MONITORING *HELICOVERPA ZEA* SUSCEPTIBILITY TO BT TOXINS: RESULTS OF 2008 STUDIES

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Abstract

Twenty-four bollworm, *Helicoverpa zea* (Boddie) populations from the southern U.S. were exposed to industry-recommended test concentrations of four Bt toxins Cry1Ac, Cry2Ab2, Cry1F and Vip3A) in diet incorporation and diet overlay assays at the University of Arkansas in 2008. A few laboratory, laboratory-selected and field populations were fed excised cotton tissues from conventional, Bollgard, Bollgard II and Widestrike cottons and observed for survival until pupation. Significant variability among colonies was found in mortality responses of the laboratory and field populations in diet incorporation and diet overlay assays. Less variability was found in mortality + stunting responses. Mortality + stunting responses at the highest test concentration of Cry1Ac diet incorporation were 100% in all assays. Average mortality + stunting responses for Cry2Ab2 in diet incorporation and Cry1F in diet overlay assays were less than those reported in 2008, but paired responses of LabZA were also less. Survival of neonates fed conventional cottons was significantly higher than those fed Bt cottons. Among the three Bt cottons, survival and pupation was least on Bollgard II cotton. Survival and pupation on Bollgard and WideStrike was similar. At pupation, survival on all Bt cottons ranged from 0 to 11%. Survival to pupation on conventional cotton ranged from 48 to 78%. Correlation analyses identified several related responses of *H. zea* to Bt toxins in diet assays. Most were associated with different test concentrations and mortality and mortality + stunting responses within a given protein. Responses to Cry1Ac were sometimes correlated to those for Cry2Ab2. A few relationships between laboratory diet responses and corrected mortality of larvae fed Bt cottons were identified, but most were not significant and several appeared to be spurious random effects. Relationships between laboratory assay results and projected field results need more study and consideration.

Introduction

Monitoring *Helicoverpa zea* susceptibility to Bt toxins is a component of industry and EPA agreements to manage potential resistance to the insecticidal proteins expressed in Bt cottons. Commercial Bt cotton cultivars include Bollgard (Monsanto Company) that expresses Cry1Ac, Bollgard®II (Monsanto Company) that expresses Cry1Ac and Cry2Ab2, and WideStrike® (Dow AgroSciences) that expresses Cry1Ac and Cry1F. Vipcot™ (Syngenta Biotech), another Bt cotton that expresses Vip3A and Cry1Ac, is expecting commercial registration.

We previously established baseline susceptibilities for monitoring Cry1Ac and Cry2Ab2 resistance in heliothines (Luttrell et al. 1999, Ali et al. 2005, 2006a, Ali and Luttrell 2007), and at the request of USDA and the biotech industry groups we began monitoring field populations of *H. zea* for resistance to Cry1Ac and Cry2Ab2 in 2006 (Ali et al. 2007). In 2007, we assayed and measured the response of over 30 field populations of *H. zea* to Cry1Ac, Cry2Ab2 and Cry1F (Ali et al. 2008). In 2008, we measured relative susceptibilities of over 20 *H. zea* field populations to the four Bt insecticidal proteins (Cry1Ac, Cry2Ab2, Cry1F and Vip3A) expressed in Bollgard, Bollgard II, WideStrike and VipCot cottons. We also measured the survival of some of these laboratory and field *H. zea* populations fed only excised tissues from conventional, Bollgard, Bollgard II and WideStrike cottons.

Materials and Methods

Insects

Eggs of 24 *H. zea* field populations were received in our laboratory from Monsanto Company's insect rearing facility at Union City, TN. Larvae from these eggs were used in assays with the four Bt insecticide proteins. These eggs originated from populations collected during April to July, 2008 in Alabama (N = 3), Arkansas (N = 5), Georgia (N = 3), Louisiana (N = 2), Mississippi (N = 5), North Carolina (N = 4), and Texas (N = 3). Most colonies were established from collections made on field corn and sweet corn (N = 19). Three colonies were collected as larvae on clover, and one was from soybean and one was from grain sorghum (Table 1). A longtime laboratory-susceptible colony of *H. zea* at the University of Arkansas (LabZA) was used as an experimental control in all assays. Monsanto Company's laboratory *H. zea* (MonZA) was also included as an occasional additional reference colony.

Bt insecticidal proteins

The source of Cry1Ac was lyophilized (freeze-dried) powder from MVP II (Mycogen Corporation, San Diego, CA) containing ~20% Cry1Ac toxin of *Bacillus thuringiensis* variety *kurstaki*). Cry2Ab2 was a Bt-corn leaf powder expressing 6 mg of Cry2Ab2 protein/g of powder, respectively. Both were supplied by Monsanto Company, St. Louis, MO. Truncated Cry1F protein containing ~14% Cry1F was provided by Dow AgroSciences, Indianapolis, IN. Lyophilized Vip3A protein (~90% potency) was supplied by Syngenta Biotech, Raleigh, NC. All materials were stored at -80°C and were allowed to warm to room temperature before weighing and use in assays.

Table 1. *Helicoverpa zea* colonies collected for Bt resistance monitoring during 2007.

Colony	Date of Collection	Place of Collection	County	State	Host Plant
S1	4/29/2008	Foreman	Little River	AR	Clover
S2	5/16/2008	College Station	Brazos	TX	Sweet corn
S3	5/02/08	Pine Bluff	Jefferson	AR	Clover
S4	5/15/2008	Possum Grape	Jackson	AR	Clover
S5	6/17/2008	College Station	Brazos	TX	Field corn
S6	6/18/2008	College Station	Brazos	TX	Corn
S7	6/17/2008	Moultrie	Colquitt	GA	Corn
S8	6/17/2008	Tifton	Tift	GA	Corn
S11	7/2/2008	Plains	Sumter	GA	Corn
S12	6/20/2008	Prattville	Autauga	AL	Sweet corn
S13	7/8/2008	Webber Farm	Artmore	AL	Field corn
S14	7/2/2008	Webber Farm	Artmore	AL	Field corn
S15	7/11/2008	Jamesville	Martin	NC	Sweet corn
S16	7/18/2008	Verona	Lee	MS	Field corn
S17	7/21/2008	Monroe	Richland	LA	Soybean
S18	7/22/2008	Ecru	Pontotoc	MS	Field corn
S19	7/15/2008	Roper	Washington	NC	Corn
S20	7/18/2008	Knightdale	Wake	NC	Corn
S21	7/22/2008	Edenton	Chowan	NC	Corn
S22	7/25/2008	Hamilton	Monroe	MS	Field corn
S23	7/28/2008	Winnsboro	Franklin	LA	Sweet corn
S24	7/31/2008	Stoneville	Washington	MS	Field corn
S25	7/3/2008	Stoneville	Washington	MS	Field corn
S26	7/30/2008	Dumas	Desha	AR	Grain sorghum
F2708	6/20/2008	Foreman	Little River	AR	Corn

Bt diet bioassays

Diet incorporation and diet overlay assays were used to measure mortality and mortality + stunting responses of the test insects to the different Bt proteins. Test concentrations for each Bt insecticidal protein studied in diet incorporation assays (100 and 250 µg/ml for Cry1Ac; 100 and 150 µg/ml for Cry2Ab2; 1 and 4 µg/cm^2 for Cry1F; and 10, 30 and 100 µg/ml for Vip3A) were recommended by each respective biotech company. Neonates were exposed to test concentrations and susceptibility was measured after 7 d as in the baseline studies of Ali et al. (2005, 2006a, 2006b, 2007) and Ali and Luttrell (2007). In all assays, neonate *H. zea* were individually exposed to Bt toxins incorporated into pinto bean diet and dispensed into 128 well bioassay trays (C-D International).

The susceptibility of field colonies of *H. zea* to two test concentrations of Cry1F (1 and 4 µg/cm^2) and two test concentrations of Cry1Ac (10 and 30 ug/cm^2) was measured in diet overlay assays (Siegfried et al. 2000, Ali et al. 2008). One ml of freshly prepared diet was poured into bioassay trays. Once the diet has cooled and solidified, 50 µl of protein solution dissolved in 0.1% Triton-X100 was pipetted onto the diet surface of a single well in the tray and allowed to dry.

For each protein, regardless of the assay method, a set of bioassay trays was prepared each week (batch). Along with assays of field populations, paired assays were conducted with LabZA as an experiment control of variable assay conditions. For additional experimental control, a test at the beginning and another at the end of the season included MonZA. For each protein treatment, an untreated control (0 µg/ml concentration) was used and there were four replicates of 16 larvae. For untreated controls, distilled water for Cry1Ac and Vip3A, lyophilized non-Bt corn leaf powder for Cry2Ab2 and 0.1% Triton X-100 for Cry1F were included.

Plant bioassay

Survival of *H. zea* larvae on excised Bt cotton tissues was studied to compare the results with those measured in diet assays and preliminary observations made in plant studies in 2007. Two conventional Bt cotton cultivars, DP147RF (Conventional-1) and PhytoGen 425 RF (Conventional-2) and three Bt cotton cultivars, DP488BGRR (Bollgard), DP143BGIIRR (Bollgard II) and PhytoGen 485WRF (WideStrike) were grown at the University of Arkansas Agricultural Experiment Station Farm, Fayetteville, AR, following standard agronomic practices. For continuous supply of different cotton tissues (leaves, squares and bolls), cultivars were planted on four different dates (1st week of May, June, July and August). Individual plots of a given cultivar were two to three rows and 320 ft in length.

Survival of larvae from six field strains and two laboratory-selected strains (colonies originated from MonZA and selected with 500 µg of Cry1Ac/ml of diet in diet incorporated and 30 µg of Cry1Ac/cm^2 in diet overlay assays) was measured when larvae were fed only different tissues from conventional and Bt cottons until pupation. Observations were made at weekly intervals. Each colony was fed cotton tissue from Conventional-1, Conventional-2, Bollgard, Bollgard II and WideStrike. The conventional cottons were genetically similar to the parent backgrounds of Bollgard II and Widestrike cottons. Tests with field and laboratory-selected strains were conducted from August to September, 2008, and included three separate replicated experiments (one in the early season, one in middle and another in late season). LabZA was included as an experimental control of day-to-day experimental variation in all studies. MonZA was also included as a reference colony in the first experiment.

Freshly-excised plant tissues were collected from field plots and placed on a thin layer of 1% agar surface in 30-mL plastic cups (Solo Cup Company, Urbana, IL). The thin layer of agar-water covering the bottom of cups helped limit desiccation of tissues. Neonate larvae were confined individually and fed terminal leaves for 14 d. There after the surviving larvae were fed young squares with bracts for seven days in 30-mL plastic cups. Surviving larvae were then fed young bolls in 4 oz clear plastic Solo containers until pupation. Plant tissues were changed on a 2-3 day interval or as needed. All larvae were reared on plant tissues until death or until pupation and were held at room temperature (~25 ±1°C). Plant tissues were collected from 40- to 90-day-old cotton plants.

All assays with conventional cottons were initiated with 25 larvae per replicate. Those with Bt cottons (Bollgard, Bollgard II and WideStrike) included 100 larvae per replicate. All tests were replicated four times. Data on larval survival were recorded at 7, 14, 21, 28 d and at pupation. Larval developmental stages (visual estimate of instars) were recorded at 7 and 21 d post-treatment. Data on length of larval developmental period, pupal weight and adult emergence were also taken but are not included in this report. Samples of all plant tisses were held at -80°C for future Bt expression analysis.

Data Analysis

Larval mortality and mortality + stunting (i.e., larvae that failed to molt to second instars) were recorded after 7 d of exposure to the treated diet. Corrected responses were computed using Abbott's (1925) formula and the 0 concentration response with each colony and batch. Response data were studied by PROC GLM (SAS 2002) within each batch and across the entire experiment for each protein.

To better understand the potential utility of different assay responses as indicators of changing Bt susceptibility, we also examined the 2008 laboratory and field data for correlated responses using the multivariate paired-correlations procedure within JMP (SAS 2002). All quantitative (i.e. eliminate data for 0 and 100% responses) data for each colony exposed to an individual protein (Cry1Ac, Cry2Ab, Cry1F and Vip3a) at a given protein concentration (one or two doses for each protein) was paired with other responses obtained for that colony. Correlation analysis was done on the paired responses across all insect colonies. Correlation coefficients were considered significant at the $P<0.05$ level of confidence. Since mortality and mortality + stunting responses are of special interest to those

involved with Bt resistance monitoring, we further studied these paired responses within each protein and across all proteins and assay procedures using simple linear regression. Relationships between laboratory diet assay responses for Cry1Ac, Cry2Ab2 and Cry1F were similarly compared to mortality of larvae fed Bollgard, Bollgard II and Widestrike plant tissues. Corrected mortality in plant assays was the inverse of survival rates relative to those on conventional cottons.

Results

Susceptibility of *H. zea* to Cry1Ac in diet incorporated assays

Mortality of laboratory susceptible *H. zea* (LabZA) ranged from 24.6 to 81.3% with a mean ± SEM of 53.7 ± 5.0% at the 100 µg concentration of Cry1Ac and 39.2 to 95% with a mean of 65.6 ± 4.4% at the 250 µg concentration of Cry1Ac across 13 test batches. Mean mortality + stunting of LabZA ranged from 50.7 to 100% with a mean of 93.81 ± 4.0% at 100 µg of Cry1Ac and ranged from 98.1 to 100% with a mean of 99.6 ± 0.2% at 250 µg of Cry1Ac. Mortality of MonZA ranged from 53.1 to 57.9% (mean of 55.5 ± 2.4%) and from 68.4 to 72.9% (mean of 70.7 ± 2.2%) at 100 and 250 µg concentrations of Cry1Ac, respectively in two test batches. Mortality + stunting for these concentrations ranged from 87.1 to 96.7% (mean of 91.8 ± 4.8%) and 98.2 to 100% (mean of 99.1 ± 0.9%), respectively. At the highest tested concentrations, mean mortalities + stunting of LabZA and MonZA were similar. For 24 field populations (26 batch-colony combinations), mean mortalities were 30.6 ± 3.8% and 46.0 ± 4.2% at 100 and 250 µg, respectively. Mean mortalities + stunting for field populations were 83.5 ± 3.4% and 97.8 ± 0.9% at the 100 and 250 µg, respectively. Figure 1 shows the variability in mortality and mortality + stunting across the different colonies and batches exposed to 250 ug Cry1Ac/ml diet.

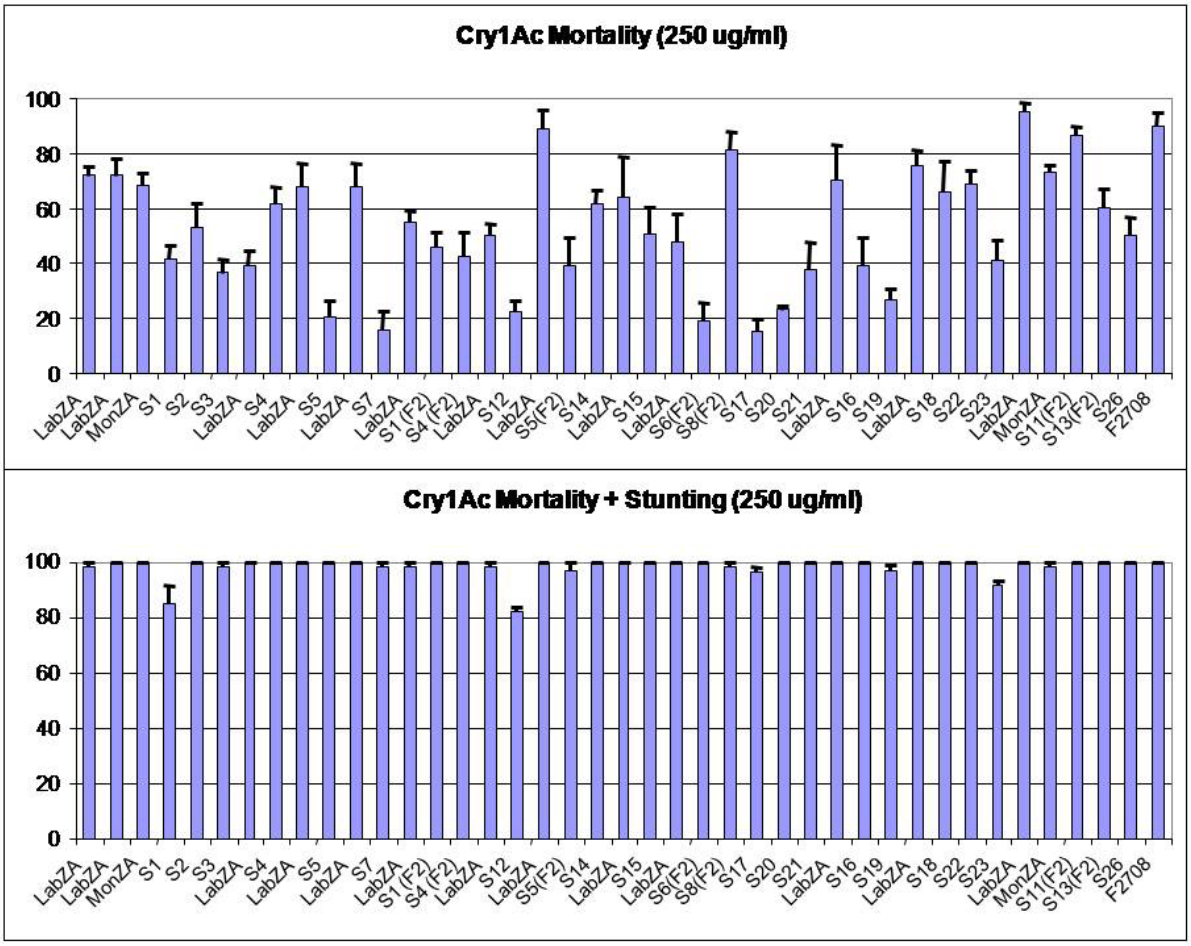

Figure 1. Mean (± SEM) percent corrected mortality and corrected mortality + stunting of *H. zea* laboratory and field colonies exposed to 250 µg/ml of Cry1Ac in diet incorporated assays in 2008.

Within Batch Mortality Observations -- Nine of 13 batches with Cry1Ac 250 diet incorporation observations at 250 ug/ml had significant mortality effects ($p<0.05$). F values ranged from 0.23 to 47.47 with corresponding p values of 0.002 to 0.66. Mortalities of LabZA and MonZA were similar in batch 1 but different in batch 16. Eleven of 26 field colonies had 7 d mortalities equal to that of LabZA in the paired within batch comparisons. Fourteen had significantly less mortality than that of the paired within batch LabZA.

Within Batch Mortality + Stunting Observations -- All LabZA within batch observations had mortality + stunting responses equal to 100% and there were no significant differences between LabZA and MonZA. Five of the 26 colonies tested had mortality + stunting responses significantly less than that of the corresponding paired LabZA response (S1 batch 2 (85%), S12 batch 9 (82%), S8 batch 12 (98%), S17 batch 12 (96%), and S23 batch 15 (92%)).

Across Batch Mortality Observations – The highest mortality observation with Cry1Ac at 250 ug/ml was that of LabZA in batch 16 (95%). The lowest was that of colony S17 in batch 12 (15%). Nine of 13 observations of LabZA across the batches had mortality levels less than that of LabZA in batch 16. The lowest mortality of LabZA was 39% in batch 3. Three of 13 LabZA batches had mortalities not significantly higher than that of LabZA in batch 3. Five observations had mortality levels no greater than that of the lowest observed (15% with S17 in batch 12). They were colonies S5 in batch 6 (15%), S7 in batch 7 (21%), S12 in batch 9 (23%), S20 in batch 12 (23%), and S19 in batch 14 (27%).

Across Batch Mortality + Stunting Observations – All laboratory colonies had mortality + stunting responses statistically equal to 100% for Cry1Ac at 250 µg/ml. Three of the 26 field colonies had mortality + stunting responses less than 100%. They were S1 in batch 2 (85%), S12 in batch 9 (82%), and S23 in batch 15 (92%).

Susceptibility of *H. zea* to Cry1Ac in diet overlay assays

Mean mortalities of LabZA exposed to Cry1Ac in diet overlay assays were 86.8 ± 4.5% (range of 62.3 to 100%) and 97.9 ± 0.6% (range of 94.67 to 100%) at the 10 and 30 µg Cry1Ac/cm^{2}concentration respectively in 10 tests. Those for mortality + stunting were 95.6 ± 2.5% (range of 75.4 to 100%) and 100 ± 0.0 (range of 100 to 100%), respectively. Mean mortalities for MonZA at 10 and 30 µg Cry1Ac/cm^2 were 100.0 ± 0.0 and 98.4 ± 0.0%, respectively. Mean mortalities + stunting were 100% at both concentrations. Mean mortalities of 23 field populations were 86.1 ± 2.5% (range of 54.2 to 100%) and 96.8 ± 1.1%, respectively at 10 and 30 µg Cry1Ac/cm^2. Mean mortality + stunting for field populations at the same concentrations were 98.3 ± 2.9% (range of 90.0 to 100%) and 99.9 ± 0.5% (range of 98.3 to 100%), respectively. Mean mortality and mortality + stunting of laboratory and field populations were similar.

Within Batch Mortality Observations – No differences were detected in 7 d mortalities of LabZA exposed to 30 ug Cry1Ac in diet overlay assays across ten batches. LabZA and MonZA mortalities were also similar. Three colonies (S6 in batch 7, S21 in batch 8, and S16 in batch 9) had mortalities (77 – 84%) less than that of the paired LabZA mortalities (98 – 100%).

Within Batch Mortality + Stunting Observations – No differences were detected among laboratory and field colonies within batches in the mortality + stunting responses of *H. zea* exposed to 30 ug/cm^2 of Cry1Ac in diet overlay assays.

Across Batch Mortality Observations – As within batch observations of mortality, across batch observations of mortality for larvae exposed to 30 ug/cm^2 Cry1Ac in diet overlay assays revealed a few differences ($F=3.10$, $p=0.001$). Four of 23 field colonies had reduced mortalities (S3 in batch 2 (90%), S6 in batch 7 (77%), S21 in batch 8 (77%) and S16 in batch 9 (84%))

Across Batch Mortality + Stunting Observations – No significant differences ($F=0.96$, $p=0.54$) in mortality + stunting were found among colonies exposed to 30 ug/cm^2 of Cry1Ac in diet overlay assays.

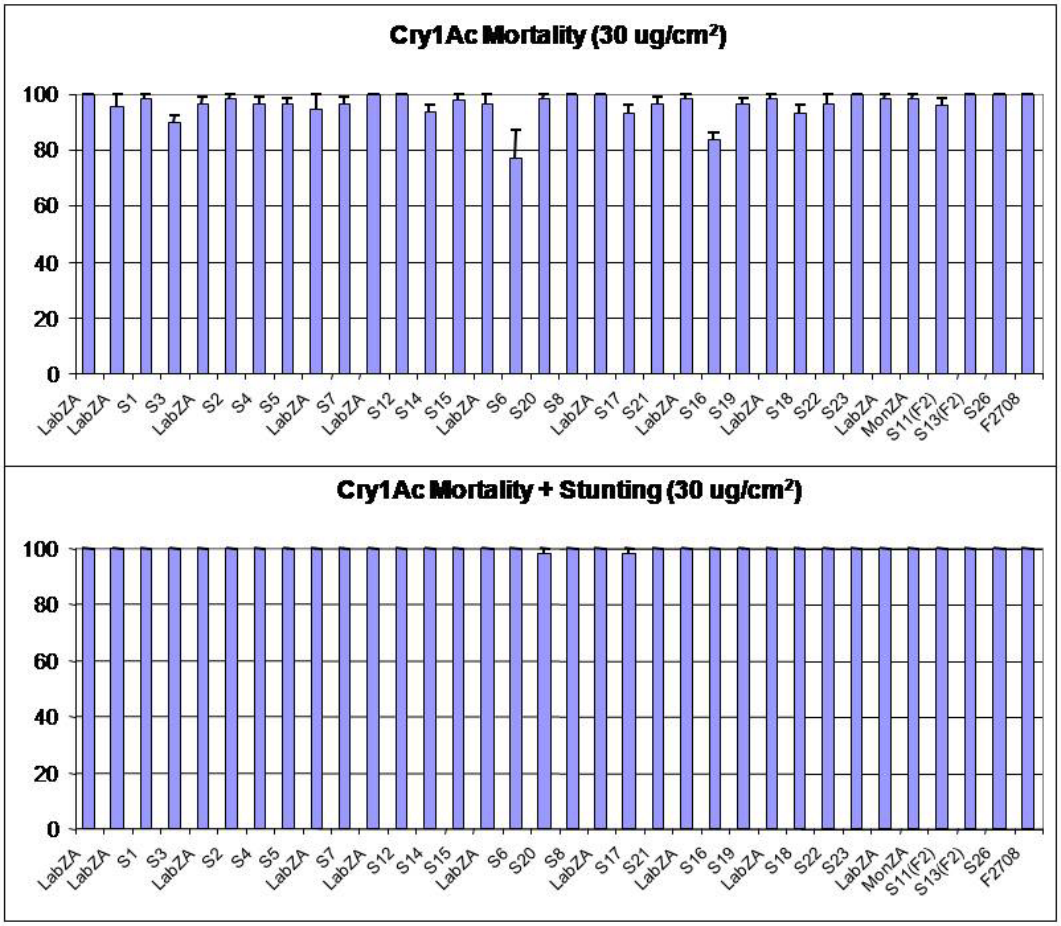

Figure 2. Mean (± SEM) percent corrected mortality and mortality + stunting of *H. zea* laboratory and field colonies exposed to 30 µg/cm^2 of Cry1Ac in diet overlay assays in 2008.

Susceptibility of *H. zea* to Cry2Ab2

Mean mortalities of LabZA exposed to 100 and 150 µg of Cry2Ab2 were 92.7 ± 2.3% (range of 63.3 to 100%) and 96.7 ± 1.1% (range of 87.0 to 100%), respectively across 12 test batches. Mean mortalities + stunting for the same concentrations were 97.0 ± 2.3% (range of 73.3 to 100%) and 99.7 ± 0.3% (range of 96.9 to 100%), respectively. Mean mortality of MonZA ranged from 86.2 to 91.9% (mean of 89.0 ± 2.8%) and 90.2 to 91.6% (mean of 90.9 ± 0.7%) at 100 and 150 µg of Cry2Ab2, respectively. Mortality + stunting ranged from 82.9 to 93.5% (mean of 88.2 ± 5.3%) and 90.2 to 93.2% (mean of 91.7 ± 1.5%), respectively. Susceptibility of MonZA exposed to the highest concentration of Cry2Ab2 was similar to that of LabZA. For field populations, mean mortalities at 100 and 150 µg of Cry2Ab2 were 64.8 ± 5.1% (range of 15.7 to 100%) and 73.0 ± 4.3% (range of 29.6 to 100%), respectively (responses to 150 µg Cry2Ab2/ml shown in Fig. 3). Across 24 field populations, mortality of *H. zea* exposed to 150 µg of Cry2Ab2 varied over 3-fold, but mortality + stunting varied only 1.2-fold.

Within Batch Mortality Observations – For Cry2Ab2 diet incorporation assays at 150 µg/ml within batch 1, LabZA and MonZA had statistically similar mortalities (97 and 90%, respectively) (F=6.15, p=0.09). Within batch 12, mortality of LabZA (98%) was similar to that of batch 1 but statistically greater than that of MonZA (92%) within the batch (F=3.07, p=0.04). Ten of 11 batches had significant differences in mortalities of colonies (range in F values from 12.30 to 98.25 and p values from 0.008 to 0.001). Fourteen of 25 batch-field-colony observations of 7 d mortality (range of 30 to 78%) were less than that of the corresponding paired LabZA mortality.

Within Batch Mortality + Stunting Observations – Both within batch comparisons of LabZA and MonZA (batch 1 and batch 12) detected significant differences (F=29.10, p=0.01 and F=6.21, p=0.003, respectively) between the two laboratory colonies in mortality + stunting response to Cry2Ab2. LabZA had 100% response in both cases. All

twelve batch comparisons had significant differences in the response of colonies to Cry2Ab2. Eleven of 25 colonies (S1 and S3 in batch 1, S4 in batch 3, S7 in batch 5, S4 and S12 in batch 6, S20 in batch 8, S17 in batch 9, S16 and S19 in batch 10, and S23 in batch 11) had mortality + stunting responses (range of 56 to 89%) less than the paired response to LabZA (100% in all batches).

Across Batch Mortality Observations – Across the 39 colony-batch comparisons, LabZA in batches 2, 7, 9, and 10 had 7 d mortality of 100%. Colony S11 in batch 12 also had a 100% mortality response. Eighteen of the 39 comparisons had mortalities statistically equal to these 100% responses. The lowest mortality observed was 30% for colony S1 in batch 2. Colony S20 in batch 8 had statistically similar mortality (34%) to that of S1. Fifteen colonies had mortalities ranging from 49 to 87% at the 150 µg/ml dose of Cry2Ab2 and were intermediate in response between those of LabZA and the colony S1.

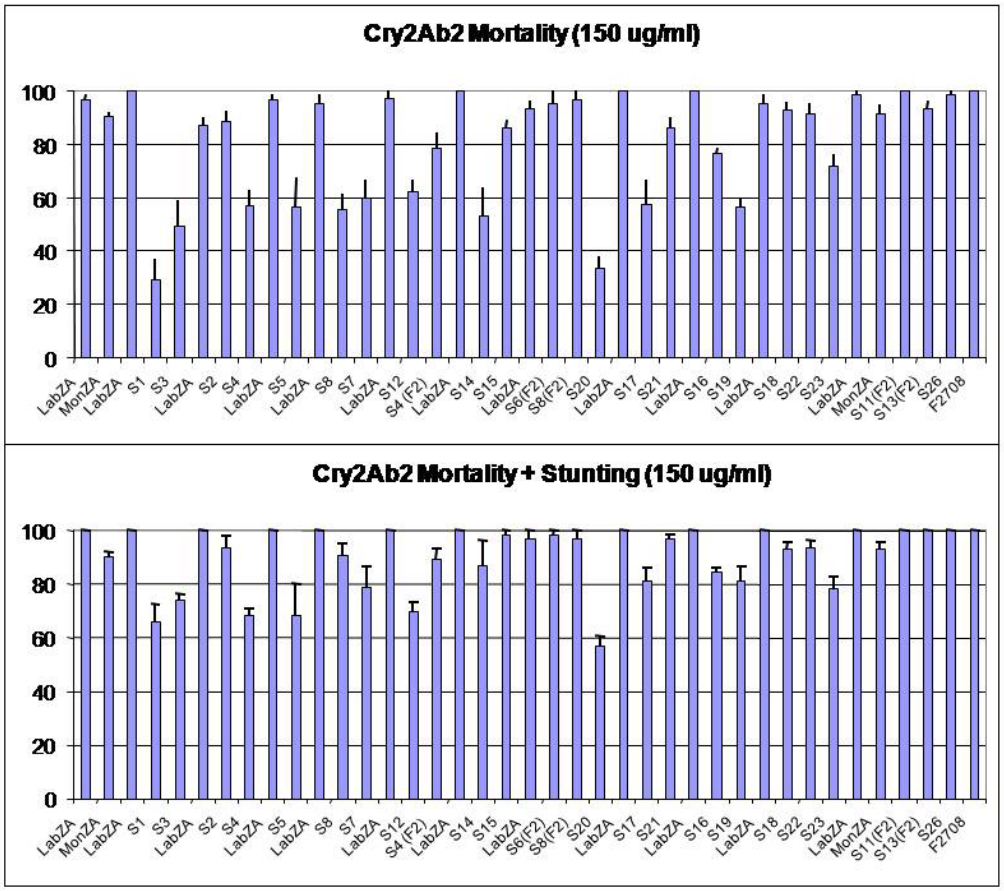

Figure 3. Mean (± SEM) percent corrected mortality and corrected mortality + stunting of *H. zea* laboratory and field colonies exposed to 150 µg/ml of Cry2Ab2 in diet incorporated assays in 2008.

Across Batch Mortality + Stunting Observations – Statistical comparisons across the batch-colony responses of mortality + stunting for Cry2Ab2 indicated no differences in the responses of LabZA and MonZA (n=14, range of 90 to 100%). Twelve of 25 field colonies had mortality + stunting responses statistically equal to that of the laboratory colonies. The lowest response was that of colony S20 in batch 8 (57%). Four colonies (S1 in batch 2 (66%), S4 in batch 3 (68%), S5 in batch 4 (68%), and S12 in batch 6 (70%)) had mortality + stunting responses similar to that of S20. All other colonies (n=9) had responses intermediate between those of LabZA and S20.

Susceptibility of *H. zea* to Cry1F

Mortalities of LabZA exposed to 1 µg/cm^2 of Cry1F ranged from 6.6 to 60.0% (mean of 27.6 ± 4.3%). Mortality + stunting of LabZA for the same concentration ranged from 8.1 to 83.3% (mean of 42.9 ± 5.6%) among 14 test batches. At the highest test concentration of Cry1F (4 µg/cm^2), mortality and mortality + stunting responses of

LabZA ranged from 21.9 to 85.1% (mean of 52.5 ± 4.7%) and from 53.0 to 97.9% (77.2 ± 3.6%), respectively. Mean mortality and mortality + stunting of MonZA at 1 µg Cry1F /cm^2 was 30.4 ± 3.1 (range of 27.3 to 33.6%) and 34.8 ± 0.7% (range of 34.1 to 35.5%), respectively. For field populations, mortality and mortality + stunting at 1 µg Cry1F/cm^2 ranged from 4.7 to 67.9% (mean of 20.2 ± 3.1%) and from 9.4 to 82.9% (mean of 30.5 ± 3.25%), respectively. At the highest tested concentration (4 µg Cry1F/cm^2), mortality of field populations ranged from 4.7 to 81.5% (mean of 35.7 ± 3.9%), and mortality + stunting ranged from 10.9 to 89.6% (mean of 54.3 ± 4.0%) (Fig. 4).

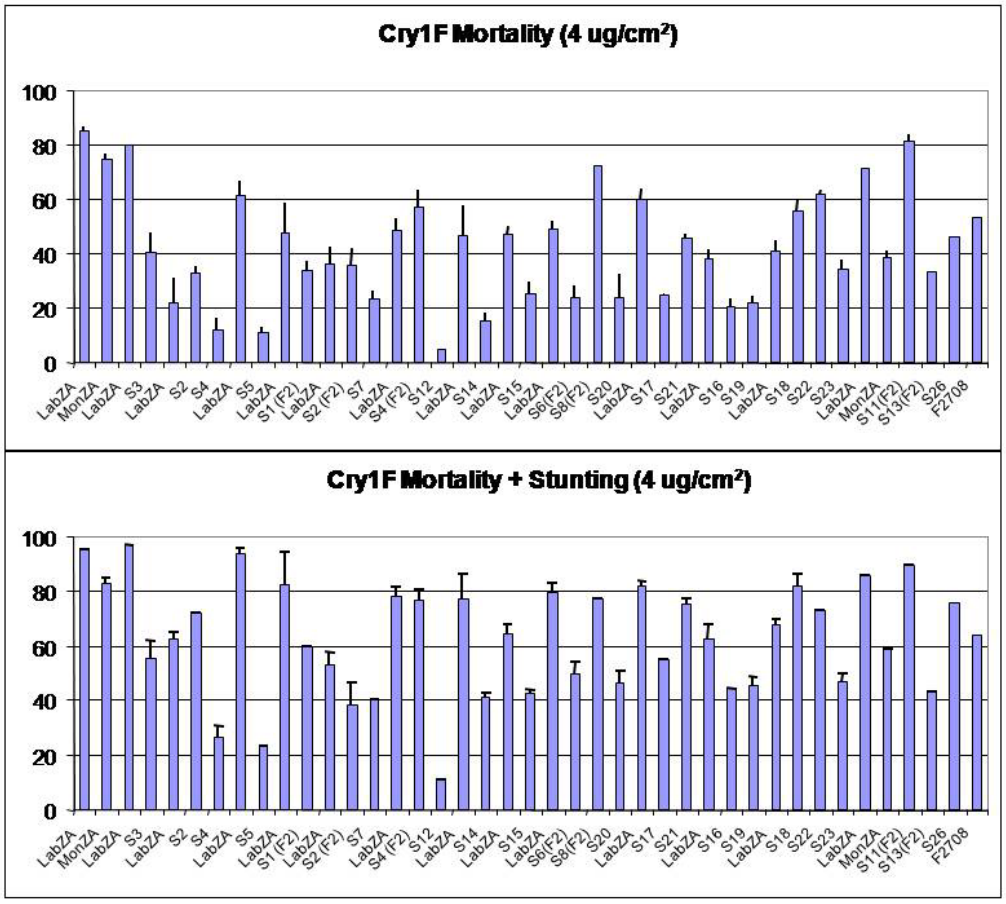

Figure 4. Mean (± SEM) percent corrected mortality and corrected mortality + stunting of *H. zea* laboratory and field colonies exposed to 4 µg/cm^2 of Cry1F in diet overlay assays in 2008.

Within Batch Mortality Observations – LabZA (85 and 72 % mortality) was more susceptible to 4 ug/cm^2 of Cry1F than MonZA (74 and 39% mortality) in two within batch comparisons (F=8.69 and 11.76, p=0.05 and 0.001). Differences in 7 d mortalities were detected among colonies in 12 of 14 batches. Twelve of 25 field colonies has 7 d mortalities (range of 7 to 46%) less than that of paired LabZA mortalities (range of 21 to 72%).

Within Batch Mortality + Stunting Observations – LabZA (95 and 85% responses) was also more susceptible than MonZA (83 and 58% responses) to 4 ug/cm^2 of Cry1F in diet overlay assays. Nine of 14 batches had significant differences in the mortality + stunting responses of colonies to Cry1F (F values range from 7.4 to 2197 and p values range from 0.008 to 0.000). Ten of 25 colonies (S3 in batch 2, S4 in batch 3, S5 in batch 4, S12 in batch 7, S14 in batch 8, S6 in batch 10, S20 in batch 10, S23 in batch 13, S13 in batch 14, and F2708 in batch 14) had less mortality at 7 d than the paired mortalities of LabZA.

Across Batch Mortality Observations – Across all colony-batches, colony S11 in batch 14 had the highest 7 d mortality (82%). Three colonies (LabZA batch 1 and batch 2, and colony S8 batch10) had mortalities similar to that of colony S11 exposed to Cry1F. The lowest mortality observed was that for colony S12 in batch 7 (5%). Eleven of the 41 colony-batches, including two LabZA batch observations, had mortalities not significantly greater

than that of S12 (range from 11 to 25%). Twenty-four of the 41, including 10 of 14 individual batch observations for LabZA, had mortalies greater than that of S12 batch 7 but less than that of S11 batch 14.

Across Batch Mortality + Stunting Observations -- Significant differences (F=6.67, p=0.001) were detected among colony-batch mortality + stunting responses to Cry1F at the highest dose tested. Twenty six of the batch-colony responses were 100% or not significantly different from 100% (range of 91 to 100%) including all LabZA and MonZA responses. The lowest observed response was that for colony S20 in batch 8 (57%). Three additional colonies (S4 in batch 3, S5 in batch 4, and S12 in batch 6) had mortality + stunting responses statistically similar to that of S20 (ranged from 68 to 70%). The remaining colonies had responses intermediate between these extremes.

Susceptibility of *H. zea* to Vip3A

Mean ± SEM mortalities of LabZA exposed to 10, 30, and 100 µg of Vip3A were 34.2 ± 5.3, 34.3 ± 5.8 and 40.0 ± 6.3, respectively. For mortality + stunting, the responses were 83.5 ± 7.2, 77.4 ± 3.6 and 91.2 ± 3.3, respectively. For MonZA, mean mortalities were 62.0 ±6.3, 70.6 ± 2.3 and 90.9 ± 0.0, respectively at 10, 30 and 100 µg of Vip3A/ml of diet. Mean mortalities + stunting for these concentrations were 91.2 ± 3.3, 98.4 ± 1.6 and 100.0 ± 0.0, respectively. Across 24 field populations, mean mortalities at 10, 30 and 30 µg of Vip3A were 47.1 ± 4.9, 51.6 ± 3.7 and 56.0 ± 4.1, respectively. Mean mortalities + stunting for these concentrations were 96.0 ± 0.0, 98.1 ±1.2 and 98.8 ± 0.6, respectively.

Within Batch Mortality Observations – Six of 11 batches had significant differences in mortality of *H. zea* larvae after 7 d exposure to 100 µg/ml Vip3A (F values range from 4.88 to 44.11 and p values range from 0.03 to 0.001). In a direct comparison to LabZA in batch 13, mortality of MonZA (97%) was significantly greater than that of LabZA (71%). Within the six batches with significant colony differences ten colonies had higher mortality than that of the paired LabZA. No colony had significantly less mortality than the paired LabZA colony.

Within Batch Mortality + Stunting – Five of 11 batches had significant differences in mortality + stunting responses to Vip3A (F values range from 3.52 to 34.84 and p values ranges from 0.03 to 0.001). In four of the five batches, MonZA had significantly lower mortality + stunting than some paired field colonies. In batch 13, the mortality + stunting response of LabZA and MonZA were equal and 100%. Colony S13 had a lower response (89%). All other field colonies had mortality + stunting responses as great or greater than that of LabZA.

Across Batch Mortality Observations – Analyis of variance across the entire dataset with 36 colony-batch mortalities indicated significant differences among colony-batches (F=15.11, p=0.001). The highest mortality was that observed for MonZA in batch 13 (97%). Four field colonies had mortalities statistically similar to that of MonZA (S15 in batch 4 (87%), S22 in batch 12 (81%), S13 in batch 13 (88%) and S26 in batch 13 (92%)). The lowest mortality observed was that for LabZA in batch 6 (19%). Five colony-batch observations (LabZA in batch 3 (25%), LabZA in batch 9 (23%), LabZA in batch 11 (24%), S6 in batch 9 (19%) and S14 in batch 9 (22%) had similar mortalities to that of LabZA in batch 6. Across the 36 colony-batch observations, 27 had mortalities between that of LabZA in batch 6 and MonZA in batch 13 (range of mortalities from 28 to 78%).

Across Batch Mortality + Stunting Observations – Across the 36 colony-batch observations of mortality + stunting for *H. zea* exposed to 100 µg Vip3A/ml, five colonies were identified with responses statistically less than 100% (F=6.41, p=0.001). Four of the five were LabZA observations (batches 5, 6, 9 and 11) with mortality + stunting responses ranging from 72 to 92%. One field colony (S11 in batch 13) also had a mortality + stunting response (89%) statistically less than 100%.

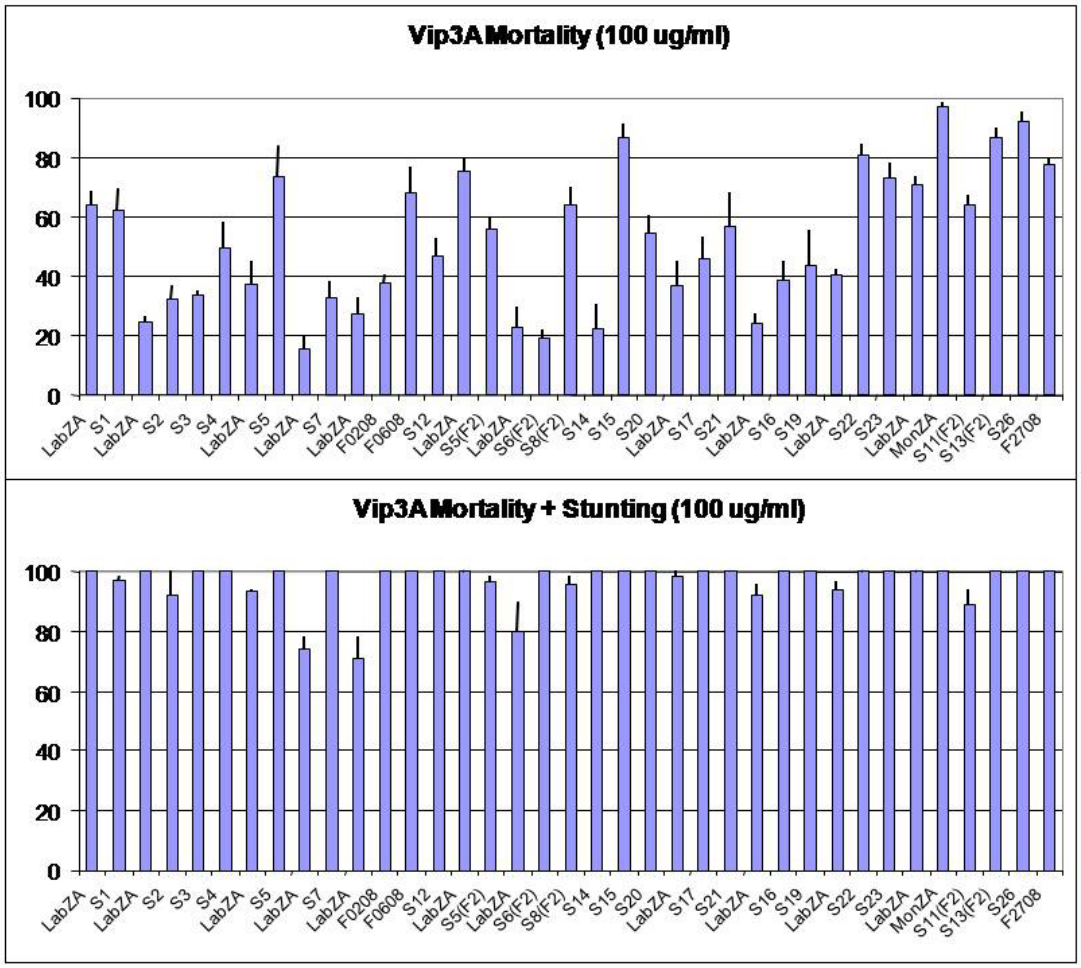

Figure 5. Mean (± SEM) percent corrected mortality and corrected mortality + stunting of *H. zea* laboratory and field colonies exposed to 100 µg/ml of Vip3A in diet incorporated assays in 2008.

Correlation of different response variables across all diet bioassays

Of the 119 response relationships examined, 38 were significantly (P=0.05) correlated. Most (29) of the 38 significant correlations were for response indicators measured within a given protein (i.e. mortality or mortality + stunting at different test concentrations). Among these, response indicators measured within Vip3A, Cry1F and Cry2Ab2 assays had more significant correlations (n=10, n=7, and n=6, respectively) than those for Cry1Ac diet incorporation (n=2) and diet overlay (n=2) assays. Significant correlations between responses of the different proteins across test colonies were most common for paired Cry1Ac and Cry2Ab2 responses (n=7). Only one significant correlation was measured between Cry1Ac and Cry1F and no significant correlation was found between responses of Cry1Ac and Vip3A across the paired responses of test colonies. No significant correlation was measured in the responses of Cry1Ac diet incorporation and Cry1Ac diet overlay assays.

Mortality and mortality + stunting responses within a protein were among the more common significant correlations observed, but many of these paired responses were not significant. Regression equations describing mortality + stunting as a function of mortality and the difference between mortality + stunting and mortality (i.e. actual measured stunting) as a function of mortality (data not shown due to page limitations), indicated that all mortality + stunting responses were significantly related to mortality (r^2 range from 0168 to 0.846, n range from 68 to 413. F values range from 16.825 to 489.12 with p values all less than 0.0001). Regressions for Cry2Ab2 and Cry1F had r^2

Table 2. Significant correlations observed in response of *H. zea* populations to Bt proteins in diet incorporation and diet overlay studies at the University of Arkansas during 2008.

Variables Significantly Correlated	n	Correlation Coefficient (r)	Significance Probability
Cry1Ac Diet Incorporation and Cry1Ac Diet Overlay Responses			
(4 of 28 possible correlations were significant, all 4 for responses within an assay method)			
Cry1Ac mortality at 100 µg/ml and 250 µg/ml	41	0.8603	<0.0001
Cry1Ac mortality + stunting at 100 and 250 µg./ml	12	0.6987	0.0115
Cry1Ac mortality and mortality + stunting. at 10 µg/cm2	13	0.6014	0.0297
Cry1Ac mortality at 10 and 30 ug/cm2	20	0.5474	0.0125
Cry1Ac Diet Incorporation and Cry2Ab2 Diet Incorporation Responses			
(15 of 28 possible correlations were significant, 8 for responses within a protein)			
Cry1Ac mortality at 100 and 250 µg/ml	40	0.8741	<0.0001
Cry1Ac mortality and mortality + stunting at 100 µg/ml	27	0.4655	0.0144
Cry1Ac mortality + stunting at 100 µg/ml and mort. at 250 µg/ml	27	0.4204	0.0290
Cry1Ac mortality + stunting at 100 and 250 µg/ml	17	0.7224	0.0011
Cry2Ab2 mortality at 100 and 150 µg/ml	26	0.9371	<0.0001
Cry2Ab2 mortality at 100 µg/ml and mort. + stunt. at 150 µg/ml	18	0.6873	0.0016
Cry2Ab2 mortality + stunting at 100 and 150 µg/ml	18	0.8932	<0.0001
Cry2Ab2 mortality and mortality + stunting at 150 µg/ml	18	0.5042	0.0329
Cry1Ac mort at 100 µg and Cry2Ab2 mort + stunt at 150 µg/ml	16	0.6958	0.0028
Cry1Ac mort. + stunt. at 100 µg and Cry2Ab2 mort at 100 ug/ml	23	0.4226	0.0446
Cry1Ac and Cry2Ab2 mortality + stunting at 100 µg/ml	20	0.5870	0.0065
Cry1Ac mort + stunt. at 100 µg and Cry2Ab2 mort. at 150 µg/ml	22	0.4666	0.0286
Cry1Ac mort + stunt at 100 µg and Cry2Ab2 mort + stunt at 150 µg/ml	14	0.6443	0.0129
Cry1Ac mort at 250 µg and Cry2Ab2 mort + stunting at 150 µg/ml	16	0.6481	0.0066
Cry1Ac mort at 250 µg and Cry2Ab2 mort + stunting at 100 µg/ml	23	0.4047	0.0554
Cry1Ac Diet Overlay and Cry1F Diet Incorporation Responses			
(9 of 28 possible correlations were significant, 8 for responses within a protein)			
Cry1Ac mortality and mortality + stunting at 10 ug/cm2	13	0.6015	0.0296
Cry1Ac mortality at 10 and 30 ug/cm2	20	0.5474	0.0125
Cry1F mortality and mortality + stunting at 1 ug/cm2	42	0.9437	<0.0001
Cry1F mortality at 1 and 4 ug/cm2	42	0.8435	<0.0001
Cry1F mortality + stunting at 1 ug/cm2 and mortality at 4 ug/cm2	42	0.8100	<0.0001
Cry1F mortality at 1 ug/cm2 and mortality + stunting at 4 ug/cm2	42	0.7361	<0.0001
Cry1F mortality + stunting at 1 and 4 ug/cm2	42	0.7634	<0.0001
Cry1F mortality and mortality + stunting at 4 ug/cm2	42	0.9005	<0.0001
Cry1Ac mortality at 30 ug/cm2 and Cry1F mort. + stunt. at 4 ug/cm2	22	0.5165	0.0138
Cry1Ac Diet Incorporation and Vip3A Diet Incorporation Responses			
(10 of 35 possible correlations were significant, all for responses within a protein)			
Cry1Ac mortality at 100 and 250 µg/ml	41	0.8603	<0.0001
Cry1Ac mortality + stunting at 100 and 250 µg/ml	12	0.6987	0.0115
Vip3A mortality at 10 and 30 µg/ml	27	0.8131	<0.0001
Vip3A mortality + stunting at 10 and 30 µg/ml	8	0.8543	0.0069
Vip3a mortality at 10 and 100 µg/ml	25	0.7672	<0.0001
Vip3a mortality at 30 and 100 µg/ml	37	0.8766	<0.0001
Vip3a mortality + stunting at 10 and 100 µg/ml	6	0.8924	0.0167
Vip3a mortality at 30 and mortality + stunting at 100 µg/ml	12	0.5980	0.0400
Vip3a mortality + stunting at 30 and 100 µg/ml	10	0.7176	0.0196
Vip3a mortality and mortality + stunting at 100 µg/ml	12	0.6214	0.0310

values generally higher than the others suggesting that variability in mortality responses may explain 80% or more of the variability measured within mortality + stunting responses for these proteins. The r^2 value for mortality and mortality + stunting responses for Vip3A was the least observed (0.168), but the regression was highly significant

(n=103, F=20.417, p<0.0001). As with the correlation results, one should consider the limits of experimental power influenced by the range of responses recorded.

Mortality response was a significant explanatory variable in the level of stunting recorded (i.e. mortality + stunting – mortality) for all protein assays except those for Cry1F (r^2=0.037, n=82, F=3.040, p=0.0851). All significant regressions (r^2 range from 0.37 to 0.90, n range from 68 to 413, F value range from 155.5 to 624.2, p values all less than 0.0001) had negative slopes indicating that as mortality level increases stunting response decreases. .

Survival of *H. zea* on conventional and Bt cotton tissues

Across all populations, survival of *H. zea* neonates on two conventional cottons at 7 d of post-treatment was 90% or higher (Table 3). Survival tended to decline at successive post-treatment observations at 14, 21, 28 d and at pupation. At pupation, percent survival ranged from 60 to 80% on the conventional cottons. Survival on two conventional cottons was similar at all post-treatment observation dates. Survival of *H. zea* neonates from different colonies on the three Bt cottons ranged from 13.8 to 63.1%, 22.5 to 89.8%, 58.5 to 85.3% and 18.0 to 42.6%, respectively for tests with LabZA, MonZA, laboratory-selected and field populations. Percent survival of larvae fed Bollgard II cotton was significantly lower than those fed Bollgard and WideStrike cottons. Percent survival of all populations declined at post-treatment dates. After 21 d post-treatment, there was no survival of LabZA on Bt cottons. At 28 d post-treatment, survival of MonZA, laboratory-selected and field population ranged from 0 to 5.8%, 0.5 to 22.1% and 1.4 to 8.1%, respectively. Across MonZA, two laboratory-selected and six field populations, the percent of larvae successfully pupating on Bt cottons ranged from 0 to 0.4% for larvae fed Bollgard II, 0 to 5.6% for larvae fed Bollgard and 0 to 11.0% for larvae fed WideStrike cottons. Across all *H. zea* populations and all post-treatment observation dates, survival on conventional cotton was significantly higher than that on Bt cottons. Among the Bt cottons, survival was least on Bollgard II. Survival of MonZA on Bt cottons was higher than LabZA and was similar or higher than that of each field colony and the laboratory-selected colonies

Observations of larval growth, larval and pupal developmental time, and moth emergence are still being analyzed. They will be included in future publications. Preliminary analyses indicated that larvae surviving to pupation on Bt cotton tissues have longer developmental time with successful pupation occurring ~2 weeks later than that of larvae fed conventional (non-Bt) cotton.

Correlation of mortality and mortality + stunting of *H. zea* in diet assays with mortality on plant tissues.

Mortality and mortality + stunting responses for two test doses of Cry1Ac diet incorporation, Cry1Ac diet overlay, Cry2Ab2 diet incorporation and Cry1F diet overlay assays (n=16 total dose-diet assay responses) were compared to corrected mortalities for larvae fed plant tissues from three Bt cottons (Bollgard, Bollgard II and Widestrike) at five time periods (7, 14, 21, and 28 d and at pupation). The total number of paired responses was 240 (80 for each of three Bt cottons). An average response for each plant assay observations was paired with the average laboratory response across the ten colonies studied in the plant assay experiments.

Of the 240 relationships examined between diet assay responses and mortality of larvae fed Bt cotton tissues after different periods of exposure, 13 significant correlations (P<0.05) were found (data are not shown due to page limitations). Based on a 5% random probability of experiment-wise error, one could have expected as many as 12 significant correlations (i.e. 0.05*240=12). Perhaps the more important observation was the lack of significant correlations observed suggesting that a range of factors other than Bt susceptibility may be involved with survival of larvae fed Bt cotton tissues. It is important to note that this was a preliminary study with a limited number (n=10) of colonies. Sample size for the paired comparisons was also reduced by the elimination of 0 and 100% response data that were outside the range of quantitative estimates. Low survival in diet and plant assays further limits the experimental power of these comparative observations.

Of the 13 significant correlations observed, only one included mortality responses on Bollgard cotton. Six were relationships between diet assay responses and mortality of larvae fed Bollgard II cotton, and six were relationships to mortality observations on Widestrike cotton. Several of the significant correlations are difficult to explain and may be associated with spurious relationships. The significant relationship between mortality of larve in Cry1F diet assays and mortality of larvae in Bollgard plant assays appears to be spurious as do the negative correlations between mortality of larvae exposed to Cry1F and mortality of larvae on Bollgard II and Widestrike cottons. Positive correlations between Cry1Ac responses and mortality of larvae on Bollgard II cotton seem logical as do

those between Cry1F and mortality of larvae on Widestrike cotton. There was no significant relationship between a diet assay response and cumulative mortality at pupation.

Table 3. Survival of laboratory susceptible, laboratory-selected and field populations of *H. zea* fed excised Bt and non-Bt cotton tissues throughout larval development at the University of Arkansas in 2008.[1,2]

Cultivar	Colony	Mean survival (%) at post-treat days				
		7day	14day	21day	28day	Pupation
Bollgard	Field	37.6 A[1] b[2]	21.2 B c	13.6 BC b	8.0 CD a	3.3 D ab
Bollgard	LabSelected	75.5 A a	39.5 B b	16.6 C b	8.2 C a	5.6 C a
Bollgard	LabZA	39.2 A b	17.4 A c	1.9 C c	0 C b	0 C b
Bollgard	MonZA	85.6 A a	78.3 A a	56.2 B a	4.9 C ab	2.2 C ab
Bollgard II	Field	17.3 A b	12.7 B bc	7.7 C b	1.4 D a	0.3 D a
Bollgard II	LabSelected	58.5 A a	31.1 B a	8.6 C b	0.5 C a	0 C a
Bollgard II	LabZA	13.8 A b	7.8 B c	0.3 C c	0 C a	0 C a
Bollgard II	MonZA	22.5 A b	20.5 A b	15.0 B a	0 C a	0 C a
Conventional-1	Field	89.2 A a	87.5 A a	86.0 A a	75.5 B a	72.2 B ab
Conventional-1	LabSelected	93.0 A a	76.0 B b	61.0 C b	54.5 C b	54.5 C c
Conventional-1	LabZA	89.7 A a	85.3 B a	80.7 BC a	84.0 BC a	78.0 C b
Conventional-1	MonZA	96.0 A a	90.0 AB a	84.0 BC a	84.0 BC a	78.0 C a
Conventional-2	Field	88.9 A a	86.5 A a	78.5 B a	67.2 C bc	65.1 C a
Conventional-2	LabSelected	88.0 A a	68.5 B b	58.0 BC b	51.5 C c	48.5 C b
Conventional-2	LabZA	89.7 A a	86.7 A a	84.0 A a	72.7 B b	63.8 C a
Conventional-2	MonZA	92.5 A a	92.5 A a	91.7 A a	89.9 A a	72.8 A a
WideStrike	Field	43.6 A c	28.9 B c	19.7 C bc	7.0 D ab	2.6 D b
WideStrike	LabSelected	85.3 A a	51.6 B b	33.6 BC b	15.4 C a	11.1 C a
WideStrike	LabZA	63.1 A b	43.7 B bc	10.8 C c	0.3 D b	0 D b
WideStrike	MonZA	89.8 A a	84.5 B a	73.0 C a	5.8 D b	3.0 D b

[1] Survival rates within a row followed by similar upper case letter(s) do not differ (P=0.05).

[2] Survival refers within a cultivars followed by similar lower case letter(s) do not differ (P = 0.05).

Discussion

Variability in mortality of laboratory susceptible *H. zea* exposed to Bt toxins was observed. This variability was as great as 2-fold in Cry1Ac diet incorporated assays, 4-fold in Cry1F diet overlay assays and 5-fold in Vip3A diet incorporated assays. With mortality + stunting responses this variability was less, about 1.5-fold in Vip3A diet incorporated and less than 2-fold in Cry1F diet overlay assays. The source of this variable response is unknown, but has been a consistent observation in our previous studies (Ali et al. 2008).

Variability in mortality response was also observed in field populations exposed to test concentrations of all four Bt insecticidal proteins. At highest test concentrations, these variabilities were about 1-fold in Cry1Ac overlay assays, 3-fold in Cry2Ab2 diet incorporating assays, 5-fold in Vip3A diet incorporated assays, 6-fold in Cry1Ac diet incorporated assays and 17-fold in Cry1F diet overlay assays. Less variability was observed when mortality + stunting responses were considered. Only 2-fold variability was observed in Cry2Ab2 diet incorporated assays and only 8-fold only in Cry1F diet overlay assays. These results suggest that using mortality + stunting response results in an increased effective dose with less variability among test populations. We have reported this previously (Ali et al. 2007, 2008). The source of the increased variability with mortality responses needs more study. Using the stunting response effectively increases the overall effective dose and may be a worthwhile measurement for major resistance gene effects, but we need to understand the factors influencing a positive or negative stunting response.

The average response of 24 field colonies to Cry1Ac 250 µg/ml of diet in 2009 was 97.9 (±0.9) with a range of 98.2 to 100. Comparative responses of 34 field populations in 2007 (Ali et al. 2008) averaged 99.9 (±0.1) with a range of 97.7 to 100. The average response of 24 field populations to Cry2Ab2 in 2008 (85.8±2.6) was less than the average response of 2007 (94.4±1.7). The range of mortality + stunting responses to 150 µg/ml Cry2Ab2 was 73 to 100% in 2007. In 2008, the range was 56.8 to 100%. Mortality + stunting responses for the higher dose of Cry1F (4 µg/cm2) averaged 77.5 (+3.8) across 22 field populations in 2007 with a range in response of 46.9 to 100%. In 2008, the average response was 54.3 (+4.0) with a range of 10.9 to 89.6%. Diet overlay assays with Cry1Ac were not conducted in 2007, and 2008 was the first year of actual monitoring for Vip3A responses. Although average responses of field populations to Cry2Ab2 and Cry1F in 2008 were less than those reported for 2007 (Ali et al. 2008), average mortality + stunting responses for LabZA exposed to the two proteins were also less than those reported in 2007. Mortality + stunting response of LabZA to Cry2Ab2 averaged 99.9 (+0.7)% in 2007. In 2008, the average response was 91.7 (+1.5)%. Similarly, the mortality + stunting response of LabZA to Cry1F in 2007 was 93.5 (+1.2)%. In 2008, the average response to 4 µg/cm2 Cry1F was 77.2 (+3.6). These differences again illustrate the importance of adequate reference colonies to standardize inherent bioassay variability over time.

Comparisons to LabZA are relative and may not actually reflect viabilities of survival for insects exposed to Bt cottons in the field, but this is largely unknown. LabZA is an important experimental reference of bioassay variability. Its use is not intended as a benchmark of field susceptibility. This has been an issue of concern and confusion for some readers of our previous reports. We include mortality estimates and experimental comparisons to LabZA because previous benchmark information is based on mortality and we need LabZA as a reference of day-to-day laboratory variability. Decreased mortality response relative to LabZA may or may not have implications for critical shifts in susceptibility. Our previous data (Luttrell et al. 1999, Ali et al. 2006, Ali and Luttrell 2007) clearly indicate wide variability in previously measured responses of *H. zea* to Bt toxins. The mortality + stunting response of the highest test concentration is probably more indicative of a theoretical high dose (Gould 2000) or a dose that would segregate resistance genotypes and measure major resistant gene effects, but it will be difficult to confirm prior to an observation of a Bt resistance event. In this and previous studies, the highest concentration tested was generally limited by the formulation of toxins used and the ability to expose larvae to very high concentration. The diet overlay assay appears to deliver a higher test dose than possible with diet incorporation methodologies used in our baseline studies.

Mortality and mortality + stunting response were generally correlated in these 2008 data as were the responses at different test concentrations. We have reported similar results with Cry1Ac (Ali et al 2006a, Ali et al. 2007) and Cry2Ab2 (Ali and Luttrell 2007 and Ali et al. 2007). Significant correlations between responses of different proteins across test colonies were most common for Cry1Ac and Cry2Ab2 . We have also reported similar results in previous studies (Ali and Luttrell 2007). Responses of larvae exposed to the other Bt proteins do not appear to be related.

Survival of laboratory-susceptible, laboratory-selected and field populations of *H. zea* on conventional and Bt cottons varied. Survival of neonates fed Bt cottons varied, ranging from about 14 to about 90% across all populations at 7 d of post-treatment. Survival of larvae fed Bt cottons also declined at all post-treatment observations. Survival to pupation for larvae fed Bt cottons ranged from 0 to 4% for MonZA, 0 to 11.0% for laboratory-selected and 0.4 to 5.6% for field populations. These results suggest that some *H. zea* can complete development and survive to pupa on Bt cottons. Interestingly, survival of MonZA was as high or higher than all laboratory-selected and field colonies. This raises additional questions about the significance of variability measured in diet assays. More work is needed to understand the relationships between laboratory assay response to Bt toxins and actual survival on toxin-expressing Bt cottons.

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