## Supplementary Materials: Pink Bollworm Resistance to Bt Toxin Cry1Ac Associated with an Insertion in Cadherin Exon 20

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**Figure S1.** Isolation of pink bollworm resistant strain AQ65. The resistant strain AQ65 of pink bollworm was originated from a single-pair cross between a field-collected male (#65) from Anqing in Anhui province of the Yangtze River Valley and a female from a Cry1Ac-resistant strain AZP-R (cadherin genotype *r1r1*) from Arizona. Their F<sub>1</sub> offspring (family #65) were screened with a diagnostic concentration of Cry1Ac (10  $\mu$ g Cry1Ac protoxin per mL diet). In view of the 40% survival of the F<sub>1</sub> progeny at the diagnostic concentration and recessive resistance to Cry1Ac of pink bollworm described before [1,2], it indicated that the male parent of family #65 carried only one recessive allele at *PgCad1* conferring Cry1Ac resistance. Sequencing of cDNA from the resistant F<sub>1</sub> offspring demonstrated that male #65 had only one transcript of cadherin allele, which we name *r16* (Genbank accession number KU254193) (Figures 1).

Survivors from family #65 are reared to adults, then each individual paired with a heterosexual adult from APHIS-S susceptible strain (cadherin genotype *ss*) from Arizona to generate resistant strain AQ65. PCR amplification was used to identify r1 allele for the parents of all single pairs in F<sup>2</sup> generation[3], and only these single pairs that their parents did not carry r1 allele (r16s) were retained, then the offspring larvae of these single pairs were selected on diet with the diagnostic concentration of Cry1Ac. The individuals survived on the diagnostic concentration were homozygotes r16r16, which were reared their offspring as strain AQ65, and feeding larvae of AQ65 with diet containing the diagnostic concentration of Cry1Ac.

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s r16	ATGECGGETGACGCCTGCATACTGETGACGGTGCTTCTACCTTCGCAACATCATTTCGGGCAAGAACAGCATCGTCGAGATGTTACTACATGACGGACG	160 160
s r16	GEACCERCIGECCTIGATCCCCEGCIGAEGCARAGEGCGIGIGCATARACGECIGGIACCCACARCICACCACCACCACCATCATCACCACATCEACAGEGAEGAEGAEGCGAEGAEGAEGAEGAEGAEGAEGAEGAEGAEGAEGAEGAEG	320 320
s r16	IGGAACCCCAGAAATIGICCAGCGGIGIIAIAGGAICIIIAACCIGCIAAGGAICCGGAAGAACCGGGAGGGA	480 480
s r16	GACGAGACTOGTGCCGCACGAGTGAGTCTCTCCATCGAAAACATTGACGATAACGACCCTATOGTCAGGGTGCTAGAGGCTTGCCAAGTGCGGGAGCCTCGACTAACTGACTG	640 640
s r16	ICGAGGCCATGACATICCGCCTCACATCAGACGTGAAGACGTACAGATATICITATGTGGAGCCAGCTCACATTACTGGTGATGGTTCAACATGCAAAATTACTATCGGTATCCTATCAGCGCTTAACITCGAAAGCAACCCGGCTGACATGGTGACGCAGGCTAGCAGAGATTACTATCGGAAGCAACCCGGCTGACATGGTGACGCAGGCAG	800 800
s r16	CRCIGCTITGGACTCCTGGCCCRACAACCATACGGTGACGGTGATGGTGCAAGTCCRGAATGTGGGAACACCGACGGCGGATGGAGGGAAGTCGGCGACGGCAGTGACGGAGGCAGGAAGGA	960 960
s r16	ACTGGCATCGGGAAAGCTATACACTATACCCTCGAGACAGATGAGGAAGAAGAATTTGTTCTTCATCGAAACACTTCCGGGCGGCCATGATGAGGCCAGCATGATGATGGGATAGGCTCCGGGGAGGAGGGAG	1120 1120
s r16	IGGCATACAAGTACGACAATGIGICCCICGCCACCCCGACACCCGICGICGICATAGTCAACGACATCAACAAGCAACCACCCGCCGCCAGAGAGAG	1280 1280
s r16	ITICTATGATGANGATTIGATCTACGCACAATTCTTGGTGGAAATACAAGGCGAGAACCCTCCAGGCGTAGAGCAAGGGTTTTATATTGCGCCCACGGAGGCTTCCAGAACTTCGCCATAGGGACTCAAGATCACCGAATGACGCGATATGAG ITICTATGATGAAGATTIGATCTACGCACAATTCTTGGTGGAAATACAAGGCGAGAACCCTCCAGGGGTAGAAGCAAGGGTTTTATTGCGCCCACGGAGGCTTCCAGAACATTCGCCATAGGGACTCAAGATCACCGAATGATGATGATGATGATGAGGATTATGAG	1440 1440
s r16	GATGTICCTITCCAABACATCAAGGTCAAGGTAATAGCAACGGACCGTGACAATACCAATIITACTGGAGTCGCGGAAGTCAACGTGAACCTGATTAATIGGAACGACGAGGAGGACGACGATCTITGAGGAAGACCAGCTGGTTGAAGGACAACGTCAAGGACAACGACGAACGA	1600 1600
s r16	IRCCRAGGACIATCACGTCGGCAGACTGAGGGCTCACGACGCGGGACATAGGAGACAGGGTTGTGCATTCCATCITGGGAAATGCGAATACATTTTAAGAATCGACGAGAAACTGGCGACATCTACGTAACTATTGATGACGGCTCGATTACGTAG IRCCCAAGGACTATCACGTCGGCAGACTGAGGGCTCACGGGGACATAGGAGACAGCGTTGGGATTCCATCITGGGAAATGCGAATACATTTTTAGGATCGACGAGAAACTGGCGACATCTACGTAGCTATTGATGACGGGTCGATTACCAAG	1760 1760
s r16	ACAGARIGARITARCATACARGTICGCGCTCAGGACACCATGTCGGAGCCAGAGTCCAGGCATACAGCGACTGCTCAGCTGGTCATAGAACICGAGGACGTCAACAACACCTCCTACTCTGAGGCTGCCGCGCGAAGTCCGAGGCGGCAGGAGAGACA ACAGARTGARITARCATACARGTICGCGCTCAGGACACCATGTCGGAGCCAGAGTCCAGGGATACAGCGACTGGTCAGGAGTGGTCATAGAACICGAGGGCGCCAACACCAC	1920 1920
s r16	GTGCCAGAGGGCTTTGAAATCAACCGGGAGATAACCGGCACGGACCCTGACACCACACGACGACGACGACGACGACGACGACGACGAC	2080
s r16	ICTICCCARACCCAGCCGACACCAGAGAAGCTGTGGGGGGGGGG	2240 2240
s r16	IRCCITCACGATAATAATAATAATAATAATAAGAACGACAATGGGCCTATCTGGGGGCGTGGTITCCTGAACCAGACCITCAGTATTCGGGAGCGATCATCTACCGGCGTCGTCATCGGGCGCCGTACGGGCTCCGAACGAA	2400 2400
s r16	CGGIACACCATIAICCCCCAGGARGAIACICCIGARGGICIAGTCCAGAIACACIIGGIACGGGGCAGAIIACAGIIGAIGAGAAGGIGCAAICGACGCIGAIAIICCACCICGIIGGCACCICAACIACAGIIAIAGCCAGCGACAAAIGIICCG CGGIACACCAIIAICCCCCAGGARGAIACICCCIGAAGGICIAGIICCAGAIACACIICGIIACGGGGCAGAIIACAGIIGAIGAGAAGGIGGAAICGIGGCGGIGAIAICC	2560 2560
s r16	ANGANANTGANGAGANCTGTCCCCCGGATCCACTGTTCTGGGGATACTCTGGGCGACATGTANTGAACATCGTGGACATAAACAAGGTCCCGGCAGCAGACCTCAGTGAGATCAACGAAACGGTGTACATTTATGAAAATGCACCCGATTTCACAAA AAGAAAATGAAGAGAACTGTCCCCGGATCCAGTGTTCTGGGGATACTCTGGGCGACAATGTAATGAACGTGGACATAAACAACAAGGTCCCGGCAGCAGACCTCAGTCGACGAAACGGTGTACATTTATGAAAATGCACCCGATTTCACAAA	2720
s r16	COTOGICARGAIATACTCCATCGACGAAGACGGAGATATATATCACGGGGGGGG	2880 2880
s r16	GATCGGGACAGAGGCGAAGACCAACACAGGATATTCATTAACCTCATTGACAACTTITATAGCGAAGGAGATGGGAAATAGAAATGTAAACACTACAGAGGTGCTGGTGATACTATTAGATGAGAATGACAACGCTCCTGAATTGCCGACTCCAGAAGGG GATCGGGACAGAGGCGAAGACCAACACAGGATATTCATTAACCTCATTGACAACTTITATAGCGAAGGAGAATGGAAATGGAAATGTAAACACTACAGAGGTGCTGGTGATACTATTAGATGAAAATGACAACGCTCCTGAATTGCCGACTCCAGAAGAG	3040 3040
s r16	IGAGIIGGAGCATIICCGAGAATIIACAAGAGGGTATAACACICGATGAGGGAAGGGA	3200 3194
s r16	ICCGAGACTICICAACAACGGCGCCTAACAACGTAACCGGATTOCTICAGACAGCAATGCCTTTGAGAGGATATIGGGGTACTACGACATAAGGTATACGGCGTCGACCACGGTATICCTCAGCAGATATCICATGAGGIGTATGGAATGGA	3360 3281
s r16	CGACCITACAAIIACLAAICCICCCCAGIICGIIIIICCIGGAAICCGGGACGAIICIACGACIGGCIIIGGAACGGGGAAAIAAIGIIIIGICACIIGIAAAGGGGGACCGGIIAGACAGGAIACAAGCAAIIGACGAGGGGAACGAIGGICIIGAIGGG GGACCIIACAAIIACAAICCICCCCAGIICGIIIIICCIGGAAICGGGGAIGICIACGACIGGGIIGGAAAGGGGGAAAIAAIGIIIIGICACIIGIAAACGGIGACCGGIIAGACAGGAIACAAGCAAIIGACGAGGGGAIGGICIIGAIGGC	3520 3441
s r16	GCGFGFGACHTCGATATTGTFGGAGATGCTGATGCATCAAACTACTTCGAAACTACATGATGACGGCGACAACTTGGAACCAGGCGCTCCCTGAGGAAGGCAAGGAACTTGGAGATGCGAACTACGAGGCGAACTAGGAACTGTGCGACACAGGCGCTCCCGAGGAAGGCAAGGAACTGGAGGCAACTAGGAGGCAACTAGGAACTGCGGAACTAGGAAGGCAACTAGGAGGCAACTAGGAGCGAGATAGGAACGCGGGAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGCAACTAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	3680 3601
s r16	accicgatchiaticaacagaciccactataacagggcicicggticggacgacggacggacggacggacggacggacgacgacgac	3840 3761
s r16	ARACTCTGCACTGACGACTGTCACGATATTTACTACAGGATCTTTGGTGGTGTGGGATTACGAGCCATTTGACCTGGACCGGACGGA	4000 3921
s r16	ATTGGCCCACAGGAGGGGGAATACCACTGCCGGGGTCTCTCTC	4160 4081
s r16	ANCICATICOGAAAAGGACAATTGACATATACCATCGAAGAGGGTGTATGGCGGGGGGCCCCCTCTGGAAGGCGGCGACGGGGGTCCATCTGAAGGGCGGGGGCGCGCGGGGGCGCGGGAGGAGGAAGACGCAGGGAGGGAGGGAGGGAGGGAGGGGGG	4320 4241
s r16	ITCGAGTICAACGTCATCGCTACTGATCCAGATGAGAAGACAGATACGGCAGAGGTGAAAGTCTACCTCATTICATCCCAAAATAGGGTGTCCTTCATATTCCTGAACGATGTGGAGACGGTTGAGAGAGA	4480 4401
s r16	ITGECITCAACATGACCIGCAATATAGATCAGGTGCIGCCGGGGCCAACGACGACGGGGGGTGATTCAGGAGGCCATGGCGGAAGTCCATGCICACITCATACAGGATAACATCCCIGIGAGCGCCGACAGTATIGAAGAGCIICGCAGGGGGGGGAGACACICAGC ITGGCITCAACATGACCIGCAATATAGATCAGGIGCIGCCGGGGCCAACGACGGCGGGGGGGGGG	4640 4561
s r16	GCTGCGCTCCGAGGTGGTGGTGGACCAACGGCTGTGGTCCGAACGACCTGGTGACGGGGGTCAGCCCTGATCTCGGCACTGCCGGGCGGCAGATCACCATCATGGCTAGCCGGGTGGTAGCCACCATCCTTGCCTTGCCTTGCTGGCCTATTCTGCTC GCTGCGCTCCGAGGTGGTGGTGGACCAACGGCTGTGGGCCTGAACGACCTGGTGACGGGGGGCAGCCCGGATCGCCGGGCGGCGCGAGTACCATCTTGTGCTAGCCGGGTGGCAGCCATCCTTGCCTTGCCTTGCCTTGCCTGGCCTGGTGGCAGCGGGGCGGGGGGCGGGGGGGG	4800 4721
s r16	ATCRATICATCGTGAGGACCCGAGGTCTGGACGGCGTITGGAAGCACTGTCGATGACGAATACGGCTGGGGATICGGGGGTGAACCGAGGGGGATAGCGGGCCCCAGGAACCAACAACAACAACGAGGCTCCAAGGAGCCAACGAGG ATCRATICATCGTGAGGACCCGAGCTGTGAACCGCCGTITGGAAGCACTGTCGATGAGGAATACGGGCGGATTCGGGGGTGAACCGAGGGGGATAGCGGGCCCCAGGAACCAACAACAACAACGAGGCTCCAACCAA	4960 4881
s r16	AGATCAAGGCCCCGGACTCGATGCCATCAGTGACGACATCGACGGAGTCTGATCGGCATCGAGGATCTACCACAATTCAAGAGCGACTATTTCCCGCTGAGGACTCGGCACCGCCGCCGTCAGGCCGCTGAGGACCGCACGCGGGAACGA AGATCAAGGCCCCGGACTTCGATGCCATCGAGGACATTGACGAGGTCTGATCGATC	5120 5041

s TEGEGCTATTECHCACHGETAGCAACAACTTCGGTTTCAACACCAGTCCTTTTAGCGCGGAGTCACCAAACAGGGGCATGCGACCAIAG r16 TEGGGCCTATTECACACAGTAGCAACAACTTCGGTTTCAACACCAGCCCCTTTTAGCGGGGGAGTCACCTAACAGGGGCGATGCGACCATAG 5129

**Figure S2.** Alignment of the full-length cDNA of *s* and *r16* alleles. Underline letters TAA and TAG indicate termination codon of *r16* and *s*, respectively.

s r16	SIG MAGDACILVTVLLTFATSVFQDETASSRCYYMTDAIPREPKPDDLPDLEWTGGWTDWPLIPAEPRDDVCINGWYPQLTSTSLGTIIIHMEEEIEGDVAIA	100
8	CR1 KLNYDGSGTPEIVQPMVIGSFNLLSPEIRNENGAWYLYITNRQDYETPTMRRYTFDVRVPDETRAARVSLSIENIDDNDPIVRVLDACQVPELGEPRLTD	200
8 8	CN2 CVYQVSDEDGRLSIEPMTFRLTSDREDVQIFYVEPAHITGDWFNMQITIGILSALNFESNPLHIFQITALDSWPNNHTVTVMVQVQNVEHRPPRWMEIFA	300
r16		300
r16	→ CR4	400
s r16	POPLODEYTISIMEETPLSLNFAELFGFYDEDLIYAQFLVEIQGENPPGVEQAFYIAPTAGFQNQTFAIGTODHRMLDYEDVPFQNIKLKVIATDRDNTN	500 500
s r16	FTGVAEVNVNLINWNDEEPIFEEDOLVVKFKETVPKDYHVGRLRAHDRDIGDSVVHSILGNANTFLRIDEETGDIYVTIDDAFDYHRONEFNIQVRAQDT	600 600
s r16	CR6 MSEPESRHTATAQLVIELEDVNNTPPTLRLPRVSPSVEENVPEGFEINREITATDPDTTAYLQFEIDWDTSFATKQGRDTNPIEFHGCVDIETIFPNPAD	700 700
8	TREAVGRVVAKEIRHNVTIDFEEFEFLYLTVRVRDLHTEDGRDYDESTFTIIIDMNDNWPIWASGFLNQTFSIRERSSTGVVIGSVLATDIDGPLYNQV	800
r16 8	DE	900
r16		900
s r16		1000
s r16	ENDNAPELPTPEELSWSISENLQEGITLDGESDVIYAPDIDEEDTPNSHVGYAILAMTVTNRDLDTVPRLLNMLSPNNVTGFLQTAMPLRGYWGTYDISI	1100 1074
s r16	LAFDHGIPQQISHEVYELEIRPYNYNPPQFVFPESGTILRLALERAVVNNVLSLVNGDPLDRIQAIDDDGLDAGVVTFDIVGDADASNYFRVNNDGDNFG	1200
8 r16	CR11 TLLLTQALPEEGKEFEVTIRATDGGTEPRSYSTDSTITVLFVPTLGDPIFQDNTYSVAFFEKEVGLTERFSLPHAEDPKNKLCTDDCHDIYYRIFGGVDY	1300
s r16	EPFDLDPVTNVIFLKSELDRETTATHVVQVAASNSPTGGGIPLPGSLLTVTVTVREADPRPVFEQRLYTAGISTSDNINRELLTVRATHSENAQLTYTIE	1400
s r16	$\Longrightarrow MPR \\ DGSMAVDSTLEAVKDSAFHLNAQTGVLILRIQPTASMQGMFEFNVIATDPDEKTDTAEVKVYLISSQNRVSFIFLNDVETVESNRDFIAETFSVGFNMTC$	1500
s r16	$\Longrightarrow TM \\ \texttt{NIDQVLPGTNDAGVIQEAMAEVHAHFIQDNIPVSADSIEELRSDTQLLRSVQGVLNQRLLVLNDLVTGVSPDLGTAGVQITIVVLAGLSAILAFLCLILL}$	1600
s r16	➡ CYT IIFIURTRALNRRLEALSMTKYGSVDSGLNRVGIAAPGTNKHAIEGSNPIWNEQIKAPDFDAISDTSDESDLIGIEDLPQFKSDYFPPEDSESAHAAFSD	1700
s r16	RTPRGNDAPIAHSSNNFGFNTSPFSAEFTNRRMRP 1735	

**Figure S3.** Predicted amino acid sequence of pink bollworm cadherin protein PgCad1 for alleles *s* (GenBank accession number MF276974) from susceptible strain APHIS-S; and *r16* (GenBank accession number KU254193) from resistant strain AQ65. The *s* was sequenced in 2015 from the subset of APHIS-S reared in China [4]. Shown are the signal sequence (SIG), cadherin repeats (CR1-CR12), membrane-proximal region (MPR), transmembrane region (TM) and cytoplasmic region (CYT). The short dashes for *r16* indicate the sequence is the same as for the *s* allele. The asterisk at position 1074 in *r16* indicates that, because of the premature stop codon (Figure S2), the *r16* allele encodes only 1073 amino acids.

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**Figure S4.** Alignment of g and cDNA sequences of *r16* and *s* alleles. The red letters indicate exon sequences. The green background represents the consistent sequence of introns in *r16* and *s*. The blue background GT/AG indicate splicing site. The yellow background ACCT indicate target site duplicates (TSDs). The grey background indicates the inserted transposon sequence. The red and underline letters TAA indicate premature stop codon in *r16*.



**Figure S5.** PCR detection of  $P_gCad1$  genotype using primers in Table S1. Primers for r16 (r16allF and r16R) generate a single band of 1211 bp in r16r16 from AQ65 (lanes 1–3) and in r16s (F1 progeny of APHIS-S × AQ65; lanes 4–6); and no band in ss from APHIS-S (lanes 7–9). Primers for s (r16allF and notr16R) generate no band from r16r16 (lanes 10–12) and a single band of 1431 bp from r16s (lanes 13–15).



**Figure S6.** Western blot of cadherin fusion proteins sPgCad1-GFP (lane 1) and r16PgCad1-GFP (lane 2) produced in Hi5 cells transfected with vectors containing the *s* and *r16* alleles, respectively.

## References

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Name <sup>a</sup>	Primer sequence (5'-3')	Template	Strain	Size (bp)
F1	CATACTGGTGACGGTGCTTCT		APHIS-S	2384
R1	GGACTTGGTTGTAAAGTGGGC	CDNA	AQ65	2384
F2	GACCTTCAGTATTCGGGAGCG		APHIS-S	2890
R2	CATGCGCCTGTTAGTGAACTC	CDNA	AQ65	2811
gF65	AGAAATGTAAACACTACAGAGGTGC		APHIS-S	2470
gR65	AACGAACTGGGGAGGATTGTAATTG	gDNA	AQ65	3664
r16allF	GATGAAAATGACAACGCTCCTG		APHIS-S	None
<i>r16</i> R	CGAGTAAGTGAGGTGCCTACAG	gDNA	AQ65	1211
r16allF	GATGAAAATGACAACGCTCCTG		APHIS-S	1431
notr16R	CTCGGAACAGTGTCCAGGTCT	gDNA	AQ65	None
	CCG <u>GAATTC</u> GCCACCATGGCGGGTGA		APHIS-S	5205
rgCADF	CGCCTGCAT	CDNA		
	TCC <u>CCGCGG</u> ACCGCCTCCGCCACCG			2210
rgCADK	CCCATATGGTAGCAAAAGATTTCCGT	CDNA	AQ03	5219

Table S1. Primers used for cloning and genotyping of PgCad1.

<sup>a</sup>F indicates forward and R reverse.

**Table S2.** Responses to Cry2Ab of pink bollworm larvae from a resistant strain (AQ65) and a susceptible strain (APHIS-S).

Strain.	Slope (SE)ª	LC <sub>50</sub> (95% FL) <sup>b</sup>	RRc
APHIS-S	2.69 (0.339)	0.157 (0.125–0.188)	
AQ65	3.42 (0.402)	0.408 (0.350-0.467)	2.60

aSlope of the concentration-mortality line with its standard error in parentheses. <sup>b</sup>Concentration killing 50% with 95% fiducial limits in parentheses, in  $\mu$ g Cry2Ab per ml diet. <sup>c</sup>Resistance ratio, the LC<sub>50</sub> for AQ65 divided by the LC<sub>50</sub> for APHIS-S.

Table S3. Genetic linkage between resistance to Cry1Ac and cadherin gene PgCad1.

	Larvae with r16r16 (%)			
<b>Backcross family</b>	<b>Control diet</b>	Cry1Ac diet		
1	43	100		
2	53	100		
3	55	100		
4	43	100		
5	46	100		
Mean	48	100		

We used PCR (Fig S3) to determine the genotype for a total of 250 larvae: 147 on control diet (30, 30, 29, 30 and 28 larvae from backcross families 1–5, respectively) and 103 on diet treated with the diagnostic concentration of Cry1Ac (20, 20, 21, 22 and 20 larvae from backcross families 1–5, respectively).

Insect	Cotton	Cotton type Bolls	Entry holes	Entry holes Survivors/	Survival(%) a	Relative
strain	type		per boll boll	boll		survival (%) <sup>b</sup>
AQ65	Bt	48	5.6 (0.3)	1.1 (0.1)	19.4 (1.6)	50.6 (2.7)
APHIS-S	Bt	35	5.6 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
AQ65	Non-Bt	44	5.2 (0.3)	2.0 (0.1)	38.3 (2.8)	
APHIS-S	Non-Bt	43	5.5 (0.2)	1.7 (0.1)	31.1 (0.9)	

Table S4. Survival of AQ65 and APHIS-S larvae reared on Bt cotton and non-Bt cotton.

Values are means with their standard errors in parentheses. <sup>a</sup>Larvae surviving per boll divided by entry holes per boll multiplied by 100%. <sup>b</sup>Survival on Bt cotton divided by survival on non-Bt cotton.