EFFECTS OF INGESTING *BACILLUS THURINGIENSIS* (BERLINER) SPORES ON DEVELOPMENTAL STAGES AND FECUNDITY OF SURVIVING *SESAMIA CALAMISTIS* (HAMPSON) (LEPIDOPTERA: NOCTUIDAE)

P.K. Baidoo[1] and M.A. Botchey[2]
[1]Department of Theoretical and Applied Biology,
Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[2]Department of Entomology and Wildlife,
School of Biological Sciences
University of Cape Coast, Cape Coast, Ghana.

ABSTRACT

Bacillus thuringiensis Berliner was isolated from dead Sesamia calamistis Hampson (Lepidoptera: Noctuidae) larvae collected from maize farms in Cape Coast, Ghana. Spores produced from the vegetative cells were incorporated into an artificial diet and fed to 2nd instar S. calamistis larvae. The duration of larval and pupal periods was calculated for both the treated and untreated larvae. Sex ratio was determined. The total numbers of eggs laid by treated and untreated females were recorded. Total generation periods for both the treated and untreated larvae were calculated. 40.8% of treated larvae survived infection and pupated, whilst 75.8% of the untreated larvae pupated. Sex ratio of pupae developing from both the treated and untreated larvae did not differ significantly (P>0.05). Female pupae developing from the untreated larvae were significantly heavier than those from treated larvae (P<0.01). More eggs (466.6 ±11.5) were laid by female moths that developed from the untreated larvae. Weights of female pupae from both the treated and untreated larvae positively correlated with the number of eggs laid. Ingestion of bacterial spores did not affect the viability of the eggs produced, since 73.5% of eggs from the treated adults and 76.9% of eggs from the untreated adults hatched. B. thuringiensis ingestion resulted in a 58.2% reduction in fecundity in adult moths that developed from the treated larvae. Total generation period of the treated larvae was increased by 5.9 days.

Keywords: *Bacillus thuringiensis, Sesamia calamistis*, egg masses, fecundity, adult longevity.

INTRODUCTION

The maize stem borer *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) is one of the major pests of maize in Ghana (Smith, 1965). This moth is responsible for considerable loss in the yield of maize planted during the minor planting seasons, September-December (Bosque-Perez and Schulthess, 1994). Presently, chemical insecticides are the most important control measures used against stem borers (Walters and Drinkwater, 1975), but majority of the small-scale farmers cannot afford purchasing these insecticides (Bosque-Perez and Schulthess, 1994). Furthermore, chemical insecticides often have a negative impact on the environment.

They are often health hazards to humans and are not compatible with other control measures such as the use of natural enemies of stem borers (Bosque- Perez and Schulthess, 1994).

The biological control agent, *Bacillus thuringiensis* Berliner is a promising alternative for the control of many agricultural pests (Ridgway *et al.* 1996). However, available data on its use to control insect pests initially focused mainly on the immediate effects of the pathogen on the treated insects. When the larvae ingest the pathogen, larval mortality leads to a reduction in the number of adults in subsequent generation as well as reduction in fecundity.

The reduction in fecundity means a reduction in the population of the pest in subsequent generations. This pathogenic effect has been recorded in insects such as *Plodia interpunctella* (Hübner) and *Aedes stimulans* Walker (Shaikh and Morrison, 1966). Delay of adult emergence due to larval infection by *B. thuringiensis* was observed in *Anagasta kuehniella* Zeller (Jacobs, 1951). Ignoffo and Gregory (1972) reported that exposure of *Heliothis zea* (Boddie) and *Trichoplusia ni* (Hübner) to Beta-exotoxin caused combined mortality of 58% and 56%, respectively, and in addition, fecundity and longevity were reduced in adults that developed from larvae exposed to Beta- exotoxin.

The study of the retarded effect of pathogens on the subsequent stages of insect pests has received the attention of researchers. Report by Li *et al.* (1995) suggested that *B. thuringiensis var kurstaki* adversely affect *Choristoneura rosaceana* (Harris) larvae surviving after ingesting the pathogen.

The effects of *B. thuringiensis on* the development of surviving larvae of *S. calamistis* have not been documented and therefore these need to be assessed in any pathogen control programme. This study investigated the retarded effects of ingesting *B. thuringiensis* spores on larval development, pupal weights, fecundity and generation period of *S. calamistis.*

MATERIALS AND METHODS

Production of spores

During the maize growing season of April to July and September to December 1998, dead and live larvae of *S. calamistis* were collected from maize fields in and around Cape Coast, Ghana. Bacteria isolated from the dead larvae were identified as *B. thuringiensis*, using features such as growth on agar, reaction with 3% hydrogen peroxide (Catalase reaction) and presence of parasporal inclusions (Lomer and Lomer, 1995). Spores were produced from the vegetative cells, using the ingredients shown in Table 1 as the growth medium. After 72 hours of incubation at 30 ± 0.5°C, the spores were recovered by the lactose co precipitation method described by Dulmage *et al.* (1970). The harvested spores were dried in an oven at 30°C, weighed and suspended in 10-ml sterilized distilled water.

Table 1: Ingredients used in the production of *B. thuringiensis* spores (g/ litre)

Constituent	Quantity
Peptone	10.00
Glucose	10.00
Yeast extract	2.00
$MgSO_4.7H_2O$	0.30
$MnSO_4.7H_2O$	0.20
$FeSO_4.7H_2O$	0.02
$ZnSO_4.7H_2O$	0.02

Artificial diet

An artificial diet was prepared, with maize stem powder, soya flour and water as the main components (Table 2). Ten millilitres of the spore suspension was added to 1000 ml of the diet and thoroughly mixed using the electric blender. The diet was dispensed into sterilized plastic vials (3cm diameter and 8 cm high).

Larval developmental period

Each vial, containing a quantity of the diet and one 2nd instar *S. calamistis* larva was kept at 25 ±

Table 2: Composition of 100ml of artificial diet used for feeding *S. calamistis* larvae

Constituent	Quantity
Soya flour	6.25g
Maize stem powder	3.00g
Glucose	1.00g
Sodium chloride	1.00g
Water	90.00ml
Potassium hydroxide (1M)	0.50ml
Ascorbic acid	1.00g
Agar	2.00g
10% formaldehyde	0.10ml
Acetic acid	0.20ml

0.5°C. A control diet of the same composition was prepared, but without the spores. One hundred and twenty larvae were used for the treated diet and the same number of larvae for the untreated diet and there were three replicates. The larvae used for the study were obtained from individuals reared in the insectary. After 7 days, the diet was changed and the numbers of dead larvae counted. This was repeated weekly until pupation, but the diet fed to the treated larvae after the first 7days did not contain the bacterial spores. The duration of larval development on both the treated and untreated diets was recorded and the means calculated.

Sex ratio and fecundity

Each pupa was weighed; its sex determined and thereafter kept in a Kilner jar until the adult emerged. The duration of life of each pupa was noted and the mean calculated for pupae from both the treated and untreated larvae. The percent adults emerging from the pupae in both cases were recorded. The fecundity of the moths was studied on 20 randomly selected female pupae from both the treated and untreated diets. After emerging from the pupa, each imago was confined in a wooden cage (50cm x 50cm x 50cm) with a newly emerged male and provided with oviposition site, consisting of a strip of white paper wound around a piece of wood. Cotton wool, soaked in 5% glucose solution, was provided as food source for the adults. Egg masses laid daily were counted, placed on moistened filter paper in petri- plates and incubated at 25°C. The time taken for the eggs to hatch was noted for adults originating from larvae that initially fed on the treated diet and adult developing from larvae fed with the untreated diet.

Adult longevity and total duration of development

Adult longevity and total duration of development were calculated. Percent reduction in fecundity of moths originating from the treated larvae was calculated using the following formula by Hornby and Gardner, (1987).

$$R = \frac{Fu - Ft}{Fu} \times 100\,\%$$

R = percent reduction in fecundity
Fu = mean fecundity of moths developing from larvae fed on untreated diet
Ft = mean fecundity of moths developing from larvae fed on treated diet.

Analysis of Data

Data were analyzed using the SPSS statistical package. A t-test was done to determine the level of significance of the parameters studied. A test for significance of the sex ratio was done, using the Chi square test.

RESULTS

Larval mortality

Seventy-one (59.2%) of the treated larvae died before pupation, leaving 49 (40.8%) to pupate. On the other hand, only 29 (24.2%) of the larvae fed on the diet without the pathogen died before pupation; the remaining 91 (75.8%) pupated. Eleven of the treated larvae passed through supernumerary moult and never pupated and these were also considered dead.

Sex Ratio

Sex ratio for the 49 pupae from the treated larvae was 26 females: 23 males. No significant

difference was observed between the sexes (χ^2 = 0.18, df=1 P> 0.05). Similarly there was no significant difference between the sexes of the 91pupae that developed from larvae fed on the untreated diet (χ^2 = 0.27; df = 1; P> 0.05) the sex ratio being 48 females: 43 males.

Weight of Pupae

Pupal weight of females developing from larvae that initially fed on the treated diet ranged from 85 mg to 193 mg,($\bar{x}$ =163 ± 16 mg) while that of the males was between 73mg and 165mg ($\bar{x}$=140 ± 16 mg). The females were significantly heavier than the males (t = 3.58; df = 19; P < 0.01). Female pupae developing from the larvae fed on the untreated diet weighed between 150 mg and 434 mg, (x= 247 ± 26 mg) while that of the males ranged from 85 mg to 195 mg (x =185 ± 13 mg). The females were significantly heavier than the males (t = 11.34; df = 19; P< 0.01).

Mean weight of female pupae that developed from larvae fed with the untreated diet was significantly heavier than that of female pupae from larvae that were initially fed with the treated diet (t = 3.18; df = 19; P < 0.01). Similarly, male pupae that developed from the untreated larvae weighed significantly heavier than those that developed from larvae initially fed with the treated diet (t = 2.98; df = 19; P < 0.01).

Duration of Pupal Period

Pupae developing from the treated larvae lived from 7-9 days with a mean of 7.4 ± 0.6 days, while those from larvae fed with the untreated diet lived from 6–8 days with a mean of 7.1± 0.4 days (Table 3). There difference was not significant (t = 0.05; df = 19; P> 0.05).

Egg Laying

Egg laying by the female adults from both the treated and the untreated started 48 hours after emerging from the pupa. Adults from the treated larvae laid an average of 25.6 eggs on day 1 whilst those from the untreated larvae laid an average of 48.2 eggs on day 1. There were sig-

Table 3: Duration of developmental stages of *Sesamia calamistis*

Developmental stage	Diet with *Bt*	Diet without *Bt*
Eggs		
Days ($\bar{x}$±s.e)	7.2 ± 0.2	6.9 ± 0.3
Range (days)	6-10	6 -8
Larvae		
Days($\bar{x}$±s.e)	32 ± 0.8	26 ± 0.5
Range (days)	27 -36	23 -32
Pupae		
Days ($\bar{x}$±s.e)	7.4 ± 0.2	7.1 ± 0.1
Range (days)	7 -9	6- 8
Adults		
Days ($\bar{x}$± s.e)	8.7 ± 0.8	9.5 ± 0.8
Range (days)	9 -11	9 –11
Total Duration of Development		
Days ($\bar{x}$± se)	55.3 ± 2.1	49.4 ± 1.9
Range (days)	49-60	45 -53

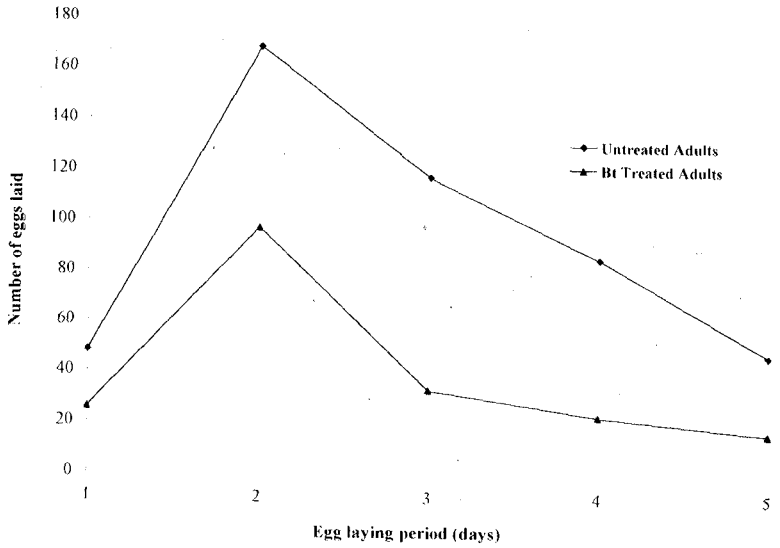

Fig. 1: Daily means number of eggs laid

nificant increases in the numbers of eggs laid on the second day, when the largest number of eggs was laid (Fig. 1). The number of eggs laid decreased from the 3rd to the 5th day (Fig.1). Egg laying stopped after the 5th day. Female adults developing from larvae fed with the untreated diet produced a mean of 466.6 ± 11.5 eggs. This was significantly more than the average of 195.2 ± 8.4 eggs laid by females that developed from larvae fed with the treated diet ($t = 20.52$, $df = 19$; $P < 0.01$). There was a positive correlation between the pupal weight and the number of eggs laid by a female ($r = 0.77$). Fecundity in moths originating from larvae fed on treated diet reduced by 58.2%, compared with fecundity in moths from larvae that were fed with the untreated diet.

Percent egg hatchability

A mean of 73.5 ± 2.3% and 76.9 ± 2.8% of eggs laid by adults from the treated and untreated hatched into larvae, respectively. There was no significant difference between the percent eggs that hatched ($t = 1.08$; $df = 19$, $P > 0.05$).

Adult longevity and total developmental period

Mean adult longevity of the treated moths was 8.7 days, as against 9.5 days for moths from the untreated larvae. The difference was not significant ($t = 1.13$; $df = 19$; $P > 0.05$). Total duration of development for the treated *S. calamistis* larvae ranged from 49 – 60 days ($\bar{x} = 55.3$ days). Total duration of development for larvae fed with the untreated diet was 45 – 53 days ($\bar{x} = 49.4$ days) (Table 3). The difference was significant ($t = 2.75$; $df = 19$, $P < 0.05$).

DISCUSSION

The recorded sex ratio suggests that one sex was not affected more than the other. The observation is similar to that by Afify and Matter (1969) who studied the retarding effect of *B. thuringiensis* on *Anagasta kuehniella* (Zeller) and observed no significant difference between the sexes when larvae were reared on artificial diet containing the toxin. Apart from causing mortality, *B. thuringiensis* had a negative effect on the survivors. This was reflected in the significant reduction in

the weight of pupae that developed from the treated larvae in both sexes. Pilcher *et al.* (1997) observed that *Papaipema nebris* (Geunee) exposed to *B. thuringiensis* treated leaf tissue were lighter than those exposed to non-*B. thuringiensis* treated leaf tissue. Female pupae were generally heavier than males probably because of the production of more body tissues during the active larval feeding stage, in preparation for egg production. The reduction in pupal weight could be attributed to the effect of *B. thuringiensis* because significant weight reduction was recorded for both the treated males and females. The reduction in pupal weights of females surviving *B. thuringiensis* infection is very important because a reduction in pupal weight means a reduction in egg production in the next generation. This is because it was established in the current study that a positive correlation exists between pupal weight and the number of eggs laid by the adult emerging from it. The reduction in weight of survivors of pathogen infection was probably due to reduced larval feeding rate. Even though surviving larvae were fed with diet without the toxin, it appears the toxin had prolonged effect on the insect beyond the initial period of exposure.

Mean larval developmental period was prolonged by 6.0 days in the treated larvae. This significant increase in larval developmental period will however be a serious drawback to stem borer control, because damage caused to maize plants occurs during the active larval feeding stage. Surviving larvae would, therefore have a longer feeding period and cause more damage to the plant. However, if *B. thuringiensis* treatment is able to reduce the pest population below the economic damaging level, then the overall damage done by surviving larvae would be reduced. If the significant reduction in pupal weight were attributed to reduction in larval feeding, then prolonging the larval period would not result in increased damage to the plant. Fecundity of moths developing from the larvae that were fed with the treated diet was reduced by 58.2% compared with the adults developing from larvae that were fed with the untreated diet. Similarly, Hornby and Gardner, (1987) reported 98.8% fecundity reduction in 3rd instar *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) reared on diet containing Beta-exotoxin of *B. thuringiensis*. Ignoffo and Gregory, (1972) also recorded reduced fecundity in *Heliothis zea* and *Trichoplusia ni* moths developing from larvae fed on toxin-treated diet. Larval ingestion of *B. thurungiensis* spores could therefore have a significant effect on the population of moths in subsequent generations since fewer and less fecund females would result in the production of relatively fewer eggs. The population of stem borers at any particular time depends on the population of the previous generation. In this study the maximum number of eggs laid by an adult *S. calamistis* was 550, but it has been reported by other workers that varying number of eggs are laid by one female (Kaufmann, 1983; Bosque-Perez and Dabrowski, 1989; Shannower *et al;* 1993). The time required for the moths to complete development from the egg till the death of the adult was longer by almost 6 days for the larvae fed on diet containing *B. thuringiensis* spores. This prolongation in generation period was the result of prolonged larval period. This would reduce the number of generations of stem borers within one growing season.

CONCLUSION

The results obtained from this study shows that *B. thuringiensis* has a great potential for the control of *S. calamistis*. Microbial control of stem borers is preferable to chemical insecticides because of its specificity and non-toxicity to other animals, including man (Dent, 1991). However, the use of this bacterial pathogen alone may not reduce the population of this borer below the economically damaging level. It may be used as a component of an integrated pest management programme. The fact that surviving female larvae developed into adults with reduced fecundity is an indication that the next generation of *S. calamistis* would have fewer numbers. The reduction

in the weights of pupae and the subsequent reduction in fecundity could reduce the overall population of *S. calamistis*, even if some larvae survive *B. thuringiensis* treatment.

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