Variability in Rice Stem Borer Populations in Their Response to *Bacillus thuringiensis* Strains and Endotoxins and Its Implications in the Development, and Possible Release of *Bt*-Engineered Rice

Cesar G. Demayo[1] and Amelita T. Angeles[2]

Abstract

This study was conducted to determine the existence of variability in rice stem borer populations in their response to an array of Bacillus Thuringiensis *(Bt) strains and endotoxins. The results showed variations in response within, between and among populations of the rice pests. Likewise, selection experiments revealed a wide range of response of insects from generation to generation indicating a great range of genetic diversity within population of the rice pests. Implication of the study in the development and possible release of the Bt-engineered rice are discussed.*

Key Words: *Bacillus thuringiensis*, engineered-rice, genetic variablity, endotoxins

Introduction

The striped stem borer *Chilo suppressalis* (Wlk) and yellow stem borer *Scirpophaga incertulas* (Wlk) are among the many insects that cause significant yield reduction throughout the rice growing world.

Controlling stem borers with biological, cultural, and chemical techniques is difficult. The use of insecticides, for example, was once perceived to have provided the only immediate, practical, and effective method of control. However, its rising cost, adverse ecological effects by indiscriminate use, and the evolution of resistance have led to worldwide concern regarding its importance to pest control (National Research Council 1986, Knight and Norton 1989. Utilization of resistant rices on the other hand cannot be relied upon. At IRRI, breeding for resistance to stem borers which started in 1973 has yet to come up with a variety which will show strong resistance to the pests (Khan et al 1991, Bernardo 1969). Continuing efforts to produce resistant rices through classical breeding and genetic engineering of pest resistant genes are still being explored at IRRI and other rice research institutes.

This work was supported by a financial grant provided by The Rockefeller Foundation (F910002; 8) and The International Rice Research Institute (IRRI)

[1] Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City

[2] Crop Protection Division, Philippine Rice Research Institute (PhilRice), Muñoz, Nueva Ecija

One of the most innovative approaches for developing resistant rice involves engineering rice plants to express genes from the bacterium *Bacillus thuringiensis (Bt)*, that code for insecticidal proteins. This strategy, while novel, has received some concerns particularly on the possibility that rice resistance to stem borers based on *Bt* toxins could break down quickly if varieties expressing the toxins are planted as monocultures over large geographical area (Bottrell et al 1992). Many workers have argued a number of general strategies that could slow down the rate at which pests adapt to classically bred or engineered plants (Gould 1988a, 1991, Raffa 1989, McGaughey and Whalon 1992). These strategies make an excellent choice of an optimal strategy for a specific crop/pest situation but is highly dependent on the genetic structure of the populations (Gould 1986a,b). Genetic population structure refers to the extent and range of population subdivision and/or intermixing. For example, large movements of susceptible individuals of stem borers into treated regions of resistant individuals will influence the development of resistance. Even the most limited gene flow may spread genes conferring resistance from one population to another. If movement among populations is small, then populations are likely to differ in many traits that directly determine pest severity. Some of these traits that may show differences among populations are the ability to survive, grow, and reproduce on different rice varieties and become resistant to insecticides including *Bt* toxins.

While there is no direct information on the ability of stem borers to adapt to the Bt toxins, there is evidence that other species of lepidoptera can adapt. Work on the Indian meal moth (*Plodia*) demonstrated limited field resistance to a strain of *Bt* that was being used to control this grain pest. When the partially resistant strains of this pest were further selected with the *Bt* in the laboratory (*McGaughey 1985* and *McGaughey and Johnson* 1988), they developed over 200 fold resistance to the bacteria (i.e., it took 200 times the concentration of the bacteria to kill the adapted meal moths as it took to kill the unadapted moths). Laboratory selection on the tobacco budworm, a major pest of cotton, soybean, tobacco and vegetables in North and South America, has lead to 70 fold resistance to a *Bt* toxin (*Stone et al, 1989*). This pest has also been successfully selected for adaptation to tobacco engineered to produce a *Bt* toxin (*Gould et al 1991*). The Colorado potato beetle, *Leptinotarsa decemliniata* has also developed resistance (30 fold) in response to laboratory selection (*Miller et al 1991*).

More significant than these findings has been the discovery that populations of the diamondback moth treated extensively with formulations of the bacteria itself have developed 30 fold resistance in less than five growing seasons (*Tabashnik et al 1990*). In a recent study, one of the resistant field populations were subjected to intense selection in the laboratory (*Tabashnik et al 1991*) and subsequently showed increased resistance in the field from 30 fold to 800 fold in less than 20 generations (i.e. approx. 10 months).

If rice stem borers possess genetic flexibility for adapting to *Bt* toxin similar to that of the species described above, then widespread use of rice that expresses a *Bt* toxin could lead to its rapid adaptation. It is therefore important to assess just how genetically flexible the stem borers are before molecular geneticists and plant breeders move much further toward releasing *Bt*-engineered rice. If it turns out that stem borers are unlikely to adapt to the *Bt* toxins then there will be reason to proceed as planned with guarded optimism and careful surveillance for possible problems. If, on the other hand, it turns out that stem borers appear to have the flexibility to adapt to the toxins, then there is a need to implement alternative strategies for the development of rice with *Bt* genes that would not cause strong selection for adaptation by the stem borers.

A theoretical framework for the development and deployment of crops in ways that will not select for rapid pest adaptation is available (Gould 1988, 1989). This framework is limited however, since it is a general framework and must be carefully examined and adjusted to fit the specific crop and pests in question. To make the necessary adjustments require studies to answer specific questions about the ecology and genetics of the organisms in the cropping systems of concern.

It is felt therefore, that prior to deployment of rice varieties with engineered and/or classically bred resistance to stem borers, it is important to know more about the nature of the pests' populations. While there has been a lot of studies on the ecology and management of stem borers, there is almost no information available on geographic variation in ecological characteristics of these pests. Studies with other rice pests have demonstrated that management programs for a specific pest developed in one area may not be appropriate for managing the same pest in a different area since local pest populations differ genetically in ecological traits relevant to control tactics. There is therefore an urgent need to assess if the same situation occurs in stem borers. Ecological traits such as response of various stem borer populations on different *Bt* endotoxins is an important characters which could be utilized in such evaluation studies. This study will help pest and crop managers to gain insights regarding geographical scale and pattern of variation in ecologically relevant characteristics of the stem borers.

Two species of stem borers were studied based on variation in sensitivity to an array of *Bt* endotoxins. Specifically, the study assessed the variability among local strains of the stem borers in their response to an array of *Bt* strains and endotoxins and determined its implication in the development and deployment of *Bt*-engineered rice.

Materials and Methods

I. Assessing Geographic Patterns of Variation in Susceptibility to *Bacillus thuringiensis* Toxins

Preparation of Bt samples for toxicity evaluation. The selected strains which contained specific crystal proteins and used in this study were: strains 83 (Cry111X), 92 (SDSCPS), 99 (Cry111X), 105 (Cry1A/Cry1C), 231 (undetermined), 261 (Cry1B), and HD73 (Cry1Ac). The growing strains were scraped from agar culture plates and dissolved in phosphate buffer saline (PBS). The suspension was then centrifuged for 15 min at 3,000 rpm. The pellet was resuspended in PBS, this was done three times. The spore/crystal protein mixture (pellet) was suspended in alkaline buffer (50 mM Na_2CO_3 and 10mM Dithiothreitol adjusting the pH to 10 with HCl) and incubated overnight at 37°C. The supernatant containing solubilized toxin was harvested and since estimated potency depends on dose and dilution of samples, protein content of the samples was determined by spectrophotometric analysis using Commasie Brilliant Blue (Bradford, 1976). Bovine serum albumin was used as standard. The protein concentration was determined by regression analysis. The pH of the sample was adjusted before trypsinization by adding 1/10 volume of 0.5M HCl. Trypsin (1mg trypsin/ml water) was added to the solubilized crystal proteins (1 ug of trypsin/20 µg of protein), mixed and incubated overnight at 37°C.

The purified endotoxins of the Cry1 (Cry1Aa, Cry1c, Cry1B, Cry1D, Cry1E) toxins were supplied by the Plant Genetics Systems (PGS) of Gent, Belgium and Department of Entomology, North Carolina State University (NCSU) while Cry1Ab, Cry1C and Cry11A toxins were supplied by Dr. William Moar of Auburn University, Auburn, Alabama, USA.

Bioassay. Since potencies of test strains and endotoxins can vary widely, it is not possible to give an exact procedure for preparation of the final diluted samples to be incorporated into the diet. However, the first (least dilute) sample was prepared to be at 50 μg. The final concentrations utilized in this study were 50 μg, 10 μg, 2 μg, 0.40 μg, and 0.08 μg. These concentrations were prepared by serial dilutions, each dilution being 1:5 of the preceding one In cases where the toxin or strain do not show any effect on these doses, the initial concentration was increased up to 250 μg. The LD50 should then fall into the mid-range of the curve.

The diet was prepared according to the protocol established by Angeles and Demayo (accompanying paper). It was cooled to about 65°C and maintained at that temperature until use. Thirty seven (37) ml diet was poured into 50 ml beaker after which a 3ml *Bt* sample containing the first diluent (50 μg) was poured into the diet and mixed thoroughly using a food mixer. Mixing of the sample into the diet was accomplished by setting the blender under low speed at first and soon increased. Mixing was done for at least 1min or until complete uniformity of the final blend was achieved. From a 40 ml preparation, 8 ml was dispensed to another beaker and 32 ml diet was again added. The mixture was again blended until uniformity was attained.

The mixture of the diet and the *Bt* samples was dispensed using a ketchup dispenser. Separate ketchup dispensers were used for each concentration and were properly washed, drained, disinfected, rinsed with distilled water, again drained and used. The containers used in this study were disposable cups obtained from BIOSERV. The mixture of the diet and samples was dispensed on these cups, allowed to solidify and dry for 1h before each cup was infested with 6 larvae. Eight cups were prepared for each dilution made. After infestation, each cup was covered with either waxed lids or plastic covers also obtained from BIOSERV. Forty-eight larvae were used for each dilution of sample and control at the beginning and end of the assay. To avoid contamination of the diet by the materials used, some precautionary measures were observed; such as, allowing the larvae to climb a plastic loop and when they were found hanging on the loop the webs were cut off by a camel's brush previously disinfected by 5% chlorox. This method allows the collection of the larvae from the culture cup without touching the body of the larvae. After infestation, the containers were incubated for four days in the rearing chamber at 28°C.

Mortality was observed daily for four days to determine which specific toxin has caused "fast-killing effect" compared with others. A microscope was used to carefully examine the physical features of the larvae. When in doubt whether the larvae were dead or alive, the larvae were touched with the tip of the camel's brush. The larvae were considered dead when not responding. If there was a response, no matter how feeble, the larva was considered alive. No consideration was given to imminent death. It was a relief that in most of the repeated assays using the artificial diet, mortality in the control was relatively low(<4%). Probit analysis was conducted in all sets of data. LD_{50} was determined from several sets of data obtained. A supplemental experiment was conducted in which the larvae were allowed to feed on the diet for 16 days after which it was dissected and the larvae counted and weighed. This was done to determine the extent of the effect of the formulations and the endotoxins used on the growth of the larvae.

The results of the bioassay determined which among the test strain formulations and endotoxins will be used for geographical variation study.

Test Insects. For *C. suppressalis*, insects were collected from rice stubbles and "whiteheads" from ricefields of different geographic locations. These were from IRRI and Calauan in Laguna, Lamut in Ifugao, and Koronadal in South Cotabato. The stubbles and "whiteheads" were cut from an infested rice field and brought to the greenhouse for dissection. The larvae collected were then cultured in Rexoro plant until emergence. The emerging moths were allowed to oviposit in an enclosed potted rice plant and the egg masses laid were allowed to incubate until darkhead stage. These were then collected by cutting the leaves where the egg masses were attached and sterilized in 5% sodium hypochlorite solution. The egg masses were then dried in paper towels and placed in rearing cups. All test larvae came from eggs laid and collected the same day. Those selected for the assay (those which hatched similarly on the same day) were reared for one day in the artificial diet.

For *S. incertulas*, moths were collected from rice stubbles in five geographically close locations in Laguna (IRRI, Victoria, Lingga, Pansol, San Antonio, Bangyas), Lian in Batangas, and Banaue in Ifugao. The populations in Laguna were at least 10-35 kilometers away from each other to reduce the effects of variation among populations due to large-scale differences in abiotic environments. The moths were directly placed in big mylar cages containing TN1 rice plants for oviposition. After one day, the rice plant containing the egg masses was removed from the cage and allowed to incubate for five days. The egg masses were cut from the leaves after six days and were sorted out by placing one large egg mass in each scintillation vial. The egg masses were then allowed to hatch and the larvae were used for testing.

Experimental Design. The design used in this study was similar to that of the experiments utilizing various rice types. Six larvae from a given egg mass were allocated to each cup of every concentration of the toxin preparations (126 larvae per egg mass were used). In the striped stem borer, however, only 5 larvae were used per cup since the cup with the diet could only accommodate up to 5 larvae. After four days, mortality per concentration/toxin/egg mass was recorded. Survival up to 16-21 days was also determined to get an idea of the extent of effects of the toxin on the physiology of the insects. Data were analyzed with a completely randomized two factor analysis of variance (ANOVA), testing for effects of toxin type, population, and a toxin by population interaction on survival. If the populations show differences in *Bt* response, the interaction term will be highly significant.

II. Feeding behavior and growth of stem borer larvae on diets containing *Bt* formulations or endotoxins.

The feasibility of transferring genes that code for pesticidal proteins from *Bt* to rice could lead to an increase in yield and decrease in pesticide use. Efficiency and management of these approaches will, however, depend on the details of the pests behavioral response to the toxins such as its feeding behavior. It was therefore the objectives of the study to investigate the growth and feeding behavior of SSB on different *Bt* formulations, determine the feeding deterrence caused by *Bt* spore-crystal complex when presented in a choice test, and to evaluate the effects of these *Bt* formulations on the growth and survival of SSB larvae.

Insects. Only the striped stem borer was utilized in the feeding behavior and growth study. There were problems encountered when the SSB set-up was used for the YSB due to susceptibility to contamination and fast hardening of the YSB diet. Striped stem borer colony maintained in Davis' modified southwestern corn borer diet was used in this study. It was also this diet that was used in the assay.

Dipel, spores and crystals of HD73 and 1715 strains, and pure endotoxins of Cry1Ac, Cry11A, and Cry1C were incorporated into the diet. The Dipel formulation had 16,000 international units per mg and is noted to contain Cry1Aa, Cry1ab, and Cry1Ac. HD73 is known to produce only one endotoxin, Cry IAc (Hofte and Whitley, 1989) and 1715 had two toxins, Cry IAc and Cry Ib.

Experiments with early instar larvae. These experiments were designed to assess the behavior and growth of the neonates of SSB when feeding solely on untreated artificial diet, treated diet and when given a choice of *Bt* treated diet or normal diet.

The experimental arenas used in this experiment were adapted from Gould et. al. (1991). For the choice tests the arena consisted of an eight ounce cup in which two aliquots of the diet were poured. One half of the area contained the control diet and the other half the spore crystals of either Dipel, HD73 or 1715, Cry1Ac, Cry11A and Cry1C.

Initial tests were made on the first instar larvae placed at the center of each cup where the control and toxin diet met. Five larvae were used for each cup. Survival and position of larvae in the choice arenas were monitored daily for seven days and subsequently three days thereafter. Larval position and condition were scored as on control diet, on *Bt* diet, on sides or top of the cup or dead. All larvae were weighed after 21 days. Four to five concentrations of the three toxins were used, Dipel (0.0025, 0.0100, 0.0200 and 0.0500 g/200 ml), and 1715 (10^1, 10^2, 10^3, 10^4 and 10^5 serial dilutions).

Experiments with late instar larvae. Since the effects of many natural and synthetic toxins decrease as larval age and size increase (Shaver and Parrot 1970), experiments were conducted to determine how *Bt* formulations and purified endotoxin would affect late instars that had not previously fed on toxic diet. To simulate the choice that might be presented to a larger larva, larger arena was used in testing for the third instar larva. This consisted of a 30 ml. plastic cup with cover provided with two holes where two test tubes containing the required diets were fitted. In the no-choice arenas, both test tubes were filled with 2 ml diet containing either the spore crystals or just plain normal diet to serve as control. Initial weights of the diet and the larvae were taken prior to testing. Daily observations on the behavior and position of the larvae in the arena were made as to whether they are dead, feeding on the control tube, on the *Bt* tube, or on either diet. Each treatment had 45 larvae for testing using only one larva placed in each cup. The amount of diet consumed for three days and the changes in weights of the larvae were also recorded.

III. Selection of *C. suppressalis* for adaptation to *Bt* Cry1Ac endotoxin

It is argued that if we devise an environmentally sound stem borer management, engineered rice plants that produce narrow *Bt* toxin, internally, have advantages over conventional insecticides. These advantages range from farmer safety to the maintenance of beneficial natural enemies of the pest insects. At one time it was hypothesized that insect pests would not adapt to it (Briese 1981). It was thought that since *Bt* had been in the environment for a long time, insects already had the opportunity to adapt to it; therefore, if they had not adapted in millions of years, they would not adapt to it on a 100-year time scale. Not only was the logic behind this thinking flawed, we now have evidence of insect adaptation to *Bt* in the laboratory (McGaughey 1985, Stone et al 1989, Gould et al 1990) and in the field (Tabashnik et al 1990). While one could argue that laboratory adaptation to *Bt* does not prove that there will be field adaptation, the current evidence for field adaptation now supports the laboratory findings. Tabashnik et al (1990) found that a population of diamondback moths on one farm where *Bt* was used intensively for 5 years was 15 times more resistant to *Bt* than an untreated field population. The fact that a population on a farm surrounded by other farms that did not use *Bt* could develop any resistance is strong evidence supporting the hypothesis that widespread use of plants that express *Bt* genes constitutively will eventually select for pest adaptation. It is therefore worth considering ways to limit the rate by which pests adapt. There were strategies hypothesized but what is missing is specific information on how insects genetically respond to various selection regimes with *Bt* toxins.

Recognizing this lack of empirical information, a laboratory selection was developed for gathering information on insect adaptation to *Bt* endotoxin Cry1Ac with chronic or acute doses. It is understood that laboratory selection may not lead to the same results as field selection; however, laboratory selection regimes will provide useful information about the pest. For most target pests, field experiment is tantamount to producing resistant pests in large regions.

An experiment was initiated with collected field material. Three populations were tested - those which were not fed with adulterated diet (control population), survivors on 25 μg Cry1Ac treated diet, and survivors of a 50 μg Cry1Ac diet. These three populations were continuously subjected to 25 and 50 μg Cry1Ac-treated diet for several generations.

Results and Discussion

I Assessing Geographic Patterns of Variation in Response to *Bt* formulations and endotoxins

The first step in engineering any crop plant with the effective *Bt* deployment system is to find gene products that are highly effective against the target pest. Measurements of specific activity of crystals of *Bt* strain isolates and endotoxins is essential in pinpointing the genes. IRRI and Plant Genetic Systems in Belgium collaborated in isolating and characterizing strains of *Bt* for potential use against the yellow stem borer and other rice insect pests. The effort accumulated almost 4,000 *Bt* isolates from different Philippine environments, and about 1,000 strains have been selected from the isolates. The crystal proteins of specific *Bt* isolates were tested. Investigations of the responses of the two stem borers on the various *Bt* strains and pure endotoxins show that Cry 1Ac, Cry11A, Cry1C, strains 105, 261 and HD73 were very effective against yellow stem borer while only Cry1Ac, strains 105 and 261 were effective against the striped stem borer (Tables1 & 2 Figure 1). The variations in toxicity among the endotoxins and strains tested in this study could be attributed to variations in plasma receptors in the gut epithelial cells of the stem borer larvae. It is believed that binding to a specific receptor on the membrane of gut epithelial cells is an important determinant with respect to differences in insecticidal spectrum of *Bt* insecticidal proteins and this had been proven in studies of selected lepidopterous pests (Hoffmann et al 1988a,b; Van Rie et al 1988,1989). It should be emphasized that the differences in activity between the endotoxins and strain formulations are not determined by differences in affinity of the toxins for a single receptor site, but correlate with differences in binding site concentration. These probably are main reasons for the variations observed regarding the toxicity differences observed between the toxins used such as those in Cry1Ac, Cry11A and Cry1C which were all observed to be effective against the yellow stem borer although variations between them are detected. The non-effectiveness of Cry1Ab, Cry1D could be interpreted as that there may be less number of binding sites specific for these toxins. The above explanation could be similarly true regarding the soluble proteins of the different strains tested.

Because of the expense and time necessary to produce transgenic *Bt* rice, it is essential that the *Bt* toxins selected for expression be effective against pest populations in as many ways as possible. On the collection of information on the variation of stem borer responses to *Bt* toxins, several populations from different parts of the Philippines were tested. Based on mean survival, significant differences were observed between populations response to the three most effective endotoxins against the yellow stem borer (Table 3, Fig. 4,5,6). A microgeographic investigation of selected populations in Laguna show that based on comparison of means of the different populations on three endotoxins, the response of IRRI and Victoria were not significantly different from each other but when compared with Calauan, Lingga and San Antonio, differences were significant (Table 4 &5). In terms of the efficiency of the toxin in effecting mortality on the yellow stem borer, Cry1Ac is as highly effective as Cry1C. Cry11A was also effective, but the efficacy was not as good as the other two (Table 6).

For the striped stem borer which was tested using only the effective Cry1Ac, the results show significant population differences in survival rates (Tables 7-8, Fig. 7). Evaluation of the weights of the surviving larvae revealed no significant differences indicating that all survivors of different populations have the same response (Table 9 & 10).

Table 1. Toxicity of selected strains and endotoxins on *S. incertulas* larvae.

STRAINS AND ENDOTOXINS	LC50 ± SE (μg/ml)	SLOPE + SE
Cry1Aa	19.68 ± 16.03	1.27 ± 0.39
Cry1Ab	84.07 ± 71.83	0.65 ± 0.25
Cry1Ac	0.24 ± 0.12	1.31 ± 0.28
Cry1B	11.15 ± 9.43	0.75 ± 0.19
Cry1C	1.99 ± 1.80	1.77 ± 0.37
Cry1D	106.40 ± 76.38	1.31 ± 0.69
Cry1E	515.09 ± 428.67	1.56 ± 0.94
Cry11A	0.55 ± 0.17	1.89 ± 0.37
strain 83 (Cry11X)	54.15 ± 38.29	0.63 ± 0.53
strain 92 (SDSCPS)	2.81 ± 1.35	0.31 ± 0.12
strain 99 (Cry111X)	2.57 ± 1.25	0.33 ± 0.14
strain 105 (cry1A/Cry1C)	0.12 ± 0.04	1.07 ± 0.30
strain 261 (Cry1B)	0.28 ± 0.09	1.07 ± 0.31
HD73 Cry1Ac	0.91 ± 0.47	0.73 ± 0.28

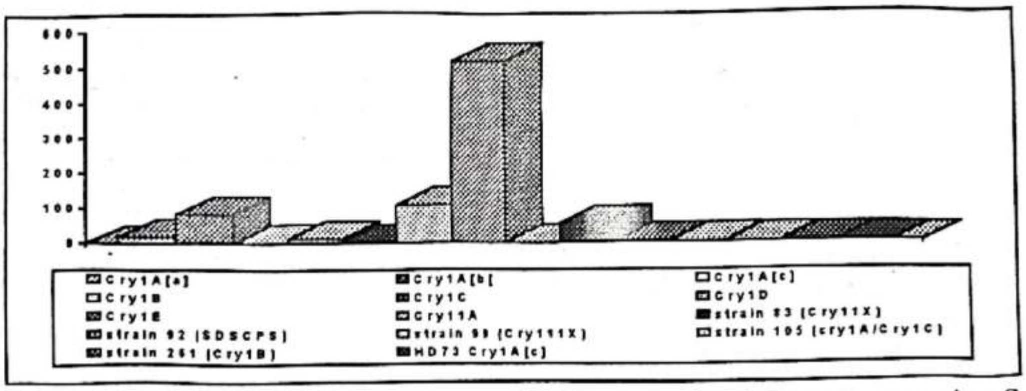

Figure 1. Toxicity of selected strains and endotoxins of *Bacillus thuringiensis* against *S. incertulas.*

Table 2. Toxicity of selected strains and endotoxins on *C. suppressalis.*

STRAINS/ ENDOTOXINS	LC50 ± SE (μg/ml)	SLOPE + SE
Cry1Ac	25.58 ± 12.02	0.73 ± 0.40
Cry1C	896.35 ± 388.32	1.205 ± 0.41
Cry11A	7.9E ± 2.5281	0.33 ± 0.08
strain 83 (Cry1111X)	5.1E ± 1270.75	0.32 ± 0.08
strain 105 (cry1A/Cry1C)	3.57 ± 1.98	1.133 ± 0.05
strain 231	17.02 ± 7.86	1.44 ± 0.14
strain 261 (Cry1B)	4.01 ± 0.09	1.28 ± 0.06
HD73 Cry1Ac	17.09 ± 0.47	1.421 ± 0.08

Table 3. Comparison of effects of high and low concentrations of selected *B. thruringiensis* strains and endotoxins on survival and growth of young *C. suppressalis* larvae.

STRAIN	TREATMENT	4-DAY SURVIVAL (%)	16-DAY SURVIVAL (%)	16-DAY WEIGHT (mg)
Strain 105	Control	100	99	0.0096
[Cry1A/Cry1c]	400	100	68	0.0001
	2,000	89	15	0.0001
	10,000	82	0	0
	50,000	18	0	0
Strain 261	Control	100	100	0.0124
[Cry1B]	400	100	87	0.0020
	2,000	96	63	0.0022
	10,000	22	0	0
	50,000	3	0	0
Strain HD73	Control	100	83	0.0072
[Cry1Ac]	400	99	69	0.0005
	2,000	96	61	0.0003
	10,000	72	1	0.0001
	50,000	49	0	0
Strain 92	Control	100	96	0.0099
[SDSCPS]	400	99	93	0.0013
	2000	99	74	0.0009
	10000	92	64	0.0003
	50000	42	0	0
Strain 83	Control	100	92	0.0091
[Cry111X]	400	97	89	0.0104
	2000	99	76	0.0077
	10000	100	76	0.0033
	50000	97	56	0.0004
Strain 99	Control	100	100	0.0111
[Cry11X]	400	99	97	0.0098
	2000	99	87	0.0108
	10000	100	90	0.0082
	50000	97	83	0.0032
Cry1Ac	Control	99	87	0.0088
	400	100	65	0.0002
	2000	92	46	0.0003
	10000	82	14	0.00001
	50000	78	4	0.00001
Cry1C	Control	100	82	0.0137
	400	96	85	0.0015
	2000	100	92	0.0005
	10000	93	58	0.0001
	50000	96	39	0.00001
Cry1C	Control	100	100	0.0127
	400	97	99	0.0007
	2000	99	79	0.0003
	10000	99	78	0.0002
	50000	99	51	0.0002

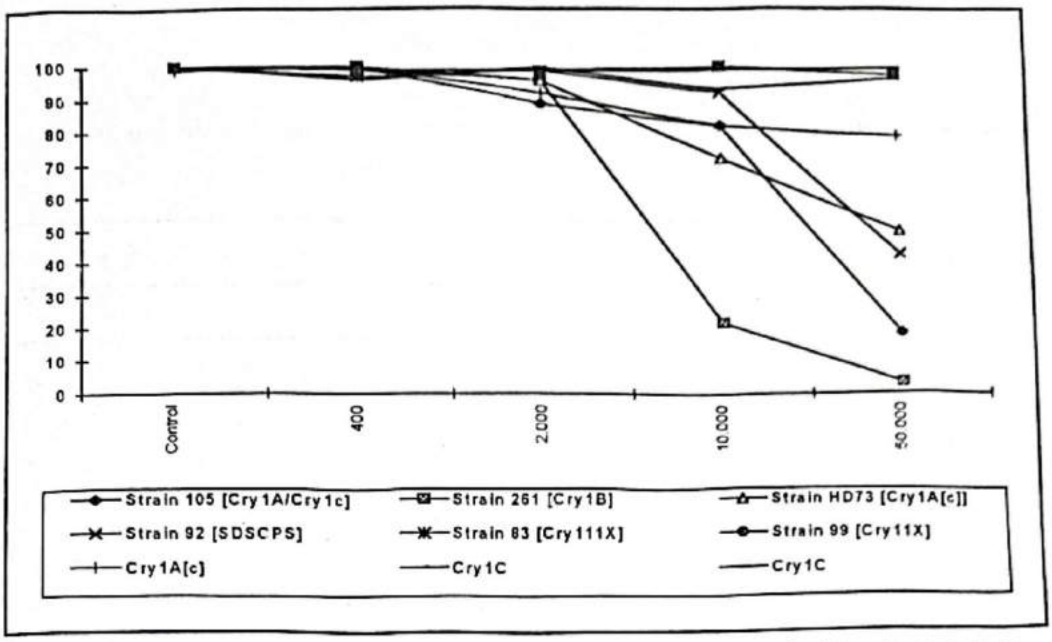

Figure 2. Four-day survival of *C. suppressalis* fed with diet treated with selected strains and endotoxins of *B. thuringiensis*.

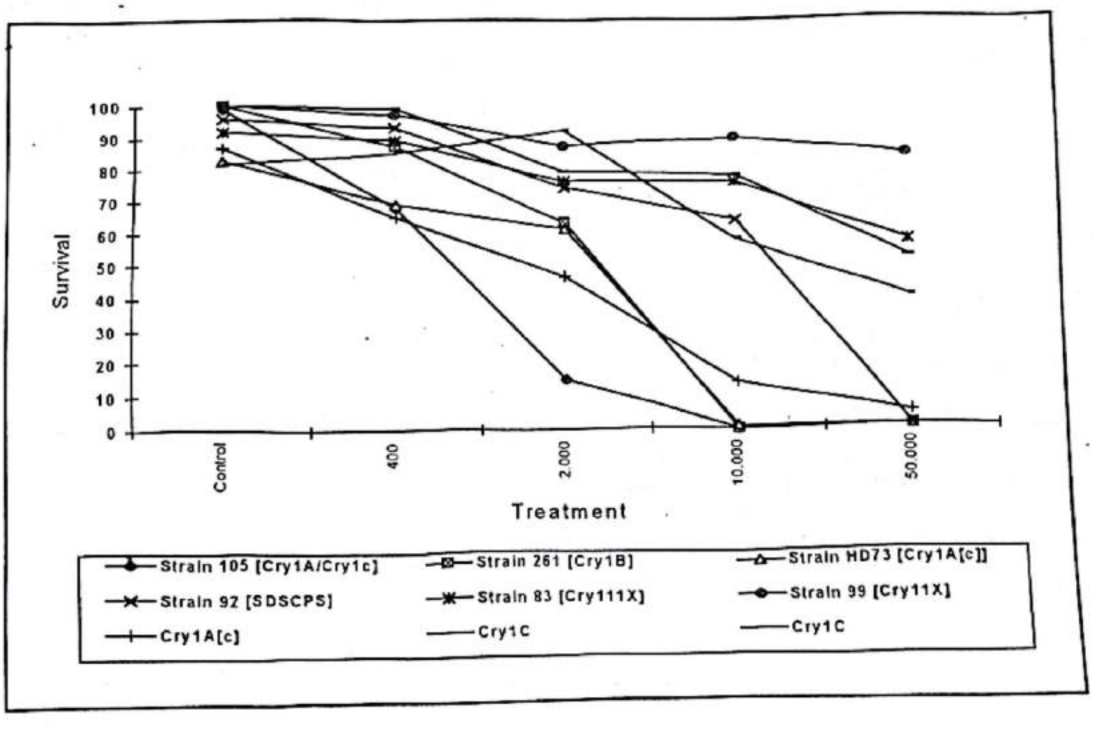

Figure 3. Sixteen-day survival of *C. suppressalis* on selected strains and endotoxins of *B. thuringiensis*.

Table 4. Effect of purified endotoxins on different strains of the *S. incertulas* from the Philippines.

POPULATIONS	LC50	SLOPE	RESISTANCE *
		CryIAc	
Banaue, Ifugao	0.0107+0.150	0.931±	most susceptible
Lian, Batangas	0.1440+0.003	0.338 ±	14x
IRRI, Laguna	0.2060+0.144	0.889 ±	21x
Victoria, Laguna	0.0990+0.026	0.839 ±	10x
Lingga, Laguna	0.6650+0.373	0.798 ±	67x
Pansol, Laguna	0.0130+0.007	0.527 ±	1x
San Antonio, Laguna	0.1910+0.112	0.662 ±	19x
Bangyas, Laguna	0.030	0.546 ±	3x
		Cry11A	
Banaue, Ifugao	0.462	0.773+0.160	3x
Lian, Batangas	0.173	0.959+0.215	most susceptible
IRRI, Laguna	1.380	0.973+0.267	8x
Victoria, Laguna	0.334	0.527+0.099	2x
Lingga, Laguna	0.607	0.655+0.032	4x
Pansol, Laguna	0.501	1.045+0.0003	3x
San Antonio,Laguna	3.456	0.668+0.079	20x
Bangyas,Laguna	1.739	1.468+0.359	10x
		CryIC	
Banaue, Ifugao	0.282	0.737+0.218	3x
Lian, Batangas	0.124	0.569+0.141	1x
IRRI, Laguna	0.288	1.060+0.043	2x
Victoria, Laguna	0.437	0.655+0.177	4x
Lingga,Laguna	0.599	1.025+0.121	5x
Pansol, Laguna	0.482	0.365+0.077	4x
San Antonio,Laguna	1.119	0.707+0.239	9x
Bangyas,Laguna	0.119	0.569+0.231	most susceptible

* Based on the most susceptible strain

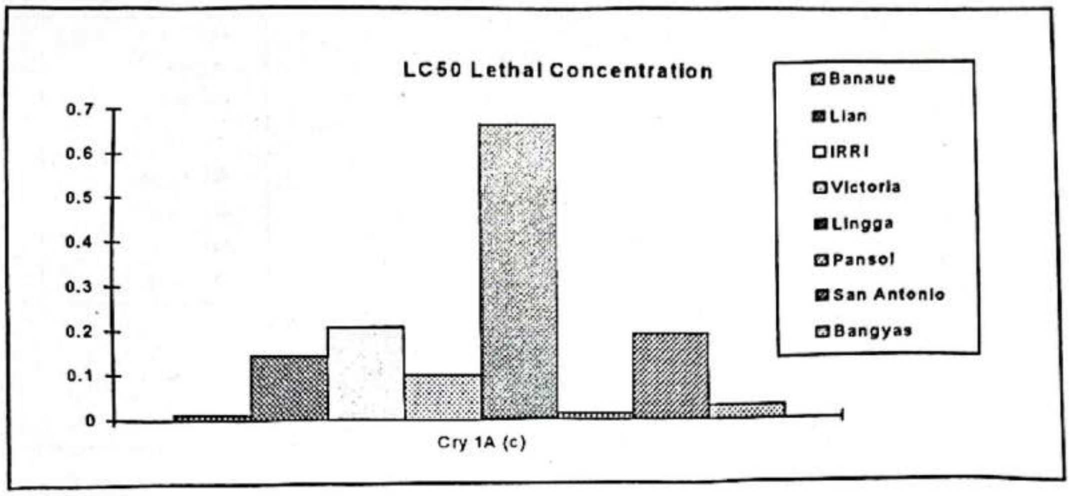

Figure 4. Variation in susceptibility of different strains of *S. incertulas* to Cry1Ac endotoxin of *B. thuringiensis*.

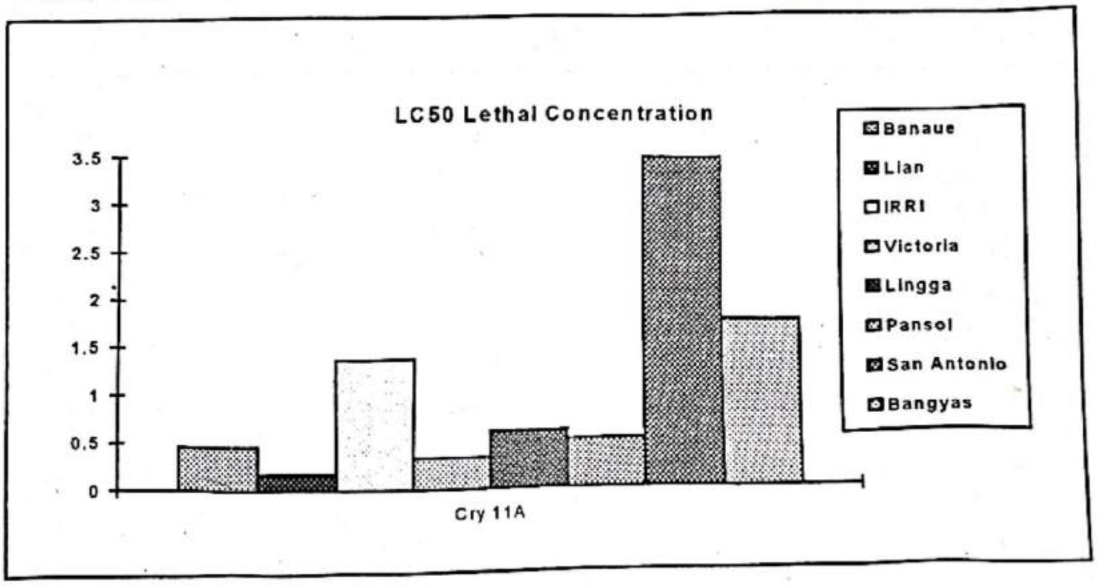

Figure 5. Variation in susceptibility of selected strains of *S. incertulas* against Cry11A endotoxin of *B. thuringiensis*.

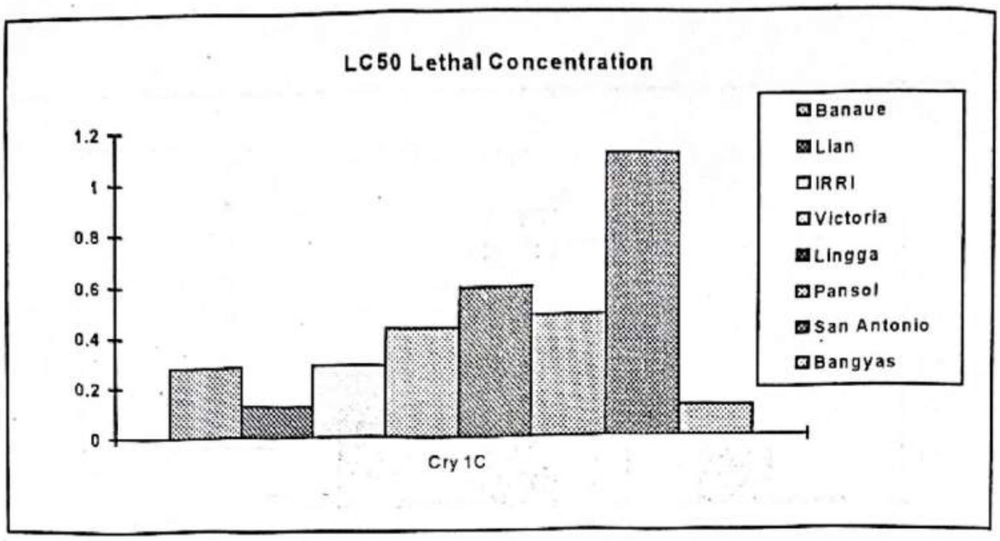

Figure 6. Variation in susceptibility of selected strains of *S. incertulas* against Cry1C endotoxin of *B. thuringiensis*

Table 5. Comparison of susceptibility to three endotoxins of *B. thuringiensis* by selected populations of *S. incertulas* from Laguna.

POPULATION	MEAN SURVIVAL	N	TUKEY GROUPING
IRRI	0.7619	75	A
VICTORIA	0.8634	75	A
CALAUAN	0.6707	75	C
LINGGA	0.7211	75	BC
SAN ANTONIO	0.6706	75	C

ANOVA

	DF	TYPE III SS	MS	F-VALUE	Pr>F
Model	37	56.933	1.540	23.11	0.0001
Error	337	22.450	0.067		
Strain	4	1.924	0.481	7.22	0.0001
Eggmass(strain)	20	1.885	0.094	1.42	0.1120
Toxin	2	2.290	1.145	17.19	0.0001
Concentration (toxin)	5	27.606	5.521	51.52	0.0001
Toxin -by-strain	8	1.263	0.157	2.37	0.0171

Table 6. Comparison of means of larval survival of populations of *S. incertulas* on the three endotoxins of *B. thuringiensis*.

ENDOTOXIN	MEAN	N	TUKEY GROUPING
Cry1Ac	0.7985	125	A
Cry1C	0.6772	125	A
Cry11A	0.7869	125	B

Table 7. Effect of purified endotoxin Cry1Ac on selected populations of *C. suppressalis* borer from the Philippines.

POPULATION	LC50 ug/ul	SLOPE	RESISTANCE*
Ballesteros, Cagayan	261.98	0.328 ± 0.07	2x
Lamut, Ifugao	587.30	0.627 ± 0.08	5x
IRRI, Laguna (1)	270.59	0.390 ± 0.06	2x
IRRI, Laguna (2)	764.68	0.584 ± 0.09	7x
Calauan, Laguna	1354.68	0.820 ± 0.11	39x
Koronadal -1, South Cotabato	320.17	0.584 ± 0.48	3x
Koronadal-2, South Cotabato	664.90	1.021 ± 0.21	6x
Ballesteros x IRRI hybrid	5161.80	0.319 ± 0.319	47x
Ifugao x IRRI hybrid (1)	111.02	0.533 ± 0.100	most susceptible
Ifugao x IRRI hybrid (2)	1711.48	0.586 ± 0.0.10	15x
Mixed Colony (IRRI, Ifugao, Cagayan)	506.99	0.417 ± 0.06	5x
Mixed Colony (all populations mixed)	4450.60	0.947 ± 0.19	40x

* based on comparison with the most susceptible strain

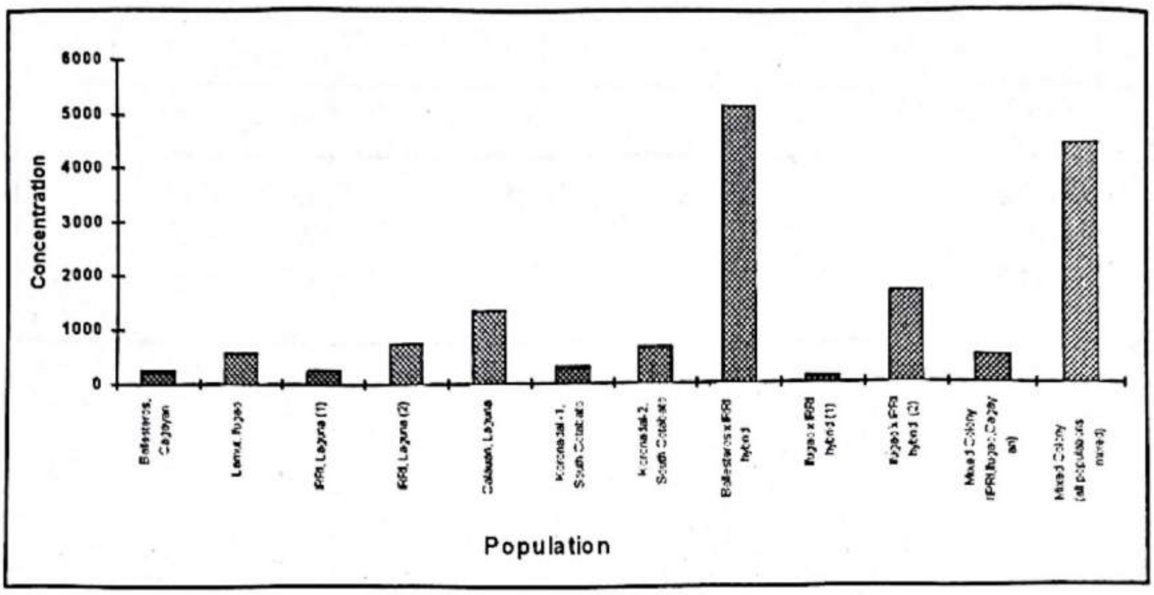

Figure 7. Effective concentration of Cry1Ac among selected strains of *C. suppressalis*

Table 8. Comparison of larval survival means of different populations of *C. suppressalis* tested on different concentrations of Cry1Ac.

POPULATION	MEAN SURVIVAL	N	TUKEY GROUPING
Ballesteros, Cagayan	1.1326	96	A
Lamut, Ifugao	0.8158	96	C
Hybrid (Ifugao x IRRI)	0.9953	96	B
IRRI	1.1356	96	A

ANOVA

	DF	TYPEIII SS	MS	F-VALUE	Pr>F
Model	37	56.933	1.540	16.71	0.0001
Error	337	22.450	0.067		
Strain	4	1.924	0.481	20.41	0.0001
Eggmass(strain)	20	1.885	0.094	10.10	0.0001
Concentration (toxin)	5	27.606	5.521	51.52	0.0001

II. Growth and feeding behavior of striped stemborer, *Chilo suppresalis* on different *Bacillus thuringiensis* formulations

The feasibility of transferring genes that code for pesticidal proteins from *Bacillus thuringiensis* to rice could lead to an increase in yield and decrease in pesticide use. Efficiency and management of these approaches will depend however on the details of the behavioral response of the pests to the toxins such as their feeding behavior.

Knowing the behavior of stem borers when allowed to feed on *Bt* formulations could have a significant impact on their management especially when the toxin gene is already incorporated in the rice genomes. If the transgenic rice developed has tissue-specific promoter sequences, the *Bt* toxin gene can be possibly engineered in highly localized damage-sensitive tissues. If the toxins deter inhibit feeding, the tendency of the insects is to feed on less damaged sensitive tissues. The same kind of situation arises when the transgenic and normal rice seeds will be planted as a mixture and the stemborer has the ability to move between plants. These situations could partially protect yield without causing intense selection pressure to the pests to adapt to the toxins. The efficacy of these approaches in controlling the stem borers will depend on details of the behavioral response of the stem borers to the toxins (Gould, 1986).

In a study where there was constitutive expression of *Bt* genes in plant parts in response to small amounts of larval feeding , it was shown that there is a potential for resistance development in target insect pests (Stone et al 1989). With this possibility, any strategy that decreases selection pressure for adaptation should be welcome. If plants for example, were developed using tissue-promotor sequences, it would be possible to have *Bt*-endotoxins present only in plant parts that were most sensitive to damage (Gould 1988). If *Bt*-endotoxins inhibit feeding, pests would be more likely to feed on less damage-sensitive tissues. The use of tissue-specific expression could at least partially protect yield without causing intense selection pressure for pest adaptation to the endotoxins. The efficacy of such approaches will depend on details of a pest's behavioral response to the toxins (Gould 1986). Assessing the extent of feeding preference caused by *Bt* strains formulations and purified endotoxins when presented in a choice test and determining the effects of the choice and no-choice treatments on the growth and survival of the larvae will help evaluate the efficacy of the above approaches in the development of a *Bt*-engineered rice crop.

Neonates. Toxins in the form of Bt formulation and spore crystal of Dipel, HD73 and 1715 affected the growth and survival of SSB larva (Table 10). Compared with the control, there was a decrease in larval growth and survival in all concentrations both in the choice and no-choice tests but with greater effect on the latter. Larval survival at 0.005 g/200 ml. dipel in the choice tests was more than five times compared to the larval survival at the same level in the no-choice test. The same trend was observed in the 21-day larval weight where the decrease in weight at 0.005 g/200 ml NC was almost fifteen times less than what resulted in the choice tests (0.0002 g in no-choice versus 0.0033 g in the choice tests) (Figure 9, 10, 11).

This pattern was duplicated in the other levels of concentration but at varying degrees. The same observation was noted in both HD73 and 1715. The difference, however, between no-choice and the choice tests at the same concentration was not as wide as in the Dipel treatment. At the lowest concentration (10^1) of HD73, for example, the effect of the choice of diet was only about less than two times both in larval survival (40.83% at no-choice versus

67.50% for choice) and weight (0.0034 g for no choice and 0.0078 g for choice).

In the tests for 1715, the larval survival is almost the same at around fifty percent both in the no-choice and choice tests but larval growth, though affected by the toxin, was more rapid. At 10 choice tests the larval weight (0.0136 g) is about six times than at the same level (0.0024 g) in the no-choice regime. As in the test using HD73 the difference between the regimes increased to nine times at the highest concentration (105). In the choice arena, a greater proportion of larvae was frequently found on control diet (Figures 11-12, Table 11). Within 24 hours all toxin-incorporated diet were avoided by the larvae even at the lowest concentration used (Dipel-0.0025 g, HD73-105 , 1715-105). No direct relationship, however, could be established between the degree of avoidance and the days of exposure because the proportion of larvae varies by day (Figures 11-12).

Larval avoidance of Dipel-treated diet reached its maximum at 0.0050 g/200 ml. (Table 11). Higher concentrations showed almost no difference on the proportion of larvae staying on the control diet indicating, perhaps, that the larvae can only tolerate concentrations below that. In HD73 and 1715 the peak of avoidance started at 101 and 105 respectively (Table 11). In the purified endotoxins, avoidance started at concentrations as low as 0.4ug/ml (Table 12)

Third Instar Larvae. All third instar larvae in both choice and no-choice regimes had feeding but those which fed on the toxins had shown weight loss ranging from 0.0039 for 0.05g toxin to 0.0073 for 0.10 g toxin used indicating that an increase on the amount of toxin resulted to more damage, presumably, to the physiological condition of the larvae, in this case expressed through weight loss (Table 13). The presence of toxin greatly reduced the food intake of the larvae. Where there is no choice the reduction is about half (0.2325 g for 0.05 g Dipel and 0.220 for 0.10 Dipel) compared to the control (0.509 g). Even if there was a choice the reduction is as much for those larvae which fed on the control 0.220 g for 0.05g Dipel and 0.354 g for 0.10 g Dipel) and also on the toxin side (0.181 g for 0.05 g Dipel and 0.245 g for 0.10 g Dipel). Probably sensing the nutritional disadvantages of the toxin most larvae seemed to be avoiding this side of the area. The frequency of the larvae on the control in both regimes (33.33% and 60%) is much greater than those found on the toxin side (11.90% and 2.55).

The result suggested that if there is no alternative diet the larvae had no choice but to feed on the toxin which affected their physiology but if there is an alternative source available the larvae would still feed on both the toxin and the diet but still with considerable damage on their survival and growth. There is, however, a bigger chance of recovery and survival because of the presence of a diet choice which is favorable to them. In the course of studying *Bt* deployment this feeding pattern of behavior of the SSB larvae could provide basic information relevant to the management aspect. This kind of feeding behavior could be presumed to provide some sort of protection on the rice plant because if the essential *Bt* gene is already deployed on the plant part favored by the SSB, and the insect either avoided or feed less on this area then the damage on the plant will probably be minimal. This is so because the larvae would tend to feed on the less favored plant part which could considerably affect their growth and survival.

Table 9. Comparison of effects of low and high concentrations of selected *B. thuringiensis* strains and endotoxins on survival and growth of young *C. suppressalis* larvae.

STRAIN	TREATMENT (ug/ul)	SURVIVAL (%)					16 d WEIGHT (mg)
		24 h	48 h	72 h	96 h	16d	
Strain 105	control	100	100	100	100	99	0.0096
	400	100	100	100	100	68	0.0001
	2000	100	96	96	89	15	0.0001
	10000	100	100	97	82	0	-
	50000	100	46	24	18	0	-
strain 261	control	100	100	100	100	100	0.0124
	400	100	100	100	100	87	0.0002
	2000	100	99	96	96	63	0.0002
	10000	100	94	76	22	0	-
	50000	100	68	57	3	0	-
HD73	control	100	100	100	100	83	0.0072
	400	100	100	100	99	69	0.0005
	2000	100	100	100	96	61	0.0003
	10000	100	90	87	72	1	0.0001
	50000	100	68	62	49	0	-
strain 92	control	100	100	100	100	96	0.0099
	400	100	100	100	99	93	0.0013
	2000	100	100	100	99	74	0.0009
	10000	100	99	99	92	64	0.0003
	50000	100	83	74	42	0	-
strain 82	control	100	100	100	100	92	0.0091
	400	100	100	99	97	89	0.0104
	2000	100	99	100	99	76	0.0077
	10000	100	100	100	100	76	0.0033
	50000	100	97	100	97	56	0.0004
strain 99	control	100	100	100	100	100	0.0111
	400	100	100	100	99	97	0.0098
	2000	100	73	99	99	87	0.0108
	10000	100	89	100	100	90	0.0082
	50000	100	87	97	97	83	0.0032
CryIAc	control	100	100	100	99	87	0.0088
	400	100	96	100	100	65	0.0002
	2000	100	100	73	92	46	0.0003
	10000	100	97	89	82	14	0.00001
	50000	100	100	87	78	4	0.00001
CryIC	control	100	100	100	100	82	0.0137
	400	100	100	96	96	85	0.0015
	2000	100	99	100	100	92	0.0005
	10000	100	100	97	93	58	0.0001
	50000	100	100	100	96	39	0.00001
CryIIA	control	100	100	100	100	100	0.0127
	400	100	100	100	97	99	0.0007
	2000	100	99	99	99	79	0.0003
	10000	100	100	100	99	78	0.0002
	50000	100	100	100	99	51	0.0002

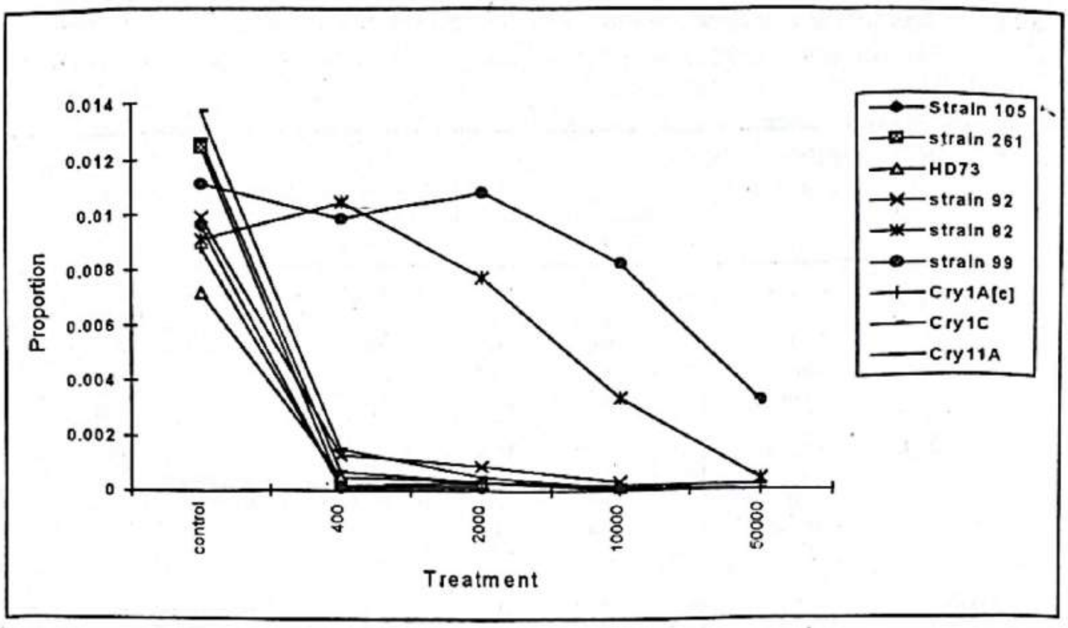

Figure 8. Sixteen-day weight of surviving larvae of *C. suppressalis* fed on different regimes of *Bacillus thuringiensis*

Table 10. ANOVA of survival and growth data of *C. suppressalis* fed by diet-treated with selected strains and endotoxins of *B. thuringiensis*.

SOURCE	DF	MS	F	Pr>F
A. Survival				
Model	53	0.64	3.01	0.0001
Error	475	0.21		
Corrected Total	528			
Toxin	8	3.51	16.59	0.0001
Eggmass	5	0.20	0.95	0.4465
Toxin - by - eggmass	40	0.12	0.55	0.9894
B. Weight				
Model	53	6.74	2.27	0.0001
Error	366	2.97		
Corrected Total	419			
Toxin	8	40.61	13.66	0.0001
Eggmass	5	0.67	0.22	0.9521
Toxin- by- eggmass	40	0.52	0.17	1.00

Table 11. Effects of high and low concentrations of selected *Bt* strain/commercial and endotoxin formulations on survival, growth and behavior of young *C. suppressalis* larvae.

STRAIN	SURVIVAL (%)			PROPORTION OF LARVAE ON Bt [%]							WEIGHT (mg)
	Day			Day							
	4	7	21	1	2	3	4	5	6	7	
				HD73							
Control*	100	100	93	-	-	-	-	-	-	-	
10^1/C	100	100	67	10	7	23	50	14	14	12	0.0231
10^2/C	100	100	92	8	2	15	6	15	5	3	0.0078
10^3/C	99	99	64	7	7	19	13	16	8	7	0.0065
10^4/C	100	100	66	13	5	15	13	15	8	4	0.0043
10^5/C	98	93	55	15	13	19	14	18	11	7	0.0092
10^1/nc	97	96	41	-	-	-	-	-	-	-	0.0073
10^2/nc	99	98	40	-	-	-	-	-	-	-	0.0034
10^3/nc	97	97	40	-	-	-	-	-	-	-	0.0032
10^4/nc	95	93	51	-	-	-	-	-	-	-	0.0022
10^5/nc	98	93	23	-	-	-	-	-	-	-	0.0006
				1715							
10^1/C	95	95	95	4	3	3	1	3	3	1	0.0136
10^2/C	99	99	78	2	1	3	1	1	1	3	0.0155
10^3/C	98	97	83	3	3	3	2	3	3	2	0.0119
10^4/C	95	94	53	3	3	3	5	2	3	1	0.0077
10^5/C	96	95	64	7	1	3	2	1	1	1	0.0057
10^1/nc	97	95	58	-	-	-	-	-	-	-	0.0024
10^2/nc	99	98	67	-	-	-	-	-	-	-	0.0037
10^3/nc	90	88	40	-	-	-	-	-	-	-	0.0034
10^4/nc	94	93	38	-	-	-	-	-	-	-	0.0006
10^5/nc	97	97	52	-	-	-	-	-	-	-	0.0006
				DIPEL							
30µg/C	96	83	20	9	6	11	3	7	6	5	0.0008
20µg/C	92	85	37	8	13	11	11	3	3	5	0.0012
10/C	98	91	38	7	9	9	3	3	5	6	0.0016
4µg/C	98	98	47	7	7	5	3	5	3	7	0.0016
2µg/C	99	97	58	7	6	9	6	6	2	4	0.0058
1µg/C	97	96	60	7	9	9	3	7	10	3	0.0033
0.5µg/C	98	97	72	3	4	3	4	3	6	6	0.0060
30µg/nc	11	40	0	-	-	-	-	-	-	-	-
20µg/nc	98	97	22	-	-	-	-	-	-	-	0.0001
10µg/nc	95	75	3	-	-	-	-	-	-	-	0.0001
4µg/nc	99	97	16	-	-	-	-	-	-	-	0.0001
2µg/nc	96	91	12	-	-	-	-	-	-	-	0.0003
1/nc	97	13	13	-	-	-	-	-	-	-	0.0002
0.5/nc	98	96	28	-	-	-	-	-	-	-	0.0003

- only 1 control was used for the experiment which was simultaneously done for the three *Bt* formulations.

C- choice; nc - no choice

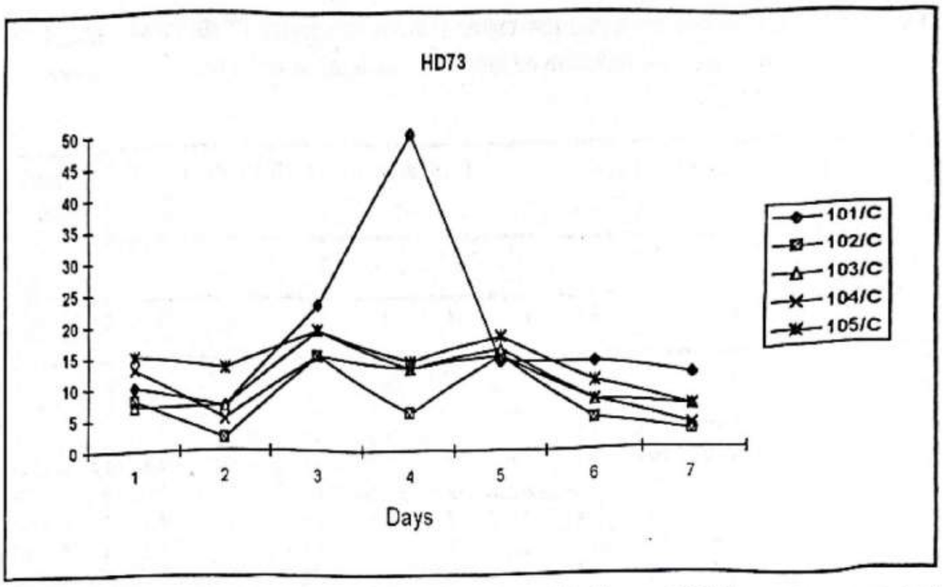

Figure 9. Proportion of *C. suppressalis* larvae observed feeding on HD73 strain -treated diet

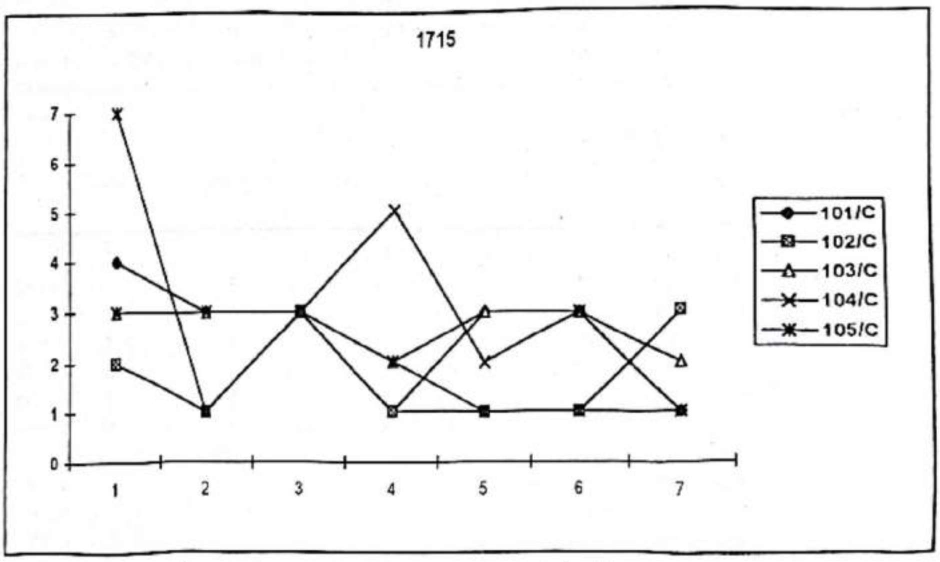

Figure 10. Proportion of *C. suppressalis* larvae observed feeding on 1715 strain - treated diet.

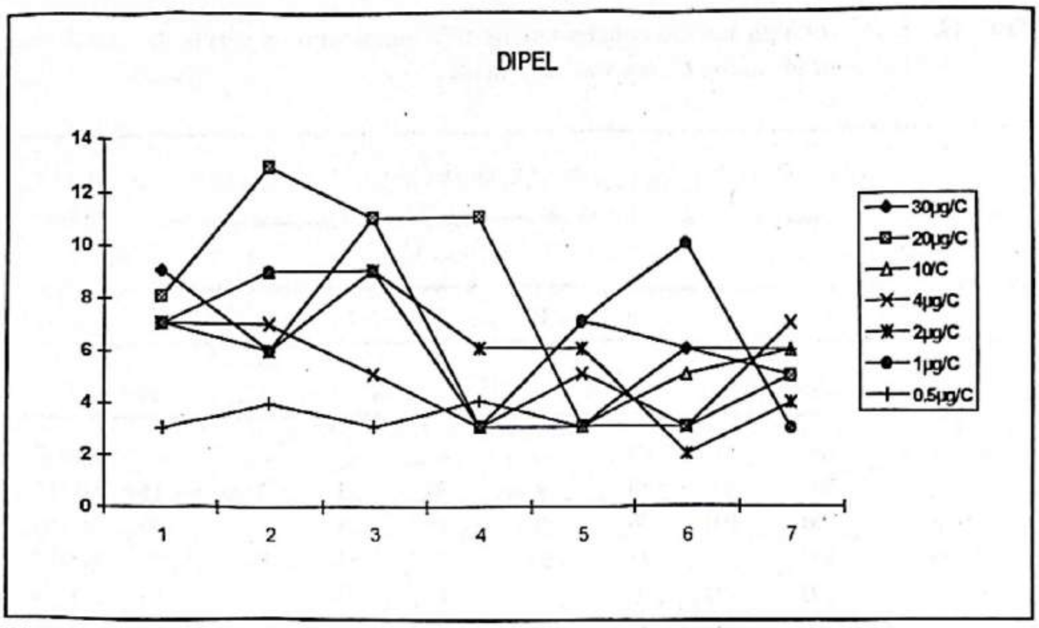

Figure 11. Proportion of *C. suppressalis* larvae feeding on Dipel-treated diet

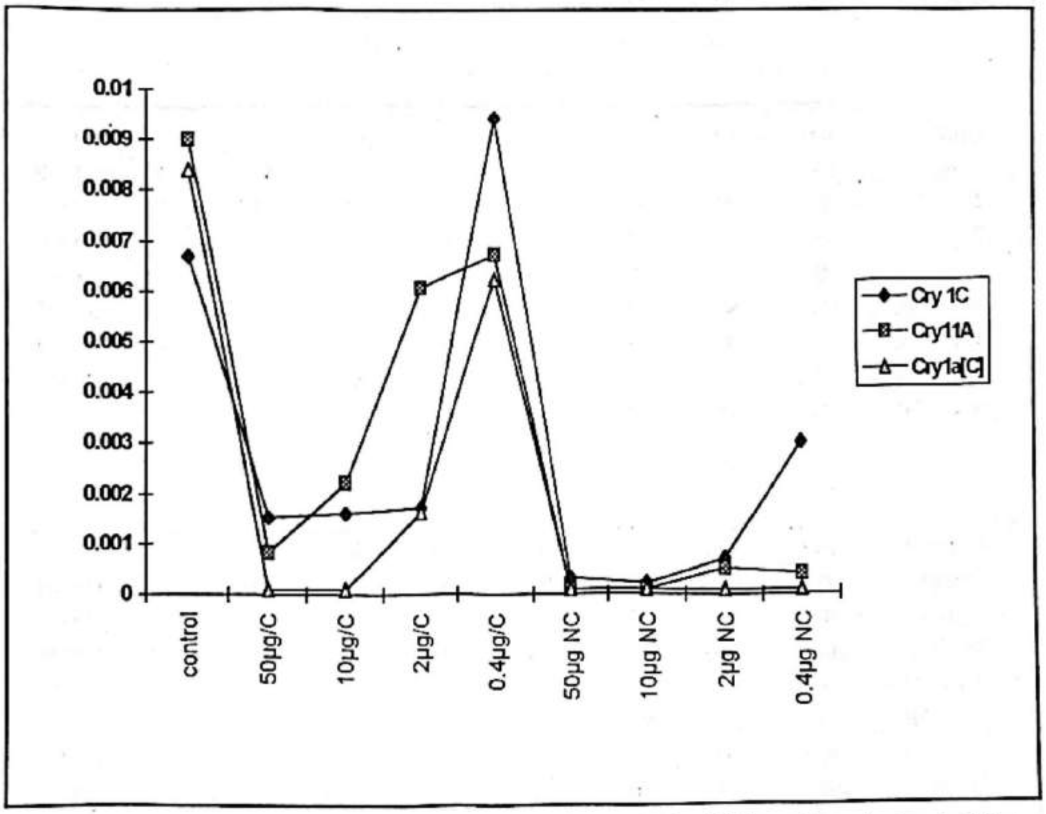

Figure 12. Weight of surviving *C. suppressalis* larvae fed with *Bt* endotoxin-treated diet

Table 12. Effects of high and low concentrations of *Bt* endotoxins on survival, growth and behavior of young *C. suppressalis* larvae.

ENDOTOXIN	SURVIVAL			PROPORTION OF LARVAE ON Bt					WEIGHT (g)
	Day			Day					
	4	7	16	1	2	3	4	7	
				Cry 1C					
Control	100	100	88	-	-	-	-	-	0.0067
50µg/C	100	100	58	40	50	53	30	15	0.0015
10µg/C	100	100	70	18	55	45	43	30	0.0016
2µg/C	100	100	45	8	70	53	38	30	0.0017
0.4µg/C	100	100	90	23	45	50	8	13	0.0094
50µg NC	88	55	5	-	-	-	-	-	0.0003
10µg NC	95	80	53	-	-	-	-	-	0.0002
2µg NC	98	98	87	-	-	-	-	-	0.0007
0.4µg NC	100	100	90	-	-	-	-	-	0.0030
				Cry11A					
Control	100	100	93	-	-	-	-	-	0.0090
50µg/C	95	93	40	13	8	8	8	15	0.0008
10µg/C	98	98	50	20	0	15	13	15	0.0022
2µg/C	95	95	88	25	8	13	20	8	0.0060
0.4µg/C	98	98	75	10	5	33	10	8	0.0067
50µg NC	90	80	50	-	-	-	-	-	0.0001
10µg NC	95	90	73	-	-	-	-	-	0.0001
2µg NC	98	98	68	-	-	-	-	-	0.0005
0.4µg NC	98	98	70	-	-	-	-	-	0.0004
				Cry1a					
Control	100	100	95	33	23	40	25	20	0.0084
50µg/C	98	98	40	8	8	8	13	3	0.0001
10µg/C	100	100	35	25	10	10	3	0	0.0001
2µg/C	100	100	45	18	15	3	8	20	0.0016
0.4µg/C	98	93	32						0.0062
50µg NC	85	82	8						0.0001
10µg NC	100	90	73						0.0001
2µg NC	93	95	40						0.0001
0.4µg NC	95	93	43						0.0001

III. Selection Experiment

There is currently no way to precisely anticipate the genetic mechanisms which will control resistance traits that develop in the field. One of the more empirical approaches is to conduct laboratory selection experiments aimed at increasing the frequency of resistance alleles in a strain so they can be studied. This study is very useful but has so many limitations. Even with the existence of these limitations, selection experiments were the most powerful approach available for gaining an understanding of how target insect species would adapt to an engineered crop(s).

Because of the absence of an artificial diet to mass rear the yellow stem borer, only *C. suppressalis* was used for the study. Prior to testing, the insects were maintained in the artificial diet for one (1) generation. Only three substrains which were selected from those which survived from the tests using 25 and 50μg/*u*l Cry1Ac-treated diet were investigated for a period of 12 generations. It was very difficult to maintain large colonies especially if the number of personnel maintaining the cultures is limited. Losses of substrains is common. In this study, the variation in number of larvae used in each regime is a function of percent mortality in that regime and the work involved in the maintenance.

After generations of selection at a *Bt* dose of 25 μg/*u*l and 50 μg/*u*l on the three substrains, it seems clear that the susceptibility of the insects to the endotoxin were almost similar (Table 14). There was no indication whatsoever that the larvae maintained either in adulterated diet or not have differences in susceptibility to the toxin.

There are so many general approaches as to how *Bt* genes should be used in pest management strategies. At least five general approaches were mentioned by Gould (1992) in the management of *Bt* engineered crops- high levels of constitutive expression of single toxins in all plants, high levels of constitutive expression in two or more toxins in all plants, spatial or temporal mixtures of plants with high levels of constitutive expression of one or more toxins and other plants with no toxin expression, low levels of expression of single toxins interacting with the pests' natural enemies and targeted gene expression. Gould (1991) explained that there some theoretical and practical advantages and disadvantages to these approaches.

Use of high levels of constitutive expression of single toxins in all plants. This approach was based on the assumption that high expression of a single toxin is so effective that only incredible fraction of susceptible or partially resistant individuals will survive. This viewpoint is naïve given the history of insect adaptation to insecticides (*Georghiou 1990*). Although some insects may not adapt to a pesticidal crop, this is not a reasonable, general expectation.

Use of multiple toxins. In a study conducted by *Van Rie et al (1990)* on *Plodia interpunctella.*, they provided evidence that resistance was mediated by heritable change in toxin membrane binding at receptor proteins in the insect's midgut. They also suggested that by using *Bt* toxins with different binding properties, in combination or sequentially, resistance could be prevented or delayed. This strategy will be highly effective only if and only if cross-resistance will not occur. *Gould et al* (1992) argued the possibility of a strain of insects resistant to one *Bt* toxin to exhibit cross-resistance to another toxin. A laboratory strain of tobacco budworm, Heliothis virescens which developed 50-fold resistance to Cry1Ac was found to be resistant to Cry1Aa, Cry1Ab, Cry1B and Cry1C. Thus managing

Table 13. Diet consumed, larval weight changes of late third instar larvae of *C. suppressalis* feeding on different regimes of Dipel, strains HD73 and 1715 of *B. thuringiensis*.

TREATMENT	DIET CONSUMED (g)	LARVAL WEIGHT CHANGES (g)	N
DIPEL			
Control	0.5090 + 0.273	0.0326 + 0.0190	47
0.05 mg/ NC	0.2325 + 0.242	0.0045 + 0.0040	24
		0.0037 + 0.0022	
0.05 / choice			
control side	0.2200 + 0.162	0.0052 + 0.0020	13
toxin side	0.1810 + 0.066	0.0031+ 0.0010	7
both sides	0.1660 + 0.217	0.0045 + 0.0030	11
0.10 mg/ NC	0.2200 + 0.275	0.0085 + 0.0060	14
0.10 choice			
control side	0.3540 + 0.301	0.0168 + 0.0150	23
toxin side	0.0245 (single)	0.0040	1
		0.0020	1
both sides		0.0181 + 0.0130	10
		0.0120 + 0.0190	4
STRAINS HD73 AND 1715			
Control NC	0.3160+ 0.3060	0..0084+. 0.0040	21
No Feeding		0.0072+ 0.0030	4
HD73 /No Choice	0.1730+ 0.1630	0.0018+ 0.0020	18
		0.0055+ 0.0035	5
HD73 Choice			
control side	0.2720 + 0.2590	0.0069 + 0.0040	10
toxin side	0.2810 + 0.2170	0.0018 + 0.0030	6
both sides	0.2570 + 0.1960	0.0037 + 0.0020	6
		0.0054	1
No feeding		0.0056 + 0.0010	2
1715 No choice	0.1810 + 0.1410	0.0030 + 0.0050	8
No feeding		0.0036 + 0.0030	16
		0.0106	1
1715 choice			
Control side	0.4060 + 0.1850	0.0070 + 0.0040	19
toxin side	0.1320 + 0.1480	0.0068 + 0.0020	2
both sides	0.4150 + 0.0360	0.0042 + 0.0030	2

Bt resistance by plain replacement of another when it becomes ineffective may not be successful. The results of this study on variability of the stem borers' response to Bt which showed significant differences in response of local populations to *Bt* supports Gould's view. The differences among populations varied ranging from double to more than 50x the susceptible population thus we could speculate for the potential cross-resistance in stem borers.

Low levels of expression of single toxins interacting with the pest's natural enemies. In this study, it was found that low levels of the toxin caused slow development in the larvae of *C. suppressalis*. This was true not only with those toxins with "knockdown effects" but also those which were not. This strategy was thought to be applicable to the management of stem borers. The use of low levels of *Bt* toxin could lead to interaction with natural enemies since the borer will remain larvae thus exposing them for possible parasitization and predation. This strategy could very much work with leaffolders but problems could be encountered with stem borers. Stem borers remain inside the stem and could continue damage the plant by remaining an immature or larva. Unlike the leaffolders which are exposed and could easily be spotted and become prey to many predators or parasitizes the stem borers inside the stem may be difficult to be controlled by natural enemies. Since the insect remain inside and in the larval form, slow but sure feeding on the tissues will still lead to considerable damage to the plant. Furthermore, this strategy could lead to big fitness differences between susceptible, moderately resistant and resistant individuals. Consider for example, resistant individuals may increase in population density since predators and parasites will affect more the susceptible individuals than the resistant ones (*Gould et al 1991*). Resistant individuals may reproduce more because they could complete more generations than that of susceptibles in which the growth is inhibited. Asynchronous development of the resistant and susceptible individuals could lead to non-random mating.

Targeted gene expression. This strategy gives the stem borers the choice between toxic and non-toxic tissues. Since the pest feeds only in the stem, then the *Bt*-toxin gene will have to be engineered to these tissues. Examination of the results of this study show that stem borers avoid the diet with the *Bt* toxin regardless of the kind. This has big implications in its management. Take for example the introduction of *Bt* genes in rice which are only expressed in the stem or in the younger tissues. When the stem borer is able to enter this engineered plant, reproductive success of the pest is not really assured. The presence of the toxin gene in this tissues will drive the larvae away from the plant thus causing no damage to the plant. Likewise, the larvae may be forced to feed on old, non-nutritional parts of the rice plant thus not only inhibiting its growth but also exposes the larvae to predation and parasitization. If the *Bt* toxins will be expressed in high doses to the stem tissues and no toxin, or extremely low levels of toxin are present in other tissues such as the leaves, this strategy could have promise.

Spatial or temporal mixtures of plants with high levels of one or more toxins and other plants with no toxin expression. Gould (*1992*) believes that mixtures have great advantages as long as two assumptions are met: first, there must be approximate random mating; second, the difference in fitness of pest genotypes in mixture must be related to the ratio of the two types of plants. The problem with this strategy however lies in the results shown in this study. Larval movement is expected since stem borer larvae can detect the presence of the toxin. In this case, movement towards the susceptible rice plants in the mixture will occur thus will still cause yield losses. There are some modifications suggested by *Gould (1992)* that to avoid this problem of movement between *Bt* and non-*Bt* plants is to

Table 14. Mortality distribution of *C. suppressalis* substrains under selection on Cry1Ac delta-endotoxin of *B. thuringiensis*

GENERATION	UNTREATED POPULATION		25µg/ul-TREATED		50µg/µl-TREATED	
	25µg/ml	50µg/ml	25µg/ml	50µg/ml	25µg/ml	50µg/ml
2	91.33	66.97	n.e.	n.e.	n.e.	n.e.
3	n.e.	n.e.	n.e.	n.e	n.e.	n.e.
4	53.22	53.33	54.12.	61.59.	20.69	55.55
5	70.18	72.85	41.05	42.02	56.93	58.20
6	78.49	86.61	66.10	77.17	21.64	50.99
7	90.88	93.19	35.70	60.96	78.14	74.41
8	n.e.	n.e.	47.95	32.16	n.e.	50.53
9	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
10	85.71	76.62	68.39	36.66	15.92	8.78
11	n.e.	n.e.	64.39	61.64	n.e.	n.e.
12	65.57	79.08	53.31	55.57	52.58	68.18

create a mixture at the field to field level and not plant by plant level. If this will be done, random mating and development of resistance will be expected to develop in *Bt*-fields.

Discussion

Recent developments in host plant resistance involves the incorporation of *Bt* endotoxin genes in rice genome through genetic engineering. This approach appeared to be a good and sound alternative source for resistance for rice since the endotoxins produced by the bacterium were perceived to be environmentally safe, highly specific, active at very low concentrations, have minimal adverse effects on non-target organisms, and compatible with other methods of control (Wilcox et al 1986). The authors however, believed that no assurance could be made that this approach will not suffer the same fate as insecticides. In the Philippines, many strains have been found to be highly effective against the Asiatic corn borer, *Ostrinia furnacalis* (Guenee), diamond backmoth, *Plutella xylostella* (L.) and mosquito (Padua et al 1980). Introduction of the *Bt* endotoxin genes in the rice genome through genetic engineering to control stem borers is believed to be highly advantageous even if the borers have found themselves inside the stem.

As early as 1987, genetic engineers had moved the gene codes for *Bt* toxins into tomato, tobacco, and potato and several laboratories have already incorporated the *Bt* genes into rice (Vaeck et al 1987). Genetic engineering of rice is now possible through direct uptake of DNA into protoplasts and regeneration of these protoplasts back into mature plants, as devised by Hodges et al (1991). This development has stimulated interest in a *Bt* Berliner deployment system for transgenic rice that mimics the insecticidal action of the insect pathogen (Sanchez-Serrano et al 1987, Gasser and Fraley 1989). If this work succeeds, it may be possible to plant rice capable of causing more than 90% mortality of the stem borers throughout Asia and Africa.

Success at engineering rice as described above could lead to significant increases in ments require studies about the ecology and evolutionary genetics of the organism in the cropping system of concern. This study attempted to collect some of the very important information on the rice-stem borer systems. It is felt that prior to deploying rice varieties with engineered *Bt* endotoxin gene against stem borers, it is important to know more about the ecology and genetics of this pest species.

Summary and Conclusion

The *Bt* gene had been successfully engineered in several crops and it will not be long that its transfer and stable expression in rice will be realized. Engineering one very effective *Bt* gene in rice does not mean a successful, sustainable agriculture. There are so many unresolved issues and straightforward scientific questions unanswered. Understanding the complexities in the interactions would mean a better chance in the deployment of the technology in a sustainable manner. To achieve this requires good evaluation for useful *Bt* genes, assessment of the potential for resistance in rice pests and in this case the stem borers, transformation technology and gene expression, field tests of strategies to delay resistance, germplasm development and distribution. Thus there is a need to understand ecological and genetic interactions between *Bt* toxins, rice and rice pests and natural enemies. The results of this study provides some answers to some of these needs.

As to the search for a useful *Bt* gene, the results of the study show that there are some *Bt* genes which could serve as candidate for introduction into the rice genome based on several criteria: lack of genetic homology with toxins of standard strains, high efficacy at killing specific stem borer pests and/unique mode of action. These are Cry1Ac for the striped stem borer and Cry1Ac, Cry11A, and Cry1C for the yellow stem borer. The striped stem borer is very susceptible to Cry1Ac but not with the other two endotoxins, Cry11A and Cry1C which were found to be as equally effective as Cry1Ac against yellow stem borer. Another important information derived from this study is that those *Bt* endotoxins which has no "knockdown effects" are also effective not by killing but by avoidance of the insects and inhibition of the growth of the larvae. This information is important to develop strategies in the proper deployment of the engineered rice.

To assess the potential for resistance in rice stem borers, a need for a transgenic rice for selection is important. Since there is no available plant at present, understanding the genetic structure of populations based on variability in some ecological characteristics such as host range and diets with specific endotoxins was done as alternative. An understanding of the pest movement based on genetic structure is essential for the logical development of resistant management strategies. The results of this study show geographical variation in response to different endotoxin *Bt*. This only shows that any *Bt*-transgenic rice will not have the assurance of complete success when deployed in all the local fields where it will be planted. It was shown in this study that resistance to the very toxic Cry1Ac varied from population to population thus there is a possibility that populations of these insects have the potential to overcome the effect of the *Bt* toxins expressed in rice. The problem will be compounded with large movements of these resistant insects to adjacent rice fields and cause big yield losses.

The practices of farmers of heavy pesticide use may contribute to the failure of the *Bt*-transgenic rice. It was found in a local survey of farmers management of rice pests that regardless of the rice variety whether it is resistant or not, these varieties are still being sprayed with insecticides. It is most likely that when *Bt*-rice will be deployed, this will be treated just like any other varieties. Considerable effort should be exerted to help farmers realize the proper way of managing rice and the pests affecting it without much reliance on pesticides. This could be done through farmers education and participatory training in pest management.

References Cited

Bernardo, E. N 1969. Varietal resistance to stem borers, pp. 101-105. In Eleventh Annu. Progress Rep. Rice and Corn Res. Program, for 1968-1969.

Bottrell, D. G., R. M. Aguda, F. L. Gould, W. Theunis, C. G. Demayo, and V. F. Magalit. 1992. Potential strategies for prolonging the usefulness of *Bacillus thuringiensis* in engineered rice. Korean J. Appl. Entomol. 31: 247-255.

Gould, F. 1988a. Evolutionary biology and genetically engineered crops. Bioscience 38: 26-33.

Gould, F. L. 1988b. Stress specificity of maternal effects in *Heliothis virescens* (Lepidoptera: Noctuidae) larvae. Mem. Can. Ent. Soc. 146: 191-197.

Gould, F. L. 1991. The evolutionary potential of crop pests. Am. Sci. 79: 496-507.

Gould, F. L. 1986. Simulation models for predicting durability of insect-resistant germplasm: Hessian fly (Diptera: Cecidomyiidae) - resistant winter wheat. Environ. Entomol. 15: 11-23.

Gould, F. L. and A. Anderson. 1991. Effects of *Bacillis thuringiensis* and HD-73 delta endotoxin on growth, behavior, and fitness of susceptible and toxin-adapted strains of *Heliothis virescens* (Lepidoptera:Noctuidae).

Gould, F. L., A. Anderson, D. Landis, and H. Van Mellaert 1991. Feeding behavior and growth of Heliothis larvae on diets containing *Bacillus thuringiensis* formulations or endotoxins. Entomol. Exp. Appl. 58: 199-210.

Gould, F.L., A Martinez-Renirez, A. Anderson, J. Terre, F.J. Silva, and L.D. Moor. 1992. Broad-spectrum to *Bacillus thuringiensis* toxins on *Helioths virescens*. PNAS (USA) 89: 7986-7990.

Hodges, T. K., J. Peng, L. A. Lyznik and D.S. Koetje. 1991. Transformation and regeneration of rice protoplasts, Chap. 7: 157-174. In: Rice Biotechnology, G. S. Khush and G. H. Toenessin eds. C.A.B. Int. Waalingford.

Hofte, H. and H. R. Whiteley 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiol. Rev. 53: 242-255.

Khan, Z. R., J. A. Litsinger, A. T. Barrion, F. F. D. Villanueva, N. J. Fernandez and L. D. Taylo. 1991. World Bibliography of rice stem borers 1794-1990. IRRI-ICIPE, Los Baños, Philippines. 415 pp.

Knight, A. L. and G. W. Norton. 1989. Economics of agricultural pesticide resistance in arthropods. Ann. Rev. Entomol. 34: 293 -313.

McGaughey, W. H. and M. E. Whalon. 1992. Managing insect resistance to *Bacillus thuringiensis* toxins. Science 258: 1451-1455.

National Research Council 1986. Pesticide resistance : Strategies and tactics for management. National Academy of Sciences, Washington D.C. : 471 pp.

Padua, L. E., M. Ohba, and K. Aizawa 1980. The isolates of *Bacillus thuringiensis* serotype 10 with a highly preferential toxicity to mosquito larvae. J. Invert. Pathol. 36: 180-186.

Raffa, K. F. 1989. Genetic engineering of trees to enhance resistance to insects. BioScience 39: 524-534.

Tabashnik, B. E. 1983. Host range evolution: the shift from native legume hosts to alfalfa by the butterfly, Colias philosidae eriphyle. Evolution 37: 150-162.

Tabashnik, B.N. Finson and M. Johnson. 1991. Managing resistance to *Bacillus thuringiensis* from the dianord backnoth (Cepidoples: Plustellida). J. Econ Entomol 84(1) 149-55

Tabashnik, B.N.. Cushing, N. Finson, M.W. Johnson 1990. Field development of resistance to *Bacillus thuringiensis* in diamond backmoth (Lepidoptera: Pyralidae) by *Bacillus thuringiensis*. J. Econ Entm. 82 (2). 416-419

Vaeck, M., A. Reynaerts, J. Hoftese, S. Jansens, M. Debeukeleer, C. Dean, M. Zabeau, M. Van Montagu, and J. Leemans 1987. Transgenic plants protected from insect attack. Nature 328: 33-37.

Van Rie, J., S. Jansens, H. Hofte, D. Deghelee, and D H. Van Mellaert. 1989. Specificity of *Bacillus thuringiensis* delta-endotoxins. Importance of specific receptors on the brush border membrane of the midgut of target insects. Eur. J. Biochem. 186:239-247.

Van Rie, J., W. H. McGaughey, D.E. Johnson, B. D. Barnett, and D. H. Van Mellaert. 1990. Mechanism of insect resistance to the microbial insecticide *Bacillus thuringiensis*. Science 247: 72-74.

Wilcox, D. R., A. G. Sghivakumar, B. E. Melin, M. F. Miller, T. A. Benson, C. W. Schopp, D. Casuto, G. H. Gundling, T. J. Bolling, B. B. Spear, and J. L. Fox 1986. Genetic engineering of biopesticides. In: M. Inoue and R. Sharma (eds), protein engineering: Applications in Science, Medicine, and Industry. Academic Press, Orlando, Florida, pp. 395-413.