Jurnal Perlindungan Tanaman Indonesia, Vol.14, No.1, 2008: 35-39

# SUSCEPTIBILITY OF THE ASIAN CORN BORER, *Ostrinia furnacalis*, TO *Bacillus thuringiensis* TOXIN CRY1AC

## *KEPEKAAN PENGGEREK BATANG JAGUNG ASIA,* Ostrinia furnacalis, *TERHADAP TOKSIN* Bacillus thuringiensis *CRY1AC*

**Aye Kyawt Kyawt Ei**
*Myanmar Agriculture Service, FAO Office Compound, Gyagone, Yangon, Myanmar*
**Y. Andi Trisyono* and Witjaksono**
*Department of Crop Protection, Faculty of Agriculture, University of Gadjah Mada, Yogyakarta*
**To whom correspondence should be addressed. E-mail: andi_trisyono@yahoo.com*

## ABSTRACT

The larval susceptibility of the Asian corn borer, *Ostrinia furnacalis* (Guenee) (Lepidoptera: Crambidae), to a *Bacillus thuringiensis* protein (Cry1Ac) was evaluated using insect feeding bioassays. The founding population of *O. furnacalis* was originally collected from the experimental station of UGM at Kalitirto and had been reared in the laboratory for three generations using an artificial diet "InsectaLf". The tested instars were exposed on diets treated with a series of concentrations of Cry1Ac for one week. The $LC_{50}$ values on the seventh day after treatment for 1st, 2nd, 3rd and 4th instars were 7.79, 21.12, 113.66, and 123.17 ppm, respectively, showing that the higher the instars the lesser the susceptibility to Cry1Ac. When the neonates were exposed to sublethal concentrations of Cry1Ac (0.0583, 0.116, and 0.5830 ppm), growth and development of the surviving larvae were inhibited. The fecundity and viability of females produced from treated larvae decreased with increasing the concentrations. These findings indicate that Cry1Ac is toxic to larva of *O. furnacalis* and has chronic effects to larvae surviving from Cry1Ac ingestion.

Key words: Asian corn borer, *Bacillus thuringiensis*, Cry1Ac, *Ostrinia furnacalis*, toxicity

## *INTISARI*

*Kepekaan larva penggerek batang jagung Asia,* Ostrinia furnacalis *(Guenee) (Lepidoptera: Crambidae), terhadap protein* Bacillus thuringiensis *Cry1Ac diuji dengan metode celup pakan. Larva berasal dari pertanaman jagung di KP-4, UGM di Kalitirto dan telah dikembangbiakkan di laboratorium menggunakan pakan buatan (InsectaLF) selama tiga generasi sebelum digunakan untuk pengujian. Larva* O. furnacalis *yang diuji dipaparkan pada pakan buatan yang telah dicelupkan pada seri konsentrasi Cry1Ac. Nilai $LC_{50}$ pada hari ketujuh setelah perlakukan untuk instar 1, 2, 3, dan 4 berturut-turut adalah 0,79; 21,12; 113,66; dan 123,17 ppm. Hal ini menunjukkan bahwa instar yang semakin tinggi tingkat kepekaannya terhadap Cry1Ac semakin menurun. Larva yang baru menetas dan diberi pakan yang telah dicelupkan pada konsentrasi sublethal Cry1Ac sebagian akan mati, sedangkan larva yang hidup pertumbuhan dan perkembangannya terhambat. Fekunditas dan vialibitas serangga betina hasil dari larva yang diperlakukan dengan Cry1Ac menurun. Penelitian ini menunjukkan bahwa Cry1Ac toksik terhadap larva* O. furnacalis *dan juga mempunyai efek kronik terhadap larva yang tetap hidup setelah memakan toksin.*

*Kata kunci:* Bacillus thuringiensis*, Cry1Ac,* Ostrinia furnacalis*, penggerek batang jagung, toksisitas*

## INTRODUCTION

The Asian corn borer, *Ostrinia furnacalis* (Guenee) (Lepidoptera: Crambidae), is one of the most destructive insect pests on corn in Asia. *O. furnacalis* reduces corn yields by 5% annually with an estimated farm value of $ 1.1 billion (INHS, 1997). The yield loss can shift to 80-100% under heavy infestation while the average loss is recently reported ranging from 10 to 30% (Klaus-Quemada, 2005). *O. furnacalis* may attack any part of the plant above ground from the early whorl stage until corn is harvested. *O. furnacalis* larvae attack corn two times during the growing season. First-brood larvae feed on plant leaves before flowering, and eventually bore into the stalk. Second-brood larvae begin feeding in the leaf sheath and collar area of corn plants after flowering. The holes and tunnels weaken the stalks and provide entry for pathogens that cause stalk rot, premature drying, broken plants, ear drop, and subsequent yield loss.

*O. furnacalis* larvae first feed on leaf tissue, but in later stages of development, they bore into the stalk, which reduces the ability of the plant to move assimilates into the grain (Mesbah *et al*., 2002). Controlling this pest through insecticide sprays is difficult in their given nature because this insect lives most of its larval stage inside the corn stem (CIMMYT, 2000). A larva will transform into a pupa and moth emerges from the stalk.

Recently, biotechnology has been seen as the advanced technologies that may contribute to maintain the high yields demanded of our food crops. Avoiding insect damage and risk associated with high use of chemical pesticide were advantages offered by the use of microbial insecticides from the soil bacterium, *B. thuringiensis* subsp. *kurstaki* (Baum *et al*., 1999). Engineering a corn plant to produce its own specific *B. thuringiensis* protein

allows for a simple system to control specific target pests such as the *O. furnacalis*. Most of *B. thuringiensis* strain produced Cry1Ab in modern gene transfer techniques in corn plant (Entwistle *et al*., 1993; He et al., 2003), and a few produce Cry1Ac protein targeted against to corn borers.

It is known that the proposed model for *B. thuringiensis* intoxication involves a three-step process: activation, binding, and pore formation (Gill *et al*., 1992). When a susceptible caterpillar, such as the corn borer, eats the food containing the crystal protein, part of it binds to receptors, penetrates and collapses the cells lining its gut, causing the larva dies from starvation (Whiteley & Hofte, 1989). The longer the Cry protein is presented to the susceptible larvae, the greater the changes for insect control. The first instar of *O. furnacalis* was more susceptible to Cry1Ab toxin than the older ones (Li-Ping Wen *et al*., 2005). If the insect is not susceptible to the direct action of the delta-endotoxin, death occurs after *B. thuringiensis* starts vegetative growth inside the insect's gut (Carrie, 1994). However, in some cases, they do not die, but their behavior may be altered causing a decrease in the reproductive abilities or generation growth rates affected (Losey *et al*., 2002). This study was conducted to examine the acute and chronic effects of Cry1Ac on the larvae of *O. furnacalis*.

## MATERIALS AND METHODS

### A. Insect

A colony of *O. furnacalis* used in this research was originally collected from research station of UGM, KP-4, at Kalitirto in July 2006. These insects were maintained on a Bio-artificial diet, "InsectaLF®", which is produced from a private company of "Nihon Nosan-Kogyo Co., Ltd," in Japan. The diets were placed in clear plastic cups using established laboratory procedures. Larvae were reared in plastic cups (5 larvae/cup) containing a cube of artificial diet. Pupae, adults and eggs were held at room temperature (25±3°C).

### B. Toxin

*B. thuringiensis* toxin Cry1Ac encapsulated in killed *Pseudomonas fluorescens* (21% AI [MVP II] San Diego, California, USA) was used. The protein was dissolved in 10 ml distilled water to make the stock solution. This solution was diluted several times to get the expected concentrations.

### C. Bioassays

#### 1. Acute toxicity of Cry1Ac

Bioassays were carried out by dipping the artificial diet in insecticide solutions. Six concentrations of Cry 1Ac (0.78125, 3.125, 12.5, 50, 200, and 800 ppm) were selected to determine the working concentrations for estimating the $LC_{50}$ values. The artificial diets were dipped in the Cry1Ac solution for 10 seconds, and air-dried for 15-20 minutes. Each concentration used 10 larvae. Larvae mortality was recorded daily from 2 to 7 days after treatment.

Based on the preliminary bioassays, six to eight consecutive concentrations of Cry1Ac ranging from 0-1500 ppm were applied against to the first to fourth instars of *O. furnacalis*. Newly hatched or moulted larvae (<1 day) were transferred individually to 12 ml plastic cups containing a cube of control or treated diet (1 $cm^3$/cube) incubated at room temperature. Ten larvae were used for each concentration and each was replicated three times. Different instars had utilized different concentrations in the bioassays to give mortality ranging from 2-98%. Larvae mortality was recorded every 24 hours for a week. Death of larvae was considered and confirmed when probed using a paint-hairbrush. Data were submitted to probit analysis using SPSS and Working Probit 5. Probit analysis was conducted only for the data of seven days after treatment.

#### 2. Chronic Effects of Cry1Ac

Three sub-lethal concentrations of Cry1Ac (0.0583, 0.116 and 0.5830 ppm) were prepared with distilled water. In addition to the control artificial diet, a natural food of corn leaves was used as a positive control to fed *O. furnacalis* larvae. Bioassays were conducted using similar procedures as described earlier. Control diet was treated with distilled water only. Ten newly hatched larvae were used for each replicate and each treatment was replicated at least 5 times. Larvae were fed with the treated or control diets. The fresh diets were filled on day 8 for all surviving larvae because the former diet was finished in one of the surviving larvae. Substitution of the diet was continued until all surviving larvae become pupae. The developments of larval, pupal and adult stages were recorded.

#### a. Larvae

Larval mortality was recorded at 7 and 14 days after treatment (DAT). Total larval mortality was computed by dividing the number of dead larvae by number of larvae used in each treatment. Surviving larvae were weighted individually after 7 and 14 days in the treated or the control diets.

#### b. Pupae

Male and female pupae were collected daily, sexed and weighed separately at two days old. Number of deformed pupae was recorded.

#### c. Adults

The effect of *B. thuringiensis* sublethal concentrations was observed until adult formation. Newly emerged males and females were paired and placed into a ventilated

220 ml of plastic cup containing a waxed paper and a nylon cloth netting covers for laying eggs. The number of pairs from the treatments of corn leaf, artificial diet, sub-lethal concentrations 0.0583, 0.116 and 0.5830 ppm of Cry1Ac were 6, 11, 6, 5, and 2, respectively. Egg masses were collected from the both nylon netting and wax paper. The number of eggs per egg mass was counted by hand-counter. The percent of hatching was calculated by dividing the number of newly hatched larvae by the number of eggs per egg masses.

**d. Data Analysis**

Larval mortality data were transformed using arcsine $\sqrt{\text{percentage}}$ transformation to stabilize variance before ANOVA using a completely randomized design (CRD). Fisher's protected LSD with $\alpha$=0.05 was applied to compare means.

## RESULTS

**A. Acute Toxicity**

The $LC_{50}$ values on the seventh day after treatment for first, second, third and fourth instars were 7.79; 21.12; 113.66; and 123.17 ppm, respectively (Table 1). The $LC_{50}$ value of 1st instar was significantly different from the third and fourth instars, where as the values for the second, third and fourth instars were not different. These results indicated that the first instar was the most susceptible to Cry1Ac and increasing the instars decreased the susceptibility to Cry1Ac.

**B. Chronic Effects**

**1. Larvae**

Application of sub-lethal concentrations (0.0583, 0.116, and 0.5830 ppm) of Cry1Ac to *O. furnacalis* larvae caused 24, 36 and 88% in larval mortality. With concentration-mortality dependent function, mortality occurred continuously in all treated larvae until they became pupae (Table 2). On the other hand, no larval mortality was observed on control treatments (corn leaf and artificial diet).

Application of sub-lethal concentration of Cry1Ac resulted in significantly lower larval weight gain at seven days after treatment compared to those of control larvae. After all surviving larvae were fed with fresh artificial diet, the treated larvae gained weight at higher rate than the control larvae resulting no differences in weights of the control and treated larvae at 14 days after treatment (Table 2).

**2. Pupae**

The application of Cry1Ac with the increasing sub-lethal concentrations caused a significant decrease in the number of pupae obtained. The successful pupation for the natural corn leaf diet control (76.7%) and artificial diet control (92%) were high. Female pupae were bigger than those of the male pupae either those produced from the treated or control larvae (Table 2).

**3. Adults**

The larval exposure to sub-lethal concentration of Cry1Ac caused a significant decrease in adult emergence. The treated larvae exhibited higher pupal mortalities, deformed adults and generally less number in normal adults than untreated larvae. The fecundity of females produced from treated larvae was decreased with increasing the concentrations. For the concentrations of 0.0583, 0.116, and 0.5830 ppm, their fecundity values greatly decreased to 20.3, 15.3 and 1.5, respectively. On the other hand, fecundity of the control females was 28.3 and 35.1 eggs per female. In addition, eggs produced from the treated females did not hatch, where as the egg hatching from these controls were 49.4 and 31.1% (corn leaf and artificial diet).

## DISCUSSION

The first instar of *O. furnacalis* was more susceptible to Cry1Ac with its $LC_{50}$ value of 7.79 ppm. This species was less susceptible to Cry1Ac than was *Helicoverpa armigera*. The $LC_{50}$ value of Cry1Ac to *H. armigera* was 4.6 ppm (Trisyono *et al*., unpublished). These differences may be due to differences in the physiological conditions of their alimentary canal. In addition, this also may be due to the lower (acidic) pH value of the mid-gut which reflects improper binding of the toxin or inactivation at all. Although the Cry1Ac exhibited toxin activity to all instars of *O. furnacalis*, the later instars of larvae resulted in a higher survival of larvae and possibly to a resistance (Storer *et al*., 2003; Tabashnik *et al*., 2003). This implies that larvae moving from the non-transgenic crops to transgenic crops at later instars could survive from the *B. thuringiensis* toxin. If this hypothesis is true, late damage could occur and refuge may not effective to prevent the development of resistance.

*O. furnacalis* was susceptible to Cry1Ac protein where the bioassays were based on larval mortality and larval growth inhibition (larval weight and adult fertility). These effects may be significant in decreasing the population of *O. furnacalis* for the following generations. Even though some treated larvae were alive at the end of their life-cycle after treatment, they were significantly smaller than control larvae. Continuous ingestion of the toxin decreased the number of larvae successful to become adults. This is due to the latent toxicity of *B. thuringiensis* toxin (Gharib & Wyman, 1991).

The weight of *O. furnacalis* larvae measured at day 7 of feeding showed a decrease that was significantly correlated with the amount of toxin presented to the larvae.

Table 1. Susceptability of Ostrinia furnacalis larvae to Bacillus thuringiensis Cry1Ac

| Instar | No. larva | Control mortality (%) | Slope (SE) | $LC_{50}$ (95% CL) ppm | | $\chi^2$ | $\chi^2$ (0.05) | Susceptibility ratio * |
|---|---|---|---|---|---|---|---|---|
| 1 | 210 | 0 | 1.14 (0.15) | 7.79 | (4.69 - 12.46) a | 6.71 | 9.49 | 1 |
| 2 | 270 | 0 | 0.37 (0.09) | 21.12 | (7.66 - 102.46) ab | 2.8 | 12.6 | 2.7 |
| 3 | 270 | 0 | 1.94 (0.28) | 113.7 | (68.76 - 235.08) b | 10.72 | 12.6 | 14.59 |
| 4 | 240 | 0 | 0.86 (0.12) | 123.2 | (38.54 - 343.71) b | 10.09 | 11.1 | 15.81 |

LC50 values were determined based on the larval mortality on 7 days exposure.
LC50 values in the column for each observation followed by the same letters are not significantly different because upper and lower limits of 95% CL are overlapped.
* Susceptibility ratio: LC50 of the instars being compared divided by the LC50 value of the first instar.

Table 2. Chronic effects of *Bacillus thuringiensis* Cry1Ac on the growth and development of *Ostrinia furnacalis* larvae

| Life Stage | Control | | Cry1Ac (ppm) | | |
|---|---|---|---|---|---|
| | Corn leaf | Artificial diet | 0.0583 | 0.116 | 0.583 |
| **No. neonate** | 30 | 50 | 50 | 50 | 50 |
| **Larvae** | | | | | |
| **Wt on 7 d (mg)** | 12.22c | 12.33c | 4.18b | 3.86b | 1.07a |
| **Wt on 14 d (mg)** | 79.98a | 71.15 a | 79.92 a | 70.50 a | 65.85 a |
| **Mortality (%)** | 23.3 b | 8.0 a | 34.0 b | 46.0 b | 88.0 c |
| **Pupae** | | | | | |
| **Wt of male (mg)** | 59.38 a | 46.89 b | 47.79 b | 50.21 b | 65.00 a |
| **Wt of female (mg)** | 87.98 a | 67.77 b | 73.98 b | 76.25 b | 73.25 b |
| **Deformed** | 0 | 0 | 10 | 10 | 0 |
| **Adults** | | | | | |
| **No. male** | 12 | 27 | 17 | 14 | 2 |
| **No. female** | 11 | 19 | 16 | 13 | 4 |
| **Fecundity (eggs/female)** | 28.3 | 35.1 | 20.3 | 15.3 | 1.5 |
| **Viability (%)** | 49.4 | 31.1 | 0 | 0 | 0 |

Wt = weight; d = day
Means followed by the same letter from each parameter are not significantly different.

However, when the fresh artificial diet was filled after 7 days exposure, some of the surviving larvae were recovered. The larvae of *O. furnacalis* survived from the treatment of sub-lethal concentrations Cry1Ac were able to pupate and become adults. Male and female pupal weight decreased with increasing treatment concentrations. Sex ratio of *O. furnacalis* did not differ significantly between the control and their applications. These results suggested that *B. thuringiensis* did not contribute in the adaptation of insect sex ratio.

The fecundity, egg number per egg mass, and egg infertility of *O. furnacalis* were also significantly different between applications and control. The egg masses from treated larvae did not hatch because there was a significant increase in pupal mortality, infertile in mating, and a decrease in the adult fertility. Generally, the insect can carry their diets over from larva feeding, such adults do not have to digest them. Chapman (1982) described that the proteins are required in larva body for egg production by female. Similarly, Pedersen (1997) who explained that less amount of proteins in larvae body reduced growth and fecundity of female.

## CONCLUSION

The acute and chronic effects of *B. thuringiensis* Cry1 Ac toxin could provide control for the corn borer pest. *B. thuringiensis* toxin was more lethal to newly hatched larvae of *O. furnacalis*, showing that the higher the instars the lesser the susceptibility to Cry1Ac. Sublethal effects of toxicant may be expressed as a decrease in the insect's

fitness. Moreover, the acute and chronic effects would cause a significant reducing in the population of next generation.

**ACKNOWLEDGEMENT**

We are especially grateful to Prof. Hironori Yasuda (Yamagata University, Faculty of Agriculture, Japan) for providing the artificial diet "InsectaLF", and PT. Monagro Kimia for providing the toxin. We thank Sriyanto, for assistance in sequencing work.

**REFERENCES CITED**

Baum, J.A., T.B. Johnson, & B.C. Carlton. 1999. *Bacillus thuringiensis*: Natural and Recombinant Bioinsecticide Products, p. 189-210. *In* F. R. Hall & J.J. Menn (eds.), *Biopesticides: Use and Delivery.* Humana Press, Totowa, NJ,

British Columbia Ministry of Health. 1992. *Bacillus thuringiensis*. Unpublished report. (December 3).

CIMMYT. 2000. World Maize Facts and Trends. Meeting Corn Need Technology. http://www.cgiar.org/cimmyt.

Chapman, R.F. 1982. *The Insects Structure and Function* 3rd Edition, Harvard University Press. Cambridge. 93 p.

Entwistle, P.F., J. S. Cory, M. J. Bailey, & S. Higgs. (eds.) 1993. Bacillus thuringiensis*, An Environmental Biopesticide: Theory and Practice*. John Wiley & Sons, New York. 311 p.

Gharib, A. H. & J. A Wyman. 1991. Food Consumption and Survival of *Trichoplusia ni* (Lepidoptera: Noctuidae) Larvae Following Intoxication by *B. thuringiensis* var. *kurstaki* and *thuringiensis*. *Jornal of Economic Entomology* 84: 436-439.

Gill, S.S., E.A. Cowles, & P.V. Pietrantonio. 1992. The Mode of Action of *Bacillus thuringiensis* Endotoxins. *Annual Review of Entomology* 37: 615-636.

He, K., Z. Wang, D. Zhou, L. Wen, Y. Song, & Z. Yao. 2003. Evaluation of Transgenic Bt Corn for Resistance to the Asian Corn Borer (Lepidoptera: Pyralidae). *Journal of Economic Entomology* 96: 935-940.

Hofte, H. & H.R. Whiteley. 1989. Insecticidal Crystal Proteins of *Bacillus thuringiensis*. *Microbiology Review* 53: 242-255.

INHS Reports January-February. 1997. European Corn Borer Management: Past and Present.

Klaus Ammann (Quemada). 2005. Introductory Overview of the Main Areas of Agricultural R&D Activities and the Broader Context of Those Activities. http:// www.pubresreg.org/index.

Li-Ping Wen, Kang-Lai He, Zhen-Ying Wang, Da-Rong Zhou, & Shu-Xiong Bai. 2005. Susceptibility of *O. furnacalis* to *Bacillus thuringiensis* and Bt Corn under Long-Term Laboratory Selection. *Agricultural Science in China* 4: 125-133.

Losey, J.E., J.J. Obrycki, & R.A. Hufbauer. 2002. Impacts of Genetically Engineered Crops on Non-target Herbivores: Bt-corn and Monarch Butterflies as a Case Study, p. 144-165. *In* D.K. Letourneau & B.E. Burrows (eds.), *Genetically Engineered Organisms: Assessing Environmental & Human Health Effects.* CRC Press, New York.

McGaughey, H. William& Whalon, Mark E. 1992. Managing Insect Resistance to *Bacillus thuringiensis* Toxins. *Science* 258: 1451-1455.

Mesbah HA, Mourad AK, el-Nimr HM, Massoud MA, & Abd el-Aziz AA. 2002. The Role of Some Agricultural Practices and Fertilizer Type on Both the Incidence of Stem Borers Infestation and Corn Yield in Egypt. *Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet* 67: 575-589.

Pedersen, A., J. Dedes, D. Gauthier & K. van Frankenhuyzen. 1997. Sublethal Effects of *Bacillus thuringiensis* on the Spruce Budworm, *Choristoneura fumiferana*. *Entomologia Experimentalis et Applicata* 83: 253-262.

Storer, N. P., S. L. Peck, F. Gould, J. W. van Duyn, & G. G. Kennedy. 2003. Spatial Process in the Evolution of Resistance in *Helicoverpa zea* (Lepidoptera: Noctuidae) to Bt Transgenic Corn and Cotton in a Mixed Agroecosystem: a Biology-rich Stochastic Simulation Model. *Journal of Economic Entomology* 96: 156-172.

Swadener, C. 1994. *Bacillus thuringiensis* (B.t.), Northwest Coalition for Alternatives to Pesticides, Eugene. *Journal of Pesticide Reform* 14: 13-20.

Tabashnik, B. E., Y. Carrière, T. J. Dennehy, S. Morin, M. S. Sisterson, R. T. Roush, A. M. Shelton, & J. Z. Zhao. 2003. Insect Resistance to Transgenic Bt Crops: Lessons from the Laboratory and Field. *Journal of Economic Entomology* 96: 1031-1038.

Whiteley, H. R. & W. R. Widner. 1990. Location of the Dipteran Specificity Region in a Lepidopteran-dipteran Crystal Protein from *Bacillus thuringiensis*. *Journal of Bacteriology* 172: 2826-2832.