MonLab instead of UALab, none of the eight colonies in 2003 and only one of 13 colonies in 2004 collected from non-*Bt* crops (F3704; collected on July 2004 from Pickens (Desha County), AR, USA) had a RR > 10 (**Table 1**). Therefore, 'resistance' from 2002 was not repeatable in 2003. F3704 was sent to Auburn University and USDA-ARS in Ames, Iowa for resistance confirmation. At Auburn, F3704 was confirmed as being highly resistant to Cry1Ac toxin but, as has been observed for other *Bt*-resistant populations of *H. zea*, went extinct due to fitness costs[5,9]. F3704 also went extinct in both the Luttrell and USDA-ARS laboratories[9]. In addition, elevated bioassay responses in field collections from Pickens (Desha County) have not been observed since 2004; if resistance truly is "field evolved," should we not have observed some change or 'shift' in efficacy in this region up to and including 2007? Therefore, even based on the definition chosen by Tabashnik *et al.* and ignoring the efficacy of commercial *Bt* cotton plants, field-evolved resistance to *Bt* cotton has not yet been detected. Furthermore, similar high levels of variability (299- to 456-fold) in responses to Cry1Ac were observed among laboratory and field-collected *H. zea* populations in 1992–1993 and in 2004 (refs. 2,3).

Collectively, reexamining these data suggests that large genetic variation in Cry1Ac-susceptibility has always been present within *H. zea* populations (at least by 1992–1993, before Bollgard commercialization), and there is no evidence to suggest that there has been a significant shift in susceptibility since the introduction of *Bt* cotton. Other comparable events have occurred that should give us caution in using these data to conclude widespread *Bt* resistance has evolved in *H. zea*. For example, Tabashnik *et al.*[10] reported that alleles for Cry1Ac resistance in *P. gossypiella* were present in surprisingly high frequencies in 1997 in *Bt* cotton fields in Arizona. However, since 1997, not only has resistance to *Bt* cotton by *P. gossypiella* not occurred in the field, laboratory-based estimates of the *Bt* resistance allele frequency in *P. gossypiella* actually have decreased. Such counterintuitive outcomes of laboratory-based resistance monitoring underscore the critical necessity to require results of field tests as the ultimate validation of resistance claims. Similarly, the range of responses to Cry1Ac currently reported in *H. zea* populations remains comparable to that when it was originally measured, and no observable change in *Bt* cotton efficacy has occurred. The primary difference in this case is that *H. zea*'s response to Cry1Ac is, and always has been, highly variable among populations, probably reflecting an inherent tolerance to *Bt* proteins and its highly polyphagous nature and annual migratory behavior. Consequently, based upon the historical and current results, it is premature to conclude that field-evolved resistance to *Bt* cotton has arisen in *H. zea*, as Randy Luttrell has noted himself (http://agfax.com/news/2008/02/btresist0208.htm).

Public scientists and the agricultural industry must continue to be vigilant and monitor for potential changes in susceptibility to *Bt* proteins. Even so, it is important to be cautious in interpreting laboratory data, particularly where comparisons are made among very complex and variable sets of data, conducted during different time periods, by multiple researchers, in different laboratories, using different susceptible colonies and with unique protein sources.

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Bruce E Tabashnik, Aaron J Gassman, David W Crowder & Yves Carrière reply:
We welcome the opportunity to confirm one of the main conclusions of our paper[1]: some field populations of a major cotton pest, *Helicoverpa zea*, evolved resistance to Cry1Ac, the *Bacillus thuringiensis* toxin (*Bt*) in first-generation transgenic *Bt* cotton (also called Bollgard cotton). This conclusion is based on extensive resistance monitoring data for 1992 to 2006 from five papers by Randall Luttrell and his collaborators[2–6], including crucial information about field efficacy and larval survival on *Bt* cotton plants from three papers not cited by William Moar *et al.* above. These data show that the field-evolved resistance documented with laboratory diet bioassays (see Table 1 below) is associated with increased survival on *Bt* cotton leaves (Fig. 1) and control problems in the field[2–6].

The primary goal of monitoring insect resistance to *Bt* crops is not to document field failures, but rather to detect resistance in field populations soon enough to enable proactive management of resistance. Thus, the US Environmental Protection Agency (EPA) mandates monitoring to provide "an important early warning sign" of resistance in field populations[7]. Evolution of resistance is defined as a heritable decrease in a population's susceptibility to a toxin[8,9]. Susceptibility is typically measured in laboratory bioassays testing the progeny of field-sampled insects for responses to the toxin. Such bioassays document resistance if one or more populations with a history of exposure to the toxin in the field are significantly less susceptible than conspecific populations that have had less exposure[9]. Because resistant individuals are most likely to be found in the field surviving on *Bt* crops, sampling insects from *Bt* crops is an essential component of resistance monitoring.

In their rigorous resistance monitoring program, Luttrell and collaborators[2–6] appropriately sampled *H. zea* larvae from *Bt* cotton and *Bt* corn, as well as from various non-*Bt* plants (Table 1). By sampling *H. zea* from *Bt* cotton fields with high boll damage and testing their progeny, Luttrell and collaborators[2–6] showed that reduced field efficacy was associated with increased larval survival on toxin-treated diet and on *Bt* cotton leaves.

Laboratory bioassays on toxin-treated diet enabled Luttrell and collaborators[2–6] to estimate survival at diagnostic concentrations and LC_{50}, the toxin concentration killing 50% of tested insects (**Supplementary Data** online). The resistance ratio (RR) is the LC_{50} of a strain divided by the LC_{50} of one or more conspecific susceptible strains. RR values >10 are most likely to reflect heritable decreases in susceptibility[9]; higher RR values provide stronger evidence of resistance. In a recent paper[10], Moar and his colleagues concluded that their laboratory-selected resistant strain of *H. zea* with a RR of ~100 was "appropriate for characterization" of resistance because this level of resistance might enable survival on *Bt* cotton and "might be appropriate for initiating alternative control strategies."

Field sampling of *H. zea* during 2003 to 2006 produced 14 strains with RR values >100, including two with RR values >1,000 (refs. 3–5) (**Table 1**). Six of these 14 resistant strains were derived from *Bt* cotton or *Bt* corn. The table presented by Moar *et al.* excludes all of the data for 2005 and 2006, as well as the data for the most resistant strains from 2002 and 2003, which were derived from *Bt* cotton.

Resistant strain F3704 derived in 2004 from Pickens, Arkansas, which is included in their table, is especially important for several reasons. This strain had an LC_{50} of 1746 µg Cry1Ac per ml diet and a RR of 578 in tests conducted at the University of Arkansas[4]. Its high level of resistance was confirmed in independent tests by Moar at Auburn University. Contrary to the claim of Moar *et al.* that "elevated bioassay responses in field collections from Pickens (Desha County), have not been observed since 2004," field collections in 2005 from this location yielded two strains with RR values >100 (**Table 1**). Collections from Fayetteville, Arkansas also generated highly resistant strains in two consecutive years (**Table 1**).

Our paper[1] cites the RR values for 2002–2004 reported by Ali *et al.*[4] based on their choice of the UALab strain from the University of Arkansas as the standard susceptible strain. This choice is appropriate, because the UALab strain was the only strain tested in all three years of the study. Furthermore, LC_{50} values (all in µg Cry1Ac per ml diet) were similar for UALab (2.8), a susceptible strain from North Carolina State University (3.2) and a susceptible strain from the US Department of Agriculture (2.2) that was infused with field-collected insects[4] (**Supplementary Fig. 1** online). In 2004, the LC_{50} of UALab was three times higher than the LC_{50} of F1004 (0.9), a susceptible field-derived strain (**Supplementary Fig. 1**). These data refute the notion that UALab had an unusually low LC_{50}.

Table 1 Field-derived strains of *H. zea* with RR values >100 for *Bt* toxin Cry1Ac

Year	Strain	Collection site	Source	RR
1992	None (maximum LC_{50} = 0.93, strain FZ)	NA	NA	NA
1993	None (maximum LC_{50} = 5.97, strain 9315Z)	NA	NA	NA
2002	None (maximum LC_{50} = 91.65, strain F3302)	NA	NA	NA
2003	F3603	Morgan City, MS, USA	*Bt* cotton	515
	F3703	Morgan City, MS, USA	*Bt* cotton	184
	F3803	Morgan City, MS, USA	*Bt* cotton	354
2004	F3704	Pickens, AR, USA	Non-*Bt* cotton	578
2005	F6605	Pickens, AR, USA	*Bt* corn	102
	F6705	Pickens, AR, USA	*Bt* corn	157
	F12105	Miller Co., GA, USA	*Bt* cotton	153
	F13305	Foreman, AR, USA	Light trap	319
	F0105	Texarkana, AR, USA	Clover	>1,000
	F5705	Foreman, AR, USA	Non-*Bt* corn	>1,000
	F7605	Fayetteville, AR, USA	Chickpea	710
2006[a]	F9206	Fayetteville, AR, USA	Chickpea	681
	F4106	Early Co., GA, USA	Non-*Bt* corn	186
	F8306	Calhoun Co., GA, USA	Light trap	254

[a]A total of seven field-derived strains tested in 2006 had less than 50% mortality at a diagnostic concentration of 250 µg Cry1Ac per ml diet[6]. LC_{50} is the concentration (µg Cry1Ac per ml diet) killing 50% of larvae. RR is the LC_{50} of a resistant strain divided by the LC_{50} of a susceptible strain. For years when no strain had a RR > 100, the highest LC_{50} for a field-derived strain is listed. Data summarized are from refs. 2–6. NA, not applicable. Larvae were collected from the plants listed. Gravid female moths were collected from light traps.

The pooled LC_{50} for UALab for 2002–2004 (2.8) also did not differ significantly from the pooled LC_{50} (5.1) for the four susceptible laboratory strains of *H. zea* tested in the 1992–1993 study[2] (**Supplementary Fig. 1**). In striking contrast to the results for *H. zea* from 2003 to 2006, the highest LC_{50} for *H. zea* in 1992–1993 was that of a laboratory strain (8.8 for strain SLZ), not a field-derived strain[2]. These results show that resistance was not detected in the strains of *H. zea* derived from the field in 1992–1993, before *Bt* cotton was commercialized.

The MonLab strain of *H. zea* provided by Monsanto (St. Louis, MO, USA) would be a poor choice as a standard susceptible strain for the 2002–2004 study because it was not tested in 2002, and its pooled LC_{50} for 2003 and 2004 (25.7) was significantly higher than the LC_{50} values of the three susceptible strains from representative geographic areas maintained by public institutions[4] (**Supplementary Fig. 1**). Nonetheless, using MonLab as the standard for comparisons for 2003 and 2004, the results still show strong evidence of field-evolved resistance, with RR values of 14 (F3703), 27 (F3803), 40 (F3603) and 88 (F3704) for four field-derived strains from these two years[4].

Results from diagnostic concentration tests also provide strong evidence of field-evolved resistance. Five field-derived strains from 2003 and 2004 had <50% mortality at a diagnostic concentration of 150 µg Cry1Ac per ml diet[4]. In 2006 studies, a diagnostic concentration of 250 µg Cry1Ac per ml diet killed 98.6% of the Monsanto strain, but caused less than 50% mortality in seven field-derived strains[6]. Mortality at 250 µg Cry1Ac per ml diet was correlated with values for LC_{50} ($r = 0.37$, df = 40, $P = 0.017$) and MIC_{50} ($r = 0.52$, df = 40, $P = 0.0004$), the concentration causing 50% inhibition of molting to second instar[6]. In sum, resistance to Cry1Ac is evident from diet bioassays based on survival at diagnostic concentrations, LC_{50} values and MIC_{50} values.

A powerful combination of experiments demonstrates that resistance in diet bioassays is linked with increased survival on *Bt* cotton plant tissues (**Fig. 1**) and control problems in the field[2–6]. In 2002, Luttrell *et al.*[3] sampled *H. zea* larvae surviving on *Bt* cotton from two fields with "unacceptable levels of boll damage," generating strain UA0233 from Mississippi and UA0234 from Arkansas. Diet bioassays revealed that these were the most resistant strains found in 2002, with RR values of 40 and 22, respectively[4]. Larval survival on *Bt* cotton leaves relative to non-*Bt* cotton leaves was higher for these two strains than for a susceptible lab strain[3] (**Fig. 1a**). This pattern was repeated when similar

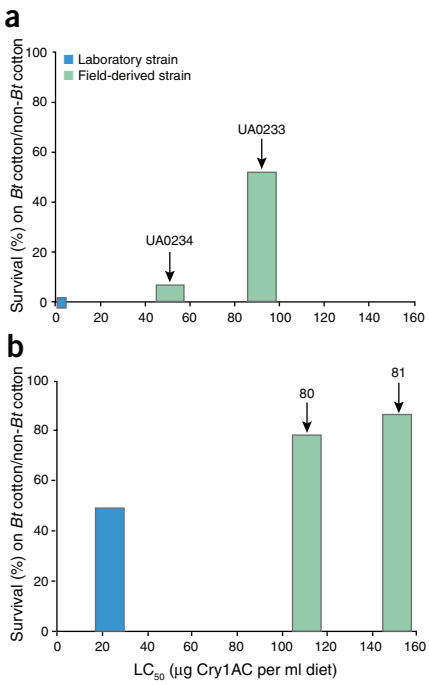

Figure 1 Association between field-evolved resistance of *H. zea* to *Bt* toxin Cry1Ac and larval survival on *Bt* cotton leaves containing Cry1Ac. LC_{50} is the concentration (µg Cry1Ac per ml diet) killing 50% of larvae. The *y* axis shows larval survival on *Bt* cotton divided by larval survival on non-*Bt* cotton × 100%. (**a**) 2002 results. Survival was recorded after larvae fed on leaves for 4 days (ref. 3); LC_{50} values are from ref. 4, in which field-derived resistant strains UA0234 and UA0233 are called F3402 and F3302, respectively (R.G. Luttrell, personal communication). (**b**) 2006 results with field-derived resistant strains, 80 from North Carolina and 81 from Georgia (ref. 5). Survival to adult emergence was recorded for neonate larvae that fed for 7 days on leaves and were transferred to a nontoxic diet. On the basis of analysis of covariance that accounts for variation between experiments in 2002 and 2006, survival on *Bt* cotton relative to non-*Bt* cotton was significantly associated with LC_{50} (one-tailed $P = 0.023$, $r^2 = 91.9\%$). The laboratory strain used in the 2002 and 2006 experiments is called UALab in ref. 4 and LabZea in refs. 3 or 4 (R.G. Luttrell, personal communication).

experiments were conducted in 2006 with two additional field-derived resistant strains[5] (Fig. 1b).

Luttrell *et al.*[3] concluded, "Survival of the more resistant colonies on Bollgard cotton confirms the phenotypic expression of the resistance trait." Luttrell and Ali[5] stated that their results suggest the low susceptibility of some field-derived strains was "heritable" and "associated with a measurable increase in survival on *Bt* plant tissue." They also noted that strains derived from larvae surviving on *Bt* cotton plants in the field "tended to have reduced susceptibility suggesting that some component of the observed field control problems may be associated with the presence of resistance genes"[5]. Moreover, University of Arkansas extension entomologist Glenn Studebaker has been reported to have observed increased damage in the field to Bollgard cotton caused by *H. zea*[11].

Resistance of fall armyworm, *Spodoptera frugiparda*, to Cry1F in *Bt* corn in Puerto Rico cited by Moar *et al.* is the second example of field-evolved resistance to a *Bt* crop documented in the United States. In this case, larvae surviving on *Bt* corn in two fields were suspected to be resistant and were collected to start two field-derived strains. In laboratory diet bioassays, the highest concentrations of Cry1F tested killed all or nearly all of the larvae from a susceptible laboratory strain, but few or none of the larvae from the two field-derived strains. The RR cannot be calculated precisely, but is estimated to be >100 for both strains. As summarized above, similar procedures applied to *H. zea* from 2003 to 2006 yielded 14 field-derived strains with RR values for Cry1Ac >100, including two with RR values >1,000 (Table 1 and Supplementary Data).

There are several implications from the above observations. Control of *H. zea* has been augmented by insecticide sprays and by rapidly increasing use of transgenic cotton that produces *Bt* toxin Cry2Ab as well as Cry1Ac[1,11]. Furthermore, Monsanto's US registration of cotton producing only Cry1Ac is scheduled to expire in September 2009. *Bt* corn producing Cry1F has been voluntarily withdrawn from the market in Puerto Rico. In contrast to the two documented cases of field-evolved resistance described above, most of the pests targeted by *Bt* crops have not evolved resistance[1] (Supplementary Data). We hope that insight gained from the first documented cases of field-evolved resistance to *Bt* crops can help to sustain the efficacy of current and future generations of transgenic crops.

Note: Supplementary information is available on the Nature Biotechnology website.

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The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/

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