Where Is Scientific Evidence in Support of Refuge Size Reduction for Pyramided Bt Crops?

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Genetically Modified Bt Corn

Genetically modified corn plants expressing insecticidal proteins originating from *Bacillus thuringiensis* (Bt) provide a powerful tool for managing insect pests. They are widely accepted by the U.S. growers and are grown over large acreages. The scope of their activity is limited to pests in the order Lepidoptera (controlled by Cry1Ab, Cry1F, Cry1A.105, Cry2Ab2, and Vip3A) toxins and order Coleoptera (controlled by Cry3A, Cry34/35Ab1, and Cry3Bb1 toxins). Most of the recent corn varieties simultaneously express two or more toxins active against the same taxonomic group of pests (pyramided plants) or against different taxonomic groups of pests (stacked plants).

Insecticide Resistance

Insecticide resistance is a serious world-wide problem, with numerous populations of different insect species becoming resistant to a broad range of insecticidal compounds (Fig. 1). This includes several Bt toxins. Bt plants proved to be rather durable compared to chemical pesticides, in large part because growers were required to follow a rigorous resistance management plan. Nevertheless, a number of field-evolved resistance cases have been already reported in Puerto Rico (*2*), South Africa (*3*), India (*4*), and Iowa (*5*). Despite their limited number, these cases already represent a serious economic concern (Fig. 2).

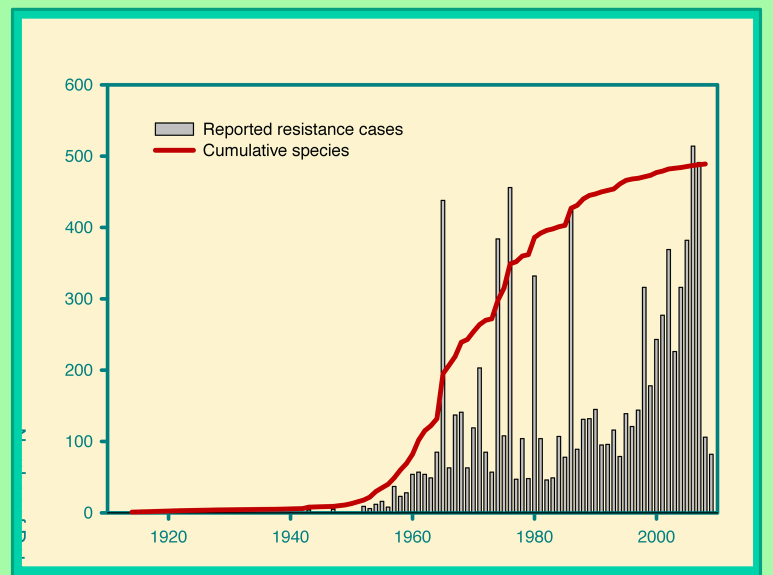


Fig. 1. Insecticide resistance since the first case was detected in 1914 (*1*). The graph is likely to underestimate actual numbers because many cases of resistance remain unreported.

Monday, August 29, 2011 B1

Monsanto Corn Plant Losing Bug Resistance

By Scott Kilman

Widely grown corn plants that **Monsanto** Co. genetically modified to thwart a voracious bug are falling prey to that very

Fig. 2. Headline on the front page of the business section of *Wall Street Journal*.

Resistance Management Requirements

Agricultural biotechnology companies are required to follow mandatory resistance management guidelines administered by the US Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). A consensus resistance management plan for corn plants was approved by the EPA in 2000 after extensive exchanges among stakeholders and following recommendations of the FIFRA Scientific Advisory Panel (SAP) comprised of independent experts. It required planting at least 20% of non-Bt refuges as blocks or strips adjacent to Bt fields.

Growing non-Bt plants adjacent to the Bt crop creates a refuge where susceptible insects can survive. Because resistance is normally inherited as a recessive trait, interbreeding between susceptible survivors in the refuge and resistant survivors in the main crop produces offspring that cannot survive on Bt plants. This strategy is only efficient when the refuge supports enough susceptible inhabitants to greatly outnumber the resistant inhabitants of the Bt crop.

Policy Changes

In the last three years, the EPA has dramatically relaxed refuge requirements for the new pyramided corn varieties that express several Bt toxins, which are different in structure but effective against the same pests. The decisions have been made either without consulting the FIFRA SAP, or directly against its recommendations (*6,7*).

- ➢In 2008, refuge size was reduced from 20% to 5% for the SmartStax® corn jointly produced by Monsanto and Dow Agrosciences
- ➢In 2010, refuge size for the Optimum® AcreMax® corn produced by DuPont was reduced to 10%. Furthermore, the growers were no longer required to plant a separate, non-Bt refuge. Instead, it was incorporated into Bt fields by pre-mixing Bt and non-Bt seeds, which were then sold to farmers (the so-called "refuge-in-bag" approach)
- ➢In 2011, refuge requirements for Genuity® SmartStax® corn by Monsanto and Dow Agrosciences, Optimum® AcreMax® Xtra corn by DuPont, and Agrisure® 3122 corn by Syngenta was reduced to 5% "refuge-in-bag" seed blends

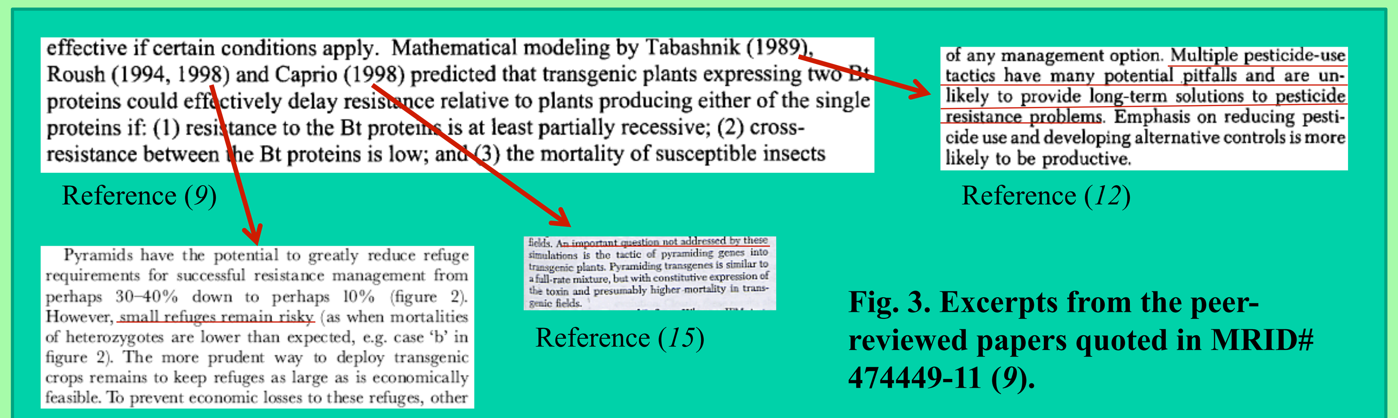
Abstract

Genetically modified plants expressing insecticidal Cry proteins originating from a soil-dwelling bacterium *Bacillus thuringiensis* (Bt) provide a powerful tool for managing insect pests. Unfortunately, insect ability to develop resistance to insecticidal proteins potentially jeopardizes its long-term efficiency. Review of registration materials submitted by plant biotech industry to the U.S. Environmental Protection Agency and the existing scientific literature shows that currently available evidence in support of reducing refuge for the pyramided genetically modified corn plants to 5% of the total crop area is essentially limited to predictions of a single unpublished mathematical model developed "in-house" by the industry scientists. Additional research and a more extensive (and open) scientific discussion will be of great benefit for ensuring sustainable use of this technology.

Scientific Evidence (or Lack Thereof)

The main argument in support of reducing the refuge size for pyramided plants is that toxins in the pyramid have different modes of action (*8,9*). Thus, a pest that has a mutation making it resistant to one toxin will be killed by another toxin expressed by the same plant. This reasoning certainly has merit. However, it lacks empirical support (at least at the present moment). Furthermore, it heavily relies on the assumption of no cross-resistance between different toxins in the pyramid.

No laboratory or field experiments have ever been conducted to test the durability of pyramided plants in the presence of a 5% refuge, even though there are laboratory microcosms allowing to do so (*10,11*). In the absence of empirical data, regulators had to rely on mathematical models predicting insect rate of adaptation to Bt plants. However, **all published mathematical models either tested larger refuges, or assumed lack of cross-resistance, or both**. Papers quoted by the industry in support of their application *(9)* never advocated a refuge of 5% (*12-15;* Fig. 3*)*. On the opposite, they often specifically cautioned about dangers of small refuges.



effective if certain conditions apply. Mathematical modeling by Tabashnik (1989), Roush (1994, 1998) and Caprio (1998) predicted that transgenic plants expressing two Bt proteins could effectively delay resistance relative to plants producing either of the single proteins if: (1) resistance to the Bt proteins is at least partially recessive; (2) cross-resistance between the Bt proteins is low; and (3) the mortality of susceptible insects

Reference (*9*)

of any management option. Multiple pesticide-use tactics have many potential pitfalls and are unlikely to provide long-term solutions to pesticide resistance problems. Emphasis on reducing pesticide use and developing alternative controls is more likely to be productive.

Reference (*12*)

Pyramids have the potential to greatly reduce refuge requirements for successful resistance management from perhaps 30–40% down to perhaps 10% (figure 2). However, small refuges remain risky (as when mortalities of heterozygotes are lower than expected, e.g. case 'b' in figure 2). The more prudent way to deploy transgenic crops remains to keep refuges as large as is economically feasible. To prevent economic losses to these refuges, other

Reference (*14*)

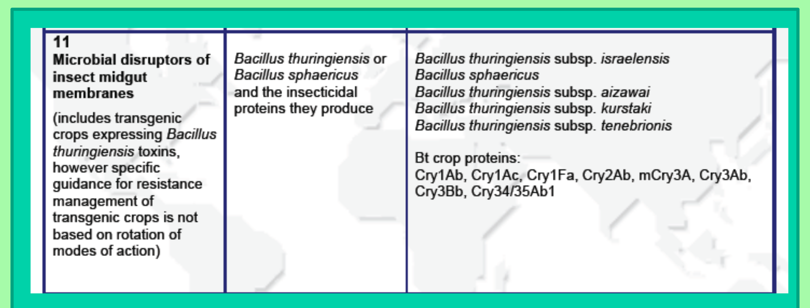
fields. An important question not addressed by these simulations is the tactic of pyramiding genes into transgenic plants. Pyramiding transgenes is similar to a full-rate mixture, but with constitutive expression of the toxin and presumably higher mortality in transgenic fields.

Reference (*15*)

Fig. 3. Excerpts from the peer-reviewed papers quoted in MRID# 474449-11 (*9*).

Issue of Cross-Resistance

Resistance to multiple structurally different chemicals is not unusual among insects. The industry argues that it is unlikely because toxins in pyramided plants have different modes of action. This, however, is not true. Insecticide Resistance Action Committee (of which all Bt plant producers are members) classifies all Cry proteins into a single group based on their mode of action (Fig. 4). It does not recognize any further subdivisions. So, it is probably better to talk about variations in a single mode of action. Furthermore, cross-resistance is known to happen even to insecticides with different modes of action. Not surprisingly, several cases of cross-resistance to different Cry toxins (or even Cry toxin and a pyrethroid) have been reported in a number of sources (Table 1),



11 **Microbial disruptors of insect midgut membranes** (includes transgenic crops expressing *Bacillus thuringiensis* toxins, however specific guidance for resistance management of transgenic crops is not based on rotation of modes of action)	*Bacillus thuringiensis* or *Bacillus sphaericus* and the insecticidal proteins they produce	*Bacillus thuringiensis* subsp. *israelensis* *Bacillus sphaericus* *Bacillus thuringiensis* subsp. *aizawai* *Bacillus thuringiensis* subsp. *kurstaki* *Bacillus thuringiensis* subsp. *tenebrionis* Bt crop proteins: Cry1Ab, Cry1Ac, Cry1Fa, Cry2Ab, mCry3A, Cry3Ab, Cry3Bb, Cry34/35Ab1

Fig. 4. Mode of action of Cry proteins (*16*).

Issue of Cross-Resistance - Continued

including industry's own submission to the EPA (Fig. 5). Positive correlations between resistance to Cry2Ab and Cry1Ac were detected in the field populations of *Helicoverpa zea*. Furthermore, *H. zea* evolved resistance in the field to Cry1Ac and Cry2Ab in pyramided Bt cotton (*21*).

Table 1. Cross-resistance to structurally different toxins reported in scientific literature.

Species	Toxins	Reference
Plutella xylostella	Cry1Ac, deltamethrin	*(17)*
Heliothis virescens	Cry1Ac, Cry2Aa	*(18)*
Pectinophora gossypiella	Cry2Ab, Cry1Ac	*(19)*
Spodoptera exigua	Cry1Ab, Cry1Ca, Cry1Da	*(20)*

Concerns over cross-resistance led to the initial rejection of the 5% refuge size by the EPA (*22*). In response, the industry developed a modification to its model (*9*) allowing for various degrees of cross-resistance between the toxins. The modified model predicted that pyramided genetically modified corn with a 5% refuge would be more durable for *Ostrinia nubilalis* and *Diatraea grandiosella* control than the single Bt products with a 20% refuge under all adoption, cross-resistance, and efficacy scenarios. Apparently, that evidence alone was deemed to sufficient for a far-reaching decision to reduce the refuge size to 5%.

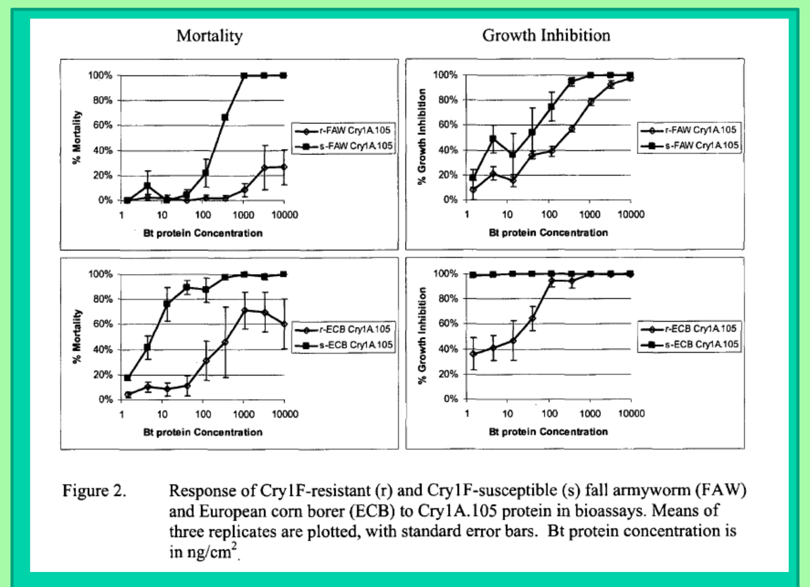


Fig. 5. Excerpt from MRID# 474449-11 (*10*).

Discussion and Conclusions

This presentation is not an attack against Bt plants or agricultural biotechnology. On the opposite, the author wants to see their sustainable use over many years. It is certainly possible that pyramiding of multiple toxins in genetically modified plants will allow a sustainable reduction in refuge size. Furthermore, the industry-developed model provides a valuable evidence in support of such an approach. **However, a single unpublished model hardly amounts to a strong foundation for making important regulatory decisions.** Additional research and a more extensive (and open) scientific discussion are essential for developing an efficient integrated resistance management plan.

References Cited

1.M. E. Whalon, D. Mota-Sanchez, R. M. Hollingworth, L. Duynslager, *Arthropod Pesticide Resistance Database*, www.pesticideresistance.org, accessed on August 25, 2011.
2.W. Moar *et al*. *Nature Biotechnology* 26, 1072–1074 (2008).
3.J. B. J. Van Rensburg, *S. African J. Plant Soil* 24, 147-151 (2007).
4.F. Huang, D. A. Andow, L. L. Buschman, *Entomol. Exp. Appl.* 140, 1-16 (2011).
5.A. J. Gassmann, J. L. Petzold-Maxwell, R. S. Keweshan, M. W. Dunbar, *PLoS ONE* 6(7):e22629 (2011) doi:10.1371/journal.pone.0022629.
6.J. E. Bailey, "Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held February 23 - 24, 2009 on the Evaluation of the Resistance Risks from Using a Seed Mix Refuge with Pioneer's Optimum® AcreMax™1 Corn Rootworm-Protected Corn" (Memorandum to Debbie Edwards, Director of the Office of Pesticide Programs, United States Environmental Protection Agency, Washington, DC, 2009).
7.S. R. Matten," Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held December 8 - 9, 2010 to Address Scientific Issues Associated with Insect Resistance Management for SmartStax™ Refuge-in-the-Bag, a Plant-Incorporated Protectant (PIP) Corn Seed Blend" (Memorandum to Steven Bradbury, Director of the Office of Pesticide Programs, United States Environmental Protection Agency, Washington, DC, 2010).
8.G. P. Head. Insect resistance management plan for second generation lepidopteran-protected corn, MON 89034. Report submitted to the EPA by Monsanto. MRID# 469514-30 (2006).
9.G. Head and N. Storer. Insect Resistance Management Plan for MON 89034 x TC1507 x MON 88017 x DAS-59122-7. MRID# 474449-11 (2008).
10.J.-Zh. Zhao *et al.*, *Nature Biotechnology* 21, 1493-1497 (2003).
11.J.-Zh. Zhao *et al., Proc. Natl. Acad. Sci. USA* 102, 8426-8430 (2005).
12.B. E. Tabashnik. *J. Econ. Entomol*. 82, 1263-1269 (1989).
13.R. T. Roush. *Biocontrol Sci. Techn.* 4, 501-516 (1994).
14.R. T. Roush. *Trans. R. Soc. Lond.* 353: 1777-1786 (1998).
15.M. A. Caprio. *J. Econ. Entomol.* 91, 1021-1031 (1998).
16.Insecticide Resistance Action Committee. 2009. IRAC Mode of Action Classification, v. 7.1. Issued June 2011. http://www.irac-online.org.
17.A. H. Sayyed *et al. Pest Manage. Sci.* 64, 813–819 (2008).
18.F. Gould *et al*. *P. Natl. Acad. Sci.* USA 89, 7986–7988 (1992).
19.B. E. Tabashnik *et al*. *P. Natl. Acad. Sci*. 106, 11889–11894 (2009).
20.Hernández-Martínez, P., Ferré, J. & Escriche, B. *Pest. Manage. Sci*. 65, 645–650 (2009).
21.B. E. Tabashnik, J. B. J. Van Rensburg, Y. Carrière. *J. Econ. Entomol.* 102, 2011–2025 (2009).
22.J. Kausch. Review of the public interest document in support of the section 3(c)7(C) Registrations of Monsanto Company's insect protected *Bacillus thuringiensis* corn products. Memorandum to Susanne Cerrelli, Regulatory Action Leader, Microbial Pesticides Branch, Biopesticides and Pollution Prevention Division, the USEPA. February 29, 2008.