Biology of the Gold-Fringed Stem Borer *Chilo auricilius* and Evaluation of Its Response to Selected Biocides

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Abstract

Developmental period, sex ratio, and pupal weights of F_1 progenies of C. auricilius *were investigated. No significant variations in larval, pupal, and hatching to emergence times were observed between the two sexes of the insect. Goodness-of-fit test showed that the population conforms with the 1:1 sex ratio. Analysis of variance for pupal weights showed female pupae are significantly heavier than the males.*

The effect of crystal protein Cry1A c on mortality and behavior of C. auricilius *was assessed using no-choice and choice tests. Mortality was directly correlated with higher concentrations but not on the choice tests.*

DIPEL, Margosan-O, and azadirachtin affected the growth and development of the larvae. It was observed that larvae avoided adulterated diets, a behavior that should be considered in the proper applications of biocides.

Key Words: *C. auricilius, B. thuringiensis*, DIPEL, Margosan-O, azadirachtin

Introduction

Rice production requires protection from insect pests like stem borers. These insects are very destructive during their larval stage. Upon hatching, they bore and feed inside the stem causing "deadheart" (the killing of the central shoot) or "whiteheads" (empty panicles). They are widely distributed and cause big losses in rice production (Calora and Reyes 1971, Pathak 1975). One of these insects is the rice gold-fringed stem borer *Chilo auricilius*.

Chilo auricilius belongs to the Order Lepidoptera, Family Pyralidae. The larvae and moths of this insect were found to be morphologically similar to the dark headed stem borer *Chilo polychrysus*. There is actually little published information on *C. auricilius* which may be due to erroneous records of *C. polychrysus* in the Philippines (Barrion et al 1990).

The control of *C. auricilius* includes cultural, physical, chemical, natural, or a combination of two or more of these means. Among these, chemical controls is the most economical and convenient (Carson 1962) but increasing costs and detrimental effects on the environment had lead to the use of biocides such as formulations from the bacterium *Bacillus*

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thuringiensis and the neem plant *Azadirachta indica*. The effective ingredients from these sources were found to be environment-safe and have a narrow host range.

Neem is one of the earliest sources of natural pesticides. It belongs to the family Meliaceae and is indigenous to India and Pakistan. It is also grown in some countries in Asia, Africa, Australia, Central and South America. For centuries, extracts from its fruits, leaves and seeds are used by farmers in India to protect their crop from insect pests. Neem derivatives such as its oil, cake powder, and others are known for their insect repellent, antifeedant, growth and reproduction inhibition properties due to the presence of compounds such as azadirachtin and many others (Lavie et al, 1967).

Bacillus thuringiensis or *Bt*, on the other hand, is a rod-shaped, gram positive, spore forming bacteria which produces several endotoxins found to have a paralytic effect when ingested by lepidopterous insects and yet harmless to beneficial insects (Smith and Couche, 1991). The different strains of *Bt* produce toxins which resemble each other in appearance and chemistry, but vary widely in their mode of action.

The use of the above-mentioned biocides against stem borer had been exploited as a good alternative to synthetic pesticides which usually cause resistance evolution, increase environmental contamination and deterioration, and are hazards to man, livestock and wild-life (Barton et al, 1987).

A study was conducted to evaluate the potential of these biocides giving emphasis on the nature of the insects' response. Since the nature of insects can affect the extent of effects of the formulations, we investigated first the nature of the insect by looking at variation in its life history prior to toxicology and behavioral studies.

Materials and Methods

Insects. Moths were collected from Batangas ricefields, and allowed to oviposit in rice leaves. Emerging larvae were infested in cups containing the artificial diet, at the rate of 3 insects/cup. During pupation, the pupae were collected, caged and allowed to emerge in a mylar cage containing a 45-day old rice plant. Emerging moths were provided with honey solution to provide nutrition inside the cage. For 6 days, moths started to lay egg masses. During blackhead stage, the egg masses were collected. These were surface-sterilized with 5% sodium hypochlorite for 5 min and rinsed 3x with distilled water for 1 min. The sterilized egg masses were placed in surfaced-sterilized petri dishes lined with moist filter paper and allowed to hatch. Thirty minutes after hatching, the newly emerged larvae were infested in the prepared diets in cups. These were stored in surfaced-sterilized racks and monitored daily until emergence. The culture was maintained at 27.5 to 28°C and photoperiod was set at 12 h dark period. The life history data was taken by monitoring the insects daily.

Effect of Bt on neonate larvae. The delta-endotoxin Cry1Ac of *B. thuringiensis* was used to determine the susceptibility of the insect larvae. The concentrations used were 0.2, 9.0, 19.0, 57.0, 114.0, 228.0, and 458.0 ug/ul. These were then added to the original diet used in culturing the insects.

For feeding behavior studies, a cup containing the unadulterated diet on one side and the adulterated one on the opposite side were prepared. Neonates position and condition were noted as follows: (1) on control diet, (2) on adulterated diet, (3) neither diet, and (4) dead. The survival and position were monitored daily for 7 days after infestation. For the no-choice test, five neonates per cup with a total of 55 larvae/concentration were used.

Effect of Bt and neem formulations on third instar larvae. Utilizing the diet incorporation bioassay, the *Bt* endotoxin Cry1Ac and the commercial formulation DIPEL, the neem-based biocides azadirachtin and Margosan-O were incorporated in the diet to assess their effects on bigger larvae of *C. auricilius*. The following concentrations were used for Margosan-O and azadirachtin: 1.0, 0.5, 0.25, and 0.10 ml/200 ml diet. For Cry1A(c), 200.0, 100.0, 50.0, and 25.0 ug/ml were prepared. DIPEL concentrations were 0.2, 0.004, 0.0008, and 0.00016 mg/ml. Control set-up made use of unadulterated diet. Test larvae were starved for 2 h and weighed before use. After 4 days of exposure to the diet, they were taken out and weighed again.

Statistical analysis of the data. Variation in sex ratios made use of G-test (Sokal and Rohlf, 1971). For other life history parameters, analysis of variance (ANOVA) was used to determine significant differences between sexes. The Tukey's Honest Studentized Design (HSD) at 0.5 level of significance was performed to compare the effects of different concentrations of *Bt* on larval mortality. This technique was also used to determine the effects of the different biocides on larval weight of the insects. Probit analysis was performed to determine effective lethal doses.

Results and Discussion

Biology of C. auricilius. Life history characters such as larval, pupal, hatching-to-emergence times, pupal weights, and sex ratio were utilized as indices in the study of the biology of *C. auricilius*. Stage differentiation could be a determinant of susceptibility to control measures and fecundity (Chi, 1988). The life cycle of *C. auricilius* consisted of egg, larva, pupa, and adult.

The moths or adults of *C. auricilius* were about 12 mm long (from the tip of the head to the tip of the abdomen). These moths have a wing expanse of about 26 mm of which the forewing has large terminal dots and the hindwing is colored light brown. It has golden fringe. Typically the female moth is larger than the male moth. As soon as the wings are fully expanded, after emergence from their pupal stage, the females begin to oviposit 2-3 days later. The female moths lay eggs in masses near the base of leaves or leaf sheaths.

The eggs of the insect are flattened, subcircular, and overlapping each other in mass. At first the eggs are whitish but soon become black. The pupa is about 7 mm long, 3.5 mm wide, brownish and characteristically without cocoon.

Variation was observed in larval development of the insect. Barrion et al. (1990) observed the larval development time of *C. auricilius* ranging from 22-24 days. In this study, the development time of the insect was shortest at 21 days and longest at 57 days. Rothchild (1971) reported the number of instars of *Chilo* species increases when reared on the insectary. This borer passes through several additional molts before pupating. This was confirmed by Pathak (1968) who noted an increase in the number of moults when under stress or in artificial conditions.

The development time for the males is 42.85$\pm$7.93 while for the females, the mean is 42.79 $\pm$ 7.81. In the field, approximate estimates of life cycle duration can be obtained from the time elapsing between peak numbers of a particular immature stage (Rothchild, 1971). The analysis of variance conducted on the mean development time of both sexes revealed insignificant differences (Table 1). This indicates that sex is not a factor for the differences observed in this stage of development.

The sex ratio conformed with the expected 1:1 ratio (Table 2). A similar result was observed by Magbanua (1993) in *C. suppressalis*. The results of Lim (1992), Diaz (1993), Ranit (1993), Olivares (1993) and Robles (1993) showed deviation from the 1:1 ratio. Fisher (1958) noted that under natural selection, the two sexes should be produced in approximately equal numbers. Their results indicate that deviation from the 1:1 sex ratio is due to the altered gene pool caused by some factors which disturbed its equilibrium. The variations observed between populations were attributed to the genetic structure of the populations investigated.

The insect had a minimum pupal development time of 2 days and maximum at 12 days. The mean pupal development time for males is 9.42 ±3.06 while for females it is 9.15 ± 2.78. Barrion et al (1990) reported the pupal development time of this insect is at 8-9 days with 1 day prepupal stage. In this study, pupal development of males was observed to be not significantly different from females (Table 1). The sex ratio of the pupae however, did not conform to the 1:1 ratio. The population deviated in favor of the females. This result differs from the larval sex ratio. This was attributed to the fact that mortality occurred in the developing larvae to pupation. It was noted that the frequency of deaths was approximately 29.6% in males and 7% in females. - an indication that selection against males is occurring in this species.

The pupal weight of insects can help predict fecundity and adult longevity in insects. In population studies, estimate of expected female emergence and egg density are calculated from the size and length of healthy pupae in the field (Morris 1963, Rothchild 1969). The mean pupal weight for males is 0.0452 ± 0.0082 and for females it is 0.0792 ±0.016 g (Table 3). These weights between sexes were observed to be significantly different at 0.05% level of significance. This indicates that the mean body weight of females is significantly greater than that of males.

Table 1. Mean and standard error for each sex of *C. auricilius* at different stages of development.

STAGE OF DEVELOPMENT	SEX	MEAN AND STANDARD ERROR
Larval	Male	42.85 ± 7.93 a
	Female	42.79 ± 7.81 a
Pupal	Male	9.42 ± 3.06 a
	Female	9.15 ± 2.78 a
Total	Male	54.00 ± 7.79 a
	Female	52.28 ± 7.19 a

In every stage of development, means with the same letter are not significantly different by ANOVA at 0.05.

Table 2. Goodness-of-fit test for the sex ratio of *C. auricilius* at different stages of development.

STAGE OF DEVELOPMENT	MALE	FEMALE	N	DF	G VALUE
Larval	27	43	70	1	3.657 ns
Pupal	19	40	59	1	7.475 *
Total	19	40	59	1	7.475 *

* - significant at 0.05 ANOVA
ns - not significant

Table 3. Mean pupal weight for each sex of *C. auricilius.*

SEX	MEAN AND STANDARD	ERROR
Male	0.0452 ± 0.0082	b
Female	0.0792 ± 0.0106	a

In a column, means with the same letter are not significantly different by ANOVA at 5%.

Looking at hatching to emergence times, the sex ratio of adults expectedly deviated from the 1:1 sex ratio in favor of the females (Table 2). Mortality observed from the development of larvae to adult is 29.6% in males and 7% in females. A similar result was noted in the development of larvae to pupae to adults. These results indicate that the population suffers from mortality in the attainment of maturity at the larval to pupal developmental stage.

Effects of Bt endotoxin Cry1Ac on mortality and behavior of neonate larvae. The choice and no-choice tests done on *C. auricilius* population provided information and data on mortality and behavior of the neonates on the Bt crystal endotoxin Cry1Ac. These tests were conducted to detect the mortality and behavior of larvae at conditions wherein a transgenic plant is already expressing the Cry1Ac endotoxin gene product in all its tissues or in its damage-sensitive tissues only.

For the no-choice tests, mortality was lowest in the control and increasing as the concentration increases. Comparison of means (Table 4) shows the effect of the different amounts of Cry1Ac on mortality. Probit analysis shown in Table 5 shows the effective doses and fiducidal limits of Cry1Ac on *C. auricilius.* A dose which falls within the range of 109.1436 to as high as 1036.469 ug can kill 90% of the borers in the population.

Table 4. Comparison of means of larval (neonates) mortality of *C. auricilius* fed with different concentrations of Bt Cry 1acendo toxin under No-Choice Test.

TREATMENT (*ug*/200 ml)	MEAN MORTALITY (7 days observation)
Control	3.381 C
0.2	12.387 C
9.0	33.714 B
19.0	44.670 AB
57.0	41.714 AB
114.0	40.241 AB
228.0	53.246 AB
458.0	64.933 A

In a column, means with the same letter are not significantly different by HSD at 0.05.

Table 5. Lethal dose value of the Bt Cry 1acendo toxin under a No-Choice.

EFFECTIVE LETHAL	DOSAGE (*ug*/ml)	FIDUCIDAL LIMITS
LD10	0.00495	0.00005 - 0.04963
LD50	1.13261	0.16431 - 3.33673
LD90	258.91690	109.14360 - 1036.46900

Larval mortality showed general increasing trend as the concentration of the toxin increases. The observed positions and feeding punctures in the diet marked the extent of recognition of the larvae of the toxin. Larval death is attributed to the lethal effect of the ingested toxin that undergoes active binding in the gut receptors. Also it might be that the toxin present in the food was immediately detected by the larvae through receptors that avoidance leading to starvation was observed. Furthermore, the high proportion of larvae feeding in the diet followed by a sudden decrease led to a conclusion that recognition of the toxin resulted to negative drive and cessation of feeding. Robles (1993) reported that the first few hours of treatment will result to attraction in the adulterated diet that feeding occurs. Furthermore, cessation of feeding on the diet occurs only at a later period when larvae recognize the toxin and undergo active binding in the gut receptors. The increasing mortality with time of exposure to the toxin indicates the longer time for the larvae to die due to minimal amount of toxin taken.

Lower mortality was observed from the choice test compared to the results of the no-choice test. The presence of unadulterated diet on one side of the cup has provided a refuge for the larvae to escape the lethal effect of the toxin. In this test, feeding occurred at both control and the treated diet. Mortality was low in the lowest concentration and increases as the concentration of the toxin increases (Table 6).

Table 6. Comparison of means of larval (neonates) mortality of *C. auricilius* fed with different concentrations of Bt Cry 1ac toxin under a Choice Test.

TREATMENT (ug/200 ml)	MEAN MORTALITY (7 days observation)	
9.0	16.914	C
19.0	22.214	BC
57.0	25.386	ABC
114.0	34.129	AB
228.0	37.557	A
458.0	35.971	AB

In a column, means with the same letters are not significantly different by HSD at 0.05.

The increasing mortality with time of exposure to the toxin is an indication that initial exposure of the larvae to the diet led them to feed on the toxin-treated formulation; and paralysis of the mouthparts avoidance was observed. Since the amount taken was minimal, it took longer for the larvae to die. Also, it might be that the toxin in the food was immediately detected by the larvae through receptors that led to avoidance.

The amount of the toxin should be minimal or at low level to avoid resistance at a higher level. Giving a choice of diet would slow adaptation to less than one fifth of the rate found in situation when the toxin is present in all of the food available (Gould and Anderson 1991).

Effects of DIPEL, Cry1A c on the growth and development of third instar larvae of C. auricilius. Utilizing diet-incorporation bioassay, the susceptibility of the insect to the commercial formulation DIPEL and the endotoxin Cry1Ac was investigated. The effect on growth and development of the larvae was manifested in the change on the weight of the insect.

In DIPEL-treated insects, the higher concentrations have affected the growth of the insects as shown by the decrease of weights of the test larvae (Table 7). The lower concentrations had slight increase in weight which is attributed to the lesser amount of the toxin in affecting the growth of the insect.

Table 7. Comparison of means of weight changes of third instar larvae of *C. auricilius* fed with different concentrations of DIPEL.

TREATMENT (*ug*/200 ml)	MEAN WEIGHT (g)
Control	(+) 0.01660 a
0.02	(-) 0.01020 c
0.004	(-) 0.00910 c
0.0008	(+) 0.00500 b
0.00016	(+) 0.01278 a

In a column, means with a common letter are not significantly different by HSD at 0.05.

(-) Loss of weight
(+) Gain of weight

All the larvae tested with Cry1A(c) did not grow. In fact, all of them showed reduction of weights. Larval infested on the unadulterated diet increased in weights (Table 8).

Table 8. Comparison of means of weight changes of third instar larvae of *C. auricilius* fed with different concentrations of Cry1AC

TREATMENT (*ug*/200 ml)	MEAN WEIGHT (g)
Control	(+) 0.01720 a
25	(-) 0.01410 b
50	(-) 0.01380 b
100	(-) 0.01150 b
200	(-) 0.01320 b

In a column, means with a common letter are not significantly different at 5% level of HSD.

(-) Loss of weight
(+) Gain of weight

The effects of the toxins could be attributed to the ingestion and binding of the toxin to receptors in the midgut of the larvae. Lysis of the cell membrane leads to the spread of the toxic substance to the different parts of the body. The insects have longer time to die if they

have ingested lesser amount of the toxin. The effects of the toxin could be manifested in the reduction of weight or in the slight increase of weight as they thrive in the toxic diet. The cessation of feeding is attributed to the paralysis of the gut and recognition of the endotoxin, thus development is disrupted. Avoidance which occurs after feeding on the diet could also cause growth retardation even if the unadulterated diet is present since the larvae refuses to feed due to learning behavior.

Rombach et al. (1989) tested some *Bt* formulations on *C. suppressalis* and found out that these formulations caused low mortality even after 180 hours of feeding and at higher doses. However, the surviving larvae which pupated had smaller pupae and the adults were deformed. Moreover, the use of *Bt* crystal protein IIA, an endotoxin which has no "knockdown effect" on the larvae of *C. suppressalis,* had affected the development of the insect (Ranit, 1993).

Effects of Neem-based biocides on the growth and development of C. auricilius. Neem-based compounds had been known to have antifeedant and repellant effects which act in disrupting and inhibiting insect feeding. It has been known that these actions are caused by azadirachtin which is the most potent of the many active components of neem (Levie et al, 1967). Neem derivatives like Margosan-O were observed to possess the same properties that several studies have been made to evaluate these compounds.

The effects of azadirachtin and Margosan-O on the weight of third instar larvae were investigated using diet-incorporation bioassay. Using azadirachtin, all treatments had been found to cause disruption of grown by the reduction of larval weight (Table 9).

Table 9. Comparison of means of weight changes of third instar larvae *C. auricilius* fed with different concentrations of azadirachtin.

TREATMENT (ug/200 ml)	MEAN WEIGHT (g)
Control	(+) 0.01850 a
0.1	(+) 0.01060 b
0.25	(+) 0.00711 b
0.5	(-) 0.00360 c
1.0	(-) 0.01320 d

In a column, means with a common letter are not significantly different at 5% level of HSD.

(-) Loss of weight
(+) Gain of weight

For Margosan-O, not one of the concentrations caused reduction of larval weight. The different treatments, however, had caused slight increase in weight indicating that growth is affected (Table 10).

Table 10. Comparison of means of weight changes of third instar larvae of *C. auricilius* fed with different concentrations of Margosan-O.

TREATMENT (*ug*/200 ml)	MEAN WEIGHT (g)
Control	(+) 0.02220 a
0.1	(+) 0.01660 a
0.25	(+) 0.00930 b
0.5	(+) 0.00950 b
1.0	(+) 0.00640 b

In a column, means with a common letter are not significantly different at 5% level of HSD.

(-) Loss of weight
(+) Gain of weight

Studies conducted on the biochemical effects of these compounds on other insects showed that these neem-based biocides affect the activities of esterases, amylase and other soluble proteins (Dalugdug, 1990, Habacon 1990).

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