Transgenic Comparisons of Pink Bollworm Efficacy and Response to Heat Stress

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Abstract

Fifteen lines from 3 different cotton families were compared. Each family had a conventional, non-transgenic standard, as well as 4 other transgenic lines. In some cases, near isogenic lines were available that theoretically only vary from their sibling lines in the presence or absence of one or more transgenes. Each Bt line was evaluated for this trait's efficacy in controlling pink bollworm under high pressure, artificial infestations. Various agronomic properties were measured including yield, micronaire, ginning properties, and fiber quality. Heat tolerance, a key goal for Arizona adapted varieties, was also evaluated using a flower rating system.

The Cry1Ac gene performed flawlessly in preventing PBW larval development when expressed alone (Bollgard®) or in combination with Cry2Ab (Bollgard II®) (i.e., 100% effective, 0 large larvae from 30185 PBW entry holes). In all cases where large larvae were found in Bollgard or Bollgard II plots, the plants bearing the infested bolls were not expressing the Cry1Ac toxin. Thus, those few times when larvae were found, it was due to contaminants in the seed supply. The novel Cry2Ab only expressing plants, produced for non-commercial testing purposes, were also very effective in controlling PBW large larval development; however, control was less than the Cry1Ac-expressing lines (99.622%, 3 large larvae from 4436 entry holes). The ramifications of this are discussed.

In terms of agronomic performance, the transgenic lines performed similarly within families and usually not different from the conventional standards. In some cases, statistically different results were found; however, in all but a few cases, performance parameters were superior in the transgenic lines when compared to the conventional standard. Even so, there are instances where characteristics of the transgenic line were inferior to the conventional standard, especially in some fiber properties. Heat tolerance was again similar throughout 2 of the cotton families (SG125 and DP50). However, for the DP5415 family, 3 of the 4 transgenic lines outperformed the conventional standard. More testing under more environmental conditions is warranted before firm conclusions are drawn.

Introduction

Varietal development is central to the success of the cotton industry. With the advent of genetic engineering techniques as applied to cotton, growers have had access to new traits not previously seen in varieties (resistance to pink bollworm; herbicide tolerance).

Cotton varieties that express insecticidal proteins derived from *Bacillus thuringiensis* var. *kurstaki* (Berl.) were commercially introduced in Arizona in 1996. This genetically engineered *Bt* cotton has already had a major impact in the

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state with ca. 64% of the cotton acreage in Arizona planted to *Bt* cotton in 1997 through 2000 (Jones and Ellsworth 2001). All commercially available *Bt* cotton varieties are based on the Bollgard® gene, which encodes for a Cry1Ac *Bt* protein. Monsanto is currently developing a second gene that encodes for a Cry2Ab *Bt* protein as a means to accomplish better control and broader spectrum of lepidopteran activity, and to manage resistance. Both genes (Bollgard II®) will be present and active at the same time. Cotton varieties may also contain the Roundup Ready® gene alone or in combination with Bollgard or Bollgard II.

Because variety choice remains a critical decision for each grower, there is a need to objectively and independently develop information on the role of these new traits on varietal performance. Varietal performance can be measured in terms of yield, micronaire and other HVI fiber properties, as well as in terms of performance of these new traits—e.g., efficacy of the *Bt* gene(s) in controlling target pests like the pink bollworm.

Some growers remain skeptical of the claims made for these new transgenic varieties. Some believe that while the traits might function as advertised, the variety itself has been changed in some integral way that may lead to poorer performance when compared to conventional relatives. This study was designed to address these concerns by objectively measuring a wide range of parameters in a unique transgenic comparison study where near isogenic lines are available for three families of cotton.

The objectives were:

1) to determine the field efficacy of two *Bt* toxins, alone or together, against PBW in several transgenic cotton lines,

2) to measure and compare the traditional agronomic performance of transgenic and related conventional varieties in terms of yield, micronaire and other fiber and ginning properties, and

3) to develop new methodologies for evaluating heat stress in cotton lines that may be important to understanding any changes that take place in new transformed varieties, and their adaptability to AZ growing conditions.

Methods

	Family	Comments
	"DP50"	
	DP50	non-transgenic
	DP50B	Bollgard gene (Cry1Ac)
isoline	15985B	Bollgard gene (Cry1Ac)
isoline	15985X	Bollgard II gene (Cry2Ab)
isoline	15985BX	Bollgard gene (Cry1Ac) + Bollgard II gene (Cry2Ab)
	"DP5415"	
	DP5415	non-transgenic
	DP33B	Bollgard gene (Cry1Ac)
	DP448B	Bollgard gene (Cry1Ac)
	DP458BR	Bollgard gene (Cry1Ac) + Round-up Ready gene
	DP33BX	Bollgard gene (Cry1Ac) + Bollgard II gene (Cry2Ab)
	"SG125"	
	SG125	non-transgenic
	SG125B	Bollgard gene (Cry1Ac)
	SG215BR	Bollgard gene (Cry1Ac) + Round-up Ready gene
isoline	SG125X	Bollgard II gene (Cry2Ab)
isoline	SG125BX	Bollgard gene (Cry1Ac) + Bollgard II gene (Cry2Ab)

Cotton Lines

Fifteen varieties (see above) were each planted and grown at the Maricopa Agricultural Center (Maricopa, AZ) in a design consisting of two main plots (spray regimes, sprayed & unsprayed for lepidopterans, i.e., PBW) each with 60 sub-plots in a randomized block design (4 replicates x 15 cotton varieties) for a total of 120 plots. All plots were 6 row x 31 ft on 40-in row spacing. Plots were planted on 26 April. The internal four rows of each plot were picked on 28

November. Grab samples were taken for gin turnouts and other fiber measurements. Hand harvests were also made for fiber measurements. Leaf and boll tissues were frozen for later analysis to confirm the presence of *Bt* proteins or genes.

Spray Regime

The no lepidopteran spray main plot was sprayed with foliar insecticides to control non-lepidopteran pests (*Lygus*, whitefly) as needed during the growing season. In addition to these sprays, the spray main plot was sprayed 3 additional times for lepidopterous pests (PBW; see table 1). The sprays effective against lepidopterous pests were applied when there were concerns about PBW moth occurrence, and not necessarily when non-*Bt* bolls reached threshold.

Artificial Infestation

We artificially infested all plots in the no lepidopteran spray main plot on two occasions (August 7 & 29). Fifty flowers per sub-plot were tagged with plant markers. Three weeks later (20–21-d old bolls), we stapled egg sheets onto the adaxial surface of the bracts of each remaining boll (n≈25). The egg sheets were approximately 2.54 cm^2 pieces of oviposition paper containing ≈200 PBW eggs each. All PBW eggs used for these artificial infestations were pharate first instars at the time of infestation which minimized losses to predation or weather. The colony is maintained by the USDA-ARS, Western Cotton Research Laboratory (Phoenix, AZ) on an artificial diet (Bartlett and Wolf 1985). We collected these bolls ca. one week after infestation. Each boll was placed in an individual container and identified with a number and plot designation. These bolls were placed in screen-ventilated plastic boxes and held in the insect rearing room at 72°F or stored temporarily in a walk-in cooler. Each boll was examined with the aid of a microscope, and all PBW larval entrance holes in the carpel exterior were counted. Once entrance holes were counted for all plots, bolls were dissected and all living and dead larval instars and pupae were counted with the aid of a microscope.

Heat Stress Evaluations

Resistance to heat stress is a complex physiological trait in cotton. As part of this study, we attempted to use pollen sterility ratings as an index of heat tolerance. In AZ, heat stress can be classified as either Level I or Level II (Brown, unpubl. data). The former is common throughout the cotton belt to varying degrees; however, Level II stress is encountered here in AZ when night time temperatures and humidities are elevated, such as during the monsoon season. Because of the developmental time lag in flower development, we examined pollen dehiscence in randomly selected white flowers ca. 2 weeks after a Level II heat stress event. This stress occurred just once in 2001 during late June. In contrast, this occurred only in late July in 2000. A rating system was developed whereby a rating of 1 denoted complete pollen dehiscence (i.e., normal pollen shed) and a 5 denoted no pollen dehiscence (i.e., sterile flowers). Ten flowers per plot for each variety in the 'sprayed' plots were examined for their levels of pollen sterility using this method. Lower ratings indicate higher levels of fertility and presumed tolerance to heat stress.

Statistical Analysis

Only the 'no lepidopteran spray' plots were artificially infested. Analyses of dependent variables from these two infestations were performed using a randomized block design. Treatment means for dependent variables were compared to the control variety (conventional non-*Bt*) for each family using Dunnett's LSD (P = 0.05). Tables are presented with colored cells indicating where significant differences occurred from the related conventional varieties.

Findings

Our findings can be quickly organized and summarized in terms of efficacy, yield, fiber quality, and resistance to heat stress.

Efficacy of New Cry2Ab Bt Trait

Table 2 shows that all lines within a variety were **equally susceptible** to PBW neonate attack as measured by the number of entry holes with few exceptions, although sometimes large numerical differences were possible. This result is expected given that larvae are not impacted by *Bt* toxins until they begin to ingest boll tissues. More larvae (dead + alive; 'All PBW') were found in some of the *Bt* lines of the DP50 family; however, overall recovery rates

were **similar among all lines** within a family and infestation date.

Table 3 documents the activity of large PBW larvae (≥ 3rd instar) in cotton bolls, in terms of number of larvae per boll ('≥3rd Live PBW'), the percentage of entry holes resulting in large larvae ('%Pressure'), and the percentage of bolls infested with large larvae ('%Infested Bolls'). In all cases, **all *Bt*-protected lines harbored fewer large larvae than the conventional comparison**. There were some interesting numerical differences among *Bt*-protected lines:

1) SG125B (non-commercial source) was known to have been contaminated with a large fraction of non-*Bt* seed. The field bioassays detected up to 37% of the bolls with large larvae.

2) We suspect strongly that the 985B isoline of the DP50 family was also contaminated with non-*Bt* plants in 2001 with up to 8% contamination. Because the isolines were open-pollenated in 2000 to produce the seed used in this trial, we suspect some level of initial contamination in 2000 may have been exacerbated by low levels of outcrossing due to our small plot structure that year.

3) Other low rates of survival of large PBW in *Bt* plots were approaching the rates of *Bt* purity assured by seed companies (ca. 2%). For example, DP448B obtained from a commercial lot had 1.25% of the bolls harboring large larvae (from 1 of 88 plants).

4) In the cases where positive finds of large larvae from bolls thought to possess the Cry1Ac (Bollgard) trait, ELISA 'gene' testing confirmed that all plants were really non-*Bt*. Thus, the finding of large larvae from Bollgard plots was not from Bollgard <u>expressing</u> plants. This was true for DP448B (1 non-*Bt* plant in 88), 985B (up to 7 non-*Bt* in 84 plants), DP458BR (1 non-*Bt* in 93 plants), 985BX (1 non-*Bt* in 85 plants). See point 6.

5) For the Cry2Ab gene alone, we found that it, too, performed very well in preventing development of PBW larvae. However, with 2 isolines, we found the presence of large larvae in bolls. For 985X, we found 2 bolls from 2 different plants (of 94 plants) that harbored large larvae <u>and</u> tested positive for the presence of the Cry2Ab protein. Furthermore, 2 bolls of SG125X (1 in 86; and 1 in 74) harbored large larvae, but only one tested positive for the presence of the Cry2Ab protein. Thus, the Cry2Ab toxin appears to be highly effective by itself in controlling PBW, but perhaps slightly less effective than the Cry1Ac toxin by itself (see below). Also, rates of stand purity could vary enough to lead to erroneous conclusions about field levels of PBW survival in Bollgard cotton.

6) Table 4 presents the calculated efficacies of the Bt lines in relation to the conventional check lines. Without knowing the specific identity of individual plants (Bt or non-Bt) as may be the case for any grower inspecting a commercial Bt field, one can significantly and erroneously underestimate the efficacy of the Bt variety (see 'Raw' in Table 4). However, with the plants identified by ELISA, we were able to estimate the true efficacy of the Bt lines in controlling the development of large PBW larvae. Only two lines allowed survival of large larvae, '985X' (99.591% effective) and 'SG125X' (99.758% effective), both expressing the Cry2Ab toxin. **All Cry1Ac (Bollgard) and Cry1Ac+Cry2Ab (Bollgard II) lines were 100% effective against PBW large larval development.** Thus, the Cry2Ab gene alone appears to be less effective than the Cry1Ac or the 2-gene combination in controlling PBW.

We conclude from this study of efficacy that our artificial infestations constitute a field bioassay technique for detecting the presence of Cry1Ac at least as well as ELISA methods. This bioassay, therefore, should allow us to determine the 'purity' of the Bollgard or Bollgard II cotton stand in any given test or location. Furthermore, we conclude that field collections of PBW larvae from Bollgard fields are more than likely obtained from non-expressing, non-Bollgard plants contaminating Bollgard fields. The source of these contaminants could be in the bag (i.e., within production tolerances), or from in-field rogue plants emerging from seed residue.

<u>Agronomic Performance – Yield & Micronaire</u>

Table 5 documents the yields in raw seedcotton and lint / A as well as some key ginning properties. In general, yields were not significantly different from their related conventional standards, and when they were different, they were higher. In only 1 case (SG125BX, no spray, seedcotton only) was the transgenic yield significantly lower than the conventional standard. Lint and seed turnouts, when different, were actually higher for the transgenic varieties.

Micronaire values were high throughout this test, which was carried late into the fall for PBW evaluation purposes. However, in most cases the transgenic varieties had similar or lower micronaire values when compared to the conventional standards. In only one case (SG215BR, no spray) was the micronaire significantly higher than the conventional comparison. In this case, however, it was also the highest yielding cultivar in the family.

We conclude that there are varying patterns of relationship in yield, micronaire, and other ginning properties between the transgenic and non-transgenic lines. However, in most cases, the transgenic lines closely resemble their within family conventional standard. Those few times when they are statistically different, they are also superior in performance to the conventional standard. SG215BR micronaire is one notable exception.

Agronomic Performance – HVI Fiber Qualities

Table 6 depicts the HVI results for the 15 cotton lines. The DP5415 family is statistically similar throughout all lines and measurements. Most lines are similar within the DP50 family; however, better lengths were found for the transgenic lines. The SG125 family showed the greatest variation in responses among lines. In most cases, the fiber properties of the transgenic variety were superior to the conventional standard (except in color grade; see 'GradeIndex'). The major exception is in SG215BR, which had significantly inferior fiber lengths when compared to the conventional standard.

We conclude from this that each new transgenic variety must be evaluated on its own, like any new variety, including the performance of the new traits. However, the general agronomic characteristics will likely resemble the genetic background from which it was derived more than other varieties. Furthermore, in most cases where statistical differences can be found, general performance parameters are improved in the transgenic varieties. There are, however, exceptions; thus, **growers should have access to independent, objective analyses of these performance parameters for each candidate variety in Arizona.**

Agronomic Performance – Heat Tolerance

Table 7 shows the results of the sterility ratings based on pollen characters in the field during 2001. The Level II heat stress occurred much earlier in the fruiting cycle in 2001 compared to 2000. The impact of this timing could be crucial in assessing the heat tolerance of any variety. Thus, it is not wise to draw major conclusions based on a single year's, single environment's results. Having said this, the DP50 and SG125 families showed no significant differences in heat tolerance within their respective families. There were, however, large differences within the DP5415 family. DP5415 had the highest rating, indicating the poorest ability to tolerate heat stress. All but DP33BX outperformed DP5415 in terms of pollen sterility or heat tolerance. These results are similar to last year's with some important exceptions. DP458BR was more similar to DP5415 in 2000 suggesting similar abilities to tolerate heat stress. The difference could be the timing of the Level II heat stress relative to fruiting loads, which also impose physiological stress on the plant.

We conclude from this study that flower ratings (i.e., of pollen sterility) are useful indicators of heat tolerance in cotton varieties. Furthermore, we found when transgenic varieties differ from their conventional counterparts, they usually show better performance under heat stress than the non-transgenic standards. More testing is needed in different years and environments to conclusively determine the relative heat tolerance of transgenic cotton lines in comparison to conventional standards.

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References Cited

Bartlett, A.C. & W.W. Wolf. 1985. *Pectinophora gossypiella*, pp. 415–430. *In* R.F. Moore and P. Singh (eds.), Handbook of Insect Rearing, Vol. 2. Elsevier Publ. Co., Amsterdam.

Ellsworth, P.C. & J. Jones. 2000. Arizona Cotton Insect Losses. The University of Arizona, Cooperative Extension. Publication #AZ1183. Tucson, AZ. URL: *http://ag.arizona.edu/crops/cotton/insects/cil/cil.html*.

Jones, J. & P.C. Ellsworth. 2001. Cotton IPM in Arizona: A decade of research, implementation & education. *In* P. Dugger & D. Richter [ed.], Proc. Belt. Cotton Conf., Cotton Research & Control Conference, Anaheim, CA. (*in press*).

Table 1. Spray log for the *Bt* isoline transgenic comparison field study (F3-M1, 2001).

Date Applied	Product	Active Ingredients	Target Pest(s)	Plots Treated
7/10/01	Knack	Pyriproxyfen 11.23%	Whitefly	Spray, No Lep Sp
7/10/01	Vydate C-LV	Oxamyl 42%	Lygus	Spray, No Lep Sp
7/16/01	Orthene	Acephate 97%	Lygus	Spray, No Lep Sp
7/25/01	Vydate C-LV	Oxamyl 42%	Lygus	Spray, No Lep Sp
7/25/01	Pix	Mepiquat Chloride	PGR	Spray, No Lep Sp
8/2/01	Orthene	Acephate 97%	Lygus	Spray, No Lep Sp
8/14/01	Applaud	Buprofezin 70%	Whitefly	Spray, No Lep Sp
8/14/01	Endosulfan	Endosulfan	Whitefly/Lygus	Spray, No Lep Sp
8/23/01	Lock on	Chlorpyrifos 22.9%	PBW	Spray
8/28/01	Warrior	Lambda-cyhalothrin 11.9%	PBW	Spray
9/6/01	Lock on	Chlorpyrifos 22.9%	PBW	Spray
9/6/01	Actara	Thiamethoxam	Whitefly	Spray, No Lep Sp

Table 2. Average number of PBW entry holes and of all PBW found (living or dead) per boll recovered from field-collected bolls of three cotton families on two dates of artificial infestation (Maricopa, AZ). '% Recovery' is 'All PBW' adjusted for the number of entry holes—i.e., an estimate of our recovery rate. For each variable, each line within a family was compared to the conventional variety ('% of Conv.').

Date	Variety	Entry holes	% of Conv.	All PBW	% of Conv.	%Recovery	% of Conv.
8/7/01	DP50	14.45	100	6.28	100	56.49	100
8/7/01	DP50B	17.60	122	11.97	191	70.84	125
8/7/01	985B	16.92	117	8.69	138	57.84	102
8/7/01	985X	18.51	128	11.79	188	65.13	115
8/7/01	985BX	21.01	145	12.18	194	60.86	108
8/7/01	DP5415	18.79	100	9.62	100	61.91	100
8/7/01	DP33B	19.09	102	9.51	99	56.24	91
8/7/01	DP448B	21.15	113	8.94	93	44.13	71
8/7/01	DP458BR	19.12	102	10.17	106	58.46	94
8/7/01	DP33BX	18.41	98	10.53	109	59.18	96
8/7/01	SG125	15.92	100	10.12	100	72.81	100
8/7/01	SG125B	17.85	112	8.48	84	56.87	78
8/7/01	SG215BR	15.65	98	9.88	98	64.69	89
8/7/01	SG125X	12.46	78	6.70	66	51.69	71
8/7/01	SG125BX	17.40	109	10.84	107	78.59	108
8/29/01	DP50	12.04	100	4.75	100	41.21	100
8/29/01	DP50B	14.11	117	7.94	167	51.52	125
8/29/01	985B	21.40	178	13.07	275	60.08	146
8/29/01	985X	9.01	75	5.19	109	53.86	131
8/29/01	985BX	18.26	152	11.70	246	64.68	157
8/29/01	DP5415	18.29	100	11.11	100	59.25	100
8/29/01	DP33B	15.97	87	7.63	69	45.54	77
8/29/01	DP448B	14.89	81	6.90	62	47.46	80
8/29/01	DP458BR	13.66	75	6.21	56	49.85	84
8/29/01	DP33BX	18.05	99	10.24	92	55.42	94
8/29/01	SG125	14.05	100	5.72	100	38.81	100
8/29/01	SG125B	18.16	129	7.40	129	45.88	118
8/29/01	SG215BR	16.51	118	7.57	132	48.44	125
8/29/01	SG125X	12.70	90	6.47	113	51.73	133
8/29/01	SG125BX	19.34	138	9.22	161	48.5	125

= significantly higher than the conventional standard (within a cotton family)

Table 3. Average number of large PBW larvae (larger than 2nd instar; including exit holes) recovered from field-collected bolls of three cotton families on two dates of artificial infestation (Maricopa, AZ). '% Pressure' is this average adjusted for the number of entry holes per boll. '% Infested Bolls' is the average % of bolls infested with PBW larger than second instar*. For each variable, each line within a family was compared to the conventional variety ('% of Conv.').

Date	Variety	≥3rd Live PBW	% of Conv.	%Pressure	% of Conv.	%Infested Bolls	% of Conv.	
8/7/01	DP50	3.48	100	25.91	100	84.77	100	
8/7/01	DP50B	0	0	0	0	0	0	
8/7/01	985B	0.61	18	2.41	9	8.06	10	* *
8/7/01	985X	0.02	1	0.21	1	2.18	3	
8/7/01	985BX	0	0	0	0	0	0	
8/7/01	DP5415	3.36	100	22.92	100	86.23	100	
8/7/01	DP33B	0	0	0	0	0	0	
8/7/01	DP448B	0.01	0	0.05	0	1.25	1	* *
8/7/01	DP458BR	0.02	1	0.22	1	1.09	1	* *
8/7/01	DP33BX	0	0	0	0	0	0	
8/7/01	SG125	4.19	100	32.6	100	91.9	100	
8/7/01	SG125B	1.26	30	10.51	32	37.39	41	* *
8/7/01	SG215BR	0	0	0	0	0	0	
8/7/01	SG125X	0.01	0	0.06	0	1.32	1	
8/7/01	SG125BX	0	0	0	0	0	0	
8/29/01	DP50	1.59	100	17.38	100	52.71	100	
8/29/01	DP50B	0	0	0	0	0	0	
8/29/01	985B	0.22	14	2.01	12	7.29	14	* *
8/29/01	985X	0	0	0	0	0	0	
8/29/01	985BX	0.05	3	0.13	1	1	2	* *
8/29/01	DP5415	1.58	100	12.65	100	64.31	100	
8/29/01	DP33B	0	0	0	0	0	0	
8/29/01	DP448B	0	0	0	0	0	0	
8/29/01	DP458BR	0	0	0	0	0	0	
8/29/01	DP33BX	0	0	0	0	0	0	
8/29/01	SG125	1.82	100	16.31	100	64.13	100	
8/29/01	SG125B	0.85	47	4.1	25	16.86	26	* *
8/29/01	SG215BR	0	0	0	0	0	0	
8/29/01	SG125X	0.03	2	0.48	3	1.67	3	* *
8/29/01	SG125BX	0	0	0	0	0	0	

= significantly better than the conventional standard (within a cotton family).

*Plants with bolls obtained from plots of *Bt* lines that harbored large larvae: 985B, 7 of 84 plants and 6 of 80 plants, all tested negative for the presence of the Cry1Ac gene; DP448B, 1 of 88 plants, negative for Cry1Ac gene; 458BR, 1 of 93 plants, negative for Cry1Ac gene; 985BX, 1 of 85 plants, negative for both Cry1Ac and Cry2Ab genes; 985X, 2 of 94 plants, each positive for the presence of the Cry2Ab gene; SG125X, 1 of 86 plants and 1 of 74 plants, the former was positive for Cry2Ab and the latter negative for Cry2Ab.

**These larvae were from non-*Bt* bolls; thus the response for this line should be 0.

Table 4. Efficacy (in %) of Bt cottons by line, family, gene, and overall in comparison to conventional, non-Bt check lines. Efficacy (in %) was calculated based on the no. of PBW larvae surviving at least to the 3rd instar and the no. of entry holes (i.e., initial larvae attacking bolls).

Variety	Raw*	Adjusted	Family	Raw*	Adjusted	Gene	Raw*	Adjusted	Bt	Raw*	Adjusted
DP50B	100	100	DP50	96.562	99.914	Cry1Ac	92.772	100	All Bts	95.555	99.952
985B	88.405	100				(Bollgard)					
985X	99.591	99.591									
985BX	99.324	100									
DP33B	100	100	DP5415	99.881	100	Cry2Ab	99.349	99.622			
DP448B	99.788	100				('X')					
DP458BR	99.536	100									
DP33BX	100	100									
SG125B	71.195	100	SG125	91.001	99.956	Cry1Ac+Cry2Ab	99.743	100			
SG215BR	100	100				(Bollgard II)					
SG125X	99.256	99.758									
SG125BX	100	100									

* = calculations based on number of larvae failing to reach 3rd instar and the number of entry holes per boll in relation to the non-Bt conventional checks; Data combined from 2 different dates of artificial infestation; 'Raw' numbers are not adjusted for those bolls that were later revealed to be negative for the presence of the gene(s) of interest (by ELISA); 'Adjusted' numbers reflect the true efficacy of Bt line(s) against PBW.

Table 5. Average yields (seedcotton / A; 480 lb. bales / A), micronaire, and ginning properties of 15 lines of cotton from 3 different families for two different spray regimes (sprayed and unsprayed for lepidopteran pests [PBW]).

Order	Variety	Sdctn/A		Bales/A		Micronaire		Turnout		%SeedT.O.		%lint	
Sprayed Leps ==>		No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
1	DP50	4342	4674	2.55	2.83	5.38	4.90	0.282	0.291	0.594	0.603	0.322	0.325
2	DP50B	4711	4745	2.99	2.95	5.30	5.00	0.305	0.299	0.596	0.605	0.339	0.330
3	985B	4629	4690	2.58	2.76	5.15	4.98	0.267	0.283	0.609	0.617	0.305	0.314
4	985X	4937	4729	2.86	2.75	5.18	5.05	0.278	0.280	0.591	0.610	0.321	0.314
5	985BX	4785	4985	2.77	2.98	5.20	5.03	0.277	0.287	0.603	0.606	0.315	0.322
6	DP5415	4289	4563	2.83	3.10	5.33	5.15	0.317	0.326	0.580	0.592	0.353	0.355
7	DP33B	4708	4721	2.89	2.89	4.93	4.73	0.295	0.294	0.615	0.619	0.324	0.322
8	DP448B	5196	5206	3.49	3.52	4.95	5.08	0.323	0.324	0.594	0.596	0.352	0.352
9	DP458BR	4229	4215	2.95	2.86	5.43	4.90	0.335	0.326	0.587	0.586	0.362	0.357
10	DP33BX	4740	4774	2.91	3.02	5.18	5.08	0.295	0.304	0.611	0.613	0.326	0.331
11	SG125	4363	3999	2.90	2.68	4.95	5.13	0.319	0.321	0.557	0.544	0.364	0.372
12	SG125B	4342	4139	2.75	2.70	5.05	5.05	0.304	0.314	0.574	0.582	0.346	0.350
13	SG215BR	4684	4629	3.28	3.43	5.45	5.40	0.336	0.356	0.566	0.580	0.371	0.380
14	SG125X	4350	4284	2.82	2.76	4.93	4.88	0.312	0.310	0.580	0.592	0.350	0.344
15	SG125BX	4031	4194	2.52	2.83	5.25	5.03	0.300	0.324	0.565	0.580	0.347	0.358

= significantly higher than the conventional standard (within a cotton family).
= significantly lower than the conventional standard (within a cotton family).

Table 6. Average HVI fiber properties for 15 lines of cotton from 3 different families for two different spray regimes (sprayed and unsprayed for lepidopteran pests [PBW]). 'GradeIndex' is a formula derived from Rd and b [b*(100-Rd)/3]; higher values indicate better color grades.

Order	Variety	Length		Uniformity		Strength		Rd		b		GradeIndex	
Sprayed Leps ==>		No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
1	DP50	1.11	1.12	81.8	81.7	28.6	28.0	77.4	76.4	7.8	7.5	58.6	58.2
2	DP50B	1.10	1.14	81.5	82.5	28.2	27.5	77.6	76.8	7.8	7.5	58.6	57.3
3	985B	1.14	1.15	82.6	83.0	28.1	28.3	75.9	77.0	8.0	8.2	64.4	62.8
4	985X	1.14	1.15	82.5	83.4	27.8	28.1	77.0	77.9	7.6	8.2	58.3	60.7
5	985BX	1.12	1.14	81.5	82.5	27.6	27.9	77.3	77.9	8.1	7.7	61.6	56.7
6	DP5415	1.12	1.14	82.0	82.5	30.5	30.6	77.1	77.8	7.5	8.1	56.9	60.1
7	DP33B	1.15	1.17	82.1	82.3	29.4	29.7	77.9	78.1	7.6	8.1	56.1	58.8
8	DP448B	1.14	1.14	82.6	83.0	29.5	29.5	77.0	77.0	8.0	8.2	61.6	62.4
9	DP458BR	1.11	1.14	81.0	81.9	30.3	30.9	76.8	78.5	8.1	8.1	62.6	58.2
10	DP33BX	1.12	1.13	82.0	81.4	29.4	28.9	77.4	77.8	7.8	8.2	58.7	60.5
11	SG125	1.11	1.11	82.1	81.9	27.8	27.7	74.0	75.0	8.2	9.0	71.1	74.7
12	SG125B	1.10	1.14	82.3	83.6	28.9	28.4	75.5	76.8	8.3	8.6	67.7	66.8
13	SG215BR	1.05	1.08	81.9	81.9	27.4	27.6	76.7	76.4	8.4	8.2	65.4	64.5
14	SG125X	1.14	1.14	81.4	81.6	27.6	26.7	76.0	77.1	8.0	8.1	64.1	61.8
15	SG125BX	1.13	1.14	82.8	82.6	27.0	27.9	74.7	76.9	7.8	8.1	65.8	62.1

= significantly higher than the conventional standard (within a cotton family).
= significantly lower than the conventional standard (within a cotton family).

Table 7. Average pollen sterility ratings of 15 lines of cotton from 3 different families. The ratings scale was subjective and based on an examination of 10 flowers per plot. A flower with no dehiscing pollen (i.e., sterile) was given a rating of 5, while one with full pollen dehiscence was given a rating of 1. So, lower ratings indicate better pollen fertility as an index of superior heat tolerance. One rating was taken about 2 weeks after a Level II heat stress event at Maricopa, AZ. Ratings sharing the same letter are not significantly different from each other (LSD; P = 0.05).

Order	Variety	Pollen Ratings	
	Sprayed Leps ==>	Yes	
1	DP50	1.25	a
2	DP50B	1.45	a b
3	985B	1.40	a
4	985X	1.53	a b
5	985BX	1.33	a
6	DP5415	2.55	d
7	DP33B	1.95	b c
8	DP448B	1.78	a b c
9	DP458BR	1.48	a b
10	DP33BX	2.15	c d
11	SG125	1.40	a
12	SG125B	1.73	a b c
13	SG215BR	1.40	a
14	SG125X	1.38	a
15	SG125BX	1.60	a b

= significantly better than the conventional standard (within a cotton family).