SELECTION OF THE FALL ARMYWORM, S*podoptera frugiperda* (SMITH) (LEPIDOPTERA: NOCTUIDAE) FOR SURVIVAL ON CRY 1A(b) *Bt* TOXIN

FRANCYS M. F. VILELLA[1], JOSÉ M. WAQUIL[2], EVALDO F. VILELA[1], BLAIR D. SIEGFRIED[3], JOHN E. FOSTER[3]

[1]*Departamento de Biologia Animal, Universidade Federal de Viçosa. CEP. 36571-000 Viçosa, MG.*
[2]*Pesquisador, Embrapa Milho e Sorgo/Bolsista do CNPq. Caixa Postal 151, CEP. 35701-970 Sete Lagoas, MG. E-mail: waquil@cnpms.embrapa.br (autor para correspondência)*
[3]*Department of Entomology/UN-L, Lincoln, NE, USA.*

Revista Brasileira de Milho e Sorgo, v.1, n.3, p.12-17, 2002

ABSTRACT - Transgenic maize hybrids expressing *Bacillus thuringiensis* (*Bt*) toxin are considered an important technology to control lepidopteran pests of maize. *Bt* maize hybrids expressing the Cry1A(b) toxin have shown a significant level to fall armyworm (FAW), *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), resistance, although larvae survival has been observed on these hybrids. In addition, *S. frugiperda* resistance to different insecticides has been reported. Increased tolerance to Cry1A(b) toxin was found on fall armyworm populations after four generations selection. This result indicates that FAW tolerance to Cry1A(b) toxin had a heritable component.

Key words: Insecta, resistance, transgenic plants.

SELEÇÃO DA LAGARTA-DO-CARTUCHO, S*podoptera frugiperda* (SMITH) (LEPIDOPTERA: NOCTUIDAE) PARA SOBREVIVÊNCIA NA TOXINA DO *Bt* CRY 1A(b)

RESUMO - Plantas transgênicas que expressam toxinas da bactéria *Bacillus thuringiensis* (*Bt*) representam um importante avanço para o controle de lepidópteros-pragas de milho. Híbridos de milho *Bt* expressando a toxina Cry1A(b) têm mostrado significativo nível de resistência à lagarta-do-cartucho (LCM), *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), embora larvas dessa espécie tenham sido observadas em campo e em laboratório sobrevivendo nesses híbridos. Resistência de *S. frugiperda* também tem sido registrada para diferentes inseticidas. Em bioensaios, seleção de insetos sobreviventes mostrou um aumento do nível de tolerância à toxina do *Bt* Cry1A(b) em populações da lagarta-do-cartucho, após quatro gerações, indicando que essa tolerância é herdada.

Palavras-chave: Insecta, resistência, plantas transgênicas.

Maize is one of the most important crops in the world agribusiness and it is cultivated all over the world. As forage or grain, corn represents about 70% of all food material for poultry, swine, beef and other small animals. Pest control is one of the most important factors to be considered in maize crops and insect resistance to pesticides is a major problem in modern agriculture (Tabashnik, 1994).

The common soil bacterium *Bacillus thuringiensis* (*Bt*) produces crystals containing proteins that are toxic to certain insects, but are harmless to most other organisms including people,

wildlife and most beneficial insects (Schnepf 1998). *Bt* toxins have been shown to be completely safe to users and the environment. The extensive laboratory studies including mammalian toxicity studies, coupled with no reported cases of human or animal disease after more than 15 years of widespread use demonstrate that the tested isolates are not toxic or pathogenic (Rechcigl *et al*. 2000). So, genes encoding *Bt* toxins have been incorporated into genomic and expressed by crop plants, thus providing environmentally benign control of insect pests. On the other hand, some maize pest species such as fall armyworm (FAW), *Spodoptera frugiperda* have presented a challenge to the Integrated Pest Management (IPM) technology using *Bt* bioinsecticide (Waquil *et al* 1982). Recently, some *Bt* maize hybrids expressing the Cry1A(b) toxin have shown to provide a significant level of control, although FAW survivors have been observed on these hybrids (Williams *et al*. 1997 and 1998). In addition, FAW resistance to different insecticides has been reported (Diez-Rodríguez, 2000).

Evolution of resistance by pests is the most serious threat to the continued efficacy of *Bt* toxins (Heckel *et al*. 1999). Although the diamondback moth (*Plutella xylostella)* is the only reported insect with resistance to *Bt* toxins in open-field populations, laboratory selection has produced resistance in several other species of Lepidoptera (Tabashnik 1994). Most models to predict *Bt* resistance in insects assume that resistance is attributed to a mutation at a single locus. In contrast, quantitative genetic models make no assumption regarding the number of genes involved, and the expression of a trait is assumed to depend on environmental as well as genetic factors (Firko & Hayes 1990). As a tool for resistance assessment, quantitative genetic enables predictions to be made regarding the speed and magnitude of genetic changes associated with resistance (Alinia *et al*. 2000). Despite their enormous economic and ecological importance, surprisingly,little is known about the genetic of most Lepidoptera (Heckel *et al*. 1999), specially the process related to the resistance against *Bt* toxins. However no information is available whether the survivors are genetically different. Thus, selection of laboratory colonies for genetic resistance provides a model to study the potential evolution of resistance.

The study was conducted at the Department of Entomology, University of Nebraska, Lincoln, NE, USA during the year of 2000.

Insect population

FAW eggs were obtained from a laboratory colony maintained by DeKalb Agricultural Research in Union City, TN. The insects were reared on artificial diet and maintained at 28° C.

Bioassays

Bt bioassays of neonate larvae were performed according to Marçon *et al*., 2000. The bioassay was performed using trays with 128-wells each. Approximately 1 mL of the diet was dispensed into each well and allowed to solidify. Each well containing diet was treated with 30 µL of a Cry1A(b) toxin concentration prepared in 0.1% Triton-X100 nonionic detergent. Control treatment was 0.1% Triton-X100 only. All treatments were allowed to dry and one neonate FAW larva was transferred to each individual well. Ten days after infestation, the treatments were analyzed by counting the number of FAW survivors and recording the weight of each surviving larva.

According to the above methodology, the LC_{50} of Cry1A(b) to neonate FAW was estimated using the following Cry1A(b) toxins concentrations: 15.62, 31.25, 62.5, 125, 250, 500 and 1000 ηg cm^{-2}. The percentage of mortality was corrected by Abbot's formula and then submitted to Probit analysis and the LC_{50} of the original lab population of FAW was estimated. The F_o population was screened on

maize plants expressing Cry1A(b) toxin, obtaining families F_1. Progenies from single pair mating were exposed to the calculated LC_{50} and families with surviving FAW larvae ≥ 60% than control larval biomass were considered tolerant to the toxin. On the other hand, larval biomass ≤ 10% than biomass of the control were considered susceptible.

This procedure was performed to establish susceptible and resistant populations based on survivorship as well as biomass increse. The proportion of surviving insects for each selected family was compared to the expected 50% of mortality by Z test.

Nineteen families were selected in F_1, eight in F_2 and six in F_3 generation (Table 1). Based on the LC_{50} of 690 ηg cm^{-2} with results of Probit analysis for the original population (F_0) (Figure 1).

Expected mortality for each family in generations F_1, F_2, and F_3 was 50%, but in the F_1 generation seventeen families showed mortality under 50% and two families above 50% (Table 1). Just one family was considered susceptible in F_2 generation and one in F_3 generation (Table 1). After four generations, the F_4 family 527C4II5III6 (not showed) was used to estimate a new LC_{50} with the same procedure, but with higher concentrations: 125, 250, 500. 1000, 2000, 4000 and 8000 ηg cm^{-2}. The LC_{50} was 3,770.98 ηg cm^{-2} with a 5 fold increase in relation to the original population (Figure 1).

FAW larvae surviving to Cry 1A(b) exposure are more tolerant and it has a heritable component. This shows a potential to increase proportion of survivors of FAW on *Bt*-toxins Cry 1A(b) through generations with the low dose strategy. However, extrapolation of such results to field conditions should be carefully considered because *Bt* maize employs a higher dose strategy and due to a significant reduction on fitness of insects based on a significant biomass reduction of surviving larvae. Also, under field conditions the possible tolerant individual to *Bt* could dilute these conditions through mating with susceptible individual. This is more limited in laboratory conditions because the population used is smaller. Adanczyk & Sumerford (2001) found increased tolerance to Cry 1A(c) transgenic *Bt* cotton but no evidence of fitness or vigor differences from FAW progeny previously fed on conventional or transgenic *Bt* cotton.

Also, it was estimated the biomass of all insects. It was observed no correlation between percentage of mortality and percentage of growth inhibition (biomass base) suggesting independent traits.

Acknowledgments

The authors thank the Brazilian Agencies "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)" and the "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)" for Scholarships and financial support. Also, we thank Terrance Spencer for helping with the bioassays. We wish to thank Monsanto for the purified toxin and Dekalb for the Fall Armyworm used in this study. And Dr. José Cola Zanuncio and Dr. José Henrique Schoereder /UFV for reviewing the originals.

TABLE 1. Family generation,biomass of control, biomass of survivor, percentage of mortality, number of insects evaluated and status of each family of FAW survivor on *Bt* toxin for 3 generations.

eneration	Family	Biomass of control	Biomass of survivor	Mortality (%)	n	Status**
F1	507	387.31	81.51	10.42	64	R
F1	514	269.48	29.01	45.87*	64	S
F1	516	254.75	92.99	9	126	R
F1	519	336.47	85.74	13.97	128	R
F1	522	294.78	160.25	0	64	R
F1	524	213.47	31.89	10.42	127	R
F1	527	332.77	112.18	6.25	127	R
F1	530	141.54	55.22	16.37	125	R
F1	602	145.17	97.49	0	39	R
F1	625	213.21	54.11	0	128	R
F1	626	304.74	101.12	4.17	128	R
F1	628	220.73	22.47	27.08	128	R
F1	629	255.35	89.65	0	128	R
F1	630	259.3	70.89	0	128	R
F1	631	191.42	43.63	13.57	128	R
F1	632	224.2	52.52	0	128	R
F1	647	150.39	0.99	78.57*	128	SS
F1	653	271.17	32.27	7.29	128	R
F1	679	278.35	20.29	8.33	128	R
F2	507C1	310.32	49.94	13.59	244	R
F2	514C2	211.76	22.7	13.66	251	R
F2	514C3	162.17	18.22	63.84*	256	S
F2	527C4	131.21	47.36	12.88	382	R
F2	527C9	242.59	49.51	8.16	256	R
F2	527C12	222.34	41.55	21.46	256	R
F2	530C4	184.34	29.53	12.69	128	R
F2	632C1	220.56	27.95	34.32	768	R
F3	507C1II1	239.75	32.04	25.73	116	R
F3	514C2II3	149.05	27.34	0	128	R
F3	527C4II1	283.33	15.44	24.11	256	R
F3	527C4II5	257.97	6.88	65.08*	256	S
F3	527C4AII10	248.05	26.87	6.73	255	R
F3	527C4BII5	303.53	20.64	17.5	76	R

*Percentages of insect mortality with no significant differences from 50 %.
**S stands for susceptibility, SS for high level of susceptibility and R for resistance.

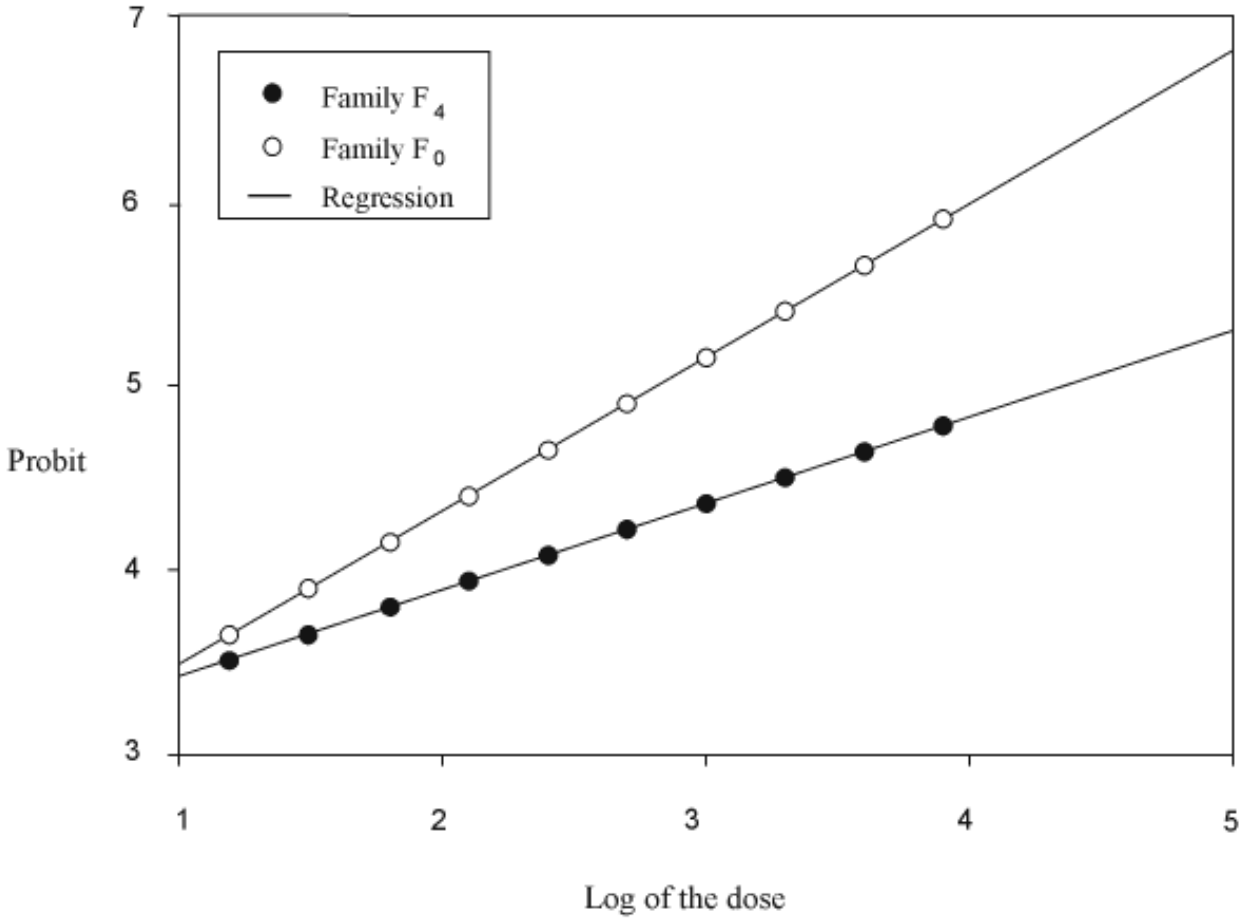

FIGURE 1. Probit analysis for F_0 and F_4 generations showing different dose response.

References

ADANCZYK, J. J. JR.; SUMERFORD, D. V. Increased tolerance of fall armyworms (Lepiodptera: Noctuidae) to Cry 1Ac δ-endotoxin when fed transgenic *Bacillus thuringiensis* cotton: impact on the development of subsequent generations**. Florida Entomologist,** Gainsville, v**.** 84, p. 1-6, 2001**.**

ALINIA, F.; COHEN, M. B.; GOULD, F. Heritability of tolerance to the Cry 1A(b) toxin of *Bacillus thuringiensis* in *Chilo suppressalis* (Lepidoptera: Crambidae). **Journal of Economic Entomology,** College Park, v. 93, p. 14-17, 2000.

DIEZ-RODRIGUEZ, G. I. **Herança e custo do valor adaptativo da resistência de *Spodoptera frugiperda* (Lepidóptera: Noctuidae) ao piretróide lambda-cialotrina.** 2000. 59 f. Dissertação (Mestrado) – Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba.

FIRKO, M. J.; HAYES, J. L. Quantitative genetic tools for insecticide resistance risk assessment: estimating the heritability of resistance. **Journal of Economic Entomology,** College Park, v. 83, p. 647-654, 1990**.**

HECKEL, D. G.; GAHAN, L. J.; LIU, Y.; TABASHNIK, B. E. Genetic mapping of resistance to *Bacillus thuringiensis* toxins in diamondback moth using biphasic linkage analysis. **Proceeding of**

National Academy of Science of the USA, Washington, v. 96, p. 8373-8377, 1999.

MARCON, P.C.R.G.; SIEGFRIED, B. D.; SPENCER, T.; HUTCHISON, W. D. Development of diagnostic concentration for monitoring *Bacillus thuringiensis* resistance in European Corn Borer (Lepidoptera: Crambidae). **Journal of Economic Entomology,** College Park, v. 93, p. 925-930, 2000.

RECHCIGL, J. E.; RECHCIGL, N. A. **Insect pest management**: techniques for environmental protection. Boca Raton: **Lewis Publisher,** 2000, 392 p.

SCHNEPF, E.; CRICKMORE, N.; VAN RIE, J.; LERECLUS, D.; BAUM, J.; FEITELSON, J.; ZEIGLER, D. R.; DEAN, D. H. *Bacillus thuringiensis* and its pesticidal crystal proteins. **Microbiology and Molecular Biology Reviews**, Washington, v. 62, p. 775-806, 1998.

TABASHNIK, B. E. Evolution of resistance to *Bacillus thuringiensis***. Annual Review of Entomology**, Palo Alto, v. 39, p. 47-79, 1994.

WAQUIL, J. M.; VIANA, P. A.; LORDELO, A. I.; CRUZ, I.; OLIVEIRA, A. C. Controle da lagarta-do-cartucho em milho com inseticidas químicos e biológicos**. Pesquisa Agropecuária Brasileira,** Brasília**,** v. 17, n. 2, p. 163-166, 1982.

WILLIAMS, W. P.; BUCKLEY, P. M.; SAGERS, J. B.; HANTEN, J. A. Evaluation of transgenic corn for resistance to corn earworm (Lepidoptera: Noctuidae), fall armyworm (Lepidoptera: Noctuidae), and southwestern corn borer (Lepidoptera: Noctuidae) in a laboratory bioassay. **Journal fo Agriculture Entomology,** Clemson, v. 15, p. 105-112, 1998.

WILLIAMS, W. P.; SAGERS, J. B.; HANTEN, J. A.; DAVIS, F. M.; BUCKLEY, P. M. Transgenic corn evaluated for resistance to fall armyworm and southwestern corn borer. **Crop Science**, Madison, v. 37, p. 957-962, 1997.