2005 Beltwide Cotton Conferences, New Orleans, Louisiana - January 4 - 7, 2005

SUSCEPTIBILITY OF FOUR HELIOTHIS VIRESCENS AND HELICOVERPA ZEA REFERENCE COLONIES TO A HOMOGENEOUS CRY1AC-INCORPORATED INSECT DIET: IMPLICATIONS FOR AN AREA-WIDE MONITORING PROGRAM

Carlos A. Blanco
USDA - Agricultural Research Service
Stoneville, MS
Ibrahim Ali and Randall Luttrell
University of Arkansas - Fayetteville
Fayetteville, AR
Sakuntala Sivasupramaniam
Monsanto
Saint Louis, MO
Jose L. Martinez-Carrillo
INIFAP
Ciudad Obregon, Sonora

Abstract

In order to assist the registrants of genetically modified cottons to fulfill their regulatory requirements in the U.S. and Mexico for *Bacillus thuringiensis* resistance monitoring, several research institutions have conducted susceptibility monitoring programs in different cotton regions of North America. Current monitoring is done at a central laboratory and duplicate population samples sent a second facility. Without a standardized methodology to compare or corroborate results across laboratories and scientists, several methodological aspects of obtaining the biological response of *Heliothis virescens* (Fabricius) and *Helicoverpa zea* (Boddie) to *B. thuringiensis* might yield discrepancies when the same insect population is tested by more than one institution / researcher. This study compared the biological response of 2 *H. virescens* and *H. zea* reference colonies from 2 different laboratories using a common standardized Cry1Ac-incorporated insect artificial diet. Mortality was significantly different among two tobacco budworm colonies but not between two bollworm colonies when tested in only one laboratory. When a common diet was tested with the same insect colony at three different laboratories, similar results were found with *H. virescens*, an insect species much more susceptible to Cry1Ac than *H. zea*, a more tolerant species, especially when mortality analysis included surviving first or second instar larvae. When the same colony was tested with 3 different insect diets in 3 different laboratories, the tobacco budworm colony responded significantly different among locations; on the other hand, more pronounced significant differences were found in the bollworm colony. Analysis of the biological response including first instar or first and second instars increased the discrepancies among the research institutions. For accurate comparison purposes among laboratories, the inclusion of a common diet and a common reference laboratory insect colony is recommended.

Introduction

Transgenic *Bacillus thuringiensis* (Berliner) (Bt) cottons produce insecticidal protein(s) from this naturally occurring soil bacterium that protects the plant from certain lepidopteran insect pests (Perlak et al. 2001). The widespread and prolonged exposure to these Bt proteins of targeted pests such as the tobacco budworm (*H. virescens*) and pink bollworm (*Pectinophora gossypiella* [Sauders]) provides a constant selection pressure, representing one of the largest selections for resistance development in insect populations the world has ever seen (Tabashnik et al.. 2003). In the United States and Mexico, the first two countries of the world that commercially planted Bt cottons in 1996, insect resistance management strategies have been mandated by the Environmental Protection Agency (E.P.A.) and the Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (Department of Agriculture, Livestock, Rural Development, Fisheries and Nutrition [SAGARPA]), respectively. Both regulatory agencies require that Bt cotton registrants monitor natural populations for Bt resistance development in specific regions. (E. P. A. 2001). Current Bt-susceptibility monitoring requirements do not specify a particular standardized methodology, and procedures already established by different research institutions vary. The potential variability in data limits the accuracy of comparisons among populations / geographies. There are numerous reports on the effect of *B. thuringiensis* on the insect species utilized in this study (tobacco budworm and bollworm [*Helicoverpa zea* Boddie]) that contain detailed methodology (Ames and Harper 1985, Dulmage et al. 1978, Luttrell et al. 1999), but only one, to our knowledge, (Rishikesh and Quélennec 1983) has addressed results made by different researchers and locations and the potential solutions to the problem of discrepancy of results. Here we

present information of a common 'standardized' methodology followed by 3 different research institutions utilizing a homogeneous Bt-incorporated insect artificial diet and 2 insect colonies for comparison purposes.

Materials and Methods

Two different *Bacillus thuringiensis*-susceptible *Heliothis virescens* and *Helicoverpa zea* reference colonies from the United States Department of Agriculture, Agricultural Research Service (USDA-ARS at Stoneville, Mississippi) and Monsanto Company (Union City, Tennessee) were used for this study. Cry1Ac (obtained from lyophilized MVP II insecticide) incorporated into wheat germ artificial insect diet[2] was prepared at USDA-ARS and sent, by overnight carrier, to all the participating laboratories, including a self-shipment back to USDA-ARS that served as indicator of the effect of overnight transportation on the diet's biological activity. [2]Diet ingredients: Nutri-soy flour (156 g), wheat germ (133 g) Wesson salt (36 g), sugar (156 g), vitamin mix (36 g), agar (85 g), ethyl paraben (3.8 g), sorbic acid (3.8 g), aureomycin (3.8g), distilled water (5.4 mL), propionic acid (4.1 mL) and phosphoric acid (0.4 mL).

Upon receiving the diet, each laboratory prepared a fresh batch of diet with their own materials and methods utilizing the same Cry1Ac concentrations. Diet utilized were wheat-germ diet in USDA-ARS and Monsanto and pinto-bean diet at the University of Arkansas. Unknown batches of MVP II were used for the first 2 *H. virescens* replications and a standard batch was used thereafter and for all the *H. zea* replications. Insecticide samples were provided by Monsanto Company.

One-night oviposition samples of *H. virescens* and *H. zea* eggs from the Monsanto's reference colony were obtained at the same time by the U.S. laboratories for each replication. These colonies were simultaneously compared with the USDA-ARS reference colonies with shipped and fresh diet at USDA-ARS only. A bioassay replicate for both insect species consisted of 15 to 32 2-mL cells (C-D International., Pitman, NJ) containing 1.0 (±15%) mL of diet per Cry1Ac concentration. We utilized 0, 0.019, 0.095, 0.19, 0.95, and 1.9 µg of Cry1Ac active ingredient per mL of diet for *H. virescens* and 0, 1.9, 9.5, 19.0, 47.5, and 95.0 µg of active ingredient per mL for *H. zea*. Cells with diet were infested with one <24-h old neonate, covered with self-adhesive membrane (C-D International., Pitman, NJ) and kept in incubators at 28 ± 2 °C, 75 ± 10% RH and 14-10 hours of light and darkness. This procedure was repeated 4 times in different dates for each insect species. Evaluations were made 7 days later by recording the number of dead larvae (those that did not respond to probing) and estimating the instar development of surviving larvae. Data where analyzed by Probit analysis (SAS Institute) utilizing number of dead larvae and surviving first or first and second instars pooled with dead larvae.

Results and Discussion

In North America, a few laboratories (USDA-ARS, The University of Arkansas and INIFAP) have been conducting for several years the monitoring of Bt-susceptibility shifts of *H. virescens* and/or *H. zea* from geographies where Bt-cotton adoption is high. At the beginning of this study, when we tried to compare results among our 4 research laboratories, many parameters were identified as potentially important in explaining the discrepancies found: insect diet, biological response of each particular colony, lapse between initiation and evaluation, mortality criteria, etc. just to mention a few. Now we have identified some of the most important components for conducting bioassays and propose a tentative method to ensure that what we obtain individually at each laboratory can be correctly interpreted as a group. Two basic biological parameters are discussed here:

A common insect colony. Baseline susceptibility information for 2 different insect colonies was obtained at one laboratory (USDA-ARS) utilizing the same insect artificial diet. The *H.* virescens colonies significantly differed in their response only when freshly prepared Cry1Ac-incorporated diet was used in the analysis (Table 1). But once surviving larvae (first or second instar) were incorporated in the analysis the differences disappeared. Bollworm response was not significantly different between the 2 colonies utilizing any evaluation parameter (diet or mortality criteria) (Table 1). This might be due to the fact that the USDA-ARS colonies were partially infused with Monsanto's colonies in 2004. Therefore, there was a great genetic similarity among the 2 colonies at the time this study was conducted. Significant differences have been found among reference colonies when tested by the same laboratory (Luttrell et al. 1999).

When 3 laboratories utilized the same reference tobacco budworm and bollworm colonies from Monsanto Company, and the standardized diet manufactured and overnight shipped by USDA-ARS, no significant differences were found when analyzing dead larvae, but significant differences were obtained in by 2 laboratories (USDA-ARS and University of Arkansas [U. of AR]) when dead and first and second instar *H. virescens* larvae were included in the Probit analysis (Table 2). Results from adding surviving larvae (first or first and second instars) to the analysis in both insect species produced significant differences among all the laboratories (Table 2). It seems possible that recognition of dead larvae presents less variability, while distinguishing 2^{nd}-3^{rd} instar worms might be very subjective and prone to affect results. Also, the intrinsic variability of bollworm colonies has been recognized by all of us. It is important that the reference colony utilized in these studies has been well characterized in terms of instar development with a particular type of artificial diet. Since the survival of larvae in these bioassays is of the utmost importance for further genetic analysis, handling them should be kept to the minimum. Therefore, determining the instar development by head capsule width, as proposed by Quaintance and Brues (1905) is not very practical; instead it might be better to include both the larval length and weight, once that these parameters have been well established for a particular colony.

Since research institutions and commercial insectaries maintain colonies for a limited time they can serve as an accurate reference. Under the best management practices in these insectaries, an unintended selection process takes place constantly, making one generation different from the previous one. Unless overwhelming procedures get implemented on these colonies and/or detailed genetic characterization at the molecular level is routinely done, the benefit from maintaining a 'reference' colony has a limited life span. However, there are research institutions where *B. thuringiensis*-susceptibility is determined in almost every generation. These are the colonies that can be most useful for comparison reasons since records of trends on susceptibility might be obtainable. This situation exists with *H. virescens* and *H. zea* colonies maintained in USDA-ARS and Monsanto Company. The possibility of obtaining a sample from these insectaries can aid scientists in other research institutions in making comparisons.

A common insect artificial diet. When the same insect colony was tested with 3 different insect diets at 3 different locations the biological responses to the same concentrations of Cry1Ac insecticide were significantly different between some laboratories (Table 3). In the case of tobacco budworm, USDA-ARS and Monsanto's data indicated no significant differences when larval stages were added to dead insects. This might be explained by the fact that both laboratories use similar wheat germ insect artificial diet. Dead bollworm analyses results were not significantly different among laboratories, but when first instar larvae were added to the mortality criteria, only 2 institutions were not significantly different. Overall, there seem to be a trend that adding a factor with subjective criteria such as estimating larval development only by looking at sizes, adds variability to the information. We have observed that these insects are capable of surviving on little or no treated diet and exhibit a repellency effect when they detect minute Bt concentrations in their diet (Gore et al. in press), therefore they can survive for 7 days without food. Considering larvae unable to molt to second instar as dead has been proposed already by Sims et al. (1996). When data were analyzed utilizing these 2 components (dead larvae and surviving first and/or second instars), the mortality values (LC_{50} and LC_{75}) were reduced but the discrepancies among laboratories increased. A more accurate determination of 1^{st} and 2^{nd} instars for each colony may aid us in solving these discrepancies. Intrinsic variations among colonies, diet components, etc. all can influence the size achieved by larvae. It has been recognized since the seminal work of Quaintance and Brues (1905) that head capsule size is the most accurate way of determining instar sizes, but this adds too much time to the evaluation of a bioassay. Perhaps the inclusion of size and weight can give us accuracy on these parameters and may not affect insect survival.

Since the migratory habits of tobacco budworm and bollworm have been documented (Beerwinkle et al. [1995], Goodenough et al. [1988], Muller and Tucker, 1986), a particular field colony of any of these pests might expand its range beyond the geographical area of most research institutions. Bt-susceptible or resistant genes therefore have the capacity to move across borders. Since cotton plantings occur simultaneously in space and time at different geographies (e.g. Texas [US] and Tamaulipas and Coahuila [Mexico]), a coordinated area-wide monitoring program can better address the vigilance of Bt resistance development. Sharing standardized Cry1Ac-incorporated artificial diet among laboratories and implementing a common protocol might be logistically and legally more feasible than moving live insects across geographies or borders if comparisons are needed. This method can be a vehicle for obtaining broader geographical information as well. Shipping effects, unintentionally delays in traffic, and the potential problem with habituation (Daly and Figueredo 2000) to new laboratory environmental conditions might negatively impact the biological performance when insects are shared for comparison reasons. For example, the adaptation of the INIFAP's *H. virescens* colony (reared for >72 generations in a desert environment) has failed in its

2 attempts to become established in a laboratory environment in Mississippi (highly humid environment), but on the other hand Monsanto's tobacco budworm and bollworm colonies kept in an environment similar to the one in Mississippi, haven't presented this type of problem. Importing Bt insecticide samples into other countries might involve its own internal legal problems, especially when certain strains are not registered in the importing country, impeding the comparison of biological material. We can conclude that using a known reference colony and a homogeneous diet are very important aspects when comparing *Bacillus thuringiensis* biological response. Similar conclusions were reported by Rishikesh and Quélennec (1983) when they utilized an internationally recognized standard *B. thuringiensis* preparation with their work with *Aedes aegyptii* mosquitoes.

Acknowledgments

We sincerely would like to thank the grants received from the USDA Foreign Agricultural. Service (IAG 60-6402-4-006) and the National Institute for Agricultural and Forestry Research (INIFAP) for their aid in organizing and partially financing an advisory committee and a group meeting held in 15-16 April, 2004. We thank the following participants for their time and contribution to our study: Craig Abel, Mike Adang, Sotero Aguilar, Mariano Berdegue, Alejandra Bravo, Enrique Campos, Mike Caprio, Marco Cotero, Tim Dennehy, Maurilio Flores, Juan de la Fuente, Rick Hellmick, Eileen Herrera, Jorge Ibarra, Sharlene Matten, Alan McCaffery, Urbano Nava, Brenda Oppert, O. P. Perera, Carlos Rodríguez, Concepción Rodríguez, Fernando Salazar, Héctor Sánchez, Katina Stamatius, Nicholas Storer, Douglas Sumerford, Patricia Tamez Guerra and Antonio Terán,

We appreciate the assistance provided by Michelle Mullen and Tabetha Stokes-Gentry (USDA-ARS), and Nancy Adams (Monsanto Company).

We are thankful to Gordon Snodgrass, Debbie Boykin, Antonio Terán for comments made to this manuscript.

Disclaimer

Mention of trade names or commercial products in this report are solely for the purpose of providing specific information and do not imply recommendation or endorsement by the U.S. Department of Agriculture, or by any of the participating institutions.

References

Ames, H. D. and J. D. Harper. 1985. Bioassay of β-exotoxin of *Bacillus thuringiensis* against *Heliothis zea* larvae. Journal of Invertebrate Pathology 46: 247-250.

Beerwinkle, K. R., J. D. Lopez, Jr., P. G. Schleider and P. D. Lingren. 1995. Annual. patterns of aerial insect densities at altitudes from 500 to 2400 meters in East-Central Texas indicated by continuously-operating vertically-oriented radar. Southwestern Entomologist 18: 63-79.

Daly, K. C. and A. J. Figueredo. 2000. Habituation of sexual. response in male *Heliothis* moths. Physiological Entomology 25: 180-190.

Dulmage, H. T., H. M. Graham, and E. Martinez. 1978. Interactions between the Tobacco Budworm, *Heliothis virescens* and the δ-endotoxin produced by the HD-1 isolate of *Bacillus thuringiensis* var. *kurstaki*: relationship between length of exposure to the toxin and survival. Journal of Invertebrate Pathology 32: 40-50.

Environmental Protection Agency. 2001. Biopesticides Registration Action Document: *Bacillus thuringiensis* Plant-Incorporated Protectants (10/16/01), posted at http://www.epa.gov/pesticides/biopesticides/pips/bt_pips.htm>

Goodenough, J. L., J. A. Witz, J. D. Lopez, and A. W. Hartstack. 1988. Patterns of occurrence of *Heliothis* spp. (Lepidoptera: Noctuidae), 1983-1985. Journal of Economic Entomology 81: 1624-1630.

Gore, J, J. Adamckzyk and C. Blanco. In press. Selective feeding of tobacco budworm and bollworm (Lepidoptera: Noctuidae) on meridic diet with different concentrations of *Bacillus thuringiensis* Berliner proteins. Journal of Economic Entomology.

Luttrell, R. G., L. Wan and K. Knighten. 1999. Variation in susceptibility of noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial. formulations of *Bacillus thuringiensis*. Journal of Economic Entomology 92: 21-32.

Muller, R. A. and N. L. Tucker. 1986. Climatic opportunities for the long-range migration of moths, pp. 61-89. *In* A. N. Sparks (ed.) Long-range migration of moths of agronomic importance to the United States and Canada: specific examples of occurrence and synoptic weather patterns conductive to migration. U. S. Department of Agriculture, Agricultural. Research Service, ARS-43.

Perlak, F. J., M. Oppenhuizen, K. Gustafson, R. Voth, S. Sivasupramaniam, D. Heering, B. Carey, R. A. Ihrig and J. K. Roberts. 2001. Development and commercial. use of Bollgard® cotton in the USA: Early promises versus today's reality. The Plant Journal. 27: 489-501.

Quaintance A. L. and C. T. Brues. 1905. The cotton bollworm. U.S. Department of Agriculture, Bureau of Entomology, Bulletin No. 50.

Rishikesh, N. and G. Quélennec. 1983. Introduction to a standardized method for the evaluation of the potency of *Bacillus thuringiensis* serotype H-14 based products. Bulletin of the World Health Organization 61: 93-97.

SAS Institute. 2001. Version 8e. SAS Institute, Cary, NC.

Sims, S. B., J. T. Greenplate, T. B. Stone, M. A. Caprio, and F. L. Gould. 1996. Monitoring strategies for early detection of Lepidoptera resistance to *Bacillus thuringiensis* insecticidal. proteins. American Chemical Society 23: 229-243.

Tabashnik, B., Y. Carrière, T. J. Dennehy, S. Morin, M. S. Sisterson, R. T. Roush, A. M. Shelton and J-Z Zhao. 2003. Insect resistance to transgenic Bt crops: Lessons from the laboratory and field. Journal of Economic Entomology 96: 1031-1038.

Table 1. Response of 2 different *Heliothis virescens* and *Helicoverpa zea* reference colonies to a freshly-prepared and overnight-shipped Cry1Ac-incorporated insect artificial. diet in the USDA-ARS Stoneville laboratory.

COLONY–DIET	N	X^2	PROB	SLOPE ± SE	LC_{50}* (95%C. I.)	LC_{75}* (95%C. I.)
DEAD *Heliothis virescens* LARVAE						
ARS-FRESH	128	35.9	0.007	0.6472 ± 0.0574	0.18 (0.14 - 0.24)	0.52 (0.39 – 0.75)
ARS-SHIPPED	128	61.8	0.0001	0.5923 ± 0.0721	0.14 (0.09 – 0.21)	0.45 (0.30 – 0.77)
MON-FRESH	128	61.2	0.0001	0.4832 ± 0.0710	0.60 (0.38 – 1.07)	2.42 (1.30 – 6.81)
MON-SHIPPED	128	37.3	0.004	0.7333 ± 0.0756	0.41 (0.31 – 0.56)	1.03 (0.74– 1.60)
DEAD + L1 *Heliothis virescens* LARVAE						
ARS-FRESH	128	22.7	0.19	0.7531 ± 0.0585	0.05 (0.04 – 0.06)	0.13 (0.11 – 0.16)
ARS-SHIPPED	128	23.8	0.15	0.7402 ± 0.0577	0.05 (0.04 – 0.06)	0.13 (0.11 – 0.16)
MON-FRESH	128	83.5	0.0001	0.9168 ± 0.1352	0.10 (0.07 – 0.14)	0.21 (0.15 – 0.34)
MON-SHIPPED	128	30.7	0.03	1.2142 ± 0.1193	0.07 (0.06– 0.08)	0.13 (0.11– 0.16)
DEAD + L1 + L2 *Heliothis virescens* LARVAE						
ARS-FRESH	128	8.3	0.97	1.1464 ± 0.1293	0.01 (0.01 – 0.02)	0.03 (0.02 – 0.04)
ARS-SHIPPED	128	0.09	1.0	4.2585 ± 8768	0.018	0.02
MON-FRESH	128	69.0	0.0001	1.2194 ± 0.2026	0.02 (0.01 – 0.03)	0.04 (0.03 – 0.07)
MON-SHIPPED	128	38.4	0.003	1.6842 ± 0.3274	0.02 (0.01– 0.02)	0.03 (0.02– 0.04)
DEAD *Helicoverpa zea* LARVAE						
ARS-FRESH	128	25.6	0.10	0.3649 ± 0.0425	54.54 (39.7- 82.8)	346.3 (194 - 835)
ARS-SHIPPED	128	105.4	0.0001	0.4740 ± 0.1044	33.59 (19.1– 74.3)	139.3 (65.5 – 811)
MON-FRESH	128	105.2	0.0001	0.4971 ± 0.1090	38.50 (22.0– 89.7)	149.5 (69.7- 888)
MON-SHIPPED	128	37.9	0.003	0.5826 ± 0.0684	44.59 (33.8– 62.9)	141.92 (93– 266)
DEAD + L1 *Helicoverpa zea* LARVAE						
ARS-FRESH	128	171.0	0.0001	0.0623 ± 0.1546	3.06 (0.63 – 6.16)	9.38 (4.31 – 21.2)
ARS-SHIPPED	128	172.3	0.0001	0.5435 ± 0.1518	2.95 (0.39 – 6.42)	10.21 (4.2 – 26.3)
MON-FRESH	128	69.8	0.0001	0.8174 ± 0.1054	8.04 (5.54 – 10.9)	18.36 (13.4– 26.8)
MON-SHIPPED	128	36.7	0.005	0.8195 ± 0.0770	8.33 (6.4 – 10.4)	18.97 (15.1– 24.5)
DEAD + L1 + L2 *Helicoverpa zea* LARVAE						
ARS-FRESH	128	91.0	0.0001	3.9953 ± 22010	1.60	1.90
ARS-SHIPPED	128	48.4	0.0001	0.9955 ± 0.3679	0.78 (0.01– 1.39)	1.53 (0.29 – 2.36)
MON-FRESH	128	43.8	0.0007	0.7782 ± 0.1644	1.10 (0.35 – 1.85)	2.62 (1.44 – 4.0)
MON-SHIPPED	128	73.6	0.0001	1.1545 ± 0.3855	1.34 (0.28 – 2.08)	2.41 (1.34– 4.41)

Micrograms (µg) of Cry1Ac per mL of diet.

Table 2. Response of the same *Heliothis virescens* and *Helicoverpa zea* colonies to the same Cry1Ac-incorporated diet in 3 different laboratories.

LABORATORY	N	χ^2	PROB	SLOPE ± SE	LC_{50}* (95%C. I.)	LC_{75}* (95%C. I.)
DEAD *Heliothis virescens* LARVAE						
USDA-ARS	128	37.3	0.004	0.7333 ± 0.0756	0.41 (0.31 – 0.56)	1.03 (0.74– 1.60)
Monsanto	112	69.9	0.0001	0.7704 ± 0.1601	0.73 (0.42 – 1.43)	1.76 (0.98– 5.44)
U. of Arkansas	128	90.3	0.0001	0.5594 ± 0.0793	0.23 (0.14– 0.36)	0.77 (0.47– 1.58)
DEAD + L1 *Heliothis virescens* LARVAE						
USDA-ARS	128	30.7	0.03	1.2142 ± 0.1193	0.07 (0.06– 0.08)	0.13 (0.11– 0.16)
Monsanto	112	26.0	0.09	1.2315 ± 0.1494	0.11 (0.09– 0.14)	0.20 (0.16– 0.26)
U. of Arkansas	128	124.9	0.0001	0.7522 ± 0.1241	0.11 (0.06– 0.17)	0.27 (0.17– 0.51)
DEAD + L1 + L2 *Heliothis virescens* LARVAE						
USDA-ARS	128	38.4	0.003	1.6842 ± 0.3274	0.02 (0.01– 0.02)	0.03 (0.02– 0.04)
Monsanto	112	31.6	0.02	1.7011 ± 0.2048	0.04 (0.03- 0.05)	0.05 (0.04– 0.07)
U. of Arkansas	128	105.2	0.0001	0.8195 ± 0.1311	0.07 (0.04– 0.11)	0.17 (0.11– 0.29)
DEAD *Helicoverpa zea* LARVAE						
USDA-ARS	128	37.9	0.003	0.5826 ± 0.0684	44.59 (33.8– 62.9)	141.92 (93– 266)
Monsanto	128	87.5	0.0001	0.8969 ± 0.1472	37.93 (27.7– 54.3)	80.47 (55– 148)
U. of Arkansas	128	55.1	0.0001	0.4996 ± 0.0979	108.65 (62– 318)	419 (176– 2923)
DEAD + L1 *Helicoverpa zea* LARVAE						
USDA-ARS	128	36.7	0.005	0.8195 ± 0.0770	8.33 (6.4 – 10.4)	18.97 (15.1– 24.5)
Monsanto	128	54.0	0.0001	1.1286 ± 0.1612	16.95 (12.7– 21.9)	30.81 (23.6– 43)
U. of Arkansas	128	77.4	0.0001	0.7139 ± 0.1187	35.15 (24.3– 54.8)	90.41 (57 – 200)
DEAD + L1 + L2 *Helicoverpa zea* LARVAE						
USDA-ARS	128	73.6	0.0001	1.1545 ± 0.3855	1.34 (0.28 – 2.08)	2.41 (1.34– 4.41)
Monsanto	128	94.6	0.0001	1.0981 ± 0.1799	4.75 (3.10 – 6.68)	8.79 (6.2– 13.3)
U. of Arkansas	128	41.3	0.001	0.9328 ± 0.0863	10.38 (8.3– 12.7)	21.39 (17.3– 27)

Micrograms (µg) of Cry1Ac per mL of diet.

Table 3. Response of Monsanto's *Heliothis virescens* and *Helicoverpa zea* colonies to 3 different freshly-prepared Cry1Ac-incorporated diets under 3 different laboratory conditions.

LABORATORY	N	X^2	PROB	SLOPE ± SE	LC_{50}* (95%C. I.)	LC_{75}* (95%C. I.)
DEAD *Heliothis virescens* LARVAE						
USDA-ARS	128	35.9	0.007	0.6472 ± 0.0574	0.18 (0.14 - 0.24)	0.52 (0.39 – 0.75)
Monsanto	112	135.3	0.0001	0.5956 ± 0.1064	0.45 (0.27 – 0.83)	1.42 (0.78 – 4.01)
U. of Arkansas	96	33.7	0.001	0.4939 ± 0.0841	1.43 (0.84 – 3.43)	5.61 (2.55 – 26.5)
DEAD + L1 *Heliothis virescens* LARVAE						
USDA-ARS	128	22.7	0.19	0.7531 ± 0.0585	0.05 (0.04 – 0.06)	0.13 (0.11 – 0.16)
Monsanto	112	179.4	0.0001	1.0106 ± 0.2144	0.13 (0.08 – 0.21)	0.26 (0.16 – 0.55)
U. of Arkansas	96	51.3	0.0001	0.6357 ± 0.1016	0.62 (0.39 – 1.08)	1.80 (1.04 – 4.58)
DEAD + L1 + L2 *Heliothis virescens* LARVAE						
USDA-ARS	128	8.3	0.97	1.1464 ± 0.1293	0.01 (0.01 – 0.02)	0.03 (0.02 – 0.04)
Monsanto	112	127.1	0.0001	0.9454 ± 0.1831	0.04 (0.02 – 0.07)	0.10 (0.06 – 0.17)
U. of Arkansas	96	91.6	0.0001	0.7272 ± 0.1364	0.33 (0.19 – 0.61)	0.85 (0.49 – 2.18)
DEAD *Helicoverpa zea* LARVAE						
USDA-ARS	128	25.6	0.10	0.3649 ± 0.0425	54.54 (39.7- 82.8)	346.3 (194 - 835)
Monsanto	112	125.1	0.0001	0.7481 ± 0.1499	44.19 (29.2 – 78.3)	108.8 (64.7- 316)
U. of Arkansas	128	119.8	0.0001	0.4164 ± 0.1194	72.25 (34.0- 533)	365.0 (112 – 26051)
DEAD + L1 *Helicoverpa zea* LARVAE						
USDA-ARS	128	171.0	0.0001	0.0623 ± 0.1546	3.06 (0.63 – 6.16)	9.38 (4.31 – 21.2)
Monsanto	112	100.0	0.0001	0.8157 ± 0.1318	13.44 (8.6 – 19.6)	30.7 (20.9 – 52.8)
U. of Arkansas	128	134.3	0.0001	0.6200 ± 0.1370	15.07 (7.70 – 27.5)	44.7 (24.8 – 137.0)
DEAD + L1 + L2 *Helicoverpa zea* LARVAE						
USDA-ARS	128	91.0	0.0001	3.9953 ± 22010	1.60	1.90
Monsanto	112	165.1	0.0001	0.8365 ± 0.1908	3.38 (1.36 – 5.74)	7.59 (4.34 – 14.6)
U. of Arkansas	128	114.8	0.0001	0.7714 ± 0.1462	4.82 (2.40 – 7.68)	11.56 (7.23- 20.3)

Micrograms (µg) of Cry1Ac per mL of diet.