the Cry1Ab toxin from *Bacillus thuringiensis*. J. Econ. Entomol. 93: 1265-1268.

Sims, S. R.; Greenplate, J. T.; Stone T., B.; Caprio, M. A. and Gould, F. L. 1996. Monitoring strategies for early detection of Lepidoptera resistance to *Bacillus thuringiensis* insecticidal proteins. Resist. Pest Manag. 9: 21-24.

Sparks, T. C.; Thompson, G. D.; Larson, L. L.; Kirst, H. A.; Jantz, O. K.; Worden, T. V.; Hertlein, M. B. and Busacca, J. D. 1995. Biological characteristics of the spinosyns: a new class of naturally derived insect control agents. *In*: National Cotton Council (eds.). Proc. Beltwide Cotton Conf. San Antonio, TX. p. 903- 907.

Sparks, T. C.; Thompson, G. D.; Kirst, H. A.; Hertlein, M. B.; Larson, L. L.; Worden, T. V. and Thibault, S. T. 1998. Biological activity of the spinosyns, new fermentation derived insect control agents, on tobacco budworm (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol. 91: 1277- 1283.

Stadelbacher, E. A.; Snodgrass, G. L. and Elzen, G. W. 1990. Resistance to cypermethrin in first generation adult bollworm and tobacco budworm (Lepidoptera: Noctuidae) populations collected as larvae on wild geranium, and the second and third larval generations. J. Econ. Entomol. 83: 1207- 1210.

Stewart, S. D.; Adamczyk Jr., J. J.; Knighten, K. S. and Davis, F. M. 2001. Impact of Bt cotton expressing one or two insecticidal proteins of *Bacillus thuringiensis* Berliner on growth and survival on Noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94: 755- 760.

Stone, T. B. and Sims, S. R. 1993. Geographic susceptibility of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctiudae) to *Bacillus thuringiensis*. J. Econ. Entomol. 86: 989-994.

Sudbrink Jr., D. L. and Grant, J. F. 1995. Wild host plants of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) in Eastern Tenessee. Entomological Society of America 24:1080-1085.

Tabashnik, B. E. 1994. Evolution of resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. 39: 47-79.

Terán-Vargas, A. P.; Rodríguez, J. C.; Blanco, C. A.; Martínez-Carrillo, J. L.; Cibrián-Tovar, J.; Sánchez-Arroyo, H.; Rodríguez-Del-Bosque, A. and Stanley, D. 2005. Bollgard Cotton and resistance of Tobacco Budworm (Lepidoptera: Noctuidae) to conventional insecticides in Southern Tamaulipas, Mexico. J. Econ. Entomol. 98: 2203-2209.

Traxler, G.; Godoy-Avila, S.; Falck-Zepeda, J. and Espinoza-Arellano, J. J. 2002. Transgenic cotton in Mexico: economic and environmental impacts. *In*: Proceedings of the 5th International Conference on Biotechnology. Ravello, Italy. p. 183- 201.

United States Environmental Protection Agency (USEPA). 2001. Biopesticides Registration Action Document (BRAD)- *Bacillus thuringiensis* Plant Incorporated Protectants. USEPA, October 15, 2001. (protocol:// www.epa.gov/pesticides/pips/bt-brad.htm.).

Usmani, K. A. and Knowles, C. O. 2001. Toxicity of pyretroids and effect of synergists to larval and adult *Helicoverpa zea*, *Spodoptera frugiperda* and *Agrotis ipsilon* (Lepidoptera: Noctuidae). J. Econ. Entomol. 94: 868-873.

Wolfenbarger, D. A.; Lukefahr, M. J. and Graham, H. M. 1971. A field population of bollworm resistant to methyl- parathion. J. Econ. Entomol. 64: 755- 756.

Wu, K. and Gou, Y. 2004. Changes in susceptibility to conventional insecticides of a Cry1Ac- selected populations of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Pest Manag. Sci. 60: 680-684.

Wu, K.; Mu, W.; Liang, G. and Gou, Y. 2005. Regional reversion of insecticides resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) is associated with the use of Bt cotton in northern China. Pest Manag. Sci. 61: 491-498.

Katina Stamatiu-Sánchez[1§]; Laura D. Ortega-Arenas[1];
Celina Llanderal-Cázares[1]; Hussein Sánchez-Arroyo[1];
Urbano Nava-Camberos[2]; Antonio P. Terán-Vargas [3]
[1]Programa de Entomología y Acarología, Instituto de Fitosanidad del Colegio de Postgraduados, Campus Montecillo, Km. 36.5 Carretera México- Texcoco, 56230, Montecillo, Texcoco; Edo. de México, México. [2]Campo Experimental La Laguna, INIFAP, Km. 17.5 Carretera Torreón- Matamoros, 27000, Torreón, Coahuila, México.
[3]Campo Experimental Sur de Tamaulipas, INIFAP, Km. 55 Carretera Tampico- Mante, 89619, Est. Cuauhtémoc, Tamaulipas, México.
[§]Author for e-mail communication: katina@colpos.mx.

Ovipositional and feeding preferences of *Helicoverpa armigera* towards putative transgenic and non-transgenic pigeonpeas

ABSTRACT: *Helicoverpa armigera* is the major constraint for pigeonpea production, and therefore, efforts are being made to develop transgenic pigeonpeas with *Bt* and *SBTI* genes to minimize the losses due to this pest. The oviposition behavior of *H. armigera* on transgenic and non-transgenic plants was studied under no-choice, dual-choice, and multi-choice conditions. No differences were observed in the number of eggs laid on the inflorescences of the transgenic pigeonpeas with *cry1Ab* or *SBTI* genes and with the non-transgenic plants. In dual-choice feeding tests, there were no differences in leaf damage, larval weights, and the number of larvae between transgenic and non-transgenic plants. The results suggested that transgenic plants have no influence on the oviposition and feeding preferences of *H. armigera*.

Pigeonpea (*Cajanus cajan* (L.) Millsp.) plays an important role in nutritional security as an important source of high quality dietary proteins. It is damaged by over 150 insect species, of which *Helicoverpa armigera* (Hubner) is the most important pest, which causes an estimated annual loss of US$ 317 million in the semi-arid tropics in pigeonpea (ICRISAT, 1992). In an effort to minimize the *H. armigera* damage, transgenic pigeonpea plants with *Bacillus tthuringiensis* (*Bt cry1Ab*) and soybean trypsin inhibitor (*SBTI*) genes have been developed recently (Sharma *et al*., 2006). Genetic transformation of crops leads to slight changes in the chemical composition, which might influence host selection and colonization by the insects. Therefore, we studied the oviposition preference by females and feeding preference by the *H. armigera* larvae on transgenic and non-transgenic plants of pigeonpea.

MATERIALS AND METHODS

The pigeonpea varieties, ICPL 88039 and ICPL 87 that were transformed using the constructs pHS 723: *Bt cry1Ab* and pHS 737: *SBTI* through *Agrobacterium tumefaciens*-mediated transformation (Sharma *et al.* 2006) were raised in a containment (P_2 level) green house at 24 to 28°C, 70 to 80% RH. The

H. armigera culture was maintained under laboratory conditions of 24^{o}C and 70% RH (Armes *et al*., 1992).

Oviposition preference

The oviposition behavior of *H. armigera* was studied under no-choice, dual-choice (in comparison to the non-transgenic control), and multi-choice conditions (all the test genotypes placed inside the cage). Fresh inflorescences (20 cm long) with flowers and tender leaves were collected from the greenhouse, and placed in a conical flask (150 ml) filled with water. A cotton swab was wrapped around the stem to keep the inflorescence in upright position. For no-choice tests three pairs, and for dual- and multi-choice tests four pairs of two-day old moths were released inside the cage. Sucrose solution (10%) in a cotton swab was offered to the adults as a food, changed on alternate days. The number of eggs laid by the moths was recorded, and the inflorescences were replaced daily. The experiments were replicated six times in a completely randomized design. Percentage of eggs laid on each plant was calculated from the total number of eggs laid. The data was subjected to analysis of variance. A Student "T" Test was used to test significance of difference in dual-choice tests.

Neonate feeding preference assay

Fully expanded tender leaves of equal size from transformed and non-transformed pigeonpea plants were collected and placed one centimeter apart in a Petri dish arena (9 cm dia) lined with moistened filter paper. Ten neonate larvae were placed in the middle of Petri dish arena. Data on leaf feeding was recorded after 72 hours on a 1 to 9 scale (1 = <10% leaf area damaged and 9 = >80% leaf area damaged). The number of larvae on each leaf and their weights were recorded separately. Each treatment was replicated five times in a completely randomized design.

RESULTS AND DISCUSSION

There were no significant differences in the numbers of eggs laid on the inflorescences of transgenic and non-transgenic control plants under no-choice, multi-choice (Fig. 1), and dual-choice conditions (Table 1). Egg densities of the tobacco budworm (*Heliothis virescens*) (Parker and Luttrell, 1998) and cotton bollworm (*H. armigera*) (Sharma and Pampapathy, 2006) have not been found to be significantly different on transgenic and non-transgenic cottons. The lack of differences in oviposition preference indicated that there are no major changes in the physico-chemical characteristics of the transgenic plants that influence oviposition behavior. This corroborates the earlier observations that the oviposition behaviour of *H. armigera* moths was independent of the presence of transgenes (MacIntosh *et al*., 1990; Orr and Landis, 1997; Ramachandran *et al.*, 1998).

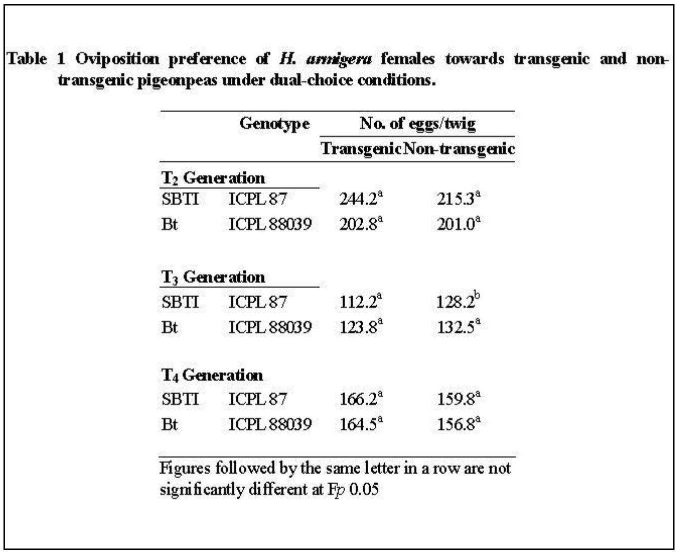


Table 1 Oviposition preference of *H. armigera* females towards transgenic and non-transgenic pigeonpeas under dual-choice conditions.

Genotype		No. of eggs/twig	
		Transgenic	Non-transgenic
T_2 Generation			
SBTI	ICPL 87	244.2^a	215.3^a
Bt	ICPL 88039	202.8^a	201.0^a
T_3 Generation			
SBTI	ICPL 87	112.2^a	128.2^b
Bt	ICPL 88039	123.8^a	132.5^a
T_4 Generation			
SBTI	ICPL 87	166.2^a	159.8^a
Bt	ICPL 88039	164.5^a	156.8^a

Figures followed by the same letter in a row are not significantly different at F*p* 0.05

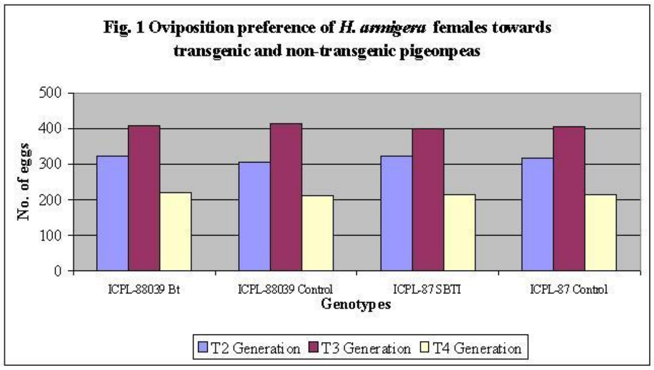
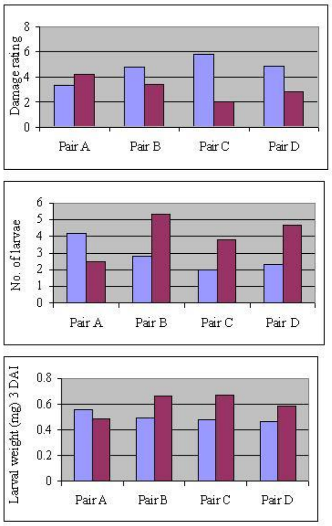


Fig. 1 Oviposition preference of *H. armigera* females towards transgenic and non-transgenic pigeonpeas

T2 and T3 generations were tested under no-choice conditions.
T4 generation was tested under multi-choice conditions.

There are no significant differences in leaf damage, larval weights, and the number of larvae that settled on leaves of transgenic and non-transgenic plants (Fig. 2). Gould *et al.* (1991) observed that *H. virescens* larvae were able to detect and avoid high levels of *B. thuringiensis* toxins in diet. Increased movement and dispersal of *H. virescens* larvae has also been observed on transgenic cotton lines (Benedict *et al.,* 1993; Parker and Luttrell, 1999). Lack of feeding preference by *H. armigera* larvae on transgenic and non-transgenic pigeonpea plants may be because of low levels of expression of toxin proteins in transgenic pigeonpeas, which do not result in perceptible changes in insect behaviour and development.

Fig. 2: Feeding preference of neonate larvae of *H. armigera* towards leaves of transgenic and non-transgenic pigeonpea plants in dual-choice tests (2002 rainy season)



Pair A : Bt ICPL 88039 and its non-transgenic control
Pair B : SBTI ICPL 87 and its non-transgenic control
Pair A : Bt ICPL 88039 and SBTI ICPL 87
Pair A : Non-transgenic controls of ICPL 88039 and ICPL 87

REFERENCES

Armes, N. J., Bond, G. S., and Cooters, R. J. 1992. The laboratory culture and development of *Helicoverpa armigera*. Natural Resources Institute Bulletin No. 57 Natural Resources Institute, Chatham, UK.

Benedict, J. H., Sachs, E. S., Altman, D. W., Ring, D. R., Stone, T. B., and Sims, S. R. 1993. Impact of delta-endotoxin-producing transgenic cotton on insect-plant interactions with *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). Environmental Entomology 22: 1-9.

Gould, F., Anderson. A, Landis, D., and Mellaert, H. 1991. Feeding behavior and growth of *Heliothis virescens* larvae on diets containing *Bacillus thuringiensis* formulations or endotoxins. Entomologia Experimentalis et Applicata 58: 199-210.

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). 1992. The Medium Term Plan, Vol.1. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India.

MacIntosh, S. C., Stone, T. B., Sims, S. R., Hunst, P. L., Greenplate, J. T., Marrone, P. G., Perlak, F. J., Fischoff, D. A., and Fuchs. R. L. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. Journal of Invertebrate Pathology 56: 258-266.

Orr, D., and Landis, D. A. 1997. Oviposition of European corn borer (Lepidoptera: Pyralidae) and impact of natural enemy piopulations in transgenic versus isogenic corn. Journal of Economic Entomology 90: 905-909.

Parker, C. D. Jr., and Luttrell, R. G. 1998. Oviposition of tobacco budworm (Lepidoptera: Noctuidae) in mixed plantings of non-transgenic and transgenic cottons expressing delta-endotoxin protein of *Bacillus thuringiensis* (Berliner). Southwestern Entomology 1998. 23: 247-257.

Parker, C. D. Jr., and Luttrell, R. G. 1999. Interplant movement of *Heliothis virescens* (Lepidoptera: Noctuidae) larvae in pure and mixed plantings of cotton with and without expression of the CryIAc delta-endotoxin protein of *Bacillus thuringiensis* Berliner. Journal of Economic Entomology 92: 837-845.

Ramachandran, S., Buntin, G. D., All, J. N., Tabashnik, B. E., Raymer, P. L., Adang, M. J., Pulliam, D. A., and Stewart, Jr. C. N. 1998. Survival, Development and Oviposition of resistant Diamond back moth (Lepidoptera: Plutellidae) on transgenic canola producing a *Bacillus thuringiensis* toxin. Journal of Economic Entomology 91: 1239-1244.

Sharma, H. C., and Pampapathy, G. 2006. Influence of transgenic cotton on the relative abundance and damage by target and non-target insect pests under different protection regimes in India. Crop Protection 25: 800-813.

Sharma, K. K., Lavanya, M., and Anjaiah, V. 2006. *Agrobacterium*-mediated production of transgenic pigeonpea (*Cajanus cajan* L. Millsp.) expressing the synthetic *Bt cry1Ab* gene. In Vitro Cell and Developmental Biology-Plant 42: 165-173.

S V S Gopala Swamy[1], H C Sharma[2], G V Subbaratnam[1] and M Peter Vijay[2]
[1] Acharya N G Ranga Agricultural University, Rajendranagar, 500 030, Andhra Pradesh
[2] International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, 502 324, Andhra Pradesh

Abstracts in Resistance Management

Genetics and Management of Whitefly Resistance to Pyriproxyfen

Selective insecticides, such as insect growth regulators, that kill pests but cause little or no harm to non-target organisms have become increasingly important in crop production systems worldwide. The insect growth regulator pyriproxyfen has been successfully used for the last decade in Arizona as part of an integrated pest management (IPM) program for the sweetpotato whitefly, *Bemisia tabaci*. *B. tabaci*, a problematic pest in Arizona and other sub-tropical regions throughout the world, damages crops due to direct feeding, transmission of plant viruses, and production of honeydew. The use of pyriproxyfen for *B. tabaci* control has decreased use of broad-spectrum insecticides, preserved natural enemies and beneficial organisms, and increased farmer profits.

A serious threat to the continued success of the IPM program in Arizona is the evolution of insecticide resistance in *B. tabaci*. Despite implementation of a rotation program designed to preserve efficacy of pyriproxyfen, laboratory bioassays tracking the evolution of resistance reveal an area-wide decline in susceptibility to this insecticide, threatening