UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

March 23, 2004

MEMORANDUM

SUBJECT: EPA Review of Corn Earworm/Cotton Bollworm (*Helicoverpa zea*) North-South Migration and Its Impact on Resistance Management for *Bt* Crops [EPA Registration Nos. 524-489, 68467-2, 67979-1, 29964-3, 524-478, 524-522] Submitted by ABSTC IRM Technical Subcommittee and Monsanto Company. ABSTC Submissions dated March 15, 2002 and May 8, 2003 [Decision Number 48527; DP Barcode: D293193; No MRIDS]; and Monsanto Submission dated January 29, 2004; MRIDs 452425-01, -02; S758117

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ACTION REQUESTED: To review the submitted data on the extent of corn earworm/cotton bollworm (*Helicoverpa zea*) north-south migration and its impact on resistance management for *Bt* (*Bacillus thuringiensis*) crops.

CONCLUSIONS

Based on the modeling studies submitted by ABSTC, parameterized using the data in Gould et al. (2002), CEW reverse migration has no signficant impact (0.05<P) on CEW adaptation to *Bt* crops. This study is an acceptable study, no further data are needed.

BACKGROUND

The *Bt* (*Bacillus thuringiens*) corn and *Bt* cotton registrants were required as a condition of registration to conduct studies or submit existing studies that evaluate the impacts of north-sourth movement of corn earworm/cotton bollworm (*Helicoverpa zea*) (Lepidoptera: Noctuidae) (CEW) from corn-growing regions to cotton-growing regions as it relates to resistance management of CEW. See EPA's 2001 Risks and Benefits Assessment of *Bt* Crops Biopesticide Registration Action Document, Section III and V. for a complete description of the terms and conditions of registration for the *Bt* corn and *Bt* cotton PIPs (EPA, 2001). Bollgard II cotton (EPA Reg. No. 524-522) has the same north-south CEW migration data requirements as Bollgard cotton (EPA Reg. No. 524-478).

This review will be divided into two basic parts: 1) studies on the extent of CEW north-south migration and implications and 2) computer modeling to evaluate the effect of north-south migration on the risk of CEW adaptation to *Bt* corn and *Bt* cotton in a mixed cropping system.. EPA's review focuses on the second ABSTC submission dated May 8, 2003 because it incorporates all of the information/data provided in the first submission dated March 22, 2002. Monsanto's January 29, 2004 is identical to ABSTC's May 8, 2003 submission.

SUMMARY OF ABSTC'S SUBMISSION

1. Studies on the extent of CEW north-south migration and implications

Appendix 1 of the May 8, 2003 submission, "Investigations into the extent of corn earworm (*Helicoverpa zea*) (Lepidoptera: Noctuidae) migration between corn and cotton growing regions of the USA and evaluation of the effects on rate of adaptation to *Bt* corn and *Bt* cotton," contains a summary of what is known about CEW migration, including published studies and a study by Gould et al. that at the time (March 2002) was unpublished but has since been published as Gould et al. (2002). Gould et al. (2002) discussed evidence for north-south migration and its potential impact on resistance management for CEW.

CEW is a highly polyphagous, highly mobile, muiltivoltine insect. The insect is a serious pest on many crops in eastern and southern United States, especially its preferred host corn (Johnson *et al.* 1975), but it also feeds on many non-crop hosts. Other host plants in which *H. zea* is an economic pest include cotton, soybean, tomato, soybean, sorghum, vetch, and peanuts. Because *H. zea* is a significant economic pest of both corn and cotton, adults moving from corn to cotton over the course of the season from and feeding on varieties expressing Cry delta-endotoxins from *Bt*, the pest may undergo selection for adaptation to Cry toxins for several generations each year.

It has previously been assumed by entomologists and in computer models (ILSI 1999, Storer 1999**)** that there is one-way CEW migration, with the insects tracking the host phenology northwards. Insects that develop on corn in the southern U.S. would in the summer migrate to flowering corn in the northern U.S. It was then assumed that these insects die in the north as they are unable to survive the winter. Alleles for adaptation (resistance) to Cry1 toxins (for example) selected on *Bt* corn in the Midwest would therefore not be transmitted to the following year's population. Gould at EPA's Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel Meeting Subpanel (SAP, 2001) suggested that a portion of the *H. zea* population in the Midwest may move back to the south in late summer and fall, and thus in theory, may be able to contribute resistance genes to the next year's population.

To select for adapation to Cry1 toxins in the Midwest that contribute to selection in the CEW population as a whole, ABSTC indicates that following conditions would have to be met:

"1. There would have to be a high level of cross-resistance to the Cry toxins in Bt corn (Cry1Ab and Cry1F) and those in cotton (Cry1Ac).
2. Weather systems would have to facilitate the long-distance dispersal from north to south in the fall. Such weather systems must occur at a time when moths are emerging from Bt cornfields in the corn-growing regions.
3. The numbers of insects returning would have to be significant compared with the number that are resident in the cotton-growing region.
4. The migrating insects must remain sufficiently fit to compete for mates with the resident population in the cotton-growing region.
5. The insects must return to a place where, and at a time when, host plants are available and suitable to maintain the next generation's larval development to pupation.
6. Such migration must be consistently successful across years.
7. Finally, for such migration to increase the rate of CEW adaptation to Cry1 toxins, the migrating population must have undergone more intense selection pressure than the resident population."

Fitt (1989) reviewed evidence for long-range migration of CEW in North America. Other noctuids, black cutworm (*Agrotis ipsilon*) and fall armyworm (*Spodoptera frugiperda*) have also been shown to migrate from southern overwintering areas to the Corn Belt. Long distance migration in all of these insects was associated with passage in weather fronts. Showers (1997) provided evidence for the return migration of *A. ipsilon* on southernly air mass flows in the fall from Iowa to Louisiana and Texas. Pair et al. (1987) presented evidence that return *H. zea* migration can occur from north Texas to the Lower Rio Grande Valley.

Gould et al. (2002) provide indirect evidence for migration of CEW moths from the other corn-growing regions to the cotton-growing regions. Data were collected from two locations – Bossier Parish, Louisiana and College Station, Texas. The timing and extent of reverse migration appears to vary considerably from year to year (Gould et al. 2002, figs. 2, 3, and 4). Gould et al. (2002) point out that at the time of apparent reverse migration, there is very little vegetation that is capable of supporting larval development in Texas. This means that it is unlikely that any migrating moths (from the north) can contribute to the local over-wintering population. However, if the moths can move further south into more tropical areas in which they would contribute to the local over-wintering population then their genes could contribute to the persisting population. There may be locally suitable host plants in the Cotton Belt other than in the area of Texas examined so a migrating population could be important.

ABSTC notes that Gould et al. (2002) did not address the point that cotton in the areas investigated is treated for CEW either by *Bt* PIPs, applied chemical insecticides, or both. *Bt* PIPs and insecticides are very effective at reducing boll damage and CEW larval survival by 80% or more. This means that the number of moths produced in cotton would be relatively small compared to the number of eggs laid in cotton. The converse situation is true for Midwest corn, which is seldom if ever treated for CEW, and 25 to 25% of which is *Bt* corn. ABSTC suggests that care should be taken when interpreting the Gould et al. (2002) data that the relative importance of selection in the Corn Belt versus the Cotton Belt not be overstated.

2. Computer Modeling

Description of the Model

If the immigrant population *Bt*-resistance frequency is different from the native population, due to different net selection pressures in the Corn and Cotton Belts, then the migration phenomenon will alter predictions of the rate of local and population-wide adaptation. ABSTC used a computer model to quantify how migration may impact adaptation rates under a range of different circumstances. The spatially-explicit model of CEW adapation to corn and cotton (Storer et al. 2003) was adapted to incorporate south-north migration in the spring and north-south migration in the summer. Effectively, two models were run in parallel for the summer generations: one for the cotton-growing region and one for the corn-growing region. The Storer et al. (2003) model focused on CEW adaptation in eastern North-Carolina; however, alternations to the adaption risk in that region are likely to be quantitatively similar to the alternations to the risk in other regions. The scenerios modeled were based on the available data and the conclusions of Gould et al. (2002). Sensitivity analyses were conducted to examine the range of possible effects, in terms of the timing and extent of migration, and the levels of *Bt* corn adoption in this region.

The key assumptions of the model include:
- corn, cotton and soybean are crop hosts for CEW;
- weed hosts are available at the start of the season and end of the season;
- complete cross-resistance among the three Cry1 toxins (Cry1Ab, Cry1Ac, Cry1F);
- additive functional dominance of allele for adaptation ($h = 0.5$);
- migration occurs pre-mating (as indicated by field studies on mating and flight behavior in *Helicoverpa* spp.); and
- migration occurs to the same extent every year.

These assumptions are highly conservative for the following reasons:
- there are other abundant crop and non-crop hosts for CEW that are ignored in the model;

- little or no cross-resistance may exist for the Cry1 toxins in corn;
- the resistance alleles in natural populations may be recessive; and
- migration extent varies from year to year and location to location (see Gould et al. 2002).

The effects of several parameters were investigated through a sensitivity analysis.
"1. *pcmigS* = nominal percent of adult CEW population in the south in the late summer that is made up of immigrants. It is nominal because it is based on the population size prior to the introduction of Bt cotton. Because Bt cotton reduced the native population, the immigrant population represents a larger proportion of the south population after Bt is introduced.
2. *returndate* = date at which return migrants actually return – this affects how successful they can be in generating a subsequent population in the south
3. *Nnnpe* = nominal density of CEW larvae in northern Corn Belt. This parameter affects the intensity of cannibalism in Midwest corn and thus the selection pressure for resistance (Storer et al. 2003). It is nominal because it is based on pre-Bt population sizes. Because Bt crops reduce the population size, the actual insect density is lower after Bt is introduced.
4. *pcmigN* = percent of CEW adult population that migrates from the south to the north for the summer
5. *BtcrN* = proportion of corn in the north that is planted to Bt varieties
6. *BtcrS* = proportion of corn in the south that is planted to Bt varieties
7. *Btct* = proportion of cotton that is planted to Bt varieties
8. *Npsoy* = proportion of CEW in the north that utilize soybean as a host rather than corn
9. *winter* = season duration (days). Like *returndate*, this influences the proportion of return migrants that can complete development before the end of the year."

Three set of runs were conducted to examine the effects of these parameters.
"A) *pcmigS* x *returndate* in a 3 x 3 factorial design to examine the interaction of these two factors on the rate of adaptation. pcmigS was set at 0, 25, and 50% returning, *returndate* was set at day 100, 105 and 110.
B) Effect of return migration (i.e. *pcmigS* = 0 or 50%) on adaptation rate at two or three values of each of parameters 3 through 9. Parameter values were chosen to capture what seemed like a reasonable range based on current knowledge. Because knowledge of several of these values is sparse, or because the real world values could change, generally the range tested was very broad. The values tested are given in **Table 1.**
C) Extreme case run, where all parameters were set at values that are likely to cause the greatest acceleration of the rate of adaptation (i.e. biggest contrast between selection in north and south). These values are given in Table 1. They are set so that selection is greatest in the north, lowest in the south, and there is the highest possible proportion of insects returning from the north and the highest probability of returning populations becoming established. The values are constrained by what is permitted (in terms of Bt crop deployment) and what is biologically feasible."

Model output was the resistance gene frequency after 15 years of deploying *Bt* corn and *Bt* cotton. Three to five runs were made at each parameter setting. Output was analyzed by ANOVA (completely randomized design) to examine the effects of return migration and interaction with other parameters on gene frequency. When significant effects or interactions were found, the magnitude of the effect was calculated as the change in the adaptation rate:

$\ln(Q_{+15}/Q_0)/\ln(Q_{-15}/Q_0) - 1,$ Eq. 1

where Q_{+15} is the r-allele frequency after 15 years with return migration, Q_{-15} is the r-allele frequency after 15 years without return migration, and Q_0 is the initial r-allele frequency. This calculation gives the proportionate change in time to a given r-allele frequency due to return migration. Despite the model for the Cotton Belt being parameterized for North Carolina, this statistic enables general inferences to be drawn on the impact of return migration on resistance evolution in other parts of the Cotton Belt.

Results and Discussion of the Model

Table 2 gives F values for all the analyses. There were two significant effects/intereactions and two weaker interactions ($0.05<P<0.01$) noted in these model runs when one parameter was varied at a time. There was no significant interaction between *pcmigS* and *returndate* meaning that even with the 50-60% CEW migrating from the north inferred by Gould et al. (2002) and producing pupae, there was no effect of return migration on gene frequency.

There was, however, a weak interaction between the percentage of *Bt* corn planted in the north (*BtcrN*) and return migration. At 80% Bt corn in the north, return migration increased the 15-year gene frequency from $2.2e^{-3}$ to $2.4e^{-3}$, an increase in adaptation rate (Eq. 1) of 2%. At 30% Bt corn in the north, return migration decreased the 15-year gene frequency from $2.3e^{-3}$ to $2.1e^{-3}$, a decrease in adaptation rate of 4%. Thus, even with extreme adoption rates of *Bt* corn of 80%, return migration had very little effect on adaptation rate in the south.

There was a trend for return migration to slow resistance evolution when investigating the percentage of insects moving north (*pcmigN*). This is due to the returning population having a lower r-allele frequency than the resident population. The main decrease in adaptation rate due to return migration was 5% ($F_{1,14}=0.05$).

Two of the parameters had significant main effects on the 15-year r-allele frequency: increasing the percentage of *Bt* corn (*BtcrS)* or *Bt* cotton (*Btct*) in the south increased the 15-year r-allele frequency. These effects are expected because the percentage of *Bt* corn and *Bt* cotton determine the intensity of selection exerted on the insects feeding in these two crops. At the highest level of *Bt* cotton deployment simulated (95%), selection in the south is most intense and return migrants are expected to reduce the adaptation rate by introducing a population that has experienced lower selection. However, this model suggests that even under this scenario, return migration would not significantly affect the rate of adaptation.

Figure 1 displays what might happen if all of the parameters were set to extreme values (**Table 1, last column**). These are the values that are expected to most enhance the effect of return migration. Under these circumstances, the effect of return migration was non-significant, but there was a trend ($0.05<P<0.1$) for this effect, $F_{1,9}=3.96$, $P=0.082$ (**Figure 1**). At these parameter setting, return migration increased the rate of CEW adaptation to *Bt* crops by 10%. For example, if *Bt* crops were expected to be effacious against CEW for 25 years without considering return migration, then in the most extreme case, with return migration, one might expect these crops to be efficacious for at least 22.5 years when return migration is considered.

Modeling Study Conclusions

This modeling study, parameterized using data on the possible extent of return migration in Gould et al. (2002), shows that north-south migration could only ever have a very limited impact on the rate of corn earworm adaptation to *Bt* crops. Under current levels of *Bt* crop deployment (30% Bt corn in the Corn Belt, 60% Bt cotton deployment in the Cotton Belt), return migration is expected to marginally slow corn earworm adaptation. Under the greatest levels of *Bt* corn deployment that are possible in the Corn Belt (80% Bt corn), this migration could marginally speed the rate of adaptation, but only under conditions where selection in the Cotton Belt is at the low end of the range investigated (60% Bt cotton).

EPA REVIEW

Gould et al. (2002) used stable carbon isotope analysis to assess alternate host use by CEW. They found that non-*Bt* C_4 plants, probably corn, in Mexico and the U.S. Corn Belt appear to serve as important alternate hosts (non-structured refuge) for CEW. Late-season CEW moths captured in Louisiana and Texas maybe migrants whose larvae developed on corn in more northern locations. These authors provide indirect evidence (they did not establish the origin of these moths, i.e., which C_4 or C_3 plants and the distances they are migrating) for migration of CEW from corn-growing areas in the northern U.S. to cotton-growing area in the southern U.S. These findings counter the prevailing hypothesis that the majority of late-season moths are produced from larvae feeding on cotton, soybean, and other C_3 plants. The authors conclude that when C_4 plants are suitable (e.g., corn), less than 10% of CEW moths are developing on cotton, and that later in the season, 50% of the moths are produced in cotton and soybean. Corn is most likely serving as the predominant C_4 host for CEW. Southern corn is probably an important C_4 host for early season; while, the Corn-Belt corn serves as an important C_4 host for mid-season. Currently, approximately 30% of the corn acreage is planted as *Bt* corn. The authors conclude that the non-*Bt* corn refuge is probably more critical to CEW resistance management than the relatively small non-*Bt* cotton structure refuge, and this non-*Bt* corn refuge should be maintained. Gould et al. (2002) state that the 50% non-*Bt* corn refuge in cotton-growing areas should be maintained. As ABSTC note in their review, Gould et al. (2002) did not discuss the impact that insecticides of *Bt* cotton might have had on the production of CEW moths in cotton as one reason why moth production in cotton is so low.

Based on the modeling studies submitted by ABSTC parameterized using the data in Gould et al. (2002), CEW reverse migration has no significant impact (0.05<P) on CEW adaptation to *Bt* crops. EPA agrees with ABSTC's analysis and conclusions. Under current levels of *Bt* crop deployment (30% *Bt* corn and 60% *Bt* cotton), return migration is expected to slow adaption by about four percent (4%). Even if *Bt* corn reached its maximum allowable level of 80% deployment in the Corn Belt, reverse migration would only increase the rate of adaptation by about two percent (2%) if *Bt* cotton was at 60% deployment, the low end of the range investigated. Even if extreme parameters were used in the model, reverse migration would be predicted to have a 10% impact on CEW adaptation. However, it is extremely remote that all of these parameter conditions would be met in the field year after year. Modeling studies indicated that the percentage of *Bt* cotton and *Bt* corn in the south significantly increases the 15-year r-allele frequency, but return migration did not significantly affect the rate of adaptation even when *Bt* cotton was at 95% deployment

This study is an acceptable study, no further data are needed.

References

Fitt, G 1989. The ecology of *Heliothis* species in relation to agroecosystems. *Annu. Rev. Entomol.* 34: 17-52.

Gould, F., N. Blair, M. Reid, T. L. Rennie, and S. Micinski. 2002. *Bacillus thuringiensis*-toxin resistance management: stable isotope assessment of alternate host use by *Helicoverpa zea*. *Proc. Natl. Acad. Sci.* 99:16581-16586.

ILSI Health and Environmental Sciences Institute. 1999. Evaluation of Insect Resistance Management in Bt Field Corn; A Science-based Framework for Risk Assessment and Risk Management. Report of an ILSI HESI Expert Panel. ILSI HESI, Washington, D.C.

Johnson, M. W., Stinner, R. E., Rabb, R. L. 1975. Ovipositional response of *Heliothis zea* (Boddie) to its major hosts in North Carolina. *Environ. Entomol.* 4: 291-297.

Pair, S.D., Raulston, J. R., Rummel, D. R., Westbrook, J. K., Wolf, W. W. Sparks, A. N., and Schuster, M. F. 1987. Development and production of corn earworm and fall armyworm in the Texas High Plains: evidence for reverse fall migration. *Southwestern Entomologist*. 12: 89-99.

Scientific Advisory Panel (SAP). 2001. Issues pertaining to the Bt plant pesticides Risk and Benefit Assessments, October 18-20, 2000. SAP Report No. 2000-07.

Showers, W. B. 1997. Migratory ecology of the black cutworm. *Annu. Rev. Entomol.* 42: 393-425.

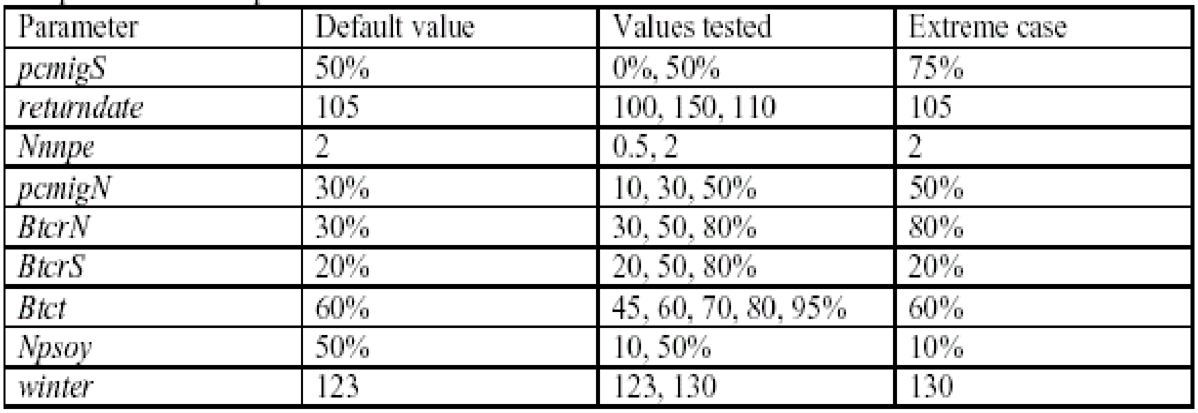
Storer, N. P. 1999. The corn earworm, Bt transgenic corn and Bt-resistance in a mixed cropping system. Ph.D. Dissertation. North Carolina State University.

Storer, N. P., S. L. Peck, F. Gould, J. W. Van Duyn, and G. G. Kennedy. 2003. Spatial processes in the evolution of resistance in *Helicoverpa zea* (Lepidoptera: Noctuidae) to Bt transgenic corn and cotton in a mixed agroecosystem: a biology-rich stochastic simulation model. *J. Econ. Entomol*. 96: 156-172.

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

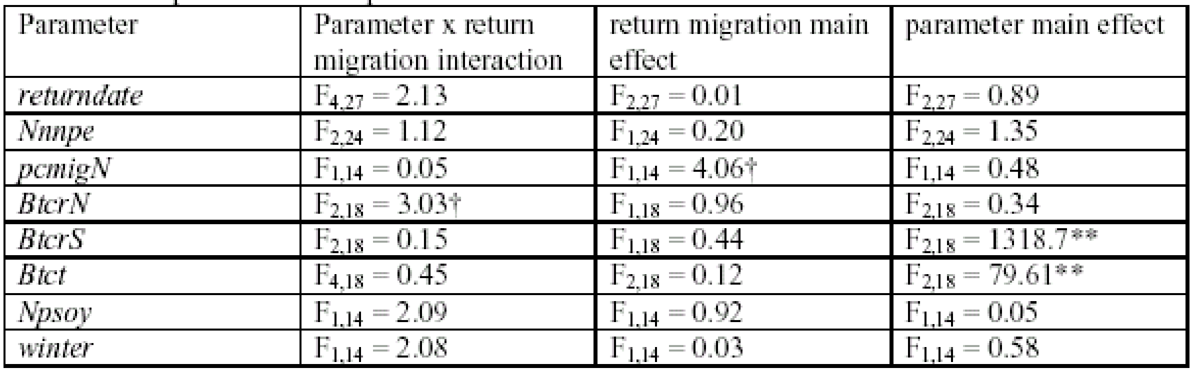
Tables 1 and 2 reprinted from p. 13 of the May 8, 2003 submission.

Table 1. Parameter values used to examine effect of return migration on corn earworm adaptation to Bt crops.



Parameter	Default value	Values tested	Extreme case
pcmigS	50%	0%, 50%	75%
returndate	105	100, 150, 110	105
Nnnpe	2	0.5, 2	2
pcmigN	30%	10, 30, 50%	50%
BtcrN	30%	30, 50, 80%	80%
BtcrS	20%	20, 50, 80%	20%
Btct	60%	45, 60, 70, 80, 95%	60%
Npsoy	50%	10, 50%	10%
winter	123	123, 130	130

Table 2. Analysis of variance for effect of return migration and input parameters on corn earworm adaptation to Bt crops



Parameter	Parameter x return migration interaction	return migration main effect	parameter main effect
returndate	$F_{4,27} = 2.13$	$F_{2,27} = 0.01$	$F_{2,27} = 0.89$
Nnnpe	$F_{2,24} = 1.12$	$F_{1,24} = 0.20$	$F_{2,24} = 1.35$
pcmigN	$F_{1,14} = 0.05$	$F_{1,14} = 4.06$†	$F_{1,14} = 0.48$
BtcrN	$F_{2,18} = 3.03$†	$F_{1,18} = 0.96$	$F_{2,18} = 0.34$
BtcrS	$F_{2,18} = 0.15$	$F_{1,18} = 0.44$	$F_{2,18} = 1318.7$**
Btct	$F_{4,18} = 0.45$	$F_{2,18} = 0.12$	$F_{2,18} = 79.61$**
Npsoy	$F_{1,14} = 2.09$	$F_{1,14} = 0.92$	$F_{1,14} = 0.05$
winter	$F_{1,14} = 2.08$	$F_{1,14} = 0.03$	$F_{1,14} = 0.58$

† weak effect (0.05 < P • 0.10)

* significant effect (0.01 < P • 0.05)

** significant effect (P • 0.01)

Figure 1. Boxplot showing the effect of return migration on the average R-allele frequency after 15 years of *Bt* crop deployment (Q_{15}). Means are indicated by the red (black if non-color printer) circle.

(Reprinted from p. 14, May 8, 2003 submission)