What Are the Major Impediments to Resistance Management for Crops in the Social Sciences and Governance?

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I am going to argue the mechanism of genetics and management theory for pesticide resistance management was well understood by about 1990–95, but 20 years later successes in delaying herbicide resistance in the field are relatively few, while there have been successes in maintaining insect resistance. After working in this area for more than 30 years, my conclusion is that the problem is not in not knowing what to do, it is in implementing the right practices. With the benefit of hindsight, I have concluded that most or all of these successes have involved some government intervention—at least to a modest level. This government involvement was proposed as early as 1989, but little or no action was taken.

I want to draw attention to Australia, where the hand of government was rather light and where intervention was quite limited. In the Australian cotton industry, innovation was driven by the growers and farmers, who then went back to the government and asked them to enforce the rules they had developed for themselves by blocking free riders from using technology without paying any of the financial and management costs to delay resistance.

In 1984 Michael Dover and Brian Croft wrote a monograph that argued that US EPA should do more to regulate pesticide resistance, because resistance would in virtually all cases lead to more environmentally risky pesticide applications and increased use. They stated that this was well within the EPA mandate, because a failure to manage resistance exacerbates the environmental impacts of pesticide use.

Also in 1984 a National Academy of Sciences meeting was held, and there was much debate about this paper. Dover and Croft had generally rejected the idea of government intervention, so demanding its involvement was an important position for them to take. Miranowski and Carlson outlined which conditions would favor resistance management at a single company level, and they concluded that for a highly profitable technology with

no potential or actual close substitutes, monitoring for resistance would be pretty easy. The monopoly permitted the company to market the technology in a way that included resistance management, and testability was such that voluntary management by growers would be economical. So if you look at the types of technologies being developed, you can talk with Charles Dickens of a "Tale of Two Monsanto Technologies." One has worked out pretty well, the other not so.

In the case of *Bt* crops, the resistance problems are relatively modest after more than 16 years of intensive use. For Roundup Ready (RR) crops, the monopoly was on the crops, technically not on the herbicide. At last count there were more than 14 weed species across the US and abroad showing resistance. Still unpublished data shows that this has resulted in varying losses to growers in excess of $99,000,000, a huge amount and actually considerably more than insect resistance costs, although even there a couple of cases beginning to show resistance. Many of the conditions that would normally favor resistance management for a single compound, as Miranowski and Carlson defined it, were met, yet there is relative success with *Bt* crops and less so far with RR crops.

I will give you a quick overview of insecticide resistance management strategies, many of which could be extrapolated to herbicide resistance and the reasons behind the strategies. I want to persuade you that this is actually pretty simple. Many rules are available for pesticide resistance management that in most cases make the determination of best practices fairly simple and straightforward. In 1989, after I had been working on this for about 10–15 years, I became aware of this and wondered why I had not figured this out sooner.

Here is a summary of my thinking since I first published it in 1989: If you look at the resistance management strategies people are discussing, you see a very short list. Should you be using high doses or low doses, or in the case of multiple compounds, should you rotate them over time, use them simultaneously as a mixture, or apply them in a mosaic, where one farmer is using one compound and his neighbor is using something else? Often overlooked in this is integrated pest management (IPM), beginning in the mid 1950s, when people realized that pesticides were never going to be a permanent solution and that the best strategy was nonchemical controls. When one technique doesn't work anymore, change your control tactics—keep the pests off balance.

The IPM strategy was based on the notion that if you could kill the heterozygotes with whatever dose you were using by controlling the length of exposure, you could delay resistance for a long time, because the heterozygotes were the most common carriers of resistance. If the initial frequency of resistance was about 10-4, which we think is pretty high, the frequency of resistant homozygotes would be small. Some populations wouldn't even have resistance from homozygotes. So if you kill all of them, you basically stop resistance in its tracks. But the strategy was more complicated than this. It depended on having a very low initial frequency of resistance. The mortality had to be high—greater than 95%—and even across the range of life stages. You might be able to kill neonate cotton boll larvae when they are barely visible, but by the time they have grown into "snakes," as we sometimes call them, once they are about an inch long, it is virtually impossible to kill them.

You also must have effective refuges that are not affected by insects migrating back and mixing with each other. For weeds we don't really have refuges in the usual sense. The strategy there is using a seed bank where some weed seeds stay in the crop for a year or two without germinating and come back out later. The basic details of all this were worked out by Tabashnik and Croft in a 1982 paper that really pulled all the analyses together in one place. Most resistance that poses a real problem is due to single major genes that give you three genotypes—septal homozygotes, heterozygotes, and resistant homozygotes. If you transform the mortality on this scale of a low dose you can see the difference between the three different genotypes. Tabashnik and Croft showed that if you increase the dose and kill everything, you can delay resistance indefinitely, but only if you have strong migration of septal homozygotes to mate with these individuals and convert their offspring heterozygotes that get killed at the dose used. In absence of migration, the time to resistance would get ever shorter, because obviously the only possible survivors would be the ones that carry both genes.

The next strategy was to see what would happen if more than one compound was used. One of the most famous of these strategies was adopted in Australia in the 1980s to try to manage resistance to pyrethrin in cotton bollworms. Australia already had had problems with DDT resistance, including spectacular failures in a new cotton growing area in North Australia, where the crop failed and farmers just walked away from the fields. We know that one of the major mechanisms of resistance to DDT is an altered nerve channel that provides resistance to pyrethroids as well. The cotton growers were very concerned about this. So they divided their cropping season into three periods, early, mid, and late, and they rotated the pesticides over time, trying to take advantage of the different susceptibilities of the beneficial species. And even though Endosulfan is considered to be a fairly noxious pesticide in many ways, it didn't have the effect of blowing out secondary pests in Australia, as did other pesticides. So they went with pyrethrin in the early season when they most needed to protect the crop and finished with organophosphates in the late season, all of which had different target sites.

One of the most interesting aspects of this strategy is that we went back to it years later to analyze what would have happened if we had taken other measures, and it looked like it bought them at least six or seven years of use of pyrethroids compared to what would have happened if they had used a laissez-faire approach. It is important to note that this strategy was adopted through a soft approach to regulation. The regulations were put on the label of pesticides, so pyrethrins were used in the middle of the season and beyond that enforcement was done not by a broad government edict but by other, more subtle actions. In Queensland, for example, where all the pesticides were being applied by air, if an aerial applicator applied a pesticide outside the target area, he risked losing his applicator license. It was a minimalist regulation that required people to participate, and a lot of compliance was voluntary, and successfully so.

Why did this work? I did some experiments with mosquitos, where I took mosquito populations that were carrying pyrethrin or DDT resistance—two different genes, two different chromosomes. I split the population and in every generation I set 10% of the

mosquitos aside. Of the others I treated half with DDT and half with pyrethrin and found an approximately twofold delay of resistance. Why should this happen? You can do this as a thought experiment: If you had a pesticide that was effective, but a single treatment would cause resistance to everything in the population, you would lose that pesticide immediately. Now let us say that you use two pesticides at the same time, so half of the population gets treated with either one or the other. Depending on dominance relationships, somewhere between 25–75% of the population will be resistant. If you had used just the first pesticide, you would at least have the second pesticide to use in the second year, giving you roughly a twofold advantage—a latent resistance. Based on this experiment it is obvious that allowing people to apply pesticides any way they want in neighboring fields must be the worst possible strategy. This is essentially what happened in Australia and in other countries.

Dave Pree in Canada has observed problems with oriental fruit moth developing resistance to various pesticides. Even though he started out in the rescue stage with very high survival from both types of insecticides, he persuaded growers to go on a rotational scheme, and over time resistance actually declined: Even though he started out with a real problem, he was able to back it off by discouraging growers from using the pesticides in neighboring orchards at the same time.

As a next strategy there are several different options. If you are prepared to accept that a rotational scheme is better than just a laissez-faire approach, what is required for mixtures to work? Once again you must have low initial gene frequency and no cross-resistance. Resistance to at least one of the two toxins needs to be somewhat recessive, and you need to get redundant killing of the septal homozygotes and maintain refuges. In order to make this work, you must be able to control the susceptible twice, because if it was susceptible to one pesticide you need to take out whatever resistant carriers might be on the second locus.

Hugh Comins in Australia and Fred Gould in North Carolina independently called this "redundant killing." The same principle works for pyramiding GM traits for *Bt* as it would for pesticides. This highlights what people have thought for a long time—that pesticides have to have equal decay rates—but that that is actually wrong. Even if they decay together over time, rare cross-resistance to one of them may occur. So the equal decay rate is less important than an abrupt drop-off of the septal homozygotes. If they are exposed to one pesticide, you have to expose them to other pesticides with extreme prejudice—you have to make sure they die.

Seen from a different perspective, if you assume the model of one locus per pesticide, then these are septal homozygotes with some resistance to both treatments. Individuals carrying both resistance genes are extremely rare. However, once you actually do the experiments you start getting survival far sooner than you expect. You get resistant individuals surviving. That is what makes the strategy fall apart. The key is redundant killing to make sure that if any individuals are carrying one genotype, they are killed by both pesticides. Resistance really starts to evolve here, and the model shows that if the residues decay or decay unequally to the point insects aren't killed, you don't really see much of an advantage for pyramiding genes or mixing compounds.

In the example of the cotton bollworms, using the same assumptions for a pyramid as for sequential use you see that you need greater than 95% mortality to get a high level of delayed resistance. The advantage of this in terms of designing a useful plant is that if you create a plant that has one gene and another plant that has another gene, you should be able to put insects on them and collect them every day. You don't have to wait until you get resistance. You can collect insects—thousands of them—on day 1, put them on the plants that incorporate the toxins, and it better kill a lot more than 95% of the insects to show that it is effective.

This is where Tony Shelton, Cornell University, enters the picture. We did these experiments with coddling moths Tony had collected in various places around the US that were resistant to one of two different *Bt* toxins. One of the toxins was identical to the one Monsanto used in cotton. We then looked at what happens if you have a mosaic strategy where half the plants get one and half the plants get the other. After 12 generations, the coddling moths were obliterating the resistant plants if they were carrying only one gene. But if we pyramided them, we went through 24 generations before we got bored and had to move on to the next thing.

So what about the herbicide-tolerant crops introduced in 1997? There was little widespread adoption of resistance management tactics as an entomologist would know them, and there was no government regulation. In Australia we did something different, because the first case of any herbicide-resistant weed in the world was actually discovered in Australia by my office neighbors, Chris Kastner and Chris Preston. An extension agent in New South Wales reported an orchard with annual rye grass that was surviving multiple applications of Roundup, and he thought something was wrong there. So he sent the seeds down to Steve Powles, Australia's weed expert. Steve tested them and walked me out to the greenhouse one day, and it was astonishing. He had dozens of susceptible plants from different locations, all sprayed with a field grade of Roundup, and several plants were dying, but the plants from that orchard were still growing.

I had been asked to serve on an advisory committee on genetic engineering and manipulation in Australia, and we started developing best practice guidelines. There were five or six steps, but the key argument was that if you are going to use herbicide tolerance, whether GM or conventional, don't include the same herbicide resistance in two different crops if you intend to use rotation. As the second company coming on the scene, you were expected to figure out the alternative to the one already in use by the first company, or at least come up with a compelling resistance management strategy, such as the traditional strategies of IPM, cleaning the equipment, etc.

RR canola was approved by the Australian federal government in 2003 but banned by states until 2008. So from 2003 to 2008 there was no GM canola grown in Australia. From 2008 until 2013 we conducted a five-year study in locations where RR canola was grown and areas of Australia where there was already widespread resistance to Roundup of annual rye grass from conventional cropping. A number of the fields we looked at had actually had RR canola planted twice, in year 1 and again in year 4. In years 2 and 5 we went through all those areas and compared the various control plots and monitored some

68 fields overall. There was no relationship between the use of RR canola and the increases in resistance in rye grass. One of the primary reasons for one of our recommendations—written by Monsanto in 1998 even though not finally put into practice until 2008—was that if you grew a RR crop you shouldn't use Roundup on the same field the following year. That would tend to stabilize and intensify resistance. This was effective in Australia.

Why did this work? Chris Preston was working on this, and by about 2000 he had found not 1 but 44 populations of Roundup-resistant rye grass in Australia in an area where Roundup had been used extensively for 15 to 20 years. This didn't have anything to do with GM. It was conventional use, since in most of Australia Roundup is applied to a bare field, after which farmers do direct drilling to avoid winter runoff and to retain moisture. The number of years of Roundup application is more important than the number of times per year. Resistance was worse in cases where no other herbicides were used. That, however, was usually correlated to farmers rotating the herbicides rather than to anything else. Another factor arose in areas with little to no tillage, where some farmers then decided to till the crop in order to kill the resistant weeds. My model showed that that tilling probably wasn't the cause, because you couldn't kill more than 95% of weeds that way. It showed that it was much more likely that tillage kept putting some seed back in the seed bank every year so selection density was lower. Tillage created a refuge.

Chris Preston did some really interesting experiments. He put Roundup-resistant and susceptible rye grass in petri dishes and put Roundup on the leaves. When we put them on a photographic plate, we saw that in the susceptible ones the Roundup quickly reached the roots. In the resistant ones it stayed right where you applied it. There must be a transport mechanism, but we didn't know exactly what it was. However, not knowing did not matter for developing a strategy. We found that the resistance was three to seven times lower. At that point I found that when I ran the data for Roundup through the model, it successfully predicted how fast resistance was evolving to other pesticides. But I couldn't get resistance to evolve fast enough for Roundup, and we finally realized that we had included fitness in the model, and there must be a spectacular fitness to this to cause this delay.

Chris took different populations of rye grass, some of them plants that were resistant to Roundup but with a small susceptibility, or he hybridized to make sure there was some susceptibility. The percentage of survival dropped from 50% to 0 in a spectacularly rapidly fashion over a period of just four generations. Even when he did this in different areas, it seemed to be most enhanced when there was high weed density. One of my hobbies has been to collect data on fitness cost, and this was the biggest one I had ever seen. It is spectacular. Usually resistance hangs on for a long time, but for Roundup it was spectacular. We concluded that the fitness level was so high without providing a great fitness advantage because people didn't use Roundup year in and year out. The conclusion we came to, one consistent with observations in the field, was that as long as you used Roundup at least one year out of every three you could pretty much hold the resistance at bay. It wasn't increasing at a fast rate. We also realized that we had to stop farmers from doing another Roundup application if the weeds got too large. As with our experience with insects, once the plants get bigger their sensitivity decreases and you are more likely to discriminate between the ones that are barely susceptible and the ones that are really resistant.

In the US things were different. Chris and I talked to people at Monsanto but were unable to persuade them of the wisdom of this practice. One of our arguments was that if they accepted this model, they would not produce any RR corn at all. You would reduce use of RR to either soybeans or cotton and you could back off on the selection intensity. Even if you created RR corn, you could argue that people should use alternative herbicides in different years to avoid a steady diet of Roundup year after year. I personally believe that the reason Roundup resistance started showing up so quickly in American weeds was not because of RR crops per se, but because of selection long before RR became available, just as in Australia. It was the RR crops in light of conventional selection that made resistance become a problem much more rapidly. We have been looking at this problem now from an economic standpoint and we have realized that if you look at the economics, RR for corn isn't nearly as valuable as for soybean or cotton. One indicator of that is that the RR trait in corn was adopted much more slowly than in the other crops.

Now let us get back to insecticide resistance management for GM crops, because there success has been better. Over the last 16 years we have had some cases where resistance to *Bt* has evolved. They include cases such as armyworm in Puerto Rico, and now there is strong evidence of it as well in large areas of Brazil. Maize stem borer in South Africa. Pink bollworm in India. There is some controversy about whether this was first documented by Timothy J. Dennehy, who was then working for Monsanto. Monsanto believed it even if some other scientists didn't. Another one is that *Bt* corn resistant to corn rootworm fails test number 1, that two genes independently don't give you anywhere near the control. This is a train wreck. Very predictably we were going to get resistance to that. And there is also the indication of increased frequency of insect resistance in various countries, but probably not in the US.

My friend Bruce Tabashnik has worked on this for a long time, and he argued in 2013 that field outcomes support the theoretical predictions that the factors delaying resistance include recessive inheritance to resistance, low initial gene frequency of resistance alleles, abundant refuges, and use of two types of *Bt* crops. This is exactly what Tony Shelton and I modeled in plants in the early 1990s. Those were the key factors in delaying resistance.

So what went wrong in these cases of control failure? They seem to be largely due to lack of any kind of government intervention in one way or another. There is an absence of structured refuges and probably low efficacy of toxins. That is exactly what was predicted to go wrong. I would argue that one of the most volatile cases in the world for resistance to evolve is cotton bollworms in Australia. A lot of cropping areas were grown with irrigation, and no sensible farmer would waste his money on irrigating anything other than cotton. We had data that showed that sometimes in the Australian system naturally occurring refuge crops were less than 5%, so we really drove the growers hard to use refuges.

Successful management strategies include large refuges and high expression of the toxins relative to the pest. Government intervention, in both the US and Australia, led to the use of refuges. Here is an example of a refuge crop. This not-Photoshopped shot

shows a real cotton crop in Queensland, both the *Bt* crop and a refuge. It shows you why, in the absence of *Bt* crops, cotton was sprayed 15–18 times a year. In Australia we have been able to keep that system going.

What happened to these two technologies? Just look at the corporate cultures driving this. At Monsanto there were Pam Marrone, who by now has set up her own company, Steve Simms, and Terry Stone. They decided internally at Monsanto to establish their own culture. They selected the first strain that was *Bt* resistant and proved to management that you couldn't ignore this possibility. They had to be taken seriously. What happened on the other side? When it came to RR crops, there was a strong view within Monsanto that resistance was nearly impossible. They used an herbicide that had been in use for around 25 years without ever failing—a much better record than any other herbicide on the market, so they might be forgiven for thinking that nothing could ever go wrong. But I don't think any entomologist would have been so bold as to say in the 1990s that resistance wasn't possible. So there were two different cultures in one company back then.

This is the backdrop to herbicide resistance. While resistance has a solid research history in entomology, here the potential problem was nearly undetectable. It was a curiosity, not really a threat to agriculture. They didn't take it seriously.

One of the key points to make here is that refuges for two *Bt* crops were essentially mandated in both the US and Australia. The USDA and the EPA were intensely lobbied to protect *Bt* on the grounds that it was a public good. In particular that resistance to *Bt* would have adverse effects on organic agriculture. Strictly speaking, it probably wouldn't have made a difference for organic agriculture, because the pests that were targeted by *Bt* in these crops were not affected by the use of *Bt* in organic culture or anywhere else. Cotton bollworms go inside of the boll. Corn borers go inside the stalk. You can't really control them with *Bt* sprays. So it wouldn't have made any difference, but it was a nice argument at the time.

Why wasn't this done for Roundup? Arguably one of the safest herbicides ever developed, so why wasn't it also seen as a public good? Another one of my key points is that in Australia the cotton growers are very concerned about resistance and the history of resistance to insecticides such as *Bt*, and they encouraged the government to adopt public sector recommendations. I remember very well when I was the one person in the room who wasn't affiliated with the Australian government, Australian growers, or Monsanto. We presented our case for a strategy in Australia to the growers, and Monsanto presented their case. At the end one of the Australian growers, the committee chair, told us that they were not interested in a short-term solution and that they were going to use our strategy in Australia, not Monsanto's. They developed and endorsed a resistance management

strategy which they then asked the government to enshrine in regulation. They took the strategy to the pesticide regulatory authority and to a gene mapping organization looking after genetically modified crops and said, this is what we would like to have you put in the rules. Soft regulation, driven by people who have an interest in going to the government, not a heavy-handed EPA crushing everybody, as seems to be the perception of the US regulation. It meant educating the growers and working from the bottom up.

I hope I have shown you that even in early cases such as rotation of insecticides in Australia, government intervention usually seems to be needed to preserve refuges and develop resistance management strategies. It has been successful for insecticide resistance management strategies but not for RR crops, and there is a vastly different outcome as a result. I think we need to try to revisit the notion of soft government intervention. At least some government intervention is required to make these things work, and maybe we can come up with a common denominator about the form that takes.

Speaker Profile: http://agsci.psu.edu/dean/biography

Q&A

A. Read, Penn State: What is it is about Australian cotton growers that make them more interested in the long term and less discounting of future developments than American growers?

Roush: Part of this is that Australian cotton growers are extremely well educated and savvy. I gave a talk in 1994, at the dawn of introducing *Bt* cotton to Australia, to cotton growers. It was at a big room at a casino, which I think is a great metaphor for pest resistance management. Ninety-five percent of Australia's cotton growers were represented, and you could hear a pin drop in the room. I gave similar talks in Mississippi and Texas, and 25–30 surly guys turned up. The atmosphere was completely different when I talked to people in Mississippi than when I talked about the same thing in Australia. Part of it was the sophistication of the growers which, of course, not all growers have. For example, some great strategies were developed to stop ticks from evolving resistance to Acaracide in Australia. They failed miserably, because cattlemen in Australia were cowboys and didn't pay attention to the rules and didn't think it would affect them. But when DDT was banned in Australia, they were left without alternative treatments for a while, and they realized that when you live on the edge of the earth you can't expect a new insecticide or herbicide to be developed just to save your 2% of the market. The same applies to herbicide resistance. They are taking herbicide resistance more seriously than elsewhere in the world because they know they are on their own. This helped drive them to be a bit more inventive.

G. Thompson, Penn State: Following up quickly, are there any differences in the relationships between the growers and the government?

Roush: There is not such a combative relationship. The government regulators keep a very low profile. They don't attend a lot of meetings.They turn up at the last minute and listen. It is much less adversarial. The growers, fully aware that they are living on the edge of the earth, have a much greater sense of personal responsibility to make sure the industry survives. Nobody else is going to come help them.

S. Fleischer, Penn State: I want you to elaborate on this. Why didn't they develop Roundup as an herbicide? Did they have the legal authority?

Roush: They questioned their legal authority, but I would say if you have the legal authority to grow *Bt* crops, why can't you use Roundup? I think it was the opposite. We were under intense pressure to do something about this new technology, and *Bt* crops seemed to be a threat to organic growers. While I doubt that that was ever the case, it made a good argument. It got lots of people engaged in the debate and stirred up. I think the agricultural community felt political pressure to do something, but just a little, not too much. I think that is how we got into trouble with some other pests since we have some existing regulation and there is a sense of we have been there, done that. We have done something and over the last few years we have marketed first and disagreed later. In some ways things are not as neat and tidy or as rigorous as they were in 2000. Basically they said Roundup is a chemical, and who is going to defend it even though it is a chemical that is so safe we will let people buy it in a store and take it home and spread it on their plants. There are few other herbicides you can do that with.

T. Harding, Lehigh Valley Organic Growers: I am curious about your recommendation for organic growers. Looking at resistance, you put some very good models up there. Many of those are things we advocate for throughout our industry, but this is not just an organic problem. This is a non-GMO problem and there is a whole industry affected by this. What do you recommend we do from the perspective of moving forward with coexistence, resistance, and meeting market demand?

Roush: I almost think the resistance can be handled as a separate issue. Organic growers can have problems with non-crops, like broccoli and cauliflower. In fact, Tony Shelton has worked on *Bt* resistance for organic growers who have similar problems in crops. We worked a lot on this issue of coexistence in Australia, and that is why I am curious about where the areas are that have run afoul of each other. We used herbicide resistance as a marker and studied first non-transgenic herbicide resistance and then Roundup resistance over a period of ten years. We looked at pollen flow for canola in Australia and found that basically we re-proved what canola growers already knew—that most pollination in canola occurs within the plant or adjacent plants and that the pollen flow was really a small factor when looking at more distance. In terms of coexistence, if people would come to some agreement on a threshold of what would be allowable, e.g., if it was less than 10%, we could tell them that 100 meters is far enough. We have data from about 100 fields in Australia that show that if you are 100 meters away, your level of pollen flow would be so low that you can't detect it by any means other than what we were doing—planting out a

vast number of seeds and measuring how many plants were transgenic. So if you allowed some kind of a threshold for that mixture, then there would be many opportunities for coexistence. The other issue in Australia is that long-distance pollination seemed to be primarily from bees, most of which were feral, displacing native bees. We didn't have too much argument from ecologists when we said that if we could identify some organic canola growers, could we just go around the neighborhood, find where the feral bees are living, haul out the eucalyptus trees, and knock out their colonies? We probably would be doing some good for the local native bees. We could adopt strategies like that. In other crops, like corn, pollen flow is still quite limited, but it is a matter of coming up with adequate distances between the fields. There is still a remote possibility that something can move long distances, so you have to have a threshold. The threshold could be below the limit of detection by any kind of routine testing currently done, ELISA etc. The way we did these pollen flow studies was to collect pounds worth of seed from canola fields. In the first round we collected and planted 58,000,000 seeds from about 50 canola fields that were upwind or downwind from 20 herbicide-resistant canola fields and planted out in fields, looking for those scattered survivors that would survive two or three applications of herbicide. That was the only way you could do it. The detection was so low that you couldn't do that by any other means. I think the main issue to be addressed is that if you really want to allow everybody the freedom to farm the way they want to, you can't have a dotted line that says no contamination at all, something which you probably can't measure anyhow. If there was a reasonable threshold value, you could work out the necessary isolation distance.

K. Merrigan, George Washington University: It is really an interesting question why EPA dealt with *Bt* but not glyphosate, and one of the things I want to share is that industry did admit that there were resistance problems with *Bt*, but that with thousands of different *Bt* strains they could always find a different one to use. But they admitted that there was a problem with glyphosate, which gave regulators a foot up. Then part of the conversation revolved around the question of if there really is such an endless diversity of *Bt* that you can go back to the shelf if the first product fails and use another one.

Roush: I agree. I once wrote a letter to EPA that I distributed to lots of people, opposing the first registration case ever performed for *Bt* corn. It was an application by Syngenta, and what really set me off was that the application proposed that if resistance to *Bt* developed they would just use another toxin. I saw red with that application. It was one where the expression in kernels was very low. For some reason they thought they could get it through the regulatory system more easily with no expression in kernels. They used a pollen promoter with fairly high expression in pollen, and this was when tests by John Losey at Cornell showed some marginal impact on monarch butterflies. He showed that only for this event, not any of the others. I opposed this application vigorously on grounds of the long history of insecticide resistance. When resistance first started occurring to insecticides, people said, don't worry, we'll find others. Curiously enough, I worked in Mississippi in an entomology program called the Clay Wild Entomology Building, and Clay Wild famously announced at the Southern Branch Meeting of the Entomological Society of America in the 1950s that taxonomists should collect all the houseflies they

were ever going to need because they were going to be driven extinct by DDT. I worked in a building with his name on it with 80 different resistances found in house flies. So when people came forward to say we don't need to worry about this, we'll come up with more *Bt* toxins, I said this was not good on the basis of history and that this application should be sent back and blocked until they came up with something more sensible. At least there was some admission of it then, but there was a perception—not just in Monsanto but I suspect among a lot of herbicide specialists—that resistance to Roundup was impossible or nearly so, when in fact it was all fluff.

T. Redick, council to the Soybean Board: At National Corn Growers we have actually looked at resistance, particularly in weeds, since there are lots of problems with that. You mentioned that you had a double stack that seemed to work a little better. We now have octostacks, and the whole soybean pipeline is quite stacked for different traits. Now that Roundup is generic, we can actually stack it for free with LL and everything else. We also have all these new forms of herbicide-resistant strains that are getting approved by the USDA after long hauls. What role do you see for these stacks to work their way into your strategies? Can you give us a little better mosaic for using these stacks, because it's going to get complicated?

Roush: The advantage with *Bt* crops was that we could control expression and dose. When some of the early *Bt*'s came out, we had mass expression, and I tested a bunch of *Bt* potato plants for algae. They had a whole range of expression levels, only some of which controlled the Colorado potato beetles successfully. The modeling showed that if you really wanted to make this work well, you had to make sure that you picked the transformation events that have good expression. For a long time people said they have to have equal decay, but that is not correct. It can either not decay all season long or decay within days, so you don't have continued emergence from the soil with insects or weeds being exposed evenly over time. The use of lower and lower doses might mean that you eventually get resistance in 20, 30, 40%. Growers often answer my question about insecticide resistance saying that they use a mixture of insecticides. When I ask them how often they find no surviving insects after spraying, they can't give me a single instance. I have to tell them that in that case it really doesn't work well. Because some of the surviving insects will be carriers of resistance. In *Bt* plants we could get pretty good expression in almost all of the seeds. But that peters off after a little while. I have been haranguing Monsanto for years to boost the expression in the first round, because it has always been weak, and they have done better since. The problem with herbicides though is that you can't control the decay. It is just like any other pesticide. So if you are going to use two herbicides in a stack, they must have very short decay periods. You might be able to get away with something like Roundup and some other herbicide that, like Roundup, has a very short decay period. Growers will spray millions of acres and plant the crop the next day. You might be able to get away with that with Roundup because almost as soon as it hits the ground it is no longer active against the weeds, but you would have to find a mixing partner that would work the same. Unfortunately I can show you data for the so-called double knockout, where mixtures with Roundup were used one right after the

other and still the level of control was not high enough. You couldn't get 95% each, so there was no benefit from doing the double knockout. So I am skeptical about these. If people are going to put multiple herbicide tolerances in crops, they should really look back at using the herbicides in rotation. They should consider that a viable option. They don't have to use Roundup all the time. Go assess what the weed problem is and pick the best herbicide for the spectrum of current weeds among the suites of tolerance that your plants have. But don't rely on mixture strategies.

M. Horack, Monsanto: Thank you very much for your talk. Please elaborate on the economic and sociological drivers for Australian cotton growers and how they are changing their practice to manage resistance.

Roush: Part of the reason why *Bt* cotton was introduced so early in Australia was that Australian cotton growers were being deluged by complaints from the public because pesticide residues were found in various places. With the salt ban, even though it was seen by the growers and pest managers as being a reasonably good option in terms of not causing pest outbreaks in cotton crops, to their great frustration salt kept turning up in some of the rivers in the area. They eventually contracted with a chemist from Sydney who determined that it wasn't runoff, as people thought, it was condensation. The rivers are cold, some of the salt gets airborne and condenses in the rivers. They barricaded the rivers to keep out runoff and they still were getting it, and it drove them mad. So Jim Peacock came along, and as soon as *Bt* technology looked like it was in the wings, he went to Monsanto and told them they had fantastic varieties, varieties which would often beat everybody else's varieties around the world in trials. He suggested using these Australian varieties for genetic modification because they wanted to get *Bt* into Australia as soon as possible. *Bt* cotton was introduced in Australia six months after it was introduced in Texas and Mississippi. It was largely driven by Jim Peacock going out and doing that deal with Monsanto. The Australian cotton industry, to its credit, decided about 1985 that it was going to try to do its best to stay out of the headlines and become not newsworthy at all, ever. And they tried to address all their environmental issues. When *Bt* cotton was first introduced, thanks to the modeling and work I did on the single genes and the low refuges, we restricted *Bt* cotton to no more than 3% of the area until the second generation of *Bt* cotton became available. The growers planted next to towns and next to streams, so they provided a buffer for the rest of the cotton operations that used *Bt* cotton. When Roundup Ready cotton came along, they were also keen to use it, because if they planted a Roundup Ready cotton crop, they could control the weeds with one spraying of Roundup and some touch-up with other sprays instead of using four or five herbicides, many of which have dubious environmental impacts. And they were keen to make sure they didn't have Roundup-resistant problems in cotton cropping and they linked with southern grain growers to develop similar strategies.