quite constant during a period of at least 2 months after which numbers dropped to no more than 10-20 larvae per 100 stems at the end of the experimental period. Results of 1975 (fig. 7A) were different: a second larval peak, higher than the previous one, occurred about 3½ months after the maize was sown.

Crops grown later in the (rain) season had a higher initial larval population than crops grown earlier: in 1973 the highest peak of larvae in maize sown 21 April was 67 larvae/100 stems; in maize sown 4 June 126 larvae/100 stems. In 1974 there were 68 larvae/100 stems in maize sown 23 April and 98 larvae/100 stems in maize sown 20 June.

Pupae almost completely disappeared at the end of the growing season. At that time quite high levels of larvae (obviously entering diapause or in diapause) could still be found. Occasionally pupation of diapausing larvae took place. This however could usually be related to rains (1973, fig. 5).

Fig. 8A shows what happened when maize was irrigated during the dry season. In 1974 irrigation was only continued till the end of January. The larval population initially increased but collapsed soon thereafter. After mid-March hardly any pupae were found. In 1975 the irrigation took place nearly throughout the observations. This resulted in an extremely high larval population density (an average of more than 5 larvae/stem) and a continuous presence of pupae.

#### 2.4.2.3 Seasonal incidence of diapause

The incidence of aestivation-diapause in mature larvae collected from the field was usually determined by both observing the cuticular pigmentation and the drought resistance. An exception is 1973 when diapause was merely based on the loss of cuticular spots. Results concerning the dormancy during the wet season in

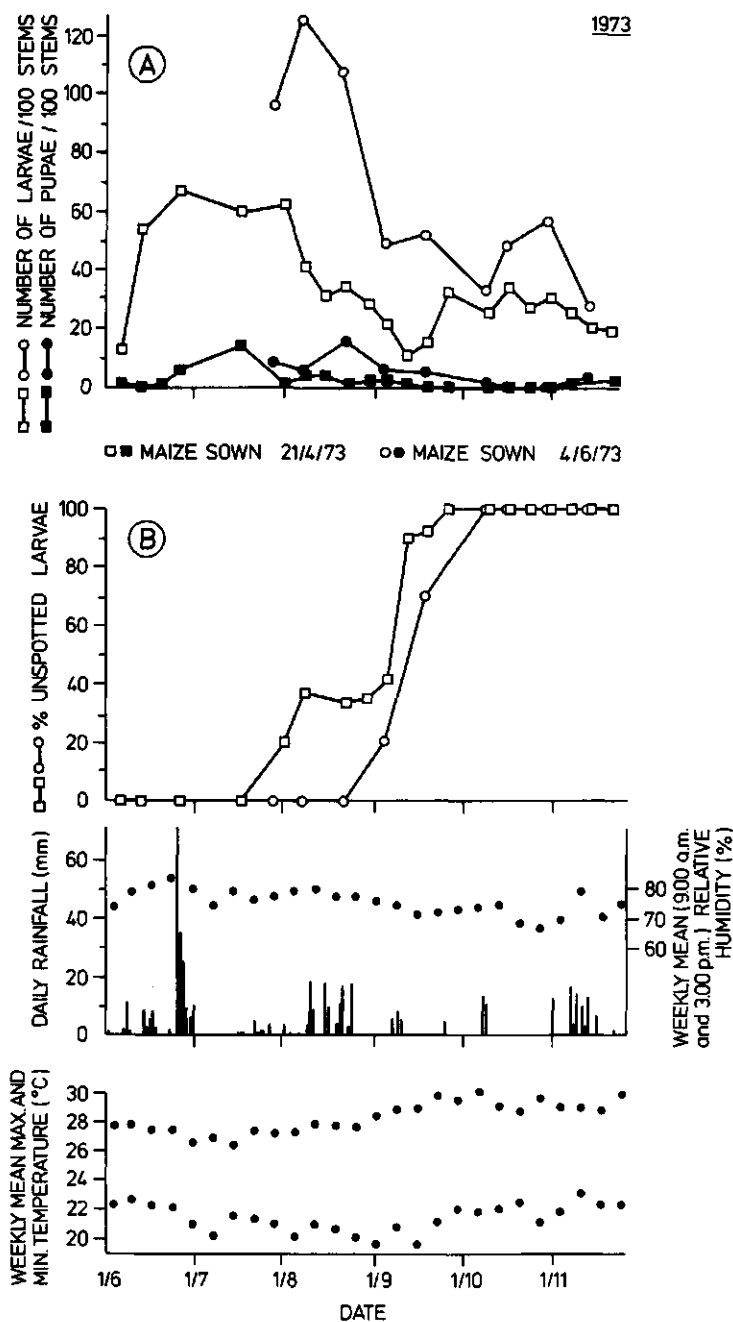


Fig.5. Seasonal fluctuations of *Chilo* stalk borers in two maize fields sown on different dates during the wet season of 1973 in relation to the climate.

A. Total number of larvae and pupae.

B. Percentage aestivating larvae.



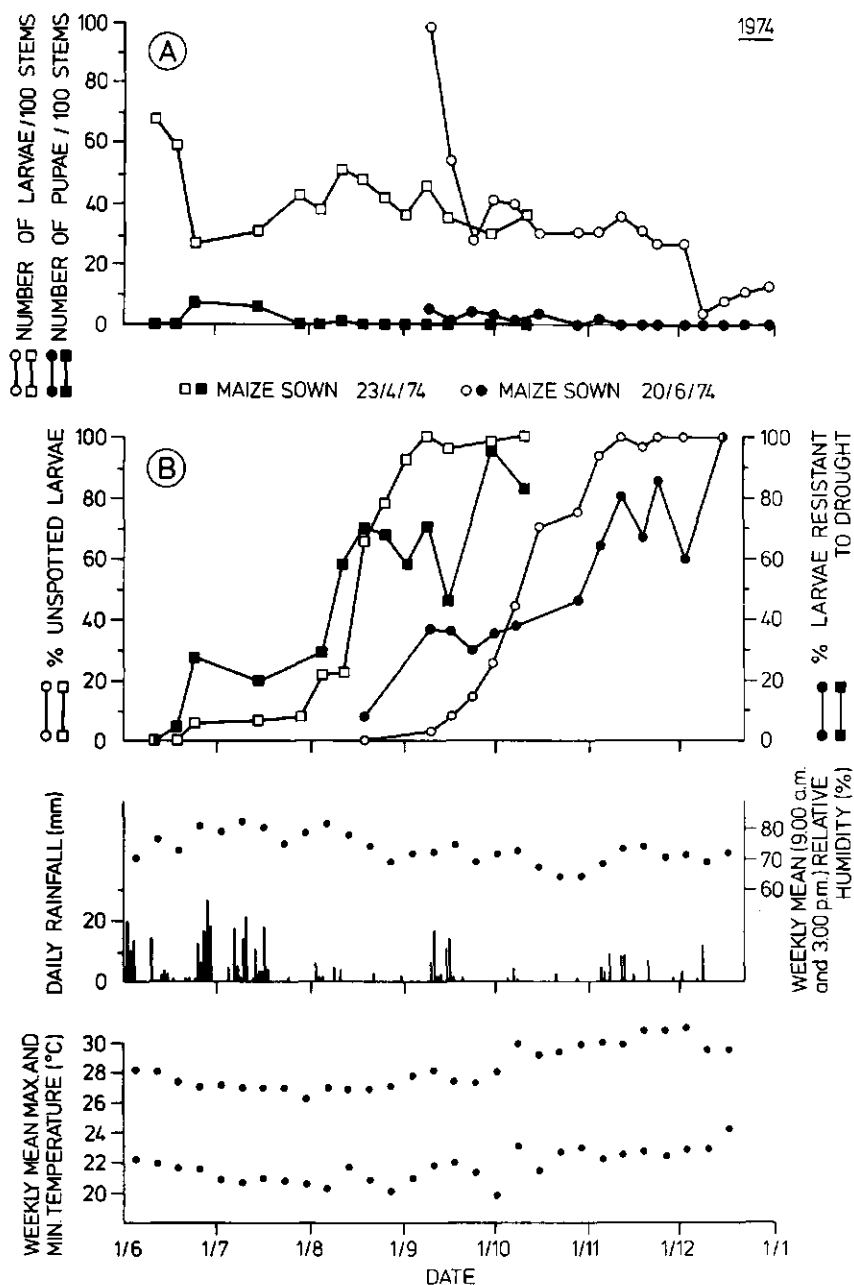


Fig.6. Seasonal fluctuations of *Chilo* stalk borers in two maize fields sown on different dates during the wet season of 1974 in relation to the climate.  
 A. Total number of larvae and pupae.  
 B. Percentage of aestivating larvae.

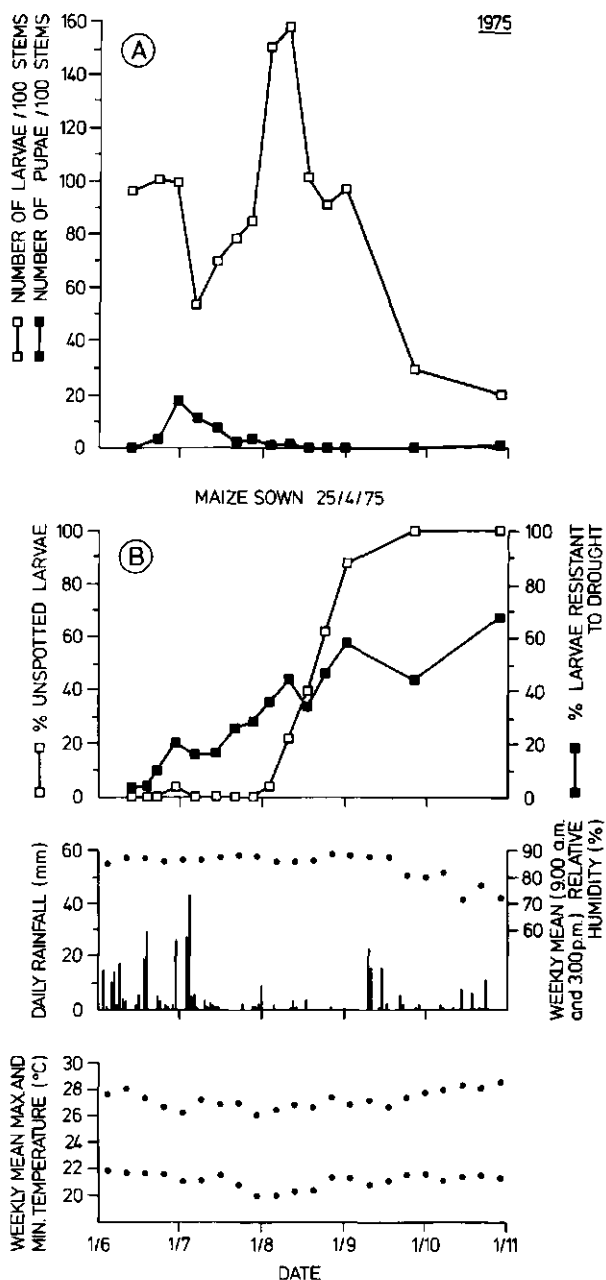


Fig.7. Seasonal fluctuations of *Chilo* stalk borers in a maize field during the wet season of 1975 in relation to the climate.

A. Total number of larvae and pupae.

B. Percentage aestivating larvae.

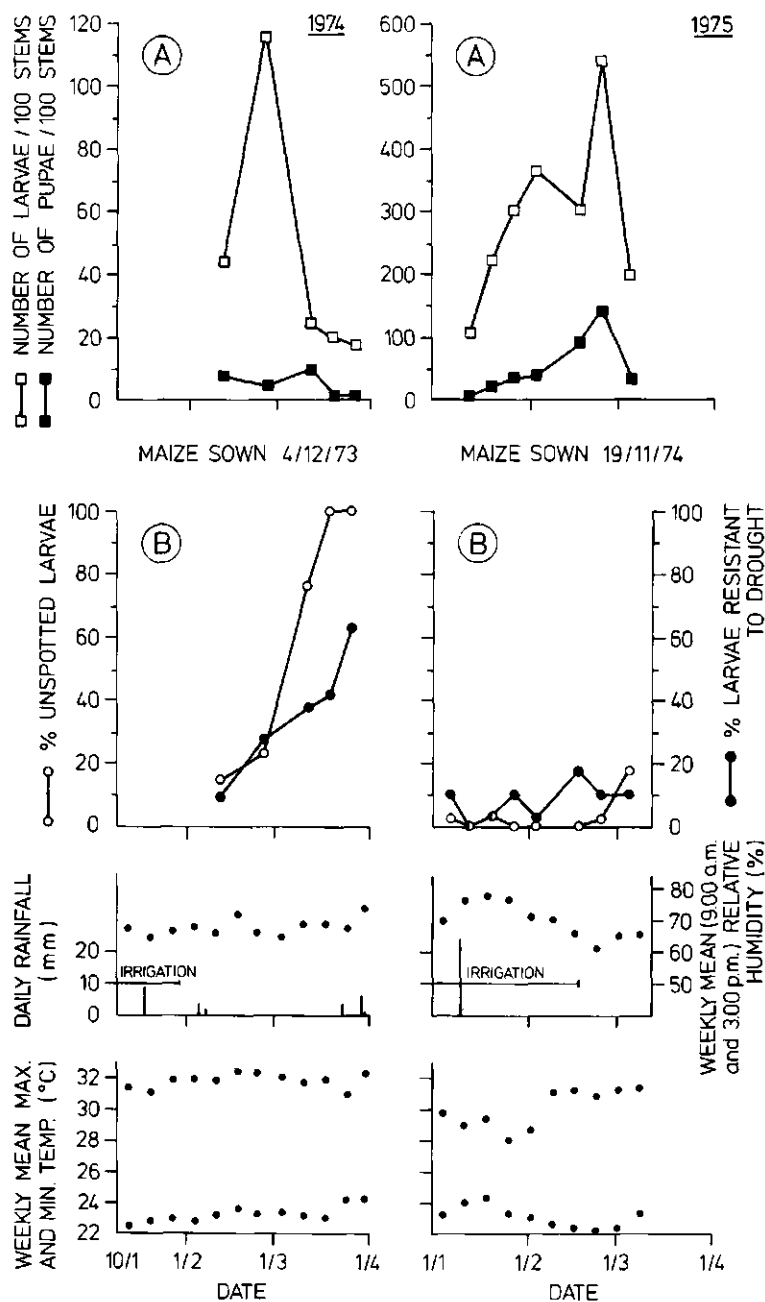


Fig.8. Seasonal fluctuations of *Chilo* stalk borers in two maize fields during the dry seasons of 1973 and 1974 in relation to the climate. The maize fields were irrigated twice a week over indicated period.

A. Total number of larvae and pupae.

B. Percentage aestivating larvae.

1973, 1974 and 1975 are given in fig. 5B, 6B and 7B respectively. In fig. 8B dry season results are shown. When considering the cuticular pigmentation, it is striking that only very little unspotted larvae could be found during the rainy period of the year. These observations include the biweekly irrigated maize field during the dry season of 1975 (fig. 8). When rains had passed, nearly always an uninterrupted gradual increase in numbers of white larvae followed. The only exception may be 1973 (fig. 5) where in the first sown crop temporarily no further increase in unspotted larvae occurred from 8 August till 5 September. This period was characterized by a high rainfall. When the percentages of aestivating larvae are considered (determined by means of their potential to resist drought) it can be seen that 20-25% aestivation may already be present during a rainy period (1974 and 1975, fig. 6 and 7). But again the most drastic increases occurred when rains were rare or absent. No correlation between other climatic factors and the incidence of diapause could be found. Diapause was observed both during the "wet" season with average minimum and maximum temperatures of 21° and 27°C respectively and relative humidities up to 88% (1975, fig. 7). But it also occurred during the dry season when minimum temperatures were about 23°C, maximum temperatures 32°C and the relative humidity was about 70% (1974, fig. 8).

#### 2.4.3 Effects of photoperiod and temperature

*Effects of photoperiod* When larvae were reared throughout larval life at photoperiods which are close to natural conditions in Kenya no effect on the rate of development was found (table 2).

Table 2. Effect of different photoperiods on the rate of development of larvae of *Chilo partellus* from first instar larva to pupa at a constant temperature of 25°C.

photoperiod	number of surviving larvae	number of days required for pupation (mean $\pm$ S.E.)
12½ L : 11½ D	51	32.2 $\pm$ 1.4
12 L : 12 D	45	33.9 $\pm$ 1.1
11½ L : 12½ D	39	33.7 $\pm$ 1.3

*Effects of temperature* The effects of temperatures on larval development were determined.

The test temperatures applied in this experiment were derived from the internal temperatures of maize during sunny days and in a field in which pre-diapause larvae were abundant. These data were obtained with thermocouples connected to a recorder which printed the temperature at  $\frac{1}{2}$  hr intervals. A period of at least 24 hrs was covered. Results are given in table 3. Maximum temperatures were 35-36°C. We therefore used 37°C as the upper limit in our experiments. Lowest temperatures in stems were around 20°C. Since these temperatures are only occurring during a period of 1-2 hrs just prior to sunrise and since the temperature during the rest of the evening and night is closer to 25°C, we used the latter as the fixed night temperature in the experiments. The length of the thermoperiod (6 hr "high" temperature: 18 hr 25°C) is rather arbitrarily but still reflects the averages met in the field (table 3).

A relation between the degree of desiccation of a maize plant and its internal temperature was suspected. Since the maize stems in table 3 are roughly grouped together according to their water content, the individual relation between moisture content and temperature is somewhat masked. A closer examination of individual stems (not shown here) revealed that such a relation is not apparent.

When we look into the effect of the different thermoperiods on the development of the larvae (fig. 9) it is clear that temperature does have an influence. The development was fastest when temperatures were 30-33°C during the 6 hr period of the 6:18 (25°C) thermoperiod. When larvae were exposed to lower or higher temperatures during this period, there was a clear increase in the time required for pupation.

Table 3. Temperatures in the interior of maize stems located in three different experimental fields with pre-diapausing and diapausing larvae of *Chilo partellus*.

date	no. of stems	water content of stems (range)	mean number of hours with the following temperatures			mean temperatures in °C (range)
			25°C	25-30°C	30°C	
early Aug. '74	4	77.7 - 84.7	14 $\frac{3}{4}$	2	7 $\frac{1}{4}$	26.2 (19.6 - 36.0)
	2	67.6 - 69.9	15	1 $\frac{3}{4}$	7 $\frac{1}{4}$	26.0 (19.1 - 35.3)
	3	67.5 - 74.6	14	2	8	26.8 (22.1 - 33.5)
mid Oct. '74	6	71.7 - 79.5	13	3	8	27.3 (19.7 - 35.4)

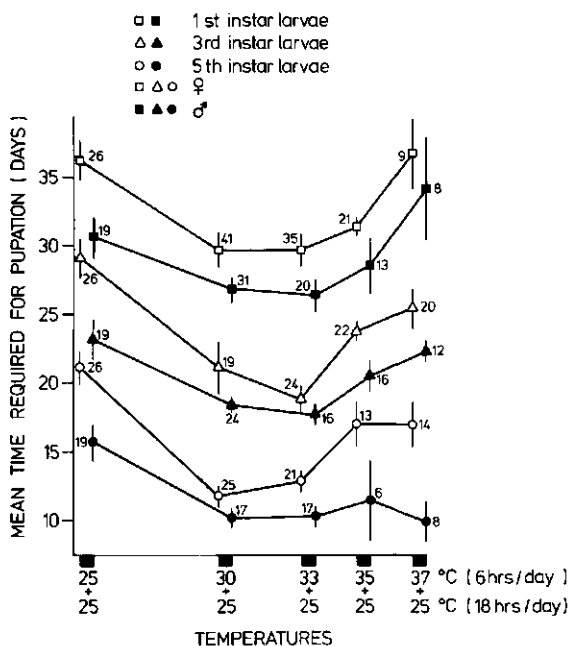


Fig.9. Time required for pupation (means  $\pm$  S.E.) of *Chilo partellus* larvae at different temperature regimes. Numbers refer to larvae remaining after pupation or death.

## 2.5 DISCUSSION

The experiments on drought resistance of larvae showed that aestivating larvae are more capable of surviving dry environmental conditions without food than non-aestivating larvae. This capacity can not be explained by the larger size and weight of the dormant larvae at the start of the experiments, because heavy non-diapause larvae were found to die (or pupate) on dry maize whereas small diapause larvae were found capable of surviving long dry periods. It is more likely that the increased drought resistance of aestivating larvae is caused by their reduced mobility within their resting sites and by physiological changes such as increased fat content, depression of the metabolism and a resistance to desiccation. In this paper some evidence for drought resistance is given. During a dry period the body weight of non-diapause larvae decreased more rapidly and to lower values than that of diapause larvae. This decrease should largely be contributed to desiccation. Non-diapause larvae contained on the average 10-15% more moisture than diapause larvae. The differences in weight decrease however were much larger and can therefore only partially be explained by the different initial moisture contents. Changed properties of the cuticle and/or in the

transpiration through the spiracles probably also have played a role. Weight loss could have been caused by the expenditure of metabolic reserves. Non-diapause larvae require more energy since they are mobile and have a relatively high metabolism. Diapausing larvae have reduced energy requirements, although the spinning of a new "diapause chamber" which was invariably done must have affected the reserves. This may explain why even undisturbed aestivating larvae lost much weight during the first ten days of exposure to dryness. During this period 25% of the body weight was lost but thereafter the weight was rather constant and only went down for another 10% during the next 80 days. In the field decrease in body weight is probably even less since aestivating larvae are better protected inside a dry maize stem than inside the gauze of the experiments. Disturbed aestivating larvae appeared to continue losing weight under dry conditions.

Before drought resistance could be applied as a criterion for aestivation-diapause it was necessary to make sure that drought (dry maize) never can induce diapause in non-diapause larvae. With a biweekly disturbance (needed for observation and essential for the final critical number of days which a larva has to survive before it can be considered as aestivating) this turned out to be the case. All non-diapause larvae either died or pupated (often precociously) under dry conditions.

During the wet season in the Coast Province of Kenya, a large variability in the size of the larval population in the different years and also during one season could be demonstrated. Similar variations were also found in the same regions by LA CROIX (1967) and MATHEZ (1972). The average numbers of larvae found in the present research (40-60 larvae/100 stems in 1973 and 1974 and about 100 larvae/100 stems in 1975) were at least half as low as the levels found by the authors mentioned above. Peaks in the larval population were found 1½-2 months after the sowing date of the maize which is in agreement with the data of MATHEZ, and perhaps also with those of LA CROIX who observed the first larvae on maize always 20-24 days after it was sown. In spite of this he invariably found the first peak of larvae in the beginning of June, independant of the sowing date of maize (in his experiments early and late April and early June). The results of this paper are not fully comparable since maize was only sown at the end of April and in June.

There is no doubt that the first peak of larvae found in the early sown (end of April) maize fields represents the progeny of the diapause generation. LA CROIX came to the same conclusion. A second peak of larvae later in the season was

usually found by both LA CROIX and MATHEZ, and in my maize fields populations tended to fluctuate similarly although the 2nd peak was not always as distinct (except in 1975). An explanation may be the great variability of the factors which are determining the rate of development at that time. The first generation could still develop rather synchronously because of 1) the similar starting point of the development (after termination of the diapause), 2) the presence of very nutritive food (young maize) of fairly constant quality and 3) the optimal weather conditions (rain). All these factors become more and more variable as the season proceeds. In particular the maize plants showed large individual qualitative differences during their development (see later chapter 4). Moreover we observed that *Chilo* larvae vary considerably, even under constant environmental conditions, in their time required for pupation. After the second "peak" the number of larvae gradually decreased. Since pupation was still extremely low or absent, it appears likely that the death rate of diapausing larvae was rather high.

A fairly low larval population was found in 1974. This may be explained by the very late start of the rainy season (25 May) whereas the maize was already sown on 23 April. A similar observation was made by MATHEZ (1972). No further relation between seasonal population fluctuations and climate could be demonstrated. Crops grown late in the rainy season were more heavily infested than crops planted earlier. The most obvious reason is of course that the number of moths which start the infestation has increased enormously compared to the previous generation of moths which originated from the diapausing population. Young maize also offers more attractive oviposition sites than more mature plants (INGRAM 1958).

The pupal population decreased during the dry periods following the rainy season. If pupation occurred late in the season it could often be associated with temporary rainfall. This means that drought tends to prevent and rain to favour pupation. This seems to be confirmed by the experiments during the dry season. As long as irrigation took place, pupae could be found. Some time after cessation of the irrigation however, pupae became rare.

The incidence of aestivation-diapause in the field, whether this is based on the colour change of the larvae or on its increased drought resistance, could not be associated with any climatic factor other than rain. Absence of rain seems to provide a stimulus for diapause. Since it was shown in this paper that diapause can not be induced by dry environmental conditions alone, the lack of rain must be perceived by the stem borer through some other medium such as the host plant. A relation between the incidence of diapause and the condition of the plant has often been suggested for many stem borers (VAN DER GOOT 1925, HYNES 1942, KEVAN



1944, SWAINE 1957, SMITHERS 1959, NYE 1960, USUA 1973, DELOBEL 1975b) and also seems to be important for *C. partellus*. This subject certainly deserves attention in future.

In an effort to separately analyse the different environmental components in the field, the influence of photoperiod and temperature on the larval development was studied under controlled conditions in the laboratory. Photoperiods in Kikambala range from 11 hrs and 50 minutes in June to 12 hrs and 18 minutes in December. With the difference of only 28 minutes between the longest and the shortest day it is not surprising to see that no effect of photoperiod could be detected. The role of photoperiod can however not be neglected altogether. It may still be that diapause is induced by a short daylength (which is permanently present in the equatorial regions) but that its effect is counteracted by diapause preventing factors of a stronger nature, e.g. rain, hostplant in full growth. Only when these factors are absent and/or other diapause inducing factors come into effect, short day influence may be felt. Experiments with long daylength - although irrelevant on these latitudes - are required to get a conclusive answer.

Temperatures only had an influence on the velocity of development, not on the development itself. Evidence that temperature is not likely to play a crucial role in the induction of aestivation-diapause was in fact already provided by the observation that diapausing larvae were found soon after the long rains (August, 21-27°C) as well as during the dry season (March, 23-32°C). Further supporting evidence is provided from experiments with thermoperiods from observations that larvae developed fastest at thermoperiods of 6 hrs 30°C: 18 hrs 25°C and 6 hrs 33°C: 18 hrs 25°C. Mean day temperatures are under these conditons 26 and 27°C respectively which is equal to the average field temperatures, measured at the moment that larvae were diapausing or entering diapause. When larvae were reared under thermoperiods with higher or lower maximum temperatures the rate of development was slower. But, the retardation represented an over-all slow down in development rather than a stage specific developmental rest.

### 3 The timing of the period of diapause induction; some behavioural, physiological and morphological aspects of aestivation-diapause

#### 3.1 INTRODUCTION

In the previous chapter I investigated the role of climate in the induction of aestivation-diapause of larvae of the spotted stalk borer *Chilo partellus* (Swinhoe) and the coastal stalk borer *Chilo orichalcociliella* (Strand). The principal outcome of this study was that the incidence of aestivation in these insects can not be associated with any climatic factor other than rain. Dry environmental conditions strongly retarded larval development. Two criteria were used to identify aestivating larvae in field collected material: disappearance of the cuticular pigmentation and the increased drought resistance of resting larvae. The second criterion was based on the failure of a dormant larva to pupate (or die) within the time limits which are normally set for non-diapausing larvae under dry environmental conditions in desiccated pieces of maize stems. When aestivation-diapause is determined this way it is very likely that only ultimate reactions to the factor(s) which are triggering the diapause are observed. Thus only larvae which have already entered dormancy are considered and not larvae which are preparing for diapause. Such observations yield only rough information on the diapause inducing factor(s). If we want to know these factor(s) more precisely it is necessary to examine larvae during the pre-diapause period when they perceive the token stimuli leading to the entrance of aestivation-diapause.

The present experiments were done for that purpose. Behavioural (construction of resting site, spinning of cocoon, feeding), physiological (ecdysial frequency, respiration, water content, fat content, heart rate and testicular development) and morphological (cuticular pigmentation, width of head capsule) changes during both the pre-diapause and the diapause period were studied.

### 3.2 LITERATURE

Many insects change their behaviour when diapause inducing factors become effective. They often start looking for a suitable place to overcome the oncoming unfavourable conditions. The larvae of the rice-borer *Scirpophaga* (= *Tryporyza*) *innotata* move within 3-4 weeks after harvest to the subterraneous parts of the rice stems where they aestivate. If drought is very severe a cocoon is made (VAN DER GOOT 1925). Tunnels of as much as 10 cm deep may be cut out in the roots (LI 1961). Similarly, larvae of the southwestern corn borer *Diatraea grandiosella* hibernate in an excavation in the base of the maize stalk below ground level (CHIPPENDALE and REDDY 1974). The larvae of the 3rd generation of the Gurdaspur borer *Bissetia steniellus* (in sugar cane) suddenly reverse their normal negative geotropic behaviour at the onset of diapause (ATWAL 1967). In South-Africa the pre-diapause larvae of the maize stem borer *Busseola fusca* also move to the lowest parts of the stem, but such a behaviour was not found in Southern-Rhodesia (SMITHERS 1959), Uganda (INGRAM 1958), Tanzania (SWAINE 1957) or Nigeria (HARRIS 1962), where diapausing larvae could be found in any part of the stem. This was also the case for *Chilo partellus* and *Chilo orichalcociliella* in East Africa (MATHEZ 1962, DELOBEL 1975a and 1975b) and for the sugarcane borer *Diatraea saccharalis* in Trinidad (KEVAN 1944). Sometimes diapause larvae occupy special structures: in Nigeria the larvae of *B. fusca* construct a chamber from frass kept together with silk inside their feeding galleries (HARRIS 1962). Diapausing larvae of the white rice borer *Rupela albinella* spin a "protective cylindrical paperlike envelope" (VAN DINTHER 1962). Larvae of *Tryporyza incertulas* and *T. innotata* form a "strong silken cocoon" in old rice stubbles (ROTHSCHILD 1971). In the temperate climates hibernacula are often constructed e.g. by the European corn borer *Ostrinia nubilalis* (BECK and HANEC 1960). The diapause cocoon of the codling moth *Carpocapsa pomonella* is not only thick walled but even has a different shape compared to the cocoon of non-diapausing larvae (HANSEN and HARWOOD 1968).

After a resting site is selected and prepared, movements and feeding activity are absent or greatly reduced. Pre-diapause *T. innotata* larvae stop feeding as soon as the subterraneous stem parts are reached (VAN DER GOOT 1925, ROTHSCHILD 1971). Once larvae had starved for several days, even fresh food is no longer accepted (VAN DER GOOT 1925). The same was observed for *C. suppressalis* by FUKAYA and MITSUHASHI (1961), but earlier KOIDSUMI and MAKINO (1958) had demonstrated reduced feeding by *C. suppressalis* on dried rice-straw even during

hibernation. A cessation of feeding of diapausing larvae of *C. orichalcociliella* and *C. partellus* in Madagascar was observed by DELOBEL (1975a and 1975b) but in India (PANT et al. 1959) and in Kenya (MATHEZ 1972) *C. partellus* larvae continued feeding during diapause, even though at a very low level. Larvae of *Diatraea lineolata* only feed in the early stages of the resting period but hardly at all during later stages (KEVAN 1944). This is also the case for *Coniesta ignefusalis* (HARRIS 1962). Diapausing larvae of *O. nubilalis* are capable of chewing, but do not ingest (BECK and HANEC 1960). Reduced feeding was observed for *D. saccharalis* during the winter on warm days (HOLLOWAYS et al. 1928) and in the laboratory (KATTIYAR and LONG 1961). Also *B. fusca* (HARRIS 1962, USUA 1970) had a reduced feeding activity when diapausing. Although the larvae mentioned above are relatively inactive during diapause, they invariably are activated when disturbed in their diapausing sites. Observed "feeding activities" after such a disturbance were sometimes believed to be associated with repair or reconstruction of the diapause chamber e.g. *O. nubilalis* (BECK and HANEC 1960) and *B. fusca* (SMITHERS 1959, HARRIS 1962).

The physiological status of diapausing insects has been investigated by numerous scientists:

Supernumerary moults have often been reported during larval diapause e.g. for *T. innotata* (VAN DER GOOT 1925), *D. lineolata* (HYNES 1942, KEVAN 1944), *D. saccharalis* (KATTIYAR and LONG 1960), *C. partellus* (MOIZ and QURESHI 1969, MATHEZ 1972, DELOBEL 1975b), *C. orichalcociliella* (DELOBEL 1975a), *B. fusca* (USUA 1970) and *D. grandiosella* (CHIPPENDALE and REDDY 1972). The stationary ecdysis is often not accompanied by growth e.g. *D. lineolata* (KEVAN 1944), *D. saccharalis* (KATTIYAR and LONG 1961) and *D. grandiosella* (YIN and CHIPPENDALE 1974).

Insects in early diapause often tend to be heavier than non-diapausing individuals. This was the case with larvae of the pink bollworm *Pectinophora gossypiella* (ADKISSON et al. 1963) and *D. grandiosella* (YIN and CHIPPENDALE 1974) and with pupae of the cabbage white butterfly *Pieris rapae* (KONO 1970). Such a weight increase is usually explained by a prolonged larval feeding period, which is quite normal for insects entering diapause (ANDREWARTHA 1952).

A reduced rate of respiration (reflecting a reduced rate of overall metabolism) is a very general quality attributed to diapausing insects. It has been observed in diapausing larvae of *C. suppressalis* (FUKAYA 1951), *O. nubilalis* (MUTCHMOR and BECKEL 1959, BECK and HANEC 1960, LYNCH et al. 1972), *Carpocapsa pomonella* (HANSEN and HARWOOD 1968, HAYES et al. 1972), *D. grandiosella* (YIN and CHIPPENDALE 1974) and *B. fusca* (USUA 1974) and in diapausing pupae of *Heliothis zea* and *H. virescens* (PHILLIPS and NEWSOM 1966) and *P. rapae* (KONO 1970). ADKISSON et al.

(1963) measured in addition to the rate of oxygen consumption also the rate of heart beat of hibernating larvae of *P. gossypiella*. Both were considerably lower compared to non-diapausing larvae.

Water and fat content of diapausing insects have also been frequently investigated. Usually there is a reduction in water content and an increase in fat content when insects enter diapause. This is very obvious in larvae of *P. gossypiella* (SQUIRE 1940, ADKISSON et al. 1963), *D. grandiosella* (CHIPPENDALE and REDDY 1972, REDDY and CHIPPENDALE 1973, YIN and CHIPPENDALE 1974) and *B. fusca* (USUA 1973). Differences were less distinct in *C. suppressalis* (FUKAYA 1951) and could not at all be associated with diapause of larvae of *C. pomonella* (HANSEN and HARWOOD 1968) and pupae of *H. zea* and *H. virescens* (PHILLIPS and NEWSON 1966).

A last well-known physiological change occurring in diapausing insects is the retarded growth of the gonads. The volume of the follicle and the testes of *C. suppressalis* remained small; spermatids are not formed since meiosis of the spermatocytes does not take place. Also development of the ovaries in female diapausing larvae was arrested (MOCHIDA and YOSHIMEKI 1962). A standstill in spermatogenesis resulting in a comparatively small testicular volume was also observed in male diapausing larvae of *O. nubilalis* (BECK and HANEC 1960, CLOUTIER and BECK 1963), *D. grandiosella* (ALEXANDER and CHIPPENDALE 1973) and *P. gossypiella* (ADKISSON et al. 1963) and pupae of *C. pomonella* (HANSEN and HARWOOD 1968), *H. zea* and *H. virescens* (PHILLIPS and NEWSOM 1966).

Lack of the pigments in the cuticular pinnaculi appears to be a common morphological change only in dormant stem borer larvae of tropical origin. Such a change has been reported for *D. lineolata* (HYNES 1942, KEVAN 1944), *T. innotata* (VAN DER GOOT 1925, ROTHSCCHILD 1971), *C. ignefusalis* (HARRIS 1962), *B. fusca* (HARRIS 1962, USUA 1970), *C. partellus* (GONCALVES 1970, MATHEZ 1972, DELOBEL 1975b), *C. orichalcociliella* (DELOBEL 1975a), *D. saccharalis* (KATYAR and LONG 1960) and *D. grandiosella* (CHIPPENDALE and REDDY 1972). Only in the last case the transition was studied more closely. In CHIPPENDALE and REDDY's work (1972) it was found that "immaculation" in these larvae most frequently occurred after (stationary) ecdysis. Ecdysis was accompanied by a reduced rate of weight loss and an increased cold-hardiness. The authors suggested that changes in hormone titers (particularly a low titer of ecdysone) may prevent the tanning of the cuticle. Others e.g. KATYAR (1960) and USUA (1970) attributed the changes in cuticular pigmentation of larvae (of *D. saccharalis* and *B. fusca* respectively) to the condition of the larval food. This of course does still not exclude endocrine involvement.

### 3.3 MATERIALS AND METHODS

#### 3.3.1 *General*

Observations on the behaviour of non-aestivating and aestivating larvae were carried out both in the field and in the laboratory. Field observations were done in experimental maize fields of the Coast Agricultural Research Station in Kikambala, Coast Province, Kenya. They were more extensively described in the previous chapter.

Aestivating larvae required for the experiments in the laboratory were obtained from Kikambala and the time of diapause initiation was estimated by using the loss of cuticular pigmentation of about 50% of the larval population as the starting point for diapause. Non-diapausing larvae were obtained from the insectary-stock bred on artificial diet (chapter 2, table 1) at a temperature of  $27 \pm 1.5^{\circ}\text{C}$ , a relative humidity between 70-85% and a 12L/12D photoperiod.

#### 3.3.2 *Behavioural studies*

The position and construction of the aestivation sites were only observed directly in the field. Later, spinning was studied more closely in the laboratory with 20 days old non-diapausing larvae (early 6th instar) and diapausing larvae which had been dormant for at least two months. All larvae were placed in small stainless gauze cages (1.5 x 0.5 cm). After four days the cage was checked for the presence of a cocoon. If a cocoon was found, a visual discrimination was made between a thin cocoon (larva visible inside) and a thick cocoon (larva not visible).

Feeding behaviour of diapausing larvae on dry maize (normal field situation) was studied with larvae that had recently entered diapause. Dry weights were recorded for frass (this includes faeces pellets and the "diapause chamber") produced by undisturbed larvae after periods of 3 days, 3 months and 8 months. These figures were compared with weights obtained for larvae disturbed twice a week by opening their stem. At that time frass was collected and the affected stem was replaced with a whole stem. The accumulated weights of "used" maize were recorded after 1,  $1\frac{1}{2}$ , 2,  $2\frac{1}{2}$  and 3 weeks. Larvae which died or pupated within these 3 weeks were not included in the data.

Feeding behaviour on moist food was investigated with 23-27 days old non-diapausing larvae (mid 6th instar) and with field-collected diapausing larvae (unspotted) that had been "stored" in the laboratory for 9 weeks before their

feeding was studied. The feeding activity was studied with the artificial diet used in the insectary. Faeces were collected approximately every 12 hours from the start of the experiment during five consecutive days. Larvae which pupated or died during these days were discarded. The period between the observations roughly coincided with the 12L/12D photoperiod prevailing in the experimental room (and also outside). Larvae were weighed each morning after faeces had been collected. However, larvae in this experiment were *not* disturbed if a cocoon was spun. In that case the weight was estimated by interpolation. Finally the feeding activity was expressed as mg dry weight of faeces/100 mg (fresh) larva/hr.

All experiments mentioned above were carried out in a constant temperature room ( $25.0 \pm 1.0^{\circ}\text{C}$ ) with a relative humidity of 70-80% and a photoperiod of 12L/12D.

### 3.3.3 Physiological studies

The physiological studies were started with non-diapause larvae of *C. partellus*. Growth rate and ecdysial frequency were observed at  $25^{\circ}\text{C}$  on artificial diet from the hatching of the egg throughout the larval period till pupation. Larvae were kept separately in glass tubes (2.5 x 7.5 cm) to prevent cannibalism. Observations started with freshly moulted 5th instar larvae (about 12 days old) selected from the insectary-stock and transferred to the constant temperature room ( $25^{\circ}\text{C}$ ). Observations on groups of 18 larvae were carried out every 2-3 days during a 3-week period until pupation was completed.

At the time of this work the diapause inducing factors were not known, thus the physiology of aestivating larvae could only be studied with larvae in which the diapause was naturally induced. These larvae were obtained from the same experimental fields used to investigate the role of the climate in the diapause syndrome (chapter 2). At one occasion diapausing larvae were collected from a new maize field situated nearby Kikambala, called 'Mtwapa'. Observations in this field started late when maize had reached full maturity. All field samples contained larvae of both *C. partellus* and *C. orichalcociliella*. Only fifth instar or further developed larvae were used. Earlier instars are not capable of entering diapause.

Oxygen consumption was measured at  $25^{\circ}\text{C}$  with a Scholander Model VR-300 micro-volumetric respirometer (SCHOLANDER 1952). Measurements of 9-18 larvae were taken at half hour intervals during the day (= light in the experimental room) for a period of 2-3 hrs. Each larva was placed into a small wire cage to prevent it from entering the compensating chamber or the KOH-tray. Vials were equilibrated

for  $\frac{1}{2}$  hr before recording the initial reading.

Moisture contents and dry weights were obtained by weighing larvae before and after 24 hrs in a 90°C drying oven.

Fats were extracted from dry material with diethyl ether in a Soxhlett apparatus for approximately 8 hrs. The percentage fat was subsequently determined by comparing the weight of fat-extracted larvae with the weight before extraction. No individual observations were done. Groups of 9-18 larvae were extracted as a whole.

Heart rates were determined by observing individual larvae under a dissecting microscope at  $25 \pm 1.0^{\circ}\text{C}$ .

The development of the testes was investigated by measuring the testicular volume of 8-12 individuals. Testes were dissected in saline and the major axis (a) and minor axis (b) were measured with a calibrated ocular micrometer. Volume (V) was calculated according to the formula  $V = \pi/6 ab^2$  assuming that the testis has an ellipsoid shape.

### 3.4 RESULTS

#### 3.4.1 *Construction of resting site and spinning behaviour*

Prepupae and pupae dissected from green maize during the rainy season were frequently found lying entirely free among frass produced during their preceeding larval stage. Occasionally the food remnants were kept together by a thin layer of silk filaments. Such a structure however was invariably encountered around aestivating larvae in dry and dead stems. Compared to the pupal chamber of non-diapausing larvae the layer of food particles which form the outside wall of the "diapause chamber" was thick (a thickness of 3 mm was quite common) and the inner wall of this layer was lined with a smooth very closely woven cover of silk. These differences in building behaviour were confirmed in the laboratory. Table 4 shows that aestivating larvae construct a thicker cocoon inside a closed metal cage than non-aestivating individuals. In such an unnatural situation (no food!) cocoons were nearly always made, even by non-diapause larvae.



Table 4. Cocoon formation of non-diapausing and diapausing larvae 4 days after transfer to a metal cage.

type of larva	n	number of larvae which had spun:		
		no cocoon	a thin cocoon	a thick cocoon
non-diapausing	21	4	17	0
diapausing	23	1	6	16

### 3.4.2 Feeding behaviour

Field observations revealed that tunneling inside drying maize stems could continue for several weeks after cessation of the rains. It was not exceptional during the dry season to find stems in which the pith was at several places completely replaced by frass (which includes faeces) for lengths of 10-20 cm. Such extensive tunneling was never found in younger fresh stems. Larvae present in such stems were all fully grown and many were unspotted. A few weeks later pupae were no longer found and almost all the larvae had constructed a diapause chamber. Thus it is apparent that pre-diapause larvae were observed. Their feeding period is considerably prolonged and thus also their total food intake must be increased during the preparations for aestivation-diapause. Judging from the construction of the diapause-chamber, it is very likely that feeding ceases as soon as this

Table 5. Accumulated amounts of frass produced by diapausing larvae on pieces of dry maize stems after different periods during which larvae were left undisturbed or were disturbed 2x week.

treatment	period after which frass was collected	mg dry weight of accumulated frass per 100 mg larva (mean $\pm$ S.E.)	no. of larvae
undisturbed	3 days	21.5 $\pm$ 0.9	20
	3 months	34.3 $\pm$ 3.7	20
	8 months	30.0 $\pm$ 4.9	15
disturbed			
2x week;	1 week	53.4 $\pm$ 1.3	22
frass	1½ week	75.1 $\pm$ 1.4	22
was removed	2 weeks	100.8 $\pm$ 1.8	22
at each	2½ weeks	118.7 $\pm$ 1.8	22
disturbance	3 weeks	133.2 $\pm$ 2.3	22

chamber is formed. This conclusion is also supported by the fact that empty diapause-chambers were never found in the field.

Further investigations on feeding behaviour were carried out in the laboratory: table 5 shows the accumulation of frass from diapausing larvae on pieces of dry maize stems. Larvae left undisturbed in a dry maize stem for 4 days had produced an average of 21.5 mg frass (dry wt.)/100 mg larva (fresh wt.). This quantity was not significantly higher ( $p = 0.05$ ) after 3 months (34.3 mg frass) or even 8 months (30.0 mg frass) of undisturbed presence. However, disturbance of the larvae in their resting sites (obtained by opening the stems) largely increased the frass production: within 1 week frass had already accumulated to a significantly higher amount ( $p = 0.05$ ) than amounts recorded for any of the undisturbed larvae in this experiment.

When diapausing larvae were provided with moist food (artificial diet) they started feeding almost immediately and as a result also started defecating. The

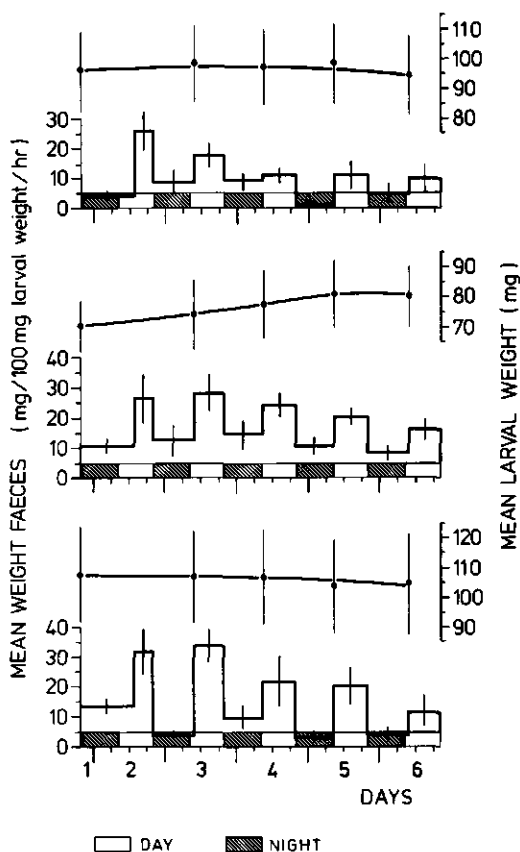


Fig.10. Day and night feeding activity (expressed in fresh wt. of faeces) of 3 groups of 8-9 aestivating larvae on artificial diet in relation to their weight. Vertical bars represent standard errors of the means.

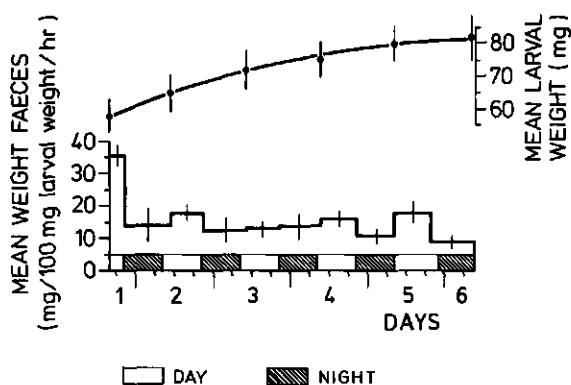


Fig.11. Day and night feeding activity (expressed in fresh wt. of faeces) of a group of 17 non-diapause larvae (L6) on artificial diet in relation to their weight. Vertical bars represent standard errors of the means.

possible effect of disturbance of the larvae could in this case not be demonstrated separately since all larvae remained active throughout the observation period once they were offered diet. Fig. 10 shows that the faecal production was fairly high for all diapausing larvae. This is even more striking when they are compared to non-diapausing larvae (fig.11) which are presumed to have a higher feeding activity. Larval weights of diapausing larvae remained rather constant during the five days of food intake (fig.10), but non-diapausing larvae gained weight (fig.11). In the same figure one can see that the feeding activity of all diapausing and - to a less extent - non-diapausing larvae is positively correlated with the light-dark rhythm. Larvae are clearly day-active.

### 3.4.3 Physiology of non-diapause larvae

Non-diapause female larvae require an average of  $36.3 \pm 1.4$  days and male larvae  $30.6 \pm 1.5$  days till pupation at a temperature of  $25^{\circ}\text{C}$ . Fig. 12 shows how the larval population is subdivided in the different instars at any given time after egg hatch. One can also see that the larval development of males and females is equal till the moult into the 6th larval instar. After this there is differentiation: the 6th larval instar is shorter for females than for males, but females normally require 7 larval instars and males only 6. An additional instar may occasionally occur: 3 out of the 26 female larvae and 3 out of the 19 male larvae had a 6th and 7th moult respectively. The widths of the head capsules for each instar are shown in table 6.

The growth of the larvae at  $25^{\circ}\text{C}$  on artificial diet was investigated in a

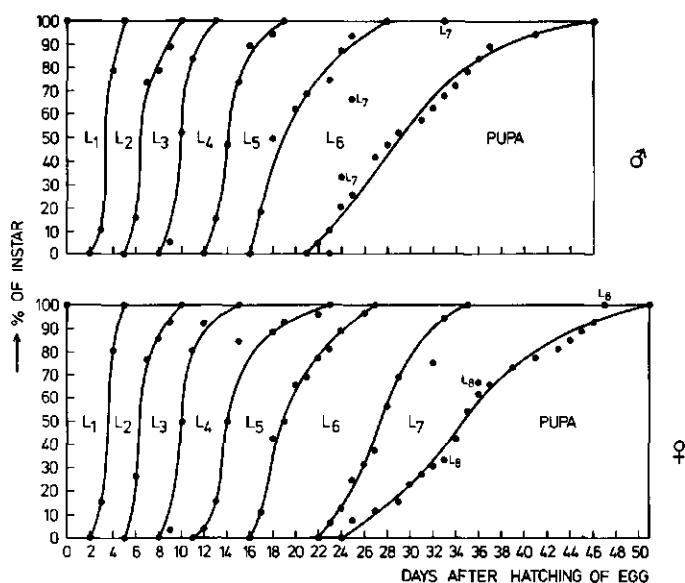


Fig.12. Larval development of non-diapause male and female larvae of *Chilo partellus* on artificial diet (25°C).

Table 6: Width of head capsules of different larval instars of *Chilo partellus* (25°C).

instar	males			females		
	mean width of head capsule (mm) $\pm$ S.E.	increase	no. of larvae	mean width of head capsule (mm) $\pm$ S.E.	increase	no. of larvae
1	0.254 $\pm$ 0.003		19	0.255 $\pm$ 0.001		26
2	0.383 $\pm$ 0.005	x 1.51	19	0.383 $\pm$ 0.005	x 1.50	26
3	0.557 $\pm$ 0.014	x 1.45	19	0.542 $\pm$ 0.010	x 1.42	26
4	0.800 $\pm$ 0.025	x 1.43	19	0.784 $\pm$ 0.018	x 1.45	26
5	1.108 $\pm$ 0.036	x 1.38	19	1.107 $\pm$ 0.026	x 1.41	26
6	1.371 $\pm$ 0.050	x 1.24	16	1.445 $\pm$ 0.041	x 1.31	26
7	1.359 $\pm$ 0.043	x 0.99	3	1.666 $\pm$ 0.028	x 1.15	16
8				1.820 $\pm$ 0.022	x 1.09	3

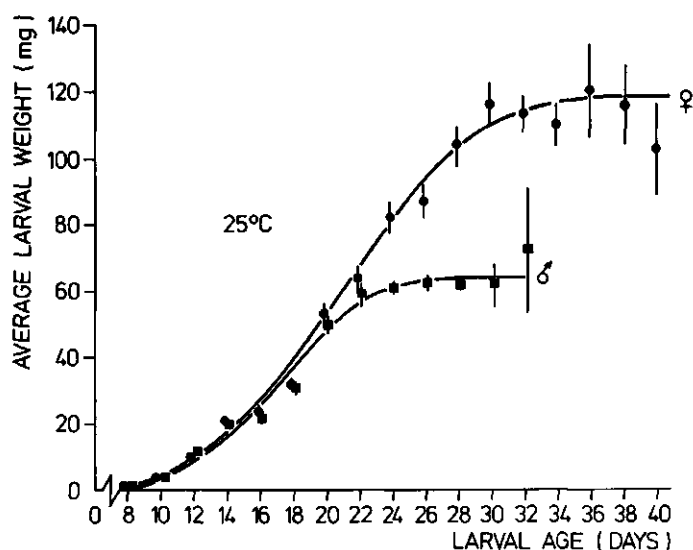


Fig.13. Larval growth of non-diapause male and female larvae of *Chilo partellus* on artificial diet (25°C) Vertical bars represent standard errors of the means.

separate experiment and is shown in fig. 13. Females and males had the same growth rate till they weighed about 50 mg on day 20. At the end of the larval development females had increased till an average of about 120 mg, while males only reached half this weight. The standard deviations of the average larval weights tended to increase at the end of the observation period due to the fact that most of the larvae had pupated: the last figures shown represent only 4 out of the initially 22 females and 2 out of the initially 26 males.

An other growth rate curve of non-diapause larvae is shown in fig. 14. In this curve, males and females were combined. Each dot represents 18 different larvae (unlike the previous figure in which we were always dealing with the same larvae). Records started with the 4th moult to 5th instar larvae. This curve is similar to the previous one (fig. 13) and is provided as a reference for the further graphs on respiration, water content and fat content of the non-diapausing larvae. The rate of oxygen consumption which is nearly  $10 \mu\text{l O}_2/\text{mg dry weight/hr}$  just after ecdysis, decreased rapidly during the further development until a level of slightly more than  $3.5 \mu\text{l O}_2$  was reached after 30 days when most larvae had pupated. Not shown in this figure is the respiration of prepupae which further went down to an average of  $2.2 \mu\text{l O}_2$ . The water content of the non-diapausing

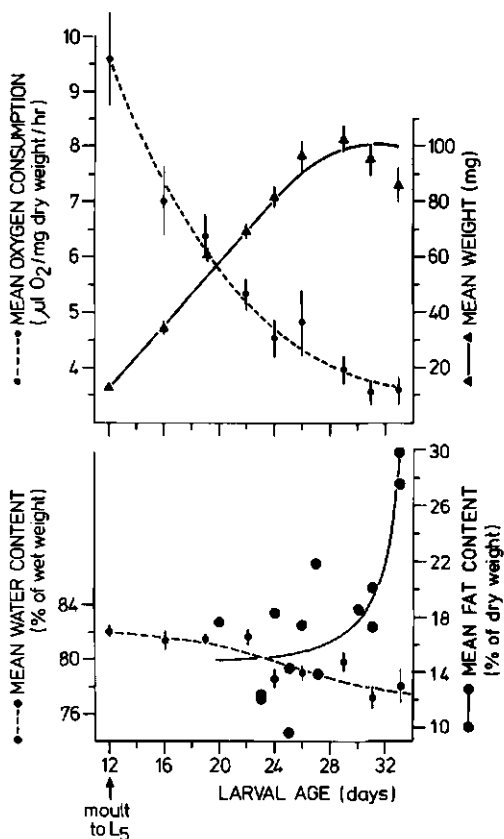


Fig.14. Growth and physiological condition of non-diapause larvae of *Chilo partellus* on artificial diet (25°C).

larvae did not change dramatically although there was a gradual decrease from 82% to 78% of water. Prepupal water contents averaged 74%. The fat content was quite variable during larval development. Lowest values of about 12% of the larval dry weight were found in larvae 11 days after the fourth larval moult when the extractions started but within 9 days fat content increased to nearly 30%.

#### 3.4.4 Physiology of larvae entering aestivation-diapause

It was not felt as useful to devote a special experiment to the moulting frequency of larvae entering diapause. The most important observation is in fact that supernumerary moults during diapause do occur. They have often been met and in all phases of the diapause. Some evidence was obtained that a larval moult may be induced by disturbance during the diapause: seldom was more than one head capsule found near to a "diapause chamber" in the field.

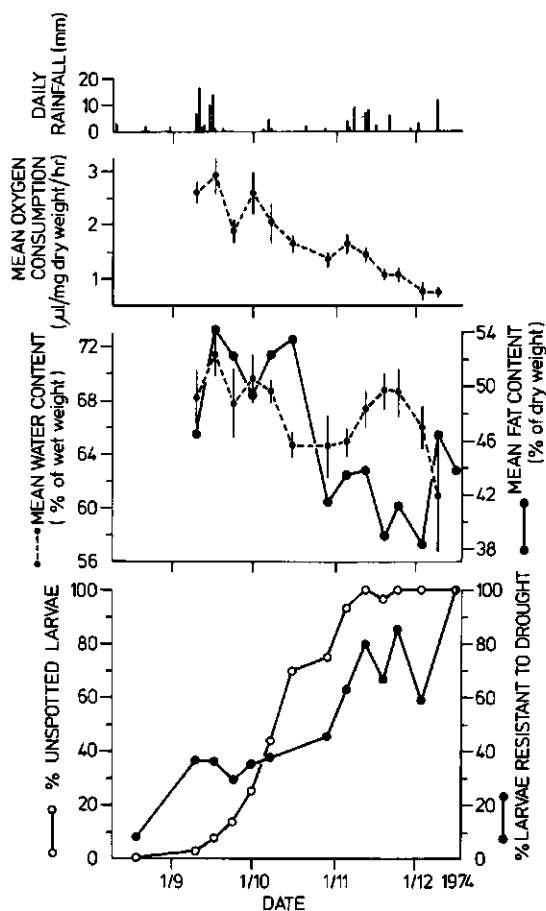


Fig.15. The physiological condition of *Chilo* sp. larvae in a maize field during the wet season of 1974 as related to rainfall and the incidence of aestivation-diapause. Vertical bars represent standard errors of the means.

The observed changes in the physiological condition of larvae during pre-diapause are shown in fig. 15 (wet season 1974), fig. 16 (wet season 1975) and fig. 17 (dry season 1975). To facilitate the survey percentages of unspotted and drought resistant larvae in the population as well as the rainfall - all already given in the corresponding figures 6, 7 and 8 - are repeated here.

The first observations were done in an experimental field during the wet season of 1974 (fig. 15). They were started as soon as the first unspotted larvae appeared (10 Sept.). At that time the larvae had an average oxygen consumption of  $2.6 \mu\text{l O}_2/\text{mg dry wt.}/\text{hr}$  and a water content of 68.2%. The  $\text{QO}_2$  gradually decreased to less than  $1 \mu\text{l O}_2$  in the course of the season. Water content also started decreasing to a minimum of 65% following dry weather. During a period of rain however the water content increased again. The fat content of the larvae in the

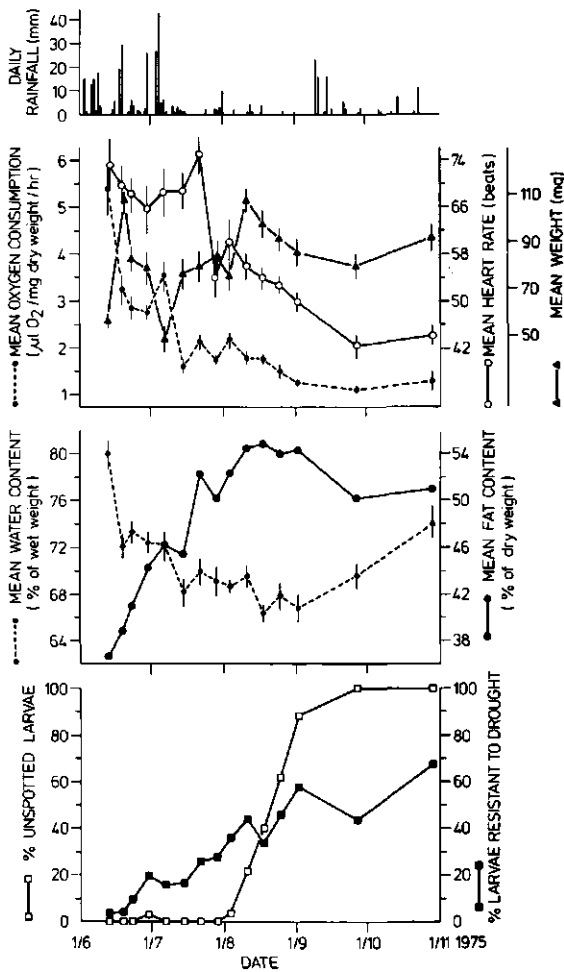


Fig.16. The physiological condition of *Chilo* sp. larvae in a maize field during the wet season of 1975 as related to rainfall and the incidence of aestivation-diapause. Vertical bars represent standard errors of the means.

first sample was already 46.4%. One week later much more fat had accumulated (average of 54.1%) and this level was constant during one more month. Thereafter the fat content decreased to reach levels between 38 and 43%. The most important result from this field experiment is that even at the start of the observations when no unspotted and an intermediate percentage (35%) of drought resistant larvae was found, the physiological condition of the larvae appeared to have already changed somewhat; oxygen consumption and water content were fairly low and fat content was high by comparison to larvae that were non-aestivating (fig. 14). Not shown in fig. 15 is that in this field also the pupation levels were very low throughout the observation period (see also fig. 6).

In order to include possible changes *before* the onset of immaculation



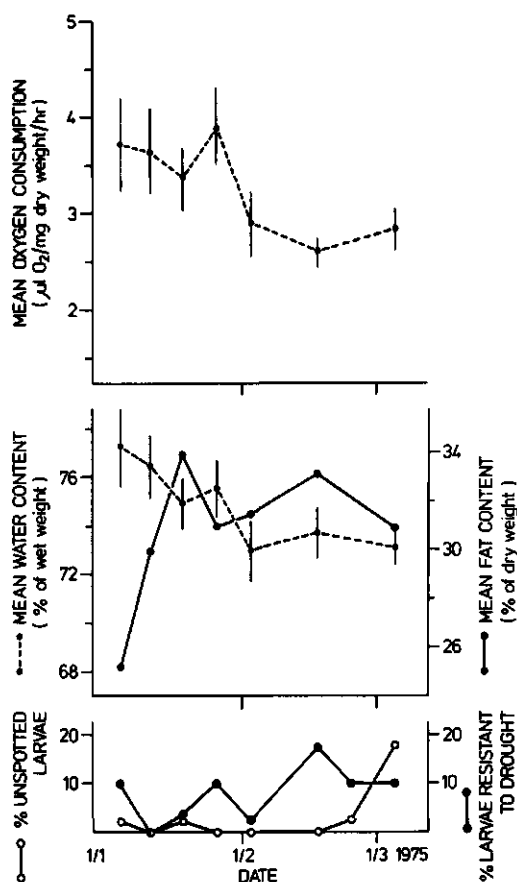


Fig.17. The physiological condition of *Chilo* sp. larvae in a maize field during the dry season of 1975 as related to the incidence of aestivation-diapause. Maize was irrigated till the end of February. Vertical bars represent standard errors of the means.

observations were started much earlier during the wet season of the following year (fig. 16). Moreover more physiological aspects were taken into account. Results were as follows: respiration decreased from an initial consumption of  $5.4 \mu\text{l O}_2/\text{mg dry wt.}/\text{hr}$  to  $1.3 \mu\text{l}$  when the last observation was made. Heart rate went down from 73 beats/min to 44. Water content decreased from 80% to a minimum of 67% and there was a subsequent increase to 74% during the last observations when also the weather was slightly more humid. The fat content gradually increased from 37% in larvae of the first sample to maximum levels of around 54% later on; from the last two samples however "only" 50% fat was extracted. Larval weights initially increased. The following decrease corresponded with a growth of the pupal population, which reached a peak at the end of June. The increase in larval weight thereafter should therefore mainly be attributed to the next generation. After this increase weights remained rather constant with an average of 85-95 mg which is normal for fully developed larvae (fig. 14). Thus, the dramatic

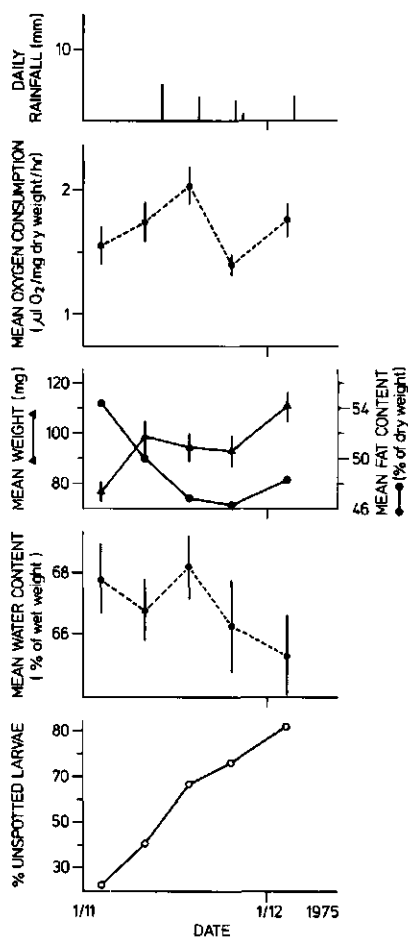


Fig.18. The physiological condition of *Chilo* sp. larvae in a maize field during the short rains of 1975 as related to rainfall and the incidence of aestivation-diapause. Vertical bars represent standard errors of the means.

physiological changes took place before any unspotted larvae could be found and sometimes even when the percentage drought resistant larvae was still below 20%.

Fig. 17 presents the physiological condition of larvae in irrigated maize stems during the dry season. Larvae in this field initially consumed  $3.7 \mu\text{l O}_2/\text{mg dry wt./hr}$ ; water content was 77.3%, fat content 25.2%. Although a gradually decreasing rate of respiration and water content and an increasing fat content was observed in the course of time, levels comparable to aestivating larvae of the previous figures were never reached.

Fig. 18 shows the data of a new maize field ("Mtwapa") which had just reached maturity at the time of the first observation. At that time also the first white larvae could be found. Of special interest are the high larval weights which were recorded in this field: up to an average of 110 mg in the beginning of December.

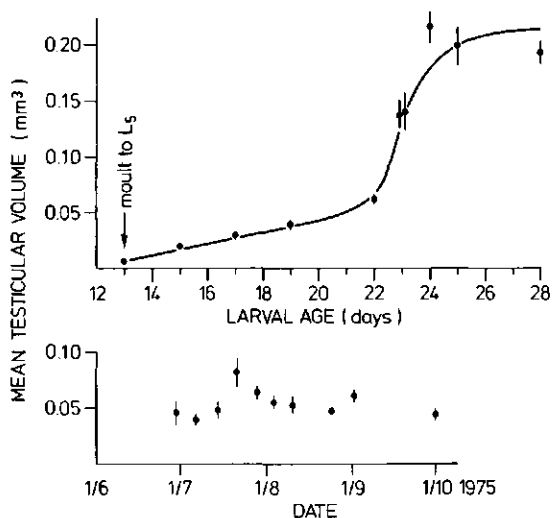


Fig.19. The development of the testes of *Chilo* sp. larvae collected from a maize field in 1975.  
Top: non-diapause larvae  
Bottom: diapause larvae  
Vertical bars represent standard errors of the means.

Oxygen consumption of the larvae was low throughout and fluctuated between 1.5 and 2  $\mu\text{l O}_2/\text{mg dry wt.}/\text{hr}$ . Water content was equally normal for diapausing larvae: 66-68%. The fat content was high (54.3%) in the first sample, and decreased gradually thereafter.

The testicular volume of non-diapausing male larvae (fig. 19) increased only slowly from less than  $0.01 \text{ mm}^3$  to about  $0.06 \text{ mm}^3$  during a period of 9 days after the 4th moult. During the following days there is however a sudden increase till an average of  $0.20 \text{ mm}^3$ . Pupation follows shortly thereafter (see also fig. 12). Measurements of the testicular volume of diapausing larvae were done with material from the 1975 wet season maize field (see also fig. 3 and 16). Unfortunately no data were obtained from the first samples. When the observations started the larvae may already have been in the pre-diapause phase. Anyway, from fig. 19 it is clear that the testes never reach a higher volume than  $0.05\text{-}0.06 \text{ mm}^3$ , which is - as shown - the volume prevailing in non-diapausing larvae just before their accelerated development which starts on day 22.

#### 3.4.5 Morphological changes in larvae entering aestivation-diapause

Table 7 shows the average width of the head capsules of larvae sampled from five experimental fields during the wet seasons of 1973, 1974 and 1975. Only head capsules of larvae completely lacking cuticular spots i.e. pre-diapause or diapause larvae were measured.

The general trend was that during the growing season in almost all fields (except

Table 7. The incidence of unspotted larvae in five experimental fields and the width of the head capsules of these larvae.

maize sown 21/4/73				maize sown 23/4/74				maize sown 25/4/75			
date	% un-spotted larvae	mean head capsule width + SE (mm)		date	% un-spotted larvae	mean head capsule width + SE (mm)		date	% un-spotted larvae	mean head capsule width + SE (mm)	
29/8	34.5	1.51+0.04		12/8	22.4	1.54+0.03		11/8	22.0	1.50+0.02	
5/9	40.9	1.51+0.03		19/8*	65.5	1.57+0.02		18/8*	40.0	1.55+0.02	
12/9	90.0	1.67+0.06		26/8	78.2	1.53+0.03		25/8	62.0	1.54+0.02	
19/9*	92.3	1.70+0.06		2/9	92.3	1.53+0.02		2/9	88.0	1.54+0.02	
26/9	100	1.72+0.04		9/9	100	1.64+0.02		27/9	100	1.53+0.02	
10/10	100	1.74+0.05		16/9	96.1	1.60+0.02					
17/10	100	1.67+0.04		30/9	97.4	1.55+0.03					

maize sown 4/6/73				maize sown 20/6/74			
date	% un-spotted larvae	mean head capsule width + SE (mm)		date	% un-spotted larvae	mean head capsule width + SE (mm)	
4/9	21.1	1.54+0.03		8/10	44.0	1.55+0.03	
18/9	70.0	1.71+0.03		16/10	70.0	1.54+0.03	
9/10	100	1.71+0.03		29/10	75.0	1.59+0.04	
16/10*	100	1.73+0.04		5/11	93.3	1.64+0.03	
30/10	100	1.79+0.03		12/11*	100	1.65+0.02	
				19/11	96.7	1.61+0.03	
				24/11	100	1.61+0.03	
				3/12	100	1.53+0.03	

\* pupation ceased after this date.

Table 8. Width of head capsules of the last larval instars of non-diapausing larvae grown on artificial diet and young maize respectively.

type of food	mean of head capsule $\pm$ S.E. (mm)		
	♀♀	♂♂	av. ♀♀ and ♂♂
artificial diet	1.67 $\pm$ 0.03 (n = 26)	1.41 $\pm$ 0.03 (n = 19)	1.56 $\pm$ 0.03 (n = 45)
young maize	1.66 $\pm$ 0.03 (n = 15)	1.41 $\pm$ 0.02 (n = 15)	1.53 $\pm$ 0.03 (n = 30)

one sown 25/4/75) a maximal average size of the head capsule was reached that was larger than the average of non-diapausing larvae grown on diet or young maize (table 8). These maxima were recorded when almost 100% of the larvae had lost their cuticular pigmentation and usually also at a time that (some) pupae were still present in the field. The observation that no increase in head capsule diameter was found in the maize field sown on 25/4/75 may indicate that such an increase does not always take place. Another explanation however may be that an increase did occur but between 2/9 and 27/9. Also in other fields mean width of the larval head capsule sometimes tended to decrease when diapause became more intense (table 7).

Table 9 represents the summarized results of the physiological condition of unspotted larvae compared to spotted and transitional individuals from the same samples. Larvae were obtained from 11 different samples. Only respiration could be related to the polymorphism; unspotted larvae consumed less oxygen. Although the difference is significant, it is by no means as big as

Table 9. The rate of  $O_2$  consumption and the water content of larvae with different cuticular pigmentation.

larval pigmentation			
spotted and transitional (n = 110)		unspotted (n = 88)	
mean oxygen consumption $\pm$ S.E. ( $\mu$ l $O_2$ /mg dry wt./hr)	water content (%)	mean oxygen consumption $\pm$ S.E. ( $\mu$ l $O_2$ /mg dry wt./hr)	water content (%)
1.81 $\pm$ 0.08*	67.36 $\pm$ 0.75	1.50 $\pm$ 0.06*	67.91 $\pm$ 0.55

\* significant difference at 1% level (t-test)

that between diapausing and non-diapausing larvae. No relation could be found between the cuticular pigmentation and the fat content.

### 3.5 DISCUSSION

In the field aestivating larvae were always found in a chamber consisting of roughly woven pieces of frass with a very smooth inner coating of silk. Such a chamber is only slightly larger than the larva which it encloses. When in the laboratory aestivating larvae were removed from their resting site, they tended - in the absence of plant material - to construct a thicker cocoon than non-diapause larvae who made no cocoon or only a thin one. Also the feeding activity of non-aestivating and aestivating larvae was observed. In the early stages of the diapause larvae continue feeding from their maize plant even though this is visibly deteriorating. This behaviour has been demonstrated for many more pre-diapause larvae and may be associated with the accumulation of reserves for the oncoming unfavourable season.

If left undisturbed in their stem, aestivating larvae dissected from their diapause-chambers in dry maize produced an equal amount of frass after 3 days or 8 months. However when disturbed this production increased considerably. From this we can conclude that aestivating larvae do not actively feed on dry maize; they only utilize this food when they need to construction or repair their diapause-chamber. Often reported feeding activities of other diapausing larvae (3.2) may also well be due to disturbance alone.

When diapausing larvae were provided with artificial diet, they invariably exhibit a high food intake (often even higher than non-diapause individuals) prior to their eventual pupation. Although the data are not shown the same results were obtained when diapausing larvae were given fresh maize stems. These data combined with the earlier observed extended feeding period of pre-diapause larvae do in fact show that diapausing larvae are very well capable of consuming food. But whether or not they do so is primarily dependent on the type of food which is available. Fresh food (maize or diet) stimulates ingestion, dry maize blocks it. It is therefore not correct to say that diapausing larvae in the field have a reduced food consumption compared to non-diapausing larvae. The difference is not the result of the state of diapause but certainly also reflects the food situation.

The artificial diet ingested by the different diapausing larvae is not converted into growth of larvae as is the case for non-diapause larvae. A similar result, was obtained with hibernating larvae of *C. suppressalis* which start to feed on

old rice stems shortly before diapause is terminated. This food intake is not expressed in an increase in body weight and was thought to serve for the production of metabolic water (KOIDSUMI and MAKINO 1958). A second explanation may be that diapause larvae only have limited means to utilize the diet. E.g. it is known that the protease activity in the midgut of overwintering larvae of *C. partellus* is almost nil compared to that of actively feeding larvae (PANT et al. 1959). Also KOIDSUMI and MAKINO (1958) stated that "the digestion and absorption of food during the hibernation period (only little) are not so remarkable as to induce further development of the larvae". They did however not mention whether this is due to the reduced consumption or to metabolic changes in the larva.

The physiological condition of stem borer larvae in the field changed considerably upon entering into diapause. A decreased rate of oxygen consumption, rate of heartbeat, water content, an increased fat content and an arrested development of the testes were always found. But the physiological condition of non-diapause larvae which develop normally changes also during the life-cycle. Generally it seems that the trends which take place during the normal larval development till the prepupal stage is reached, are further continued when a larva enters diapause. For example, the  $QO_2$  gradually decreased to  $3.5 \mu l O_2/mg$  dry wt./hr prior to pupation. A similar trend was also recorded for larvae of the European corn borer *O. nubilalis* (BECK and HANEC 1960, LEWIS et al. 1971, LYNCH et al. 1972), the corn ear worm *Heliothis zea* (EDWARDS 1970) and the Egyptian cotton leafworm *Spodoptera littoralis* (ABOUL-NASR et al. 1976). Other recorded changes in non-diapause larvae include a decrease of water content (from 82% in early 5th instar larvae to 78% in fully grown larvae) and an increase of fat content (from 12 to 30%). In table 10 figures are shown on the physiological condition of non-diapause and diapause (mid-diapause) larvae of some Heterocera as compared to *C. partellus*. Figures are in close agreement.

In chapter 2 we have already demonstrated the close relationship between the incidence of diapause and the rainfall. Supplementary evidence was now obtained from the present studies of the physiological condition of the stem borer larvae: in 1974 (fig. 15) observations only started when the first unspotted larvae were noticed. At that time the larvae consumed an average of  $2.6 \mu l O_2/mg$  dry wt./hr, had a water content of 68.2% and a fat content of 46.4%. All these figures were in fact different from the characteristics of non-diapausing larvae and were more similar to those of aestivating larvae. We concluded therefore that the larvae in this field had already entered the prediapause phase when the observations started. The first sample in 1974 was taken after a long period (11 weeks) of almost complete drought. This caused a standstill in the growth and development

Table 10. The oxygen consumption, water content and fat content of last instar non-diapausing and diapausing (mid-diapause) larvae of *Chilo partellus* compared to some other Lepidoptera larvae.

insect	environment from where larvae were obtained	mean oxygen consumption		mean water content (% of fresh wt.)	mean fat content (% of dry wt.)	author
		expressed as published	$\mu\text{l O}_2/\text{mg dry wt./hr}$			
<u>non-diapausing larvae:</u>						
<i>Chilo partellus</i>	diet field (maize)*		3.5-4.0	78	28-30	SCHELTES
<i>Busseola fusca</i>	4-9 weeks old maize	600 $\mu\text{l/g}$ fresh wt./hr	3.3-5.4	73-80	36	
<i>Pectinophora gossypiella</i>	diet, 27°C, 16 hrs light		3.05	80.3	33.0	USDA (1973, 1974)
<i>Diatraea grandiosella</i>	diet, 30°C, 12 hrs light		3.63	-	32.4	ADKISSON et al. (1963)
<i>Chilo suppressalis</i>	field (rice)		2.32	73	40.3	YIN and CHIPPENDALE (1974)
<i>Ostrinia nubilalis</i>	field (maize) diet, 30°C, no light	1070.4 $\mu\text{l/g}$ fresh wt./hr	3.15	66	47-49	FUKAYA (1951)
			2.34	74.5	45.3	
			2.20	76.6	39.0	BECK and HANEK (1960)
<u>diapausing larvae:</u>						
<i>C. partellus</i>	field (maize)**		1-2	65-68	46-54	as for non-diapausing larvae
<i>B. fusca</i>	10-15 weeks old maize	150 $\mu\text{l O}_2/\text{g}$ fresh wt./hr	0.45	66.9	49.0	
<i>P. gossypiella</i>	diet, 27°C, <13 hrs light		0.61	-	43.0	
<i>D. grandiosella</i>	diet, 23°C, 12 hrs light		0.45	67	52.1	
<i>C. suppressalis</i>	field (rice stalks)	399.6-446.9 $\mu\text{l/g}$ fresh wt./hr	1.18-1.31	66	50-53	
<i>O. nubilalis</i>	field (maize) diet, 26°C, 9 hrs light		1.21	60.3	50.2	
			1.05	65.4	-	

\* data from fig. 16 (first observation on young maize) and fig. 17 (irrigated maize field)

\*\* data from fig. 15, 16, 18 (mid-diapause larvae from old maize in dry conditions)



of the maize plants; they never developed beyond the tasseling stage.

Apparently also the stem borers inhabiting these plants were affected. They had changed physiologically although this was not yet reflected in the pigmentation and only little in the drought resistance of the larvae.

The experimental field of 1975 (fig. 16) differed from 1974 in two aspects:

1. there was more rainfall during the growth of the maize which resulted in a complete maturity of the plants, 2. the observations were started amply before any sign of cuticular colour change or drought resistance in the larvae could be found. The physiological condition of larvae in the first sample was as follows: respiratory rate was  $5.4 \mu\text{l O}_2/\text{mg dry wt.}/\text{hr}$ , water content 80% and fat content 36%, all of which suggest non-diapause larvae. After the first observation most physiological qualities were fairly constant during the rainy period. Only fat content showed a continuous increase. As soon as the rains ceased on 8 July major changes took place: respiratory rate dropped from  $3.5 \mu\text{l}$  to  $1.6 \mu\text{l O}_2/\text{mg dry wt.}/\text{hr}$ , water content from 72 to 68%, heartbeat rate decreased from 75 to 54 beats/min and the fat content increased from 45 to 52%. Obviously once again, drought appeared to be a major factor in the induction of diapause. The fact that (little) pupae were found even during the 2 week period after cessation of the rain (fig. 7) indicates that at least a small proportion of the larval population, most likely the oldest larvae, was not effected by the weather change. As in 1974, the change in cuticular pigmentation only became visible after the major physiological changes had occurred.

Convincing evidence of the rain dependence of aestivation came from the experiment in which maize was irrigated during the dry season (fig. 17). Larval oxygen consumption and water content remained high and fat content low throughout the irrigation period.

Although both the respiratory rate and the water content gradually decrease when larvae diapause and also during the diapause itself, these processes do not always follow a smooth curve. Again rain seems to be the influential factor. Some caution should be exercised with this interpretation, since the quantity of rain is not the only factor which determines the moisture condition of the field. The duration of the rainfall and the rate of evaporation (dependent on the sun/clouds ratio) are equally important.

Larval fat content increases during the first phase of diapause and this is possibly the result of the continued feeding of the larvae during the pre-diapause period for which some field evidence was presented in 3.4.2. Fat contents always started decreasing after some time presumably due to a slow utilization of this food reserve. No convincing evidence was obtained that the body weight of

diapause larvae exceeds that of non-diapause larvae, but width of the head-capsules often appeared to be larger for aestivating larvae.

We finally return to the question which we asked ourselves in the introduction: "when do larvae perceive the token stimuli which lead to the entrance of aestivation-diapause?". From the results mentioned in the previous chapter it was already clear that the diapause inducing factors most likely have to be looked for in the condition of the plant. The present results suggest that we might succeed in finding the factor(s) if we investigate the plant before the larvae are turning unspotted and/or are becoming drought resistant i.e. when the first changes in the physiological condition of the larvae appear. The asynchronous manifestation of changes in the physiological condition of the larvae and the loss of their body pigments was demonstrated in 3.4.5. It is true that unspotted larvae were found to have a lower rate of respiration than spotted individuals of the same sample. But these results have to be regarded with caution since also unspotted larvae of the same samples had a highly reduced rate of respiration as compared to non-diapause larvae indicating that the spotted larvae had already entered the pre-diapause stage. No relation at all could be found between the polymorphism and the water or fat content. From these results it is not clear what causes the loss of cuticular pigmentation. It may be a direct result of the changed condition of the larval food as is suggested for *D. saccharalis* by KATIIYAR (1960) and for *B. fusca* by USUA (1970). But it may also be that the factor(s) primarily inducing aestivation-diapause (i.e. the host plant) only play an indirect role. This is for example the case with larvae of *D. grandiosella* which enter diapause (and turn "immaculate") when the temperature decreases and the daylength shortens. Diapause could however be induced directly with juvenile hormone (YIN and CHIPPENDALE 1974). In this case juvenile hormone seemed to regulate not only the polymorphism but also the physiological processes involved in the diapause. Similar mechanisms have been found for many other diapausing insects (LEES 1956, BECK 1968, CHIPPENDALE 1977) and may well be valid for *C. partellus* as well.

## 4 The incidence of aestivation-diapause as related to the condition of the host plant (maize)

### 4.1 INTRODUCTION

In the previous chapters it was shown that the only climatic factor affecting aestivation-diapause of the larvae of the spotted stalk borer *Chilo partellus* (Swinhoe) and the coastal stalk borer *Chilo orichalcociliella* (Strand) is rain. After a period of dry weather physiological and subsequent morphological changes could be observed. It was thought that the most likely medium through which a stem borer detects drought is the condition of its host plant. The present research was carried out to investigate whether there are grounds for this reasoning. Water, protein and sugar content of the food plant were considered as possible signals leading to the initiation of aestivation-diapause in the above mentioned stalk borers.

### 4.2 LITERATURE

Although diapause in the temperate and subtropical regions is primarily controlled by photoperiod and temperature, food has often been found to be a factor which influences the percentage of diapause. Well known is the relation between the composition of the cotton boll and the diapause in the pink bollworm *Pectinophora gossypiella*. SQUIRE (1940) first suggested that diapause is promoted by an increase in fat content and a decrease in water content of the cotton boll, but this relationship was only convincingly proved much later (ADKISSON 1961, BULL and ADKISSON 1960 and 1962, RAINA and BELL 1974). CROWDER et al. (1975) observed that early crop maturity (induced by irrigation cut-off) increased the incidence of diapause. The maturity of the food plant also enhanced the incidence of diapause in larvae of the rice stem borer *Chilo suppressalis* (PATHAK 1968) and of the codling moth *Laspeyresia pomonella* (PHILLIPS and BARNES 1975).

In all these cases however (short) photoperiod and (low) temperature had effects overriding those of food. Also the type of food may be important: larvae may enter diapause when feeding on one host plant, but may - under the same environmental conditions - not do so on another. Examples of this are the Indian meal moth *Plodia interpunctella* (WILLIAMS 1964) and the plum fruit moth *Grapholita funebrana* (SARINGER and DESEO 1968).

In the tropics the condition of the food is often referred to as the primary diapause inducing factor. The data on the exact impact of the host plant on diapause are however fragmentary and incomplete. VAN DER GOOT (1925) reported that the dormancy of the white rice borer *Scirpophaga innotata* (= *Tryporyza innotata*) on Java is initiated by the ripening of the rice stem after the ears start developing and that this dormancy is independant of the rainfall and the moisture of the plant. Observations of ROTHSCILD (1971) in Malaysian Borneo with the same borer as well as with *T. incertulas* seemed to support this idea. No research was done to find the actual plant factor(s) responsible for the arrested development of the *Tryporyza* larvae. HYNES (1942) described the resting stage of the larva of *Diatraea lineolata* on Trinidad and suggested that the quiescence, which coincided with the dry period of the year, was due to the dry condition of the maize stem. Evidence for this idea was provided by the observation that resting larvae pupated when they were transferred from dry old maize stems to fresh green stalks (a quick response) or to dead wetted stems (a slower response). When the larvae were kept in the dry pith they stayed in the resting stage. KEVAN (1944) working with the same insect believed that "it is the lack of suitable food rather than the food itself which is the cause of the diapause". As evidence he mentioned that spotted larvae when transferred from green stems to moistened "dry" stalks or to green stalks which had been allowed to become rotten usually lost their spots and became yellow. KEVAN suggested that chemical changes in the stem were the most important factors. However also "larvae that wander away from the food provided enter a prolonged resting stage" and thus total lack of food may also result in diapause. Similarly, the diapause of the maize stem borer *Busseola fusca* is thought to be induced by the host plant. The drying out of the maize plant was mentioned as a factor initiating the diapause by SWAINE (1957) in Tanzania and by SMITHERS (1959) in southern Rhodesia. This was derived from the field observation that diapause increased as the food started drying out and that no diapausing larvae were found in irrigated maize or in tillers of wild grasses and sorghum (which contain high water contents). USUA (1973) working in Nigeria with the same stem

borer mentioned that other factors in the ripening maize stem, apart from water, are involved i.e. the increasing carbohydrate content and the decreasing protein content. But he did not give conclusive evidence that these factors are critical. It was only demonstrated that more larvae enter diapause as the maize plant matures. Equally little is known on the spotted stalk borer *Chilo partellus*. Resting larvae are present in old dry stalks or stubble remaining in the field at the end of the growing season. This may be in winter at relatively low temperatures e.g. India (PANT and KALODE 1964, KHAN and KHAN 1968), Pakistan (MOIZ and QURESHI 1967) or in "summer" at the end of the wet season in East Africa: NYE (1960) in Tanzania, SCHMUTTERER (1969) in Sudan, MATHEZ (1972) in Kenya and DELOBEL (1975b) in Madagascar. The breeding of *C. partellus* seemed to be continuous in Uganda, but during the dry season when larvae (and pupae) are found in sorghum trash and stubble "the development is probably slowed down" (INGRAM 1958). Later NYE (1960) did come across aestivating larvae during the dry season in Uganda. The larvae were found in dry maize stems and in the higher parts of sorghum plants. At the same time active larvae were observed in the lower parts. The author concluded that *C. partellus* "only undergoes a resting stage when its host plant dries out completely" and "that the lower parts of sorghum remain sufficiently moist for the larvae to continue breeding during the dry season". GONÇALVES (1970) working in Madagascar could find the spotted stalk borer throughout the year, but observed "a low activity" during the four months of the dry season. Only DELOBEL (1975b) made an effort to explain the incidence of diapause in this borer. He suggested that the extremely long development of last instar larvae was partially caused by the deterioration of the nutritive environment (sorghum). Again, no conclusive experiments were performed.

#### 4.3 MATERIALS AND METHODS

##### 4.3.1 Handling of samples

Samples of maize stems (Coast Composite) were collected during the morning hours in experimental fields in Kikambala. Fields and sampling methods were previously described in 2.3.3. On the day of sampling the average growing stage of the plants was determined by using criteria outlined at the bottom of fig. 20. All larvae dissected from the field collected stems were then dispatched to Nairobi inside stem pieces, which had been randomly taken from the sample. Only bottom and central parts of the stem were included since top parts hardly contain larvae. If possible the drying procedure for the determination of the water

content in this stem material was started immediately after the arrival of a sample which was usually 24 hrs after the stems were cut. Otherwise samples were sealed in a plastic bag and stored in the deep freezer ( $-20^{\circ}\text{C}$ ). For all samples water content was determined and most samples were analysed further to determine total sugars, reducing sugars and crude proteins. For this, dried material was ground in a Wiley mill to 40-mesh size and, if not used directly, stored in a small air-tight container in a cool dark place. Since all stems in one sample were mixed, only the mean chemical composition of the stems of this sample was obtained.

Larval material was handled as described in chapter 2. For reasons of convenience percentages of diapausing larvae i.e. drought resistant and unspotted larvae - also shown in chapter 2 - were repeated in the different figures.

In order to obtain an impression of the compositional changes occurring in maize grown under normal conditions (i.e. with sufficient water available throughout the growth) stems obtained from a private field close to Nairobi were analysed from the early vegetative stage to beyond maturity.

#### 4.3.2 *Analysis of stem material*

*Determination of water contents* After recording the wet weight, the stem pieces were heated for 30 min at  $100^{\circ}\text{C}$  to inactivate enzymes and kill the plant cells (this procedure was neglected if stems had been frozen). The drying continued at  $70^{\circ}\text{C}$  in an oven with moving air till a constant weight was reached. The percentage moisture was calculated from the loss of weight after drying and expressed as percentages of the fresh weight.

*Determination of soluble sugars* Sugars were extracted (in duplicate) with 80% ethanol as described by SMITH and GROTELUESCHEN (1966). Deproteinization of the extract (with neutral lead acetate) was not done since preliminary experiments had shown that this step could be omitted. Aliquots of the extract were checked for their reducing power before hydrolysis with  $\text{H}_2\text{SO}_4$  (giving the content of reducing sugars) and thereafter (total soluble sugars). Non-reducing sugars such as sucrose could then be obtained by subtracting the reducing sugars from the total sugars. The reducing power of a solution was determined by the Shaffer-Somogyi copper-iodometric method as described by HEINZE and MURNEEK (1940). Sugar contents were expressed as glucose, which was used as a standard.

*Determination of crude protein* Total nitrogen was determined by the micro-Kjeldahl procedure. The determination (in duplicate) was directly carried out with ground stem material and was not preceded by any fractionation. Multiplication of total nitrogen by 6.25 gave the amount of crude protein.

#### 4.3.3 *Consumption and utilization of maize stems*

The feeding behaviour of non-diapause larvae on the pith of stems of maize plants (Hybrid 512) of different growing stage was studied. The following indices were used: consumption index (C.I.), relative growth rate (G.R.), efficiency of conversion of ingested food to body substance (E.C.I.), conversion of digested food to body substance (E.C.D.) and approximate digestibility (A.D.). The indices are explained in more detail by WALDBAUER (1968). C.I.'s were calculated as fresh weight of food eaten/day/ mean larval fresh wt. during feeding period (C.I.<sub>fresh</sub>) and as dry weight of food eaten/day/mean larval fresh wt. during feeding period (C.I.<sub>dry</sub>). For the rest all indices were calculated on the basis of dry weights. Dry weights at the end of a feeding period were determined directly. Dry weights at the start of an experiment had to be calculated; this was done by determining the water content of equivalent control material.

To preclude moulting during the observations only 6th instar larvae which had recently moulted (head capsules not yet tanned) were used. For each experiment 10-15 larvae were observed separately over a feeding period of 24 hrs.

### 4.4 RESULTS

#### 4.4.1 *Diapause and stage of maize*

Figures 20-23 represent the incidence of diapause in relation to the growing stages of maize plants in the course of time. The growing conditions for the maize fields were quite different: when enough water for growth of the plants was available (i.e. when maize was sown early in the growing season at the end of April) a normal development occurred resulting in fully mature plants of stage 6 (fig. 20 and 23). When maize was sown later in the growing season full maturity was seldom reached. For example: plants in the field represented in fig. 22 (sown at the end of June) ceased to develop soon after tasseling (stage 4). When maize was grown during the dry season, plants had a very slow growth rate and could - whether the irrigation period was short (fig. 21a till the end of January) or longer (fig. 21b till the end of February) - not develop beyond the

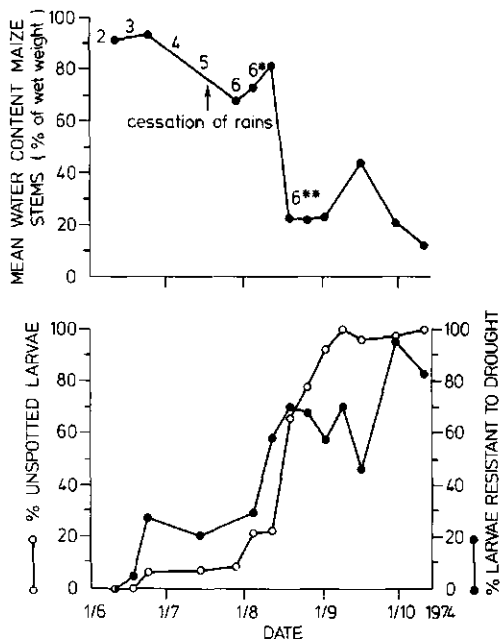


Fig. 20. The seasonal incidence of diapause in larvae of *Chilo* sp. as related to development stage and water content of the maize plant during the wet season. Maize was sown 23 April 1974. Numbers refer to the developmental stages of the maize:

- stage 1: all stages before appearance of 4th leaf  
 2: collar of 8th leaf visible; early leaves may be dead; leaf growth  
 3: tips of tassels visible  
 4: pollen shedding; silks visible; ears start developing  
 5: ears fully grown, grains maturing  
 6: grains mature and drying

Asterisks indicate the appearance of the maize plant

no asterisk: leaves and stem succulent and green (wilting may occur)

one asterisk: leaves dry, stem green

two asterisks: leaves and stem dry, plant dying.

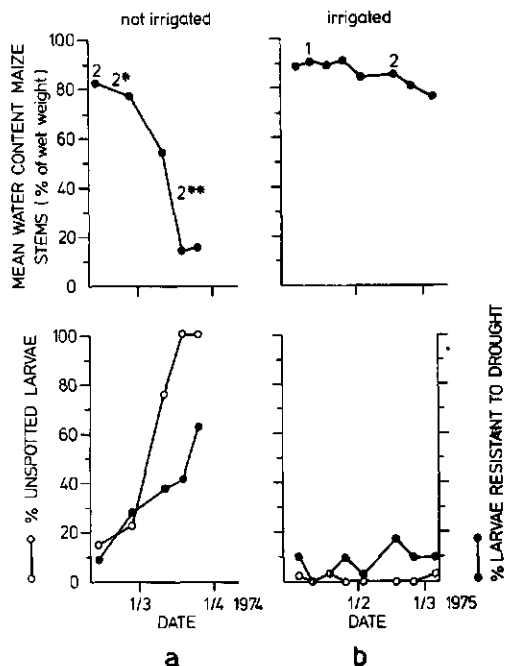


Fig. 21. The season incidence of diapause in larvae of *Chilo* sp. as related to developmental stage and water content of the maize plant during the dry season. a. maize sown 4 December 1973 and not irrigated beyond 28 January 1974, b. maize sown 19 November 1974 and irrigated till 20 February 1975. Numbers refer to developmental stages of the maize and are explained in fig. 20.



vegetative stage 2. No doubt due to the extremely high evaporation rate during that season. It can be seen that larvae were able to enter aestivation-diapause in all these different stages of the maize plant. This indicates that the induction of diapause is not dependent on the actual stage of the maize plant. In other words: the plant factors which trigger the diapause may appear in any developmental stage of the host plant. Larvae from the field shown in fig. 21b did not enter diapause due to continued irrigation (see also 2.6).

#### 4.4.2 *Diapause and chemical composition maize stem*

Aestivation-diapause of stem borers in the tropics has often been related to the moisture content of the food plant. The present results show that attention should be paid to the stem when this is still green (leaves may be wilted) and contains a fair amount of water. The figures (20-23) show that some aestivating larvae are found at any time, but that the first significant increases in percentage unspotted and drought resistant larvae take place in stems which contain 70-80% of water, well before the water content decreases steeply. A study of the physiological condition of these larvae (insets fig. 22 and 23) confirmed this by demonstrating that the first preparations for diapause are made before the appearance of unspotted or drought resistant larvae. The larval water content, the respiratory rate and the rate of heart beat had dropped notably, and the fat content had increased when the moisture content of the stems had only slightly decreased, i.e. from 80 to 75 % in 1974 (fig. 22) and from 80 to 70 % in 1975 (fig. 23). In both cases plants were water stressed because of the lack of rainfall.

Samples from two experimental fields were analysed for reducing sugars, total sugars and crude proteins (fig. 22 and 23). The sugar contents of the maize stems from the two fields were very different. Total sugar content during the period of diapause induction was about 6 % of the stem fresh weight (29 % of the dry weight) in 1974 (fig. 22) whereas in 1975 (fig. 23) never more than 2.5 % (fresh wt.) or 9 % (dry wt.) was found. In other experimental fields similar levels have been recorded. The large differences may be due to soil and cultivation; the two fields were sown at a different location of the Coast Agricultural Research Station, at a different time of the year and in different years. In both fields the quantity of reducing as well as non-reducing sugars was highest in the pre-diapause phase of the larvae.

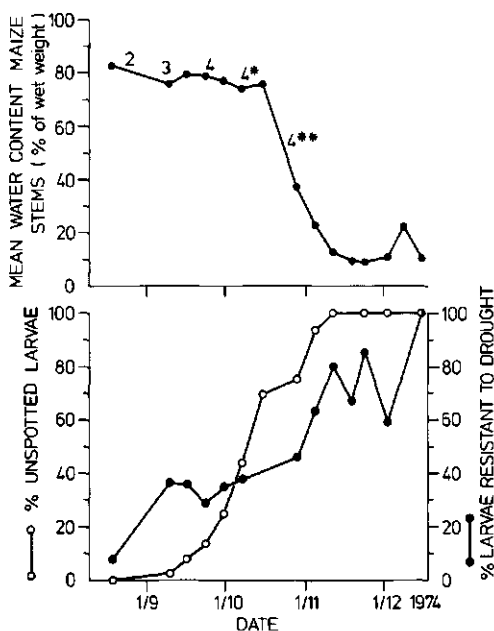
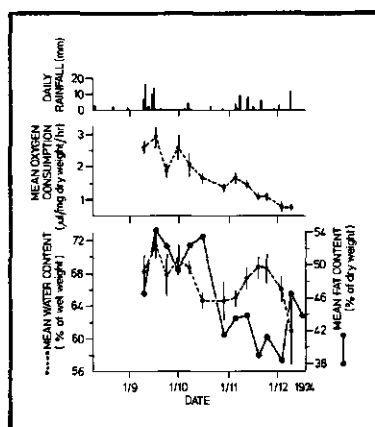
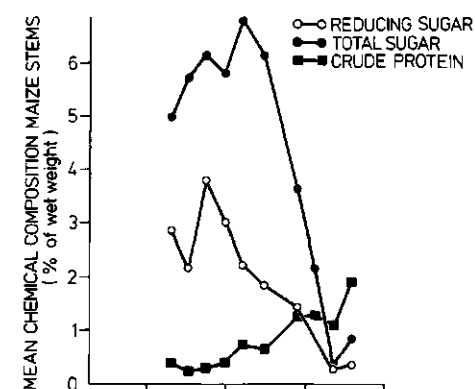


Fig. 22. The seasonal incidence of diapause in larvae of *Chilo* sp. as related to developmental stage and chemical composition of the maize plant during the wet season. Maize was sown 20 June 1974. Numbers refer to developmental stages of the maize and are explained in fig. 20. The inset shows the physiological condition of the larvae during the same period.

During that period protein levels varied between 0.5 - 0.9% of the fresh wt. of the stem or 2 - 3% of the dry wt. In another experimental field larvae preparing for diapause were found in stems containing 1.3% protein (fresh wt.) or 4.2% (dry wt.). As a result of the rapidly decreasing water content of the stem when the plant dies, protein contents of the "fresh" stems ultimately increase. But at that time larvae have already entered diapause.

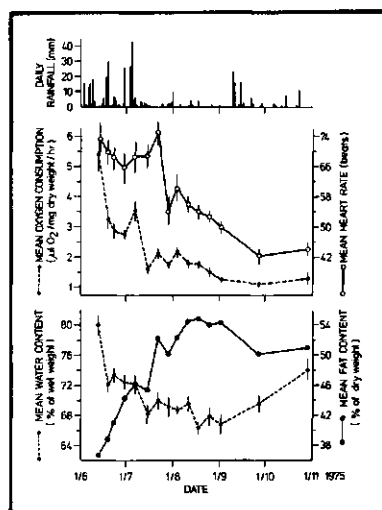
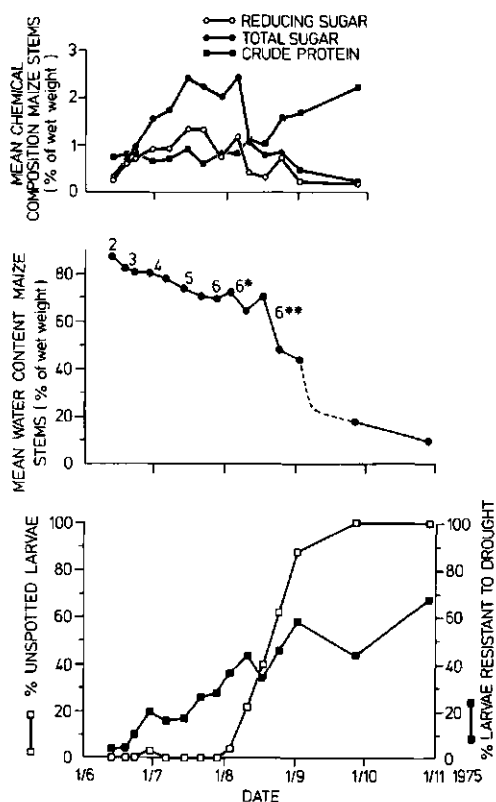


Fig. 23. The seasonal incidence of diapause in larvae of *Chilo* sp. as related to developmental stage and chemical composition of the maize plant during the wet season. Maize was sown 25 April 1975. Numbers refer to developmental stages of the maize and are explained in fig. 20. The inset shows the physiological condition of the larvae during the same period.

#### 4.4.3 Composition of the maize stem during its normal development

The chemical composition of a maize stem changes considerably during its growth. Table 11 shows that in the early (vegetative) stages stems had a high water content, a high protein content and a low sugar content. During the further vegetative growth the water content remained high, whereas the protein content gradually decreased and the sugar content increased. The major changes however occurred when the plant started its generative development with the formation of the male inflorescence, the tassel: the plant reduced the water content of its stem from 90 to 80 %, and the sugar content increased from 15.1 to 35.8 % of the stem dry weight or 1.5 to 7.1 % of the fresh weight. When expressed as percentages of dry wt. also the protein content changed at that time but because of the simultaneous decrease in water content, the percentage

in the fresh stems remained constant. It is remarkable that only minor differences were found between stems from almost mature plants and stems 4-5 weeks beyond maturity.

Table 11. Composition of maize stems (Hybrid 512) at different stages of growth.

stage of growth	length of stem till point of growth (cm)	n	mean water content (%)	mean sugar and protein content *		
				reducing sugars	total sugars	crude proteins
6-8 leaves	5	5	91.5	2.8 (0.24)	6.7 (0.57)	21.4 (1.82)
8-9 leaves	25	5	90.8	6.8 (0.66)	15.1 (1.39)	16.0 (1.47)
9-10 leaves	60 - 80	3	90.3	7.1 (0.69)	15.1 (1.46)	13.4 (1.30)
tassel well developed; pollen shed; ears developing	110 - 130	5	80.2	13.6 (2.69)	35.8 (7.09)	6.9 (1.37)
plants 4-5 weeks beyond maturity; leaves dry, stem green	160 - 200	5	79.7	11.3 (2.29)	39.7 (8.06)	6.4 (1.03)

\* expressed as percentage of dry weight and in parentheses as percentage of fresh weight of stem.

#### 4.4.4 Consumption and utilization of maize

The consumption of the pith of maize stem obtained from plants in different growing stages is shown in table 12. Although larvae consumed less and less fresh stem material as the plant aged, a significant difference could only be demonstrated between the consumption of fresh aged maize (stage V) and the other maize stages. Because of the simultaneously decreasing water content, significantly *more* dry weight of the older stems was ingested, which in turn resulted in higher growth rates. Most remarkable is the difference in food intake by larvae feeding on stems of almost mature maize (stage IV) and larvae feeding on aged stems (stage V). Although the chemical composition of these stems was almost equal, larvae consumed significantly less of the fresh or dry aged maize stems and in consequence had a lower growth rate.

Figures on the digestibility and utilization of the various maize stems are given in table 12 and are graphically shown in fig. 24. The A.D., E.C.D. and

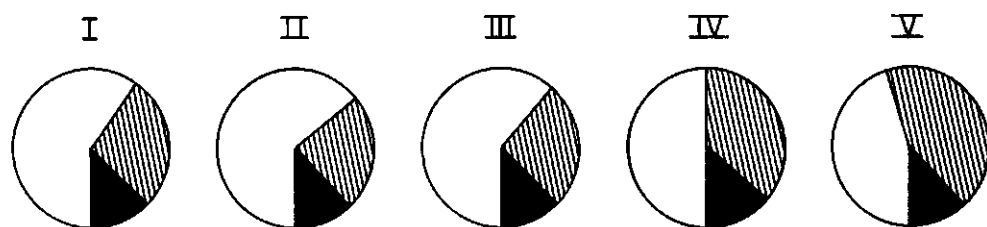


Fig. 24 Digestibility and utilization of the pith of maize stems in different stages of growth by 6th instar larvae of *Chilo partellus*. Numbers refer to the stages of the plant and are explained in table 12.

proportion of undigested food (faeces)  
 proportion of digested food metabolized for energy  
 proportion of digested food used for body substance

E.C.I. of the three vegetatively growing maize stages I, II and III were very much alike. Compared to these stages a higher proportion of stems of further developed plants (stage IV and V) was digested. But since this digested material was less efficiently converted into body weight than the digested material of the younger stems, the efficiency of conversion of ingested food was approximately the same for all stems. The significantly different A.D's, E.C.D.'s and E.C.I.'s of maturing (stage IV) and aged (stage V) maize material indicate that a larva perceives these materials in different ways.

#### 4.5 DISCUSSION

As long as the weather remained dry aestivating larvae could be found in the field in plants of almost any stage of growth: during the vegetative stage (fig. 21a), when shedding pollen (fig. 22) and when fully mature (fig. 20 and 23). In chapter 2 it was demonstrated that climatic factors other than rain can be excluded as diapause inducing factors. The present findings that larvae are capable of entering aestivation-diapause independent of the growing stage of the maize plant they are inhabiting, suggest that the effects of drought on the plant may be similar for all developmental stages. Some evidence supporting this hypothesis was given by LEVITT (1972) and HSIAO (1973) both reviewing the general responses of plants - irrespective of the developmental stage - to water stress. Any following information on this subject is derived from these papers.

The most commonly affected process in a water stressed plant is the growth rate. Due to a lack of turgor, cells do not expand and also the number of cell

Table 12. Consumption, growth rate, digestibility and utilization of the pith of maize stems in different stages of growth by 6th instar larvae of *Chilo partellus*.

growing stage of maize plant (1)	composition of pith			n	C.I. fresh	C.I. dry	G.R.	A.D	E.C.D.	E.C.I. 3)
	Water content	total sugars (2)	Crude proteins (2)							
I	92.8	12(0.9)	18(1.3)	10	6.39+0.6 <sup>a</sup>	0.46+0.04 <sup>c</sup>	5.5+0.9 <sup>f</sup>	40.7+4.4 <sup>h</sup>	35.3+6.4 <sup>k</sup>	12.8+2.3
II	93.2	15(1.4)	16(1.5)	14	5.88+0.4 <sup>a</sup>	0.40+0.03 <sup>c</sup>	4.6+0.5 <sup>f</sup>	36.5+2.7 <sup>h</sup>	37.9+6.0 <sup>k</sup>	12.0+1.1
III	91.6	20(1.7)	11(0.9)	14	5.24+0.2 <sup>a</sup>	0.44+0.02 <sup>c</sup>	5.2+0.4 <sup>f</sup>	38.9+3.1 <sup>h</sup>	38.1+6.3 <sup>k</sup>	12.2+1.2
IV	84.4	37(5.8)	7(1.1)	14	5.32+0.3 <sup>a</sup>	0.83+0.05 <sup>d</sup>	11.0+0.6 <sup>g</sup>	49.6+1.6 <sup>i</sup>	27.5+1.1 <sup>k</sup>	13.5+0.5
V	84.4	40(6.2)	6(0.9)	15	3.78+0.2 <sup>b</sup>	0.59+0.03 <sup>e</sup>	6.6+0.6 <sup>f</sup>	54.1+1.3 <sup>j</sup>	21.1+1.5 <sup>l</sup>	11.4+0.8

1) stage of maize: I vegetative stage with 7-8 leaves

II vegetative stage with 9 leaves

III 11 leaves, plants almost tasseling

IV plants near maturity, cobs almost developed

V aged plants, 1 month after maturity, dry leaves, but stem still green

2) expressed as % of dry weight and in parentheses as % of fresh weight of pith

3) no significant differences between any of the E.C.I's except between IV and V

a-l) maize having the same letter within one column is equal and differs significantly from other }  
maize within that column }  
t-test,  
p=0.05

divisions may seriously be reduced. A maize plant being a stenohydric plant which can only grow within a narrow range of water stresses may particularly suffer. The flowering stage of the plant is most sensitive. In the experimental field mentioned in this paper the growth retardation after the start of the dry weather was obvious.

It was found that the water content of the plants usually decreased somewhat after cessation of the rains, but in general water turned out to be remarkably well held within the plant, probably due to stomatal closure and consequent reduced transpiration. Soon after the complete desiccation of all leaf tissues the stems started drying out at a much faster rate than when green leaves were still present. The size of the aestivating larval population was clearly inversely proportional to this rapidly decreasing water content. But it is questionable whether this dramatic water loss of the plant induces the larvae in the plant to enter diapause. The first rises in number of drought resistant and unspotted larvae were found before this rapid fall of the water content of the maize stem. This means that larvae entered a pre-diapause phase when stems contained 70-80% of water. Earlier studies on the physiological condition of the larvae confirmed this. Major changes in the physiology of the larvae were found to take place soon after the drought starts (fig. 22 and 23, see also chapter 3). Thus, if the water condition of the host plant provides the token-stimulus for a larva to enter aestivation-diapause, it is certain that this is while the water stressed plant still contains a large amount of water.

Sugar contents of maize stems normally rise gradually during the development of the plant. This was demonstrated by JONES and HUSTON (1914) and confirmed by the present analyses (table 11). Due to stomatal closure photosynthesis declines in water stressed plants. Since growth (for which sugars are required) is affected by water stress more quickly than photosynthesis (producing sugar) is, sugar contents of the plant may even increase in the early stages of the stress. In the experimental fields represented in fig. 22 and 23 it is shown that the sugar content remained fairly constant (but at very different levels) for more than one month after the start of the dry period. This may be advantageous for the accumulation of fat reserves by larvae preparing for diapause. HIRANO (1964) found that larvae of *C. suppressalis* grown on carbohydrate rich food became very fatty and highly tolerant to environmental resistances. The variability of the sugar content in stems containing pre-diapause larvae in the field suggests that the quantity of sugar in a stem is not essential to prevent diapause to take place and thus does not seem to play a role in the

induction of diapause.

Protein contents of maize stems are always highest in the earliest stages of their growth. Under normal growing conditions there is a gradual decrease during the vegetative stage and a very marked reduction soon after the start of the generative stage due to a rapid translocation of nitrogen to the young ear. Thereafter protein levels gradually fall again till low levels at harvest. These trends shown by JONES and HUSTON (1914) were also found in Kenya. It is not certain whether drought accelerates the processes of protein reduction in the maize stem or not. HSIAO mentioned that generally dehydration of a plant tends to retard synthetic reactions and to favour hydrolytic reactions. He gave some evidence that this statement also holds for the protein metabolism in maize. In connection to the present research more information would certainly be interesting.

Protein contents of the stems in the two experimental fields in Kikambala were invariably low during the period of diapause induction: 0.5 - 0.9% (fresh wt.) or 2 - 3% (dry wt.). Slightly higher contents of 1.3% (fresh wt.) or 4.2% (dry wt.) also allowed diapause. In one of the experimental fields grown during the dry season, diapause developed in the young maize plants soon after the irrigation was ceased (fig. 21a). Stems of plants in this stage normally have a high protein content and it would have been interesting to know whether such a content was maintained during the subsequent period of drought. The quantity of protein has often been proved to be of great importance for the growth and development of a large number of insects (HOUSE 1961). Normally protein requirements of stem borers are high: e.g. *C. suppressalis* (HIRANO 1964), *D. grandiosella* (REDDY and CHIPPENDALE 1972). *O. nubilalis* larvae need much protein in the early instars and only little when further developed (BECK 1956). But such requirements were seldom related to diapause. An exception to this may be the diapause of the maize stalk borer *B. fusca* (USUA 1973). The present experiments only show the simultaneous presence of pre-diapause larvae and low protein contents of the stem in which they feed at that time.

Influences of host plants on the physiological condition of insects are often related to differences in chemical composition of the plant. The components are frequently expressed as percentage of plant *dry* weight (HIRANO 1964, USUA 1973, McCAFFERY 1975). Conclusions from such observations are only valid if it can be assumed that 1. the compared food is similar except for the component under investigation and 2. the investigated component is not influencing the quantity of food consumed. Such conditions are seldom fulfilled for plant material. Thus,



when physiological responses of insects to plants are investigated, the feeding behaviour of the insect on whole fresh plants has to be incorporated in the research and if specified components are studied they may better be expressed in percentage of *fresh* weight of the plant. Several examples are known of insects making their food intake conditional to physical or chemical qualities of the food (see reviews of WALDBAUER 1964, GELPERIN 1971 and BARTON-BROWNE 1975). One example is the larva of *Celerio euphorbiae* which, when reared on artificial diets with different concentrations of nutrients, progressively consumed more fresh food as the water content of the diet increased (and the nutrient concentration decreased). The growth rate of the *Celerio* larvae appeared to not be affected (HOUSE 1965). HOEKSTRA and BEENAKKERS (1976) found large differences in dry weight consumption of five different grasses by *Locusta migratoria*. After conversion of the dry weight figures into fresh weights, a highly positive correlation could be demonstrated between the amount of fresh grass consumed and the water content of the grass. Apart from water, it is possible that other components which were different between the five grasses have regulated the feeding. Growth was again not affected by the different food intake.

Similar results as above were observed for the consumption of fresh maize of different developmental stages by *Chilo partellus* larvae. Progressively less pith material of a stem was eaten as the maize plant developed further and contained less water. It was very interesting to find that significantly less fresh stem material was consumed from a maturing plant than from an aged plant, in spite of the very similar chemical composition of the stems. Even the water content of the stems is equal, so the larva does not need to compensate for decreased nutrient concentrations as was shown for *Celerio* larvae (HOUSE 1965). It is more likely that a structural component of the stem has resisted feeding.

The observation gives rise to a hypothesis so far overlooked. It was found that in nature *Chilo* larvae gradually cease feeding when entering aestivation-diapause (chapter 3). However, the causal relationship between feeding and diapause is not known. It may be true that the reduced feeding is a result of the diapause, but the reverse may be equally possible. It is known that many plants react to water stress by early maturation of tissues, shown in the formation of a more compact structure and thicker cell walls and cuticles (KRAMER 1959). In the present research, an indication was obtained that a structural differentiation of a maize plant may lead to reduced feeding of larvae in such a plant. With consistent quantities of nutrients in the food plant, reduced feeding results

Table 14. Development of 4th, 5th and 6th instar larvae of *Chilo partellus* after different periods of residence in aged maize stems or artificial diet (control).

food	larval stage	sampling period											
		0 - 14 days				15 - 28 days				29 - 42 days			
		no. introduced	no. surviving	larvae (%)	pupae + pupal exuviae (%)	no. introduced	no. surviving	larvae (%)	pupae + pupal exuviae (%)	no. introduced	no. surviving	larvae (%)	pupae + pupal exuviae (%)
aged maize	L4	94	52	100	0	44	19	84.2	15.8	30	5	100	0
	L5	87	59	98.3	1.7	43	19	73.7	26.3	30	19	78.9	21.1
	L5/L6	20	12	100	0	40	22	50.0	50.0	30	16	12.5	87.5
diet	L4	50	40	100	0	50	40	52.5	47.5	50	40	15.0	85.0
	L5	50	48	97.9	2.1	50	48	35.4	64.6	50	48	2.1	97.9
	L5/L6	50	41	78.1	21.9	50	41	29.3	70.7	50	41	4.9	95.1

3-7 samples from stems or diet were taken at different times during the indicated periods. Opened stems could not be reused, so different stems were used for each observation. Age larvae L4: 8 and 9 days, L5: 12 and 13 days, L5/L6: 18 days.

Table 15. Pigmentation of 4th, 5th and 6th instar larvae of *Chilo partellus* after different periods of residence in aged maize stems.

larval stage 2)	sampling period								
	0 - 14 days			15 - 28 days			29 - 42 days		
	n <sup>1)</sup>	% TR <sup>1)</sup>	% US <sup>1)</sup>	n	% TR	% US	n	% TR	% US
L4	52	17.3	9.6	16	0	56.2	5	0	100
L5	58	20.7	24.1	14	7.1	92.9	15	6.7	93.3
L5/L6	12	33.3	58.1	11	0	100	2	0	100

- 1) n - number of recovered and surviving larvae  
 TR - transitional larvae  
 US - unspotted larvae

2) L4: 8 and 9 days old, L5: 12 and 13 days old, L5/L6: 18 days old.

### 5.4.3 Larval growth and pupation on diets

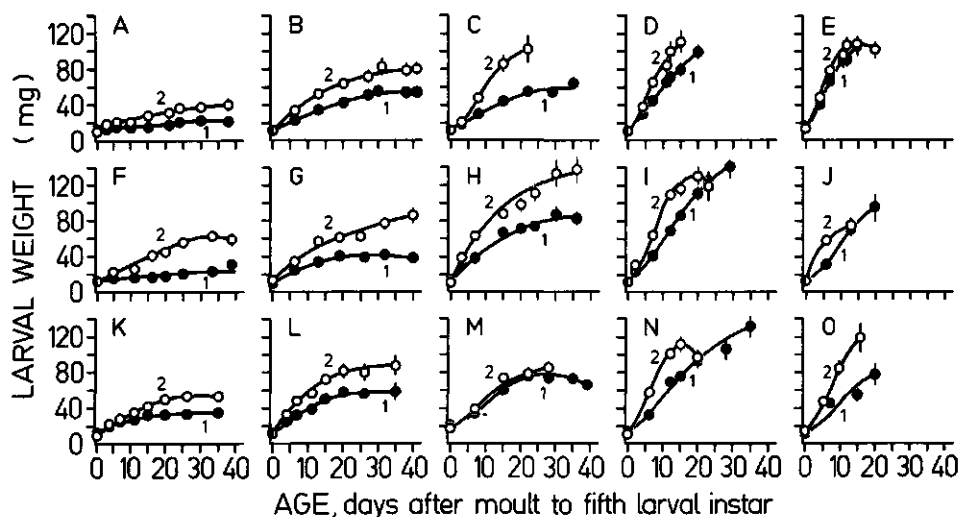


Fig. 28. Growth curves for larvae of *Chilo partellus* on artificial diets of different chemical composition. See for composition table 13.

The effect of various diets on larval body weight are shown in fig. 28. Proteins turned out to play a dominant role in the weight gain. Growth rate was extremely slow when diets contained less than 0.45% protein (diets A, F and K); after an experimental period of 40 days none of the larvae on these diets weighed more than 40-60 mg. On the average normal growth was obtained when the casein contents were 1.7-1.8% or more of the diet fresh weight. Water and sugar seemed to be rather ineffective. Larval growth on diets with about the same sugar and protein contents but with different quantities of water (e.g. 1-H and 2-B or 1-I and 2-C) proved to be the same. Likewise, diets with different sugar contents and otherwise identical quantities of water and protein, did not affect the larval weight increase. Even a sugar content of 1.4% was sufficient for a normal growth.

The effects of the artificial diets on pupation were in line with the above mentioned effects on body weight (fig. 29). Larvae grown on diets with little protein did not pupate in large numbers whereas pupation rates were normal when more protein was ingested. The pupation rate of larvae feeding on low protein diets remained very slow beyond the period of 40 days shown in the figure; two weeks later only a few more pupae were formed. An important observation was that

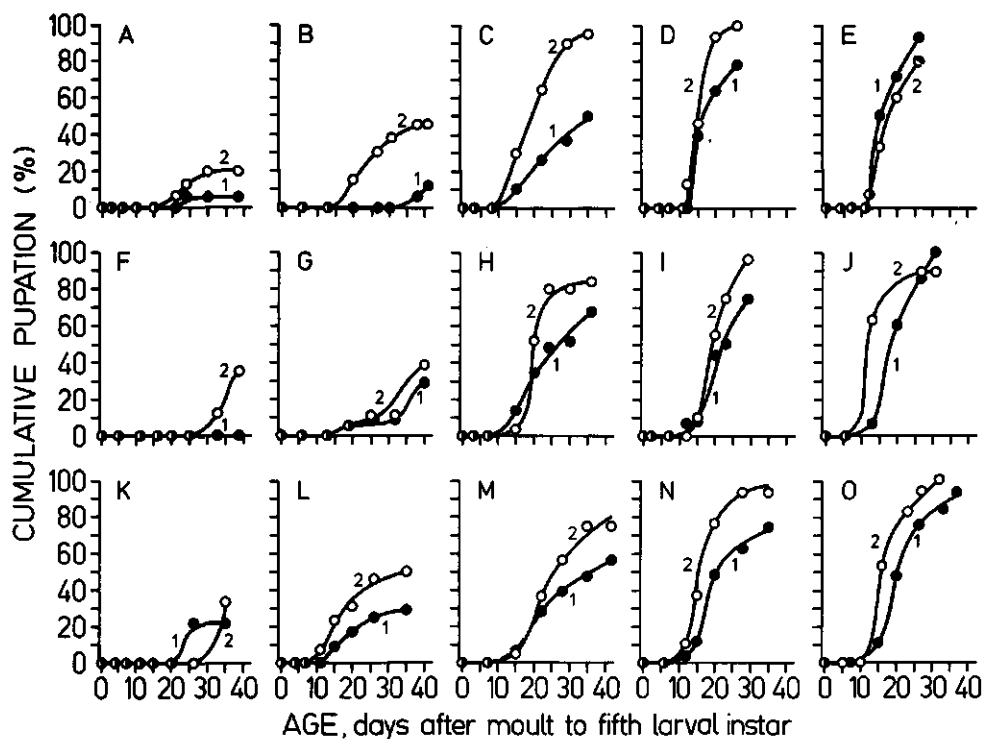


Fig. 29. Pupation of larvae of *Chilo partellus* on artificial diets of different chemical composition. See for composition table 13.

moulting continued to take place. Again, no significant influence was observed of water or sugar on the larval development.

#### 5.4.4 Pigmentation and physiology of larvae on diets

During the previous experiments I noticed on some diets substantial numbers of unspotted larvae. Since loss of cuticular pigmentation was considered as an element of the aestivation-diapause syndrome (chapter 2) it was thought appropriate to have a closer look into this phenomenon. This was done in a new experiment in which the behaviour of larvae was observed on a number of freshly prepared diets of the same composition as the diets mentioned in 5.3.3. Pupation rates were in agreement with those in fig. 29. The changes in the cuticular pigmentation of the remaining larvae are given in table 16. No signs of change were observed during the first 15 days of larval feeding on any of the diets. Larvae reared on diets containing 0.45% protein hardly turned unspotted. When

Table 16. Changes in cuticular pigmentation of 30 spotted 5th instar larvae after a feeding period of 10, 20 and 30 days on artificial diets of different chemical composition.

diet	chemical composition of the diet (% of fresh wt.)			larval pigmentation* (numbers)											
	% water	% sugar	% protein	after 10 days				after 20 days				after 30 days			
				n	SP	TR	US	n	SP	TR	US	n	SP	TR	US
1-F	86	2.8	0.3	30	30	0	0	21	21	0	0	13	13	0	0
1-G	86	2.8	0.6	27	27	0	0	24	24	0	0	22	13	6	3
1-H	86	2.8	1.1	28	28	0	0	20	12	5	3	14	6	1	7
1-I	86	2.8	1.7	27	27	0	0	14	8	2	4	6	2	1	3
1-K	86	4.2	0.3	27	27	0	0	24	24	0	0	20	20	0	0
1-L	86	4.2	0.6	27	27	0	0	21	18	3	0	10	4	5	1
1-M	86	4.2	1.1	26	26	0	0	21	11	5	5	16	3	2	11
1-N	86	4.2	1.7	27	27	0	0	14	9	5	0	9	1	2	6
2-F	70	6.0	0.45	30	30	0	0	24	24	0	0	17	15	2	0
2-G	70	6.0	0.9	26	26	0	0	23	12	3	8	21	5	3	13
2-H	70	6.0	1.8	26	24	2	0	12	4	3	5	5	0	0	5
2-I	70	6.0	2.7	29	29	0	0	13	8	1	4	1	0	0	1
2-K	70	9.0	0.45	29	29	0	0	29	22	7	0	26	19	5	2
2-L	70	9.0	0.9	29	29	0	0	27	14	3	10	23	2	6	15
2-M	70	9.0	1.8	27	27	0	0	22	14	4	4	14	0	1	13
2-N	70	9.0	2.7	28	28	0	0	7	4	0	3	2	0	0	2

\* SP - spotted      TR - transitional      US - unspotted

more protein was presented, increasingly more unspotted larvae were formed. The highest yield of unspotted larvae was obtained with diets containing intermediate levels of protein (0.9 - 1.1%). It is remarkable that also larvae which had consumed diets with higher protein contents regularly turned unspotted. Since pupation rates of these larvae were high, only few larvae were unspotted after 30 days. No relation with water and sugar contents could be found.

After a feeding period of 33-39 days water contents and respiratory rates were determined of those larvae which had retained their larval stage in sufficient numbers. Diets 1-F and 2-F were not taken into consideration because of the poor condition of many larvae reared on it. Results are shown in table 17.

Table 17. Water content and respiratory rate of larvae of *Chilo partellus* after a feeding period of 33-39 days on artificial diets of different chemical composition.

Diet	Chemical composition of the diet (% of fresh wt.)			days after moult to 5th larval instar	mean water content + S.E. (%)	mean oxygen consumption + S.E. ( $\mu$ l O <sub>2</sub> /mg dry wt./hr)
	% water	% sugar	% protein			
1-G	86	2.8	0.6	36	72.3 $\pm$ 1.2 (19)	2.33 $\pm$ 0.15 (11)
1-K	86	4.2	0.3	33	72.3 $\pm$ 1.0 (18)	2.68 $\pm$ 0.38 ( 8)
1-L	86	4.2	0.6	33	67.2 $\pm$ 1.3 (18)	2.46 $\pm$ 0.11 (12)
1-M	86	4.2	1.1	39	69.6 $\pm$ 1.7 (10)	2.31 $\pm$ 0.13 (10)
2-G	70	6.0	0.9	36	66.0 $\pm$ 1.4 (15)	1.93 $\pm$ 0.10 (12)
2-K	70	9.0	0.45	33	63.7 $\pm$ 0.9 (20)	1.41 $\pm$ 0.14 (12)
2-L	70	9.0	0.9	33	63.6 $\pm$ 1.0 (20)	1.83 $\pm$ 0.11 (12)
2-M	70	9.0	1.8	39	62.3 $\pm$ 1.8 (12)	1.76 $\pm$ 0.15 (12)

Differences between diets 1 and 2 significant with t-test at  $p = 0.05$ . Numbers between brackets refer to number of observations.

#### 5.4.5 Sensitivity of larvae to diet

All previous experiments were made with fifth instar larvae. The effects on larvae of other ages were studied in the present experiment. For this we selected diet 2-G on which larvae had been produced with qualities nearest to those of aestivating larvae from the field. This diet contained 70% water, 0.9% protein and 6.0% sugar. Fifth instar larvae reared on this diet retarded their growth and development (fig. 28 and 29), lost their cuticular pigments (table 16) and showed a reduced respiration (table 17).

Growth curves are given in fig. 30. Maximum larval weights were about the same for all larvae and similar to weights of larvae reared on the basic diet (fig. 13). Growth of 15 and 20 days old larvae was completed within the observation period of 34 days. Younger larvae continued to gain weight throughout this period.

The rates of pupation (fig. 31) were in line with this; the development of 4th instar and early 5th instar larvae hardly showed any progress. Only few pupae were formed whereas mortality was high as compared to more aged larvae.

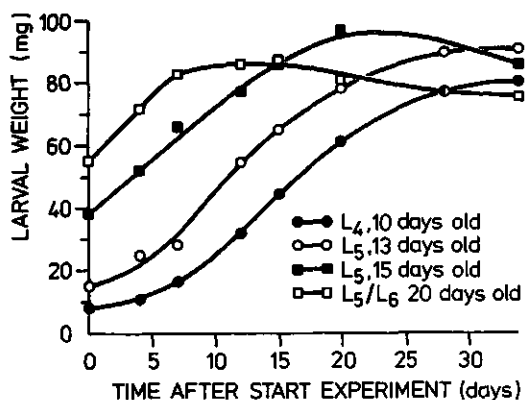


Fig. 30. Growth curves for *Chilo partellus* larvae of different age on an artificial diet containing 70% water, 6.0% sugar and 0.9% protein.

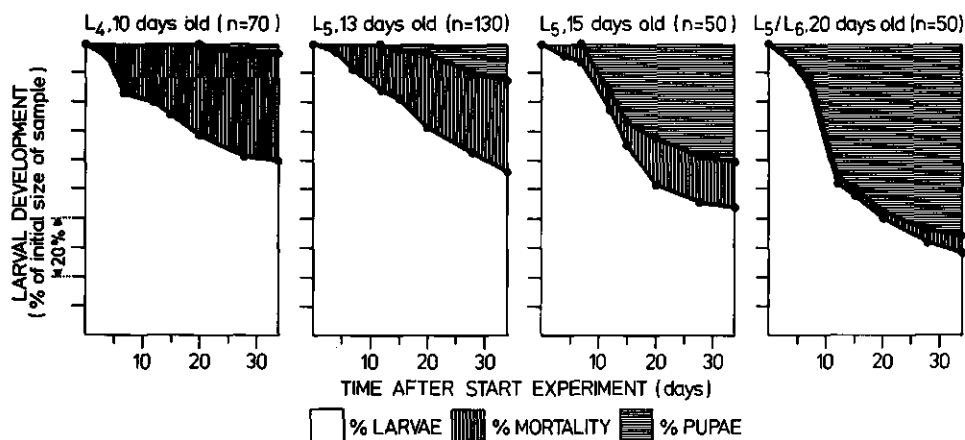


Fig. 31. Development of *Chilo partellus* larvae of different age on an artificial diet containing 70% water, 6.0% sugar and 0.9% protein.

The changes in cuticular pigmentation are shown in table 18. All larvae ultimately tended to turn from the spotted into the unspotted morph.

A final observation was done on the drought resistance of larvae. It is known that 95% of non-diapause larvae die or pupate within 16 days when placed in dry pieces of maize stem at 25° C (chapter 2). When 4th and 5th instar larvae

Table 18. Changes in cuticular pigmentation of spotted larvae of different instar and age after a feeding period of 20 and 34 days on an artificial diet containing 70% water, 0.9% protein and 6.0% sugar.

larval stage and age at start of experiment (n)	larval pigmentation* (numbers)								% US of initial number of larvae
	after 20 days				after 34 days				
	n	SP	TR	US	n	SP	TR	US	
L4, 10 days (70)	48	35	5	8	42	10	8	24	34.3
L5, 13 days (130)	93	53	15	25	73	9	12	52	40.0
L5, 15 days (50)	26	14	2	10	22	1	1	20	40.0
L5/L6, 20 days (50)	20	9	1	10	14	1	1	12	24.0

\* SP - spotted      TR - transitional      US - unspotted

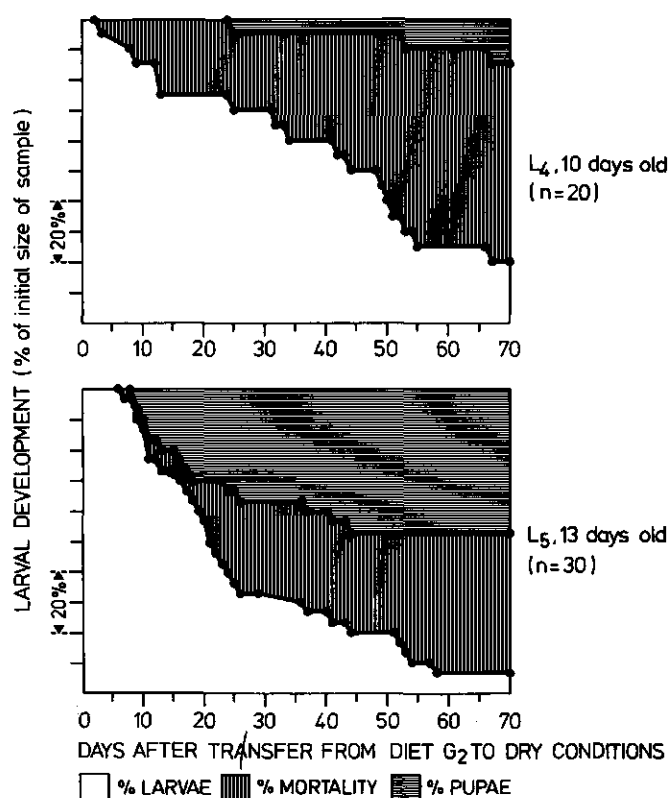


Fig. 32. Drought resistance of *Chilo partellus* larvae after feeding on diet 2-G during preceding period of 34 days.



which had been feeding on diet 2-G for a period of 34 days were placed in similar dry conditions (petri dish with dry filter paper) they survived this period for the greater part (fig. 32). From this it seems that the drought resistance of larvae which had been feeding on the experimental diet had indeed increased.

## 5.5 DISCUSSION

A large number of non-diapause fifth instar larvae of *Chilo partellus* entered diapause after they were introduced into stems of aged maize plants (5.4.1.). Pupation was not much affected during the first five weeks of presence in the stems, thereafter pupation was retarded and only few pupae were formed. A certain rate of pupation normally occurs among aestivating larvae and has also been found to take place in the field (chapter 2). Further evidence that larvae had entered diapause came from the disappearance of the cuticular pigmentation and from the low respiratory rate and water content which were all also demonstrated in field-collected aestivating larvae (chapter 3).

At the time of introduction, stems contained about 75% water, 8% sugar and 1.3% protein; tissues were relatively hard. All these conditions of the stems were comparable to stems in which larvae were found to enter aestivation-diapause in the field (chapter 4). The above mentioned results confirm the field data, but do not give conclusive evidence on the importance of the chemical or structural nature of the stem for the induction of diapause. The different responses of larvae to different section of the aged plants with relatively high and low moisture contents (5.4.1) seem to indicate a possible involvement of water, but it may also be that these differences are the result of a slightly different chemical composition or - as was shown in 4.4.5 - a difference in feeding behaviour of the larva itself.

It was shown that different larval instars have a different capacity to respond to diapause inducing cues (5.4.2). When fourth instar larvae were introduced into aged maize, few larvae pupated and few entered aestivation-diapause. The mortality of this stage was high. A high proportion of early fifth instar larvae entered aestivation-diapause, but most late fifth and sixth instar larvae pupated. It is probable that these older larvae had proceeded too far with the preparations for the pupal moult and were not sensitive to the diapause inducing factors in the aged maize. A loss of cuticular pigmentation was observed in all three larval stages introduced. Due to their high pupation rate,

only few unspotted larvae originating from old larvae could be found after six weeks. Therefore the total number of unspotted larvae obtained from younger larvae was considerably higher.

The chemical composition of maize stems which had been found to induce the highest incidence of aestivation-diapause in the field (4.4.2) and in the laboratory (5.4.1) was simulated in 30 artificial diets with varying protein, sugar and water contents.

Protein appeared to be the crucial food factor regulating weight increase and rate of pupation. Casein contents below 0.45% of the diet fresh weight were inadequate and resulted in very poor growth and extremely low pupation. An increase to 0.9-1.1% provided some improvement whereas normal growth and development was obtained at a minimal protein content of 1.7-1.8%. This is precisely the requirement for the larvae of the European corn borer, *O. nubilalis* (BECK 1956).

Sugars were less crucial: a sugar content of 1.4% (lower than any of the maize stems encountered in the field during diapause induction) permitted normal development as long as proteins were sufficiently available. *O. nubilalis* larvae require 1.8% glucose in their diet (BECK 1956) and the growth of *D. grandiosella* larvae was only slightly retarded on diet supplemented with 1.65% glucose (CHIPPENDALE and REDDY 1974).

If a low water content would have played a dominating role in the induction of aestivation-diapause, a difference should be found between larvae grown on diet 1 (86% of water) and diet 2 (70% of water), independent of other components of the diet. No difference was found on larval growth or pupation even if the proportion of the different components was identical. However, respiratory rates and water contents were lower of larvae from the diets containing reduced water contents (table 17). This result strengthens the above mentioned similar results of larvae from "moist" bottom sections and "dry" middle sections of the aged maize plants (5.4.1).

The protein content of the diet not only affected growth and development but also appeared to influence cuticular pigmentation. Larvae grown on diets deficient in protein (< 0.45%) always remained spotted. As already mentioned larvae on these diets hardly grew and they seldom moulted. CHIPPENDALE and REDDY (1972) demonstrated that moulting is necessary for larvae of the southwestern corn borer *D. grandiosella* to change from a spotted non-diapause form into an unspotted diapause form. A similar phenomenon was observed for *C. partellus* (unpublished results). When diets with a protein content higher than 0.45% were ingested by

larvae, moults could take place and many larvae changed to the unspotted form. On high-protein diets (> 1.7-1.8%) pupation rate was high with few unspotted larvae remaining after 30 days, but on diets with intermediate levels of protein (0.9-1.1%) less pupae and more unspotted larvae were found.

Different larval instars supplied with a low water (70%)/low protein (0.9%) diet (5.4.5) appeared to react to this diet in a similar way as to aged maize stems (5.4.2): Late 5th and 6th instar larvae were developed too far to be sensitive to the diapause inducing stimuli of the diet and pupated. The development of younger instars was increasingly retarded, but the mortality rose. Unspotted larvae were obtained from any instar; largest numbers from 5th instars. It was shown that 4th and 5th instar larvae reared on the above mentioned diet had an increased resistance to dry environmental conditions, which is a normal quality of aestivating larvae (chapter 2).

The results of the experiments with artificial diets indicate that aestivation-diapause is induced by the condition of the food. More data are required, e.g. on the physiological condition of the larva, to substantiate and further define these findings.

On protein deficient food (i.e. aged maize stems, table 15 or diet, table 18) not only young larvae but also late fifth and sixth instar larvae lost their cuticular pigmentation even though pupating soon thereafter. In previous chapters it was demonstrated that spotted larvae could exhibit physiological conditions resembling that of diapause larvae (fig. 15, 16, 18). These observations indicate that the loss of cuticular pigmentation itself may not be a criterion for diapause as reliable as previously assumed. Diapausing larvae of *D. grandiosella* are normally unspotted. The diapause is induced and maintained by an intermediate titre of juvenile hormone (YIN and CHIPPENDALE 1974, 1976). A slight increase of the JH titre in unspotted diapausing larvae resulted in ecdysis into the spotted form without termination of the diapause (CHIPPENDALE and YIN 1976). The observations on *C. partellus* agree with the results of CHIPPENDALE and YIN that diapause and cuticular pigmentation are related but not inseparable physiological processes. Considering this, I conclude that one should be careful when using the disappearance of pigments as criterion for diapause as long as the precise factors regulating the cuticular pigmentation are not known. It would be more preferable to combine the pigment characteristic with several physiological criteria as demonstrated in the present study (chapter 3).

## 6 Hormonal involvement in aestivation-diapause

### 6.1 INTRODUCTION

The larvae of the spotted stalk borer *Chilo partellus* and the coastal stalk borer *Chilo orichalcociliella* enter a stage of aestivation-diapause when oncoming adverse conditions for further development are announced by the cessation of rains (chapter 2). Only when rains reappear, diapause is terminated.

The induction, maintenance and termination of diapause has been demonstrated to be directly controlled by endocrine mechanisms in a wide range of insects (LEES 1956, DE WILDE 1970, CHIPPENDALE 1977). Previous observations that diapausing larvae of the above mentioned *Chilo* sp. are capable of undergoing stationary moults without terminating diapause suggest that such larvae have 1) at least periodically active prothoracic glands and 2) a juvenile hormone titre which is sufficiently high to prevent pupal ecdysis.

The present research was carried out to investigate this hypothesis.

### 6.2 LITERATURE

The hormonal regulation of larval diapause has recently been reviewed by CHIPPENDALE (1977). Evidence is accumulating that the induction and maintenance of larval diapause in many insects is regulated by the corpora allata (CA) which continue to actively secrete juvenile hormone (JH) during the diapause. Examples of this are now known of the rice stem borer, *Chilo suppressalis* (FUKAYA and MITSUHASHI 1961, YAGI and FUKAYA 1974), the southwestern corn borer, *Diatraea grandiosella* (YIN and CHIPPENDALE 1973, 1974, 1976), the European corn borer, *Ostrinia nubilalis* (YAGI and AKAIKE 1976) and the slug moth, *Monema flavescens* (TAKEDA 1978). In all these cases diapause was terminated when the activity of the CA decreased. Most evidence for the involvement of JH was derived from

surgical operations and from application of juvenoids or injection of ecdysones. In some cases the activity of the CA was measured directly by determining the size of the CA and/or the JH titre in the haemolymph. Details will be given in paragraph 6.5 if connected to the present research.

CHIPPENDALE and YIN (1976) postulated that the larval diapause of *D. grandiosella* is in fact not merely controlled by JH but rather by the interaction between the cerebral neurosecretory (NS) system and the CA. They suggested that intermediate levels of JH found in diapausing larvae of *D. grandiosella* inhibit the NS cells of the brain involved in the production, transport or release of ecdysiotropin. A high or a low titre of JH may activate these NS cells and thus induce pupal ecdysis. A similar mechanism is thought to be involved in the larval diapause of *M. flavescens* (TAKEDA 1978). In both cases however no conclusive evidence exists. The feedback of JH to the NS cells of the brain as well as the control of the CA itself by the brain require further studies.

### 6.3 MATERIALS AND METHODS

#### 6.3.1 Juvenile hormone titre

In larval material obtained from the experimental maize field sown on 25 April 1975 JH titre was determined to follow the changes during the induction of the diapause. Data on pupation in this field were given in fig. 7 and on the physiological condition of the larvae in fig. 16. From samples of 25-30 larvae taken at regular weekly intervals, haemolymph was removed with a micro capillary tube and pooled. Quantities obtained amounted to 0.5 - 1.0 ml.

The JH titre of diapausing larvae exposed to diapause terminating conditions (moist filter-paper) was determined with larvae in which diapause had been induced by growing them on aged maize stems (chapter 5). All larvae had been in diapause for about 1 month and were therefore in an early-diapause stage. Pooled haemolymph samples from 14-20 larvae (300-600  $\mu$ l) were obtained following exposure to moist filter-paper.

JH was extracted from the haemolymph with ether/ethanol and extracts were quantitatively assayed on fresh pupae of a local strain of *Galleria mellonella*. The procedures are described in more detail by DE WILDE et al. (1968). Dilutions of extracts were always tested on a minimum of 15 pupae. One *Galleria* Unit (G.U.)

equals the quantity of JH which provokes a positive reaction in at least 50 % of the pupae to which the JH containing dilution was applied. In our experiments, the G.U. was found to correspond with  $6 \times 10^{-6}$   $\mu$ g Cecropia JH.

### 6.3.2 Head ligatures and injections with $\beta$ -ecdysone

Unspotted larvae were obtained from the field at the beginning of the dry season. They were subsequently kept in the laboratory under dry conditions for three more weeks to assure their diapause condition. The effect of isolation of body parts from JH supply was studied by ligation of the cervical region with a fine silk thread. Non-ligated larvae served as controls. The effect of  $\beta$ -ecdysone on ligated and non-ligated diapause larvae was investigated by injecting a single dose of 1 and 4  $\mu$ g ecdysone in 1  $\mu$ l of distilled water one and seven days after the ligature was made. Control injections were carried out with pure water. The performance of ecdysis was followed during 5 days after each treatment. An ecdysis was only considered as progressive when a visible differentiation of wing buds was detected and when the anal plate, prolegs and crochets had transformed into an (irregular) cremaster as was described for prothetelic *Chilo suppressalis* larvae by FUKAYA and HATTORI (1957). Usually such changes are accompanied by sclerotization and tanning of the cuticle of especially the abdominal parts.

### 6.3.3 Experiments with JH-analogue

The effects of the juvenile hormone analogue (JHA) ZR 619 on growth, development and moulting of non-diapause larvae were studied. Larval material was obtained from the insectary where larvae of *Chilo partellus* were routinely bred on an artificial diet (see 2.3.1). Three groups of 50 late fifth instar larvae were topically treated with 2  $\mu$ g JHA (in 2  $\mu$ l acetone) twice a week, 2  $\mu$ g JHA once a week and 2  $\mu$ l acetone twice a week (control). The applications were continued throughout the observation period of 40 days. Records were taken on larval body weights (as a group), pupation and stationary ecdyses.

In one experiment the effect of JHA was followed on two qualities of aestivating larvae which earlier had been used as criteria to determine aestivation-diapause in the field: loss of cuticular pigmentation and drought resistance. Five groups of about 30 larvae each were treated topically twice a week with 0.5, 1.0, 2.0 and 4.0  $\mu$ g JHA (ZR 619) in one  $\mu$ l acetone during 3½ weeks

of growth on diet. An application of 1  $\mu$ l acetone served as a control. Pupation, death and larval pigmentation was determined 3 days after the last application was carried out. The remaining larvae of each group were then subdivided in two equal groups of which one was transferred to pieces of dry maize stem and another was kept on diet. Survival and pupation was recorded one month later.

## 6.4 RESULTS

### 6.4.1 Juvenile hormone titre during diapause induction, maintenance and termination

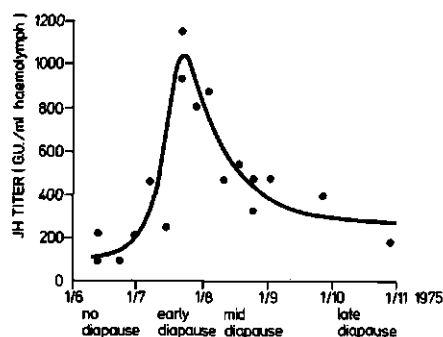


Fig.33. JH titres in the haemolymph of field collected larvae of *Chilo* sp. before and during the incidence of aestivation-diapause.

Fig.33 shows that the JH content in the haemolymph of field collected larvae was relatively low (100-200 G.U./ml) as long as the majority of the larvae was in a non-diapause condition. Larvae were considered as non-diapause because 1. samples contained pupae and very low percentages of drought resistant and unspotted larvae (fig. 7) and 2. the respiratory rate and water content of the larvae were not depressed (fig.16). When entering diapause JH levels increased to about 1000 G.U./ml and this level gradually decreased to about 200 G.U./ml as diapause continued. These findings suggest that relatively high levels of JH are responsible for the induction of diapause in *Chilo* larvae whereas the diapause is maintained by intermediate levels of this hormone. The aestivation-diapause of *Chilo* larvae is terminated by exposure of the larvae to moist conditions. Fig.34 shows that -although 40 % of the larvae died after a period of 20 days- the remaining 60 % had pupated during that period. Half of these larvae had already pupated within 11 days. Table 19 demonstrates that moist conditions induce a rapid decrease in JH content of the haemolymph of diapausing larvae, allowing the larvae to terminate the diapause and pupate.

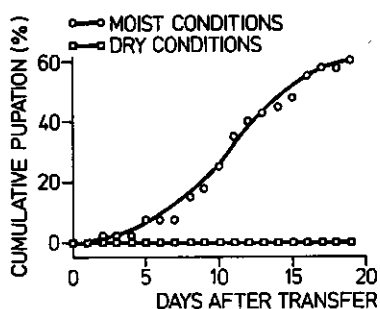


Fig.34. Effect of moisture on the termination of diapause in larvae of *Chilo* sp. Both experiments were started with 40 larvae.

Table 19. Effects of moisture on the JH content in the haemolymph of diapausing larvae of *Chilo* sp.

experiments no.	days after transfer to moist conditions	JH titre (G.U./ml haemolymph)
1	0	750
	3	80
2	0	1280
	3	230
	8	130

#### 6.4.2 Effects of head ligatures and/or $\beta$ -ecdysone on diapausing larvae

Table 20 shows that very few stationary and no progressive ecdyses occurred in non-ligated and ligated larvae, which had been untreated or injected with distilled water. Most larvae remained unaffected. This result suggests an inactivity of the prothoracic glands of the larvae used for this experiment. However when the level of ecdysone was artificially increased by injection, moulting did occur. In non-ligated larvae stationary moults were abundant and progressive moults rare. I concluded that the quantity of JH circulating in the haemolymph of these larvae is sufficiently large to maintain the state of diapause. This was also the case when ecdysone was injected one day after the neck ligature was made. Apparently JH is still effective. In contrast, when larvae had been ligated 7 or more days before the ecdysone injection took place, only progressive ecdyses were observed, indicating that JH titre had dropped to a level which no longer prevents pupation.



Table 20. Effects of neck ligation and/or  $\beta$ -ecdysone on ecdysis of diapausing larvae of *Chilo* sp.

ligature	treatment	no. of larvae	situation 5 days after treatment		
			no. of larvae unaffected	no. of stationary ecdyses	no. of progressive ecdyses
-	untreated	42	37	2	0
-	1 $\mu$ l water	15	11	0	0
-	1 $\mu$ g ecdysone	20	0	13	0
-	4 $\mu$ g ecdysone	20	1	14	1
+	untreated	29	22	4	0
+	1 $\mu$ l water (1) <sup>*</sup>	14	13	0	0
+	1 $\mu$ l water (7)	15	14	0	0
+	1 $\mu$ g ecdysone (1)	17	0	11	3
+	4 $\mu$ g ecdysone (1)	20	8	9	2
+	4 $\mu$ g ecdysone (7)	15	2	0	13
+	4 $\mu$ g ecdysone (9)	12	3	0	5
+	4 $\mu$ g ecdysone (15)	14	5	0	7

\* number of days between ligation and treatment with ecdysone.

#### 6.4.3 Effects of JHA on non-diapause larvae

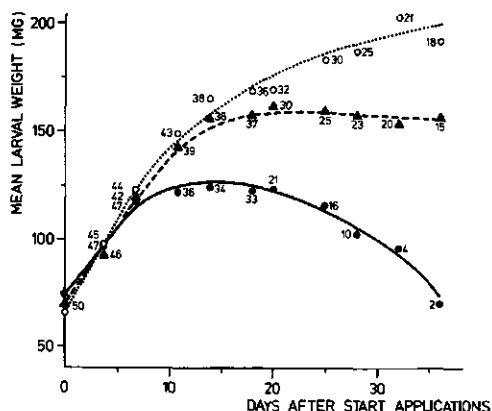


Fig.35. Effect of the JHA ZR 619 on body weight of late 5th instar larvae of *Chilo partellus* during a period of 36 days of treatment.  
Code: ●—● 2  $\mu$ l acetone twice a week, ▲—▲ 2  $\mu$ g JHA once a week, ○—○ 2  $\mu$ g JHA twice a week. Numbers refer to numbers of surviving larvae.

The effect of JHA on live weight of 5th instar larvae is shown in Fig. 35. No significant differences in larval weights were observed during the first week of treatments. Thereafter however larvae treated with JHA continued to gain weight, whereas the weights of acetone treated larvae remained constant and later

started to decline. This decline could be associated with the formation of pupae in this control group (Fig. 36). Twenty days after the start of the JHA applications larvae which were treated once a week ceased gaining weight, possibly because of reduced feeding. The maximal weights obtained were about 1.3 x those of the control larvae. The group of larvae treated twice a week with JHA even became heavier: after 30 days larvae weighed over 200 mg, i.e. about 1.7 x the maximum weight of the controls.

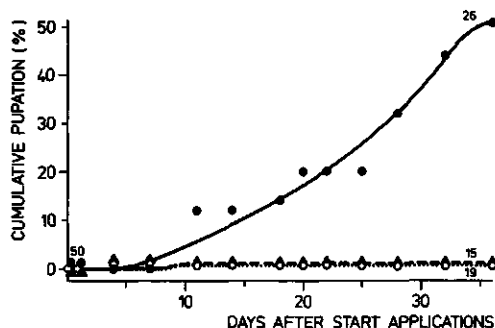


Fig. 36. Effect of JHA ZR 619 on development of late 5th instar larvae of *Chilo partellus* during a period of 36 days of treatment.  
Code: ●—● 2 µl acetone twice a week, ▲—▲ 2 µg JHA once a week, ○—○ 2 µg JHA twice a week. Numbers refer to numbers surviving (larvae + pupae).

Fig.36 shows that only one (abnormal) pupa was formed in either of the hormone treated groups of larvae. In contrast: 26 pupae were formed in the control group while 24 larvae died. In both of the hormone treated groups even more larvae had died after 36 days of treatment: 31 larvae after an application twice a week and 35 larvae after an application once a week.

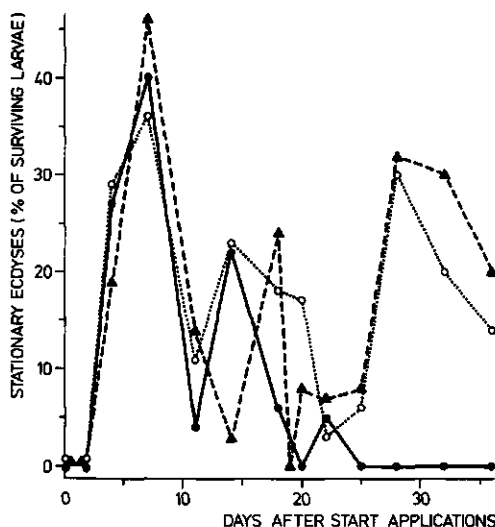


Fig. 37. Effect of the JHA ZR 619 on stationary ecdyses of late 5th instar larvae of *Chilo partellus* during a period of 36 days of treatment.  
Code: ●—● 2 µl acetone twice a week, ▲—▲ 2 µg JHA once a week, ○—○ 2 µg JHA twice a week. The stationary ecdysis was expressed by taking the number of head capsules found since the previous observation as % of the number of surviving larvae.

Fig. 37 shows that stationary moults in all three groups of larvae initially followed the same pattern. A very large number of head capsules per larva was found between days 4 and 7 and little moulting took place thereafter till day 11. Intermediate levels of head capsules were found during the following period till day 21 when only few stationary moults were observed. Differences between the groups developed thereafter: larval ecdysis ceased in the acetone treated larvae whereas it was obvious that the prolonged development of hormone treated larvae is accompanied by extra moults.

No significant differences in the pigmentation of hormone treated and control larvae were observed after 3½ weeks of applications (table 21).

Table 21. Effect of JHA on development and larval pigmentation of early fifth instar larvae of *Chilo partellus*.

no. of larvae used	JHA treatment	no. of larvae after treatment	larval pigmentation <sup>2)</sup> after treatment		
			SP	TR	US
29	1 µl acetone	24	17	3	4
31	0.5 µg JHA	28	5	20	3
29	1.0 µg JHA	27	10	17	0
30	2.0 µg JHA	29	7	22	0
29	4.0 µg JHA	29	16	8	5

1) JHA (ZR 619) was topically applied twice a week during 3½ weeks period

2) SP-spotted TR-transitional US-unspotted

These findings suggest that the cuticular pigmentation is not directly related to juvenile hormone.

Table 22 shows that larvae which had received a JH-treatment did not survive any better on dry maize than solvent treated controls. Death rates were high in all groups, but some larvae which had received acetone or a low dosage of 0.5 µg JHA pupated. In other groups JH titres probably remained sufficiently high to prevent pupation.

When larvae were transferred to diet after the JHA treatment, survival was generally better. Also more larvae pupated: first only acetone and 0.5 µg JHA treated larvae and later - when JHA had lost its effectivity - some larvae treated with higher dosages. It was concluded that drought resistance - a quality belonging to the diapause syndrome of natural aestivating larvae - is not governed by JH.

Table 22. JHA treatment\* on larvae of *Chilo partellus* subsequently kept on different substrates.

no. of larvae used	JHA treatment	substrate post-JHA treatment	effects after 16 days post-JHA treatment			effects after 30 days post-JHA treatment		
			no. larvae	no. pupae	no. death	no. larvae	no. pupae	no. death
12	1 µl acetone	dry maize	5	1	6	0	4	8
14	0.5 µg JHA		4	0	11	1	5	8
15	1.0 µg JHA		4	0	11	1	0	14
15	2.0 µg JHA		2	0	13	1	0	14
15	4.0 µg JHA		4	0	11	0	0	15
12	1 µl acetone	diet	4	5	3	0	8	4
14	0.5 µg JHA		4	4	6	1	4	9
12	1.0 µg JHA		7	0	5	0	2	10
14	2.0 µg JHA		14	0	0	2	3	9
14	4.0 µg JHA		12	0	2	5	3	6

\* Treatments were carried out by topical application of the JHA ZR 619 during a period of 3½ weeks, where upon the larvae were transferred to the two substrates.

## 6.5 DISCUSSION

To elucidate the role of JH in the regulation of aestivation-diapause of *C. partellus* and *C. orichalcociliella* the JH titre in the haemolymph of field collected larvae was determined before, during and at the end of diapause. Non-diapause larvae contained an average JH quantity of 100-200 G.U./ml haemolymph. This content seems to be low as compared to titres found in related stalk borers. For example, non-diapause larvae of *D. grandiosellla* contain 300-3000 G.U./ml (YIN and CHIPPENDALE 1976) and in last instar non-diapause larvae of *C. suppressalis* 2400 G.U./ml were found (YAGI and FUKAYA 1974). In both insects JH titres rapidly dropped to 60 G.U./ml and 30 G.U./ml respectively shortly before the pupal moult. It was known that the early samples of larval material collected from the experimental field contained a fairly large number of pupae. This probably explains the low overall JH titre in the pooled haemolymph of the non-diapause larvae in the early samples. The lack of pupae in the next samples as well as the depression of the larval metabolism and water content (fig. 16) indicated the onset of diapause. During this period of diapause induction a relatively high JH content of about 1000 G.U./ml was

found, which had dropped to about 400 G.U./ml one month later and ultimately reached a level of 200 G.U./ml in the late diapause larvae. These results clearly demonstrate the continuous activity of the CA during the diapause. An elevated level of JH and a subsequent decrease during the course of the diapause were also reported for *C. suppressalis* (YAGI and FUKAYA 1974), *D. grandiosella* (YIN and CHIPPENDALE 1976) and *M. flavescens* (TAKEDA 1978). The finding that the JH titre of aestivating *Chilo* larvae decreased rapidly (i.e. within 3 days) to sub-diapause levels after exposure of the larvae to moist conditions in addition to the fact that pupation is started soon thereafter, support the idea that *C. partellus* and *C. orichalcociliella* belong to the group of insects in which larval diapause is regulated by JH.

Further evidence for this was obtained from the different reactions of non-ligated and ligated diapausing larvae to  $\beta$ -ecdysone. Within the five days of observation very few ecdyses at all took place in both the non-ligated and ligated untreated or solvent treated controls. Because of the short observation period it is not known whether the termination of diapause in the ligated larvae would ultimately have been accelerated as was found for *D. grandiosella* after a 30 days period (YIN and CHIPPENDALE 1973).

Injection of  $\beta$ -ecdysone provoked ecdysis in the majority of the diapausing larvae, whether they were ligated or not:

In non-ligated larvae stationary ecdyses were predominant. This result may reflect the early diapause stage of the larvae, which had been collected shortly after rains in the field had ceased. It has been shown for *D. grandiosella* (YIN and CHIPPENDALE 1973), for *C. suppressalis* (YAGI and FUKAYA 1974) and for *M. flavescens* (TAKEDA 1978) that more larvae tend to pupate after treatment with ecdysone as diapause proceeds. Once more the decreasing JH content during the period of diapause was held responsible for this changing response to ecdysone. In ligated diapausing larvae the type of ecdysis appeared to be dependent on the time elapsed between the ligation and the injection of ecdysone. If this period was only one day, most larvae moulted into a next larval stage. It was concluded that the JH had not yet dissipated. If however this period was 7 days or more, only progressive ecdyses could be provoked. This result suggests that at that time the JH titre in the haemolymph had sufficiently decreased to allow pupation to take place.

Several experiments were conducted to determine the effect of a JH analogue (JHA) on non-diapause larvae of *Chilo partellus*. It was demonstrated that the larval stage of non-diapause larvae may considerably be prolonged during the

period of JHA applications and that extra moults occur. The JHA did not appear to activate the CA of the recipient larvae since pupae were formed when the JHA treatments ceased. Similar results had been obtained for *D. grandiosella* (YIN and CHIPPENDALE 1973, 1974, 1976), *C. suppressalis* (YAGI and FUKAYA 1974) and *O. nubilalis* (YAGI and AKAIKE 1976). The result of the JHA experiments confirm the previous results mentioned in this paragraph.

No further evidence was obtained that JHA affects any other aspect of the diapause syndrome. It was found that larvae treated with JHA attained more than normal weights, but - although heavy diapausing larvae were frequently collected from the field - an extra weight could never be associated with aestivation-diapause since also small diapausing larvae were often observed. JHA has not been found to influence any of the aspects of diapause which have been used in the present research as criterion for diapause namely the cuticular pigmentation and the drought resistance. Some evidence was obtained that also the respiratory rate is not affected. YIN and CHIPPENDALE (1974) clearly demonstrated that larvae of *D. grandiosella* with hormonally (JHA) induced diapause were morphologically (pigmentation) and physiologically comparable to larvae with environmentally induced diapause. This situation differs from larval diapause of *Chilo partellus* and *Chilo orichalcooiliella*. To obtain the complete syndrome belonging to their diapause (chapters 2 and 3) more is required than JH only. On the other hand, several experiments in this paragraph have demonstrated that JH is at least involved in the regulation of the larval diapause of *Chilo* sp. in Kenya.

## Summary

Stalk borers are highly destructive to a large number of important graminaceous crops all over the world. Some examples of economically important stalk borers and a general description of their life-cycle are mentioned in chapter 1. In the same chapter difficulties in controlling the insects are described. The crucial role of aestivation-diapause in the life history of tropical stalk borers is elucidated and the importance of further research on this subject is demonstrated.

Aestivation-diapause in two Pyralid stalk borers, *Chilo partellus* (Swinhoe) and *Chilo orichalcociliella* (Strand) was investigated under field and laboratory conditions.

The relation between diapause and climate during three consecutive years is described in chapter 2. Yearly and seasonal fluctuations in the larval and pupal populations of the two stalk borers in maize appeared to be considerable. As long as the water conditions for plant growth were suitable, insects had a continuous development. Under these conditions larvae had pigmented spots and could not survive dry conditions. Soon after cessation of the rains (or irrigation) rates of pupation decreased. At that time larvae lost their cuticular pigmentation and became resistant to drought. Comparison of the incidence of aestivation in the field with the prevailing climatic conditions showed that only lack of rain could be associated with the arrested larval development. No effects of temperature, relative humidity or photoperiod could be found. These results indicate that the host plant may be involved in the induction of diapause.

Chapter 3 is concerned with characteristics of pre-diapause and diapause larvae. Evidence was obtained that under natural conditions larvae do not feed during diapause as long as they are not disturbed. The physiological condition of field-collected stem borer larvae changed considerably upon entering diapause: a decreased rate of oxygen consumption, rate of heart beat and water content, an increased fat content, and arrested development of the testes were found. These

changes normally occurred before larvae were turning unspotted and/or were becoming resistant to drought.

The condition of the host plant in relation to diapause induction is described in chapter 4. Diapause could be induced inside maize plants of different developmental stages. It was shown that the first (physiological) signs of the diapause syndrome appear in larvae feeding in stems containing 70-80% water and very little (< 1.3% of the fresh wt.) protein. The considerable variation in the sugar content of stems containing pre-diapause larvae suggests that sugar is not important in the induction of diapause.

Marked differences were found in the consumption and utilization of stems of maize plants in different developmental stages. Its possible relevance to diapause is discussed.

In chapter 5 experiments are described on the induction of aestivation-diapause by varying the food condition. Most early 5th instar larvae of *C. partellus* entered diapause after being introduced into aged maize stems, containing 75% water, 8% sugar and 1.3% protein (fresh wt.). Pupation rate, cuticular pigmentation,  $QO_2$  and water content of these larvae were comparable to values obtained from field-collected aestivating larvae. Larvae which had developed beyond the early 5th instar were less sensitive to the diapause inducing factors of the aged maize stems: most of them pupated.

Test of 30 different diets with varying protein, sugar and water contents, indicated that diets containing 0.9-1.1% protein and 70% water were best in inducing diapause. Early 5th instar larvae on diets with the above mentioned protein content grew slowly (but reached normal weights), moulted into the unspotted form and had a retarded rate of pupation. Larvae on diets with lower protein contents hardly developed at all, whereas on diets with higher protein contents larvae pupated normally. Larvae reared on diets in which the water content had been reduced from the normal level of 86% to 70%, resulted in a reduction of the larval water content and respiratory rate, close to values normal for field-collected diapause larvae. Evidence was obtained that larvae reared on diapause inducing diets attained a certain degree of drought resistance. Early 5th instar or younger larvae were the most sensitive stages to diapause induction by diet.

Many larvae on aged maize stems and artificial diets turned unspotted even though pupating soon thereafter. The relevance of the cuticular pigmentation as a criterion for aestivation-diapause is discussed.



The endocrine involvement in the aestivation-diapause is described in chapter 6. From juvenile hormone titre determinations and ligation experiments evidence was obtained that the diapause is regulated by an intermediate level of JH. Application of JH to non-diapause larvae prevented pupation of these larvae but did not evoke other aspects of the diapause syndrome.

## Samenvatting

Stengelboorders zijn zeer schadelijke insekten voor een groot aantal belangrijke grasachtige gewassen in de gehele wereld. Enige voorbeelden van economisch belangrijke stengelboorders en een algemene beschrijving van hun levenscyclus zijn genoemd in hoofdstuk 1. In hetzelfde hoofdstuk is melding gemaakt van moeilijkheden bij de bestrijding van deze insekten. Aestivatie-diapauze is van uitermate groot belang in de levensloop van tropische stengelboorders. Een uitgebreid onderzoek over dit onderwerp werd gewenst geacht.

Aestivatie-diapauze in twee stengelboorders behorende tot de Pyralidae, nl. *Chilo partellus* (Swinhoe) en *Chilo orichalcociliella* (Strand), werd onderzocht in het veld en in het laboratorium.

De relatie tussen diapauze en klimaat gedurende drie opeenvolgende jaren is beschreven in hoofdstuk 2. De jaarlijkse- en seizoens schommelingen in de larvale en pupale populaties van de beide stengelboorders in mais bleken groot. Zolang de plant voldoende water ter beschikking had, konden de insekten zich continue ontwikkelen. Onder die omstandigheden hadden de larven gepigmenteerde vlekken en waren ze niet instaat droogte te doorstaan. Spoedig na het ophouden van de regens (of van de irrigatie) nam het verpoppings-percentages onder de larven af, ging de kutikulaire pigmentatie geleidelijk verloren en werden de larven resistent tegen droogte. Uit een vergelijking tussen het optreden van aestivatie in het veld en de daarbij heersende klimatologische omstandigheden bleek dat slechts gebrek aan regen met de vertraagde larvale ontwikkeling in verband gebracht kon worden. Temperatuur, relatieve vochtigheid en daglengte waren niet van invloed. Deze resultaten wijzen op een mogelijke rol van de waardplant in de inductie van de diapauze.

In hoofdstuk 3 worden enige kenmerken gezien van larven in pre-diapauze en in diapauze. Onder natuurlijke omstandigheden nemen de larven gedurende de diapauze geen voedsel op tenzij ze worden gestoord. De fysiologische gesteldheid van stengelboorders uit het veld veranderde sterk wanneer de diapauze intrad.

Waargenomen werden een verlaging van zuurstofkonsumptie, hartslag en watergehalte, een verhoging van het vetgehalte en een stilstand in de ontwikkeling van de testes. Al deze veranderingen traden gewoonlijk op voordat de larven ongevekt en/of droogte resistent werden.

De relatie tussen diapauze induktie en de toestand van de waardplant is beschreven in hoofdstuk 4. Diapauze kon worden geïnduceerd in maisplanten in verschillende stadia van hun ontwikkeling. De eerste (fysiologische) tekenen van het diapauze syndroom bleken zich voor te doen in larven die zich bevonden in stengels met een watergehalte tussen 70 en 80% en een laag eiwitgehalte ( $< 1.3\%$  van het vers gewicht). Het suikergehalte in de verschillende stengels was zeer uiteenlopend. Suiker is daarom vermoedelijk niet bij de diapauze induktie betrokken.

Grote verschillen werden gevonden in de konsumptie en de benuttingsgraad van stengels van maisplanten in verschillende ontwikkelingsstadia. Het mogelijke verband met diapauze is besproken.

In hoofdstuk 5 zijn experimenten beschreven handelend over de induktie van diapauze door middel van verschillende soorten voedsel. Larven in het begin van het 5e stadium, geïntroduceerd in oude maisstengels met 75% water, 8% suiker en 1.3% eiwit (vers gewicht) gingen grotendeels in diapauze. De mate van verpopping, pigmentatie van de kutikula, zuurstofverbruik en watergehalte van deze larven waren vergelijkbaar met die, gevonden voor aestiverende larven uit het veld. Larven die verder ontwikkeld waren dan het begin van het 5e stadium waren minder gevoelig voor de diapauze inducerende factoren van de oude mais stengels: de meesten verpopten.

Uit experimenten met 30 verschillende dieten waarin eiwit, suiker en water gehalten werden gevarieerd, bleek dat vooral de dieten met 0.9-1.1% eiwit en 70% water diapauze konden induceren. Larven in het begin van het 5e stadium groeiden langzaam op dieten met bovengenoemd eiwitgehalte maar bereikten een normaal eindgewicht. Na vervelling verloren ze hun pigmentatie en de verpopping was sterk geremd. Larven op dieten met een lager eiwitgehalte ontwikkelden zich vrijwel geheel niet, terwijl op dieten met een hoger eiwitgehalte de verpopping normaal was. Larven gevoed met dieet waarin de hoeveelheid water was verminderd van het normale gehalte van 86% tot 70%, bleken een watergehalte en ademhalings-quotiënt te bezitten, dichtbij die van diapauze larven uit het veld. Aanwijzingen werden gevonden dat larven opgekweekt op diapauze inducerende dieten, tevens een zekere mate van droogte resistentie verkregen. Larven jonger dan begin 5e stadium waren het gevoeligst voor diapauze induktie door dieet.

Gedurende de experimenten met oude maisstengels en met kunstmatige dieten werden dikwijls larven aangetroffen die hun pigmentatie verloren hadden terwijl ze kort daarna toch verpopten. De waarde van de kutikulaire pigmentatie als criterium voor aestivatie-diapauze is besproken.

De rol van hormonen bij de aestivatie-diapauze is het onderwerp van hoofdstuk 6. Uit titer bepalingen van juveniel hormoon en uit snoeringsexperimenten kon worden afgeleid dat diapauze wordt gereguleerd door intermediaire hoeveelheden JH. Een JH applicatie bij niet-diapauze larven voorkwam verpoping maar leidde niet tot het ontstaan van andere aspecten van het diapauze syndroom.

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## References

- Aboul-Nasr A.E., Esaac E.G. and El-Gogary S. (1976) Oxygen consumption by larvae and pupae of Spodoptera littoralis (Boisd.) reared on different host plants. Z. angew. Ent. 81, 78-85.
- Adkisson P.L. (1961) Effect of larval diet on the seasonal occurrence of diapause in the pink bollworm. J. econ. Ent. 54, 1107-1112.
- Adkisson P.L., Bell R.A. and Wellso S.G. (1963) Environmental factors controlling the induction of diapause in the pink bollworm, Pectinophora gossypiella (Saunders). J. Insect Physiol. 9, 299-310.
- Alexander B.R. and Chippendale G.M. (1973) Spermatogenesis of the southwestern corn borer, Diatraea grandiosella. 1. Comparison of rates in prediapause and nondiapause larvae. Ann. ent. Soc. Am. 66, 747-752.
- Andrewartha H.G. (1952) Diapause in relation to the ecology of insects. Biol. Rev. 27, 50-107.
- Ankersmit G.W. and Adkisson P.L. (1967) Photoperiodic responses of certain geographical strains of Pectinophora gossypiella (Lepidoptera). J. Insect Physiol. 13, 533-564.
- Atwal A.S. (1967) Diapause among insect pests of crops. Trop. Ecol. 8, 1-16.
- Barton-Browne L.B. (1964) Water regulation in insects. Ann. Rev. Ent. 9, 63-82.
- Barton-Browne L. (1975) Regulatory mechanisms in insect feeding. Adv. Insect Physiol. 11, 1-116.
- Beck S.D. (1956) The European corn borer, Pyrausta nubilalis (Hübner), and its principal host plant. II. The influence of nutritional factors in larval establishment and development on the corn plant. Ann. ent. Soc. Am. 49, 582-588.
- Beck S.D. (1968) Insect Photoperiodism. Academic Press, New York, 288 pp.
- Beck S.D. and Hanec W. (1960) Diapause in the European corn borer, Pyrausta nubilalis (Hübner). J. Insect Physiol. 4, 304-318.
- Beckwith R.C. (1976) Influence of host foliage on the Douglas-fir tussock moth. Environ. Entomol. 5, 73-77.
- Bottger G.T. (1951) Sugars and protein in the corn plant as related to nutrition of the European corn borer. J. econ. Ent. 44, 40-44.
- Bull D.L. and Adkisson P.L. (1962) Fat content of the larval diet as a factor influencing diapause and growth-rate of the pink bollworm. Ann. ent. Soc. Am. 55, 499-502.
- Butani D.K. (1955) The influence of temperature on the development of insects, with special reference to Chilo zonellus. Indian J. Ent. 17, 280-282.
- Chatterji S.M., Siddiqui K.H., Panwar V.P.S., Sharma C.G. and Young, W.R. (1968) Rearing of the maize stem borer Chilo zonellus Swinhoe on artificial diet. Indian J. Ent. 30, 8-12.
- Chippendale G.M. (1977) Hormonal regulation of larval diapause. Ann. Rev. Entomol. 22, 121-138.

- Chippendale G.M. and Reddy A.S. (1972) Diapause of the southwestern corn borer, Diatraea grandiosella: transition from spotted to immaculate mature larva. Ann. ent. Soc. Am. 65, 882-887.
- Chippendale G.M. and Reddy A.S. (1973) Temperature and photoperiodic regulation of diapause of the southwestern corn borer, Diatraea grandiosella. J. Insect Physiol. 19, 1397-1408.
- Chippendale G.M. and Reddy A.S. (1974) Diapause of the southwestern corn borer, Diatraea grandiosella: low temperature mortality and geographical distribution. Environ. Entomol. 3, 233-238.
- Chippendale G.M. and Reddy G.P.V (1974) Dietary carbohydrates: role of feeding behaviour and growth of the southwestern corn borer, Diatraea grandiosella. J. Insect Physiol. 20, 751-759.
- Chippendale G.M. and Yin C.-M. (1976) Endocrine interactions controlling the larval diapause of the southwestern cornborer, Diatraea grandiosella. J. Insect Physiol. 22, 989-995.
- Cloudsley-Thompson J.L. (1975) Adaptations of Arthropoda to arid environments. Ann. Rev. Ent. 20, 261-283.
- Cloutier E.J. and Beck S.D (1963) Spermatogenesis and diapause in the European corn borer, Ostrinia nubilalis. Ann. ent. Soc. Am. 56, 253-255.
- Coaker T.H. (1956) 'An experiment on stem borer control on maize'. E. Afr. agric. J. 21, 220-221.
- Crowder L.A., Watson T.F. and Langston D.T. (1975) Diapause of the pink bollworm as related to crop maturity. J. econ. Ent. 68, 110-112.
- Danilevskii A.S. (1961) Photoperiodism and seasonal development of insects. English translation 1965. Oliver and Boyd, Edinburgh.
- Das Y.T. (1976) Some factors of resistance to Chilo suppressalis in rice varieties (Lep., Pyralidae). Ent. exp. & appl. 20, 131-134.
- Delobel A. (1975a) Chilo orichalcociliellus Strand (Lepidoptera, Pyralidae), foreur des tiges du sorgho et du maïs à Madagascar. II. Premières données biologiques. Cah. ORSTOM, sér. Biol. X (1), 11-16.
- Delobel A. (1975b) Une population hivernante de Chilo partellus (Lepidoptera, Pyralidae) sur la côte ouest de Madagascar. Cah. ORSTOM, sér. Biol. X (1), 17-23.
- Dinther J.B.M. van (1962) Flight periods of the white rice borer Rupela albinella (Cr) in Wageningen, Surinam (Sth.America). Meded. LandbHooges. OpzoekStns Gent, 27, 829-836.
- El-Sayed T.M. and Rustom Z.M.F. (1960) Factors affecting the initiation of diapause in the pink bollworm Pectinophora gossypiella Saunders. Bull. Soc. Ent. Egypte. 44, 253-264.
- Fukaya M. (1951) On the theoretical bases for predicting the occurrence of the rice stem borer in the first generation. Ber. Ohara Inst. 9, 357-376.
- Fukaya M. (1967) Physiology of the rice stem borers, including hibernation and diapause. In: Proceedings of a symposium on the major insect pests of the rice plant, 14-18 Sept. 1964, Los Banos, Laguna, Philippines. Chapter 8, 213-227. John Hopkins Press, Baltimore.
- Fukaya M. and Mitsuhashi J. (1961) Larval diapause in the rice stem borer with special reference to its hormonal mechanism. Bull. Nat. Inst. Agr. Sci. Ser. C 13, 1-32.
- Gahukar R.T. (1976) Nutrition de la pyrale du maïs Ostrinia nubilalis Hübner (Lepidoptera: Pyraustidae). Ann. Zool.-Écol. anim. 8, 119-128.
- Galichet P.E. (1964) Diparopsis watersi Rothschild, Lepidoptera, Noctuidae, Ravageur du cotonnier en Afrique Centrale. Monographie, écologie des populations, étude expérimentale de la diapause. Cot. Fibr. trop. 19, 437-472.
- Gelperin A. (1971) Regulation of feeding. Ann. Rev. Ent. 16, 365-378.

- Gonçalves L. (1970) A broca do milho Chilo partellus Swinhoe (Lepidoptera, Crambidae) em Moçambique. Contribuição para o seu estudo. Agron. moçamb., Lourenço Marques 4 (4), 239-246 (summary in english and french).
- Goot P. van der (1925) Levenswijze en bestrijding van den witten rijstboorder op Java. Meded. Inst. Plziekten (Buitenzorg) 66, 308 pp.
- Hansen L.D. and Harwood R.F. (1968) Comparisons of diapause and non-diapause larvae of the codling moth, Carpocapsa pomonella. Ann. ent. Soc. Am. 61, 1611-1617.
- Harris K.M. (1962) Lepidopterous stem borers of cereals in Nigeria. Bull. ent. Res. 53, 139-171.
- Hassanein M.H. and Galal A. (1969) Studies on the diapause of the pink bollworm Pectinophora gossypiella Saund. Bull. Soc. Ent. Egypte 53, 69-78.
- Hayes D.K., Horton J., Schechter M.S. and Halberg F. (1972) Rhythm of oxygen uptake in diapausing larvae of the codling moth at several temperatures. Ann. ent. Soc. Am. 65, 93-95.
- Heinze P.H. and Murneek A.E. (1940) Comparative accuracy and efficiency in determination of carbohydrates in plant material. Missouri Agr. Exp. Sta. Bull. 314, 1-23.
- Hill D.S. (1975) Agricultural insect pests of the tropics and their control. Cambridge Un. Press, Cambridge.
- Hirano C. (1964) Studies on the nutritional relationships between larvae of Chilo suppressalis Walker and the rice plant, with special reference to the role of nitrogen in nutrition of larvae. Bull. Nat. Inst. Agr. Sci., Ser. C 17, 103-180.
- Hirano C. and Noguchi H. (1963) Food utilization by the last instar larvae of the cabbage armyworm, Mamestra brassicae L., fed on plant leaves of different food values. Jap. J. appl. Ent. Zool. 7, 311-315.
- Hoekstra A. and Beenackers A.M.Th. (1976) Consumption, digestion, and utilization of various grasses by fifth-instar larvae and adults of the migratory locust. Ent. exp. & appl. 19, 130-138.
- Holloway R.L. and Smith J.W. (1976) Lesser cornstalk borer response to photoperiod and temperature. Environ. Entomol. 5, 996-1000.
- Holloway T.C., Haley W.E. and Loftin H.C. (1928) The sugar-cane moth borer in the United States. U.S.D.A. Techn. Bull. 41, 1-76.
- House H.L. (1961) Insect nutrition. Ann. Rev. 6, 13-26.
- House H.L. (1965) Effects of low levels of the nutrient content of a food and of nutrient imbalance on the feeding and the nutrition of a phytophagous larva, Celerio euphorbiae (Linnaeus) (Lepidoptera: Sphingidae). Can. Ent. 97, 62-68.
- Hsiao T.C. (1973) Plant responses to water stress. Ann. Rev. Plant Physiol. 24, 519-570.
- Hummelen P.J. (1974) Relations between two rice borers in Surinam, Rupela albinella (Cr.) and Diatraea saccharalis (F.), and their Hymenopterous larval parasites. Meded. Landb. Hooges. Wageningen 74-1, 89 pp.
- Hynes H.B.N. (1942) Lepidopterous pests of maize in Trinidad. Trop. Agriculture, Trin. 19, 194-202.
- Ingram W.R. (1958) The Lepidopterous stalk borer associated with Gramineae in Uganda. Bull. ent. Res. 49, 367-383.
- Inouye H. and Kamano S. (1957) The effect of photoperiod and temperature on the induction of diapause in the rice stem borer, Chilo suppressalis. Jap. J. appl. Ent. Zool. 1, 100-105.
- Jacquemard P. (1976) La diapause de Diparopsis watersi (Roths.) (Lep., Noctuidae) dans le nord du Cameroun. Cot. Fibr. trop. 31, 297-311.
- Jepson W.F. (1954) A critical review of the world literature on the Lepidopterous stalk borers of tropical graminaceous crops. London, Commonw. Inst. Ent. 127 pp.



- Jones W.J. and Huston H.A. (1914) Composition of maize at various stages of its growth. Bull. Agr. Exp. Sta. Purdue Univ. 175, 599-629.
- Kalode M.B. and Pant N.C. (1966) Studies on the susceptibility of different varieties of sorghum, maize and bajra to Chilo zonellus (Swinhoe) under field and cage conditions and the methods of determining it. Indian J. Ent. 28, 448-464.
- Katiyar K.P. (1960) Diapause in the sugarcane borer Diatraea saccharalis (F). PhD dissertation Louisiana State University Library, Baton Rouge, Louisiana, 148 pp.
- Katiyar K.P. and Long W.H. (1961) Diapause in the sugarcane borer, Diatraea saccharalis. J. econ. Ent. 54, 285-287.
- Kevan D.K.M. (1944) The bionomics of the neotropical corn stalk borer, Diatraea lineolata, Wlk. (Lep., Pyral.) in Trinidad, B.W.I. Bull. Ent. Res. 35, 23-30.
- Khan M.R. and Khan B.M. (1968) Biology and control of maize stem borer (Chilo partellus Swinhoe) in Peshawar. Sci. Ind. (Pak.) 6, 1-2, 124-130.
- Kishino K. (1969) Ecological studies of the local characteristics of seasonal development of the rice stem borer, Chilo suppressalis Walker. I. Effects of photoperiod and temperature on diapause induction. Jap. J. appl. Ent. Zool. 13, 52-60.
- Koidsumi K. and Makino K. (1958) Intake of food during hibernation of the rice stem borer, Chilo suppressalis Walker. Jap. J. appl. Ent. Zool. 2, 135-138.
- Kono Y. (1970) Photoperiodic induction of diapause in Pieris rapae crucivora Boisduval (Lep: Pieridae). Appl. Ent. Zool. 5, 213-224.
- Kramer P.J. (1959) Transpiration and the water economy of plants. In: F.C. Steward (ed.), Plant physiology. Academic Press, New York 2, 607-709.
- La Croix E.A.S. (1967) Maize stalk borers in the Coast Province of Kenya. E. Afr. agric. for. J. 33, 49-54.
- Lees A.D. (1955) The physiology of diapause in Arthropods. Cambridge University Press. 151 pp.
- Lees A.D. (1956) The physiology and biochemistry of diapause. Ann. Rev. Ent. 1, 1-16.
- Lees A.D. (1968) Photoperiodism in insects. In Photophysiology, vol IV (Ed. A.C. Giese), 67-138. Academic Press, New York.
- Levitt J. (1972) Responses of plants to environmental stresses. Chapters 13-16: Water stress, 322-446. Academic Press, New York and London.
- Lewis L.C., Mutchmor J.A. and Lynch R.E. (1971) Effect of Perezia pyraustae on oxygen consumption by the European corn borer Ostrinia nubilalis. J. Insect Physiol. 17, 2457-68.
- Li C.S. (1961) Bionomics of the white rice borer Tryporyza innotata (Walker). IRC, Working Party on Product. Protect., New Delhi, 13 pp.
- Lynch R.E., Brindley T.A. and Lewis L.C. (1972) Influence of photophase and temperature on survival and oxygen consumption of diapausing European corn borers. Ann. ent. Soc. Am. 65, 433-436.
- Mathez F.C. (1972) Chilo partellus Swinh., C. orichalcociliella Strand (Lep., Crambidae) and Sesamia calamistis Hmps. (Lep., Noctuidae) on maize in the Coast Province, Kenya. Mitt. schweiz. ent. Ges. 45, 267-289.
- McCaffery A.R. (1975) Food quality and quantity in relation to egg production in Locusta migratoria migratorioides. J. Insect Physiol. 21, 1551-1558.
- Menakor M. and Gross G. (1965) Effect of fluctuating temperature on diapause induction in the pink bollworm. J. Insect Physiol. 11, 911-914.
- Matcalf C.L. and Flint W.P. (1967) Destructive and useful insects. 4th ed. Tata McGraw-Hill, New Delhi.
- Mochida O. and Yoshimeki M. (1962) Relations with development of the gonads, dimensional changes of the corpora allata, and duration of post-diapause period in hibernating larvae of the rice stem borer. Jap. J. appl. Ent. Zool. 6, 115-123.

- Moiz S.A. and Qureshi M.S. (1969) Life history of maize stem borer (Chilo zonellus Swinhoe) at Tandojam. Agriculture Pakistan XIX (2), 130-149.
- Moreau J.P. and Gahukar R.T. (1975) Importance des méthodes expérimentales dans les études des relations plantes-insectes. Ann. Zoo. Ecol. anim. 7, 119-136.
- Mutchmor J.A. and Beckel W.E. (1959) Some factors affecting diapause in the European corn borer, Ostrinia nubilalis (Hbn.) (Lepidoptera: Pyralidae). Can. J. Zool. 37, 161-168.
- Norris M.J. (1965) The influence of constant and changing photoperiods on imaginal diapause in the red locust (Nomadacris septemfasciata Serv.). J. Insect Physiol. 11, 1105-1119.
- Nye I.W.B. (1960) The insect pests of Gramineous crops in East Africa. Colon. Res. Stud. 31, 48 pp.
- Oliver B.F. and Gifford J.R. (1975) Weight differences among stalk borer larvae collected from rice lines showing resistance in field studies (Lep., Pyralidae). J. econ. Ent. 68, 134.
- Pant N.C. and Kalode M.B. (1964) Pests of wheat, maize and millets. In: Entomology in India, silver jubilee number of the Indian J. Ent., 279-292.
- Pant N.C., Srivastava P.D. and Ghai S. (1959) Physiology of digestion in the larvae of Chilo zonellus (Swinhoe). Indian J. Ent. 21, 238-245.
- Pathak M.D. (1968) Ecology of common insect pests of rice. Ann. Rev. Ent. 13, 257-294.
- Pearson E.O. (1958) The insect pests of cotton in tropical Africa. Commonwealth Inst. of Entomology, 355 pp.
- Phillips P.A. and Barnes M.M. (1975) Host race formation among sympatric apple, walnut and plum populations of the codling moth, Laspeyresia pomonella (Lep., Olethreutidae). Ann. ent. Soc. Am. 68, 1053-1060.
- Phillips J.R. and Newsom L.D. (1966) Diapause in Heliothis zea and Heliothis virescens (Lepidoptera: Noctuidae). Ann. ent. Soc. Am. 59, 154-159.
- Prevett P.F. (1971) Some laboratory observations on the development of two African strains of Plodia interpunctella (Hübner) (Lepidoptera: Phycitidae) with particular reference to the incidence of diapause. J. stored Prod. Res. 7, 253-260.
- Raina A.K. and Bell R.A. (1974) Influence of dryness of the larval diet and parental age of diapause in the pink bollworm, Pectinophora gossypiella (Saunders). Environ. Entomol. 3, 316-318.
- Reddy A.S. and Chippendale G.M. (1973) Water involvement in diapause and the resumption of morphogenesis of the southwestern corn borer, Diatraea grandiosella. Ent. exp. & appl. 16, 445-454.
- Reddy G.P.V. and Chippendale G.M. (1972) Observations on the nutritional requirements of the southwestern corn borer Diatraea grandiosella. Ent. exp. & appl. 15, 51-60.
- Rothschild G.H.L. (1971) The biology and ecology of rice-stem borers in Sarawak (Malaysian Borneo). J. Appl. Ecology 8, 287-322.
- Saringer G. and Deseo K.V. (1968) The effects of various fruit species on the development and diapause of the plum fruit moth (Grapholita funebrana Tr., Lepidoptera, Tortricidae). Acta Phytopathol. Acad. Hung. 3, 365-372.
- Schelte P. (1976) The role of graminaceous host plants in the induction of aestivation-diapause in the larva of Chilo zonellus Swinhoe and Chilo argyrolepis Hmps. Symp. Biol. Hung. 16, 247-253.
- Schmutterer H. (1969) Pests of crops in Northeast and Central Africa. Gustav Fisher Verlag, Stuttgart.
- Scholander P.T., Claff C.L., Andrews J.R. and Wallach D.F. (1952) Microvolumetric respirometry: methods for measuring O<sub>2</sub> consumption and CO<sub>2</sub> production by cells and enzymic reactions. J. gen. Physiol. 35, 375-395.

- Skopik S.D. and Bowen M.F. (1976) Insect photoperiodism: an hourglass measures photoperiodic time in Ostrinia nubilalis (Lep., Pyralidae). J. comp. Physiol. 111, 249-259.
- Smith D. and Grotelueschen R.D. (1966) Carbohydrates in grasses. I. Sugar and fructosan composition of the stem bases of several northern-adapted grasses at seed maturity. Crop science 6, 263-266.
- Smithers C.N. (1959) Some recent observations on Busseola fusca (Fuller) (Lep., Noctuidae) in Southern Rhodesia. Bull. ent. Res. 50, 809-819.
- Squire F.A. (1940) On the nature and origin of the diapause in Platyedra gossypiella, Saund. Bull. ent. Res. 31, 1-6.
- Swaine G. (1957) The maize and sorghum stalk borer, Busseola fusca (Fuller) in peasant agriculture in Tanganyika Territory. Bull. Ent. Res. 48, 711-722.
- Takeda N. (1978) Hormonal control of prepupal diapause in Monema flavescens (Lepidoptera). Gen Comp. Endocrinol. 34, 123-131.
- Usua E.J. (1968) Temperature and relative humidity effects on the development of the immature stages of the maize stemborers Busseola fusca and Sesamia calamistis. J. econ. Ent. 61, 1091-1093.
- Usua E.J. (1970) Diapause in the maize stemborer. J. econ. Ent. 63, 1605-1610.
- Usua E.J. (1973) Induction of diapause in the maize stemborer, Busseola fusca. Ent. ent. & appl. 16, 322-328.
- Usua E.J. (1974) Observations on the physiology of diapause and non-diapause larvae of Busseola fusca (Fuller) (Lep., Noctuidae). Bull. ent. Res. 63, 513-518.
- Waldbauer G.P. (1968) The consumption and utilization of food by insects. Adv. Insect Physiol. 5, 229-288.
- Watson T.F., Lindsey M.L. and Slosser J.E. (1973) Effect of temperature, moisture and photoperiod on termination of diapause in the pink bollworm. Environ. Entomol. 2, 967-970.
- Wilde J. de (1970) Hormones and the environment. Mem. Soc. Endocrinol 18, 487-514.
- Wilde J. de, Staal G.B., de Kort C.A.D., de Loof A. and Baard G. (1968) Juvenile hormone titer in the haemolymph as a function of photoperiodic treatment in the adult colorado beetle (Leptinotarsa decemlineata Say). Proc. K. ned. Akad. Wet. 71C, 321-326.
- Williams G.C. (1964) The life-history of the Indian meal moth Plodia interpunctella (Hübner) in a warehouse in Britain and on different foods. Ann. appl. Biol. 53, 459-475.
- Yagi S. and Akaike N. (1976) Regulation of larval diapause by juvenile hormone in the European corn borer, Ostrinia nubilalis. J. Insect Physiol. 22, 389-392.
- Yagi S. and Fukaya M. (1974) Juvenile hormone as a key factor regulating larval diapause in the rice stem borer, Chilo suppressalis (Lepidoptera: Pyralidae). Appl. Ent. Zool. 9, 247-255.
- Yin C.-M. and Chippendale G.M. (1973) Juvenile hormone regulation of the larval diapause of the southwestern corn borer, Diatraea grandiosella. J. Insect Physiol. 19, 2403-2420.
- Yin C.-M. and Chippendale G.M. (1974) Juvenile hormone and the induction of larval polymorphism and diapause of the southwestern corn borer, Diatraea grandiosella. J. Insect Physiol. 20, 1833-1847.
- Yin C.-M. and Chippendale G.M. (1976) Hormonal control of larval diapause and metamorphism of the southwestern corn borer Diatraea grandiosella. J. exp. Biol. 64, 303-310.

# Curriculum vitae

Paul Scheltes, geboren 5 mei 1943 te Waarle. Middelbare School opleiding: "Het Nieuwe Lyceum" te Hilversum (1955-1962). De daarop volgende studie aan de Landbouwhogeschool te Wageningen (studierichting Planteziektenkunde) werd in 1971 voltooid. Hoofdvak was: entomologie (verzwaard); bijvakken: zoölogie (populatiodynamica) en sociologie van de niet-Westerse gebieden. Het in dit proefschrift vermelde onderzoek werd in de periode 1971-1976 verricht aan het "International Centre of Insect Physiology and Ecology" te Nairobi, Kenya. Werkgevers waren achtereenvolgens de Landbouwhogeschool (1971-1974), het ICIPE (1975) en de DITH (1976). Voor de afronding van dit proefschrift werd in 1977 door de Landbouwhogeschool de gelegenheid gegeven.