
Vulnerability and Environmental Risk Assessment

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Over the past half century, environmental risk assessment (ERA) has become increasingly important in political decisions. It forms an important basis within the World Trade Organization's Sanitary and Phytosanitary Agreement (SPS) and is an important touch point within the Convention on Biological Diversity. The scope of ERA is expanding rapidly both in the issues that it covers and in its role in policy formation. Classic environmental risk issues—starting in the 1960s—include air and water pollution and pesticide usage. However, new ecological challenges include hormones in the environment, invasive species and the GM issue. Also, new technologies are becoming the focus of ERA. Importantly, the way that ERA is used in policymaking is changing as well. Historically, ERA was used primarily as a decision-support tool. In other words, risk-assessors provided information that decision-makers took into account to guide policy. However, in the last few decades, ERA has been increasingly used to legitimize environmental policy; when the public knows that a risk assessment was done, it is likely to legitimize the eventual decision. Thus, the process now is important in terms of acceptability of the decision, which can create tension if the risk-assessment data are lacking in some regard. In addition, risk assessment has been expanded in ways to set environmental policy. In the developing area of comparative risk assessment, certain policy options are favored, rather than certain policy decisions, which has raised additional challenges for risk assessment. The expansion of the scope of these issues has increased vulnerability because, as we address new problems, we have to keep revising our *modus operandi* merely to keep up.

GENETICALLY MODIFIED CROPS

It is clear that, overall, some GM crops are better for the environment than non-GM crops; one clear example is *Bt* cotton in Arizona where reduction in pesticide use has been substantial. On the other hand GM crops do require local risk assessment. Three dimensions vary around the world in terms of governmental frameworks that consider risks associated with GM crops and these are whether or not the following are examined:

- only direct or both direct and indirect effects of the crop
- only non-agricultural or both agricultural and non-agricultural effects
- effects mediated by both humans and non-humans or non-humans only.

In Australia, agriculture is dealt with through a separate ERA process, so GM-ERA only addresses non-agricultural effects. In contrast, most of the European frameworks consider agriculture as part of the environment and, therefore, farming issues are a part of the overall assessment of risk. In the United States, only some aspects of agriculture are considered part of the environment. So there is variation on that point around the world. As far as direct and indirect issues are concerned, genetically engineered herbicide tolerance provides a good example. Herbicides are applied to many crops to control weeds. With herbicide-tolerant crops, we examine the direct effects of the transgene on the environment; every regulatory system around the world considers this. However, those regulatory systems vary in terms of indirect effects on the environment. Of the many possible indirect effects, one is the accompanying switch in herbicide product. USDA/APHIS defines the transgenic plant as the transgene itself and any change in the herbicide is not required when using the GM plant, and, as a result, it and its consequences are not regulated. In contrast, the European system considers the change in the herbicide as part of the regulatory process. In the United States, because herbicides are already regulated, it is believed that there is no reason to regulate them again. On the other hand, we do know that when we switch herbicides we alter selection pressures on weeds, potentially causing development of evolutionary resistance, which has occurred in the southeast of the United States, leading to new patterns of weed development. In the United States we have not regulated the evolution of weed resistance, in Europe some of these issues are considered. Thus, the many indirect effects may or may not be considered under different regulatory processes. Factors considered important or unimportant in the United States may or may not be considered similarly in other countries.

With regard to possible environmental effects of GM crops, three categories garner attention:

- transgene flow and subsequent effects including increased weediness in the recipient
- resistance evolution
- unintended effects on organisms and ecosystems.

The high-dose refuge approach towards *Bt* resistance has been a great success. We have never had an insect pest-control method used as widely that has not resulted in resistance evolution. Of course, we will continue to monitor this approach for many years. Low-dose

situations are not quite as successful; all of the examples of resistance evolution occurring in *Bt* crops around the world are associated with low-dose crops. However in this talk, I will focus instead on unintended effects on organisms.

THE VALUE OF CULTURE

Bt maize produces a protein that is toxic to many Lepidoptera. Pollen containing the toxin, dispersed in the wind, falls onto the leaves of plants that other insects—including monarch-butterfly larvae—eat and then may die, possibly threatening the insect population. Although that's the risk story involving monarch butterflies, several published studies have shown that *Bt* corn does not constitute a serious risk for monarch butterflies. Fortunately, the varieties of *Bt* maize that were commercially successful have very low toxicity in the pollen, whereas some of the varieties that were less successful actually have higher toxicity, otherwise the outcome of the story may have been different. Monarch butterflies are exposed to many risks, but only minor ones related to GM maize.

Why was there so much uproar about *Bt* maize and monarchs in the United States? It's just a butterfly, of which many types exist. Monarchs aren't endangered, nor is their conservation value considered very high. Part of it is that monarchs are amazing in that they migrate from Canada and the United States to Mexico for the winter, as well to parts of California and Florida. They aggregate in huge numbers in certain locations in Mexico. Programs such as Monarchs in the Classroom elicit great interest among school children: their larvae are grown and allowed to pupate and emerge and then are set free, which has a considerable emotional impact on youngsters. Monarchs are even on US postage stamps, reflecting their cultural significance.

In fact, issues of cultural significance are actually quite important *vis-à-vis* environmental risk. We need to expand our approaches to ERA to include cultural dimensions. The bald eagle is also important in the United States, and I have talked to people around the world and found that other species have special cultural significance in other countries, creating heterogeneity in what are seen as important parts of the environment.

So, my first conclusion is that cultural significance needs to be incorporated into ERA.

ASSESSING SUSTAINABILITY

Sustainability has been a buzz word for some time and has permeated universities and industry, and in some ways it's a "mom and apple pie" type of thing. Those of us who work in the area know that it's actually quite complicated to accomplish. I'm not going to go into this in its tremendous complexity, but rather focus on one element: risk-assessment associated with chemical toxins, which goes back to the beginning of ERA in the 1960s. This approach to ERA has been promoted by several stakeholders for application to GM crops. I will argue that this approach doesn't really get at the problem of sustainability.

The general model is that, to make an ecological risk assessment, you conduct toxicology tests in the laboratory—which are easier and less expensive than field tests—using exposure rates that are much higher than normally applied. If an effect is observed, then the logical step is to continue testing. If there is no effect, the basic logic is that because

the toxin levels are higher than would be seen in the environment, then you can stop. However, one has to ask how good are the tests. In particular, one doesn't want a "no effect" conclusion when there actually were effects that were undetected.

Biological Control of Pests

Biological methods of controlling insect pests employ insects or other organisms that feed on pests and thus control them. Figure 1 shows a few examples that, in addition to pathogenic micro-organisms, are biological control agents.



Figure 1. Examples of natural and biological control of insect pests.

Meta-Analysis

I should note that there is ongoing scientific dispute on what I will present here. One of the areas under debate is meta-analysis, which provides a means of combining information from multiple studies to show overall trends. It is regarded as less subjective than summarizing summaries because it uses data that are statistically significant as well as data that are non-statistically significant. So, for example, suppose I studied the effect of Cry1Ac, one of the *Bt* toxins, on a species of ladybird beetle, *Cycloneda munda*, and suppose I come up with two non-significant effects on development and growth, both with *p* values of 0.09. If I did this study, I would conclude that I wouldn't see any effect of Cry1Ac on *Cycloneda munda*. Now, supposing ten studies showed similar results. Now the null-hypothesis of no effect across the ten studies would be rejected at a very low *p* value of 0.0029, meaning that even though none of the studies showed a significant effect, there really were significant responses. It would be wrong to conclude that lots of non-significant effects implies that the effect is non-significant, which is counter-intuitive. Meta-analysis shows that lots of non-significant effects may imply that there are significant effects.

Considering how we do meta-analysis, there are two kinds of null hypothesis. There is a null hypothesis that is common within medical meta-analysis literature and then there is a null hypothesis that hasn't really been well recognized in the ecological literature. The main difference is whether or not you assume that the data you are looking at are underlain by one basic response or multiple responses. In medical literature you basically study the effect of some chemical on humans and it's the same chemical and basically the same protocols, therefore you expect that all of the studies are measuring the same things. Everybody is trying to measure one response, which is either positive or negative, and as a consequence—which is a key assumption within meta-analysis—we say that if study one shows evidence for a significant positive effect and study two shows evidence suggestive of a significant negative effect then, combined, the two studies show that there was no significant effect because it can't be both positive and negative at the same time. Technically, the null hypothesis is that if there is one real response then the data and evidence are distributed in a standard normal distribution around the real response value, whatever that real response value is. If there are many responses, the problem is different. The real responses can be positive and negative, and with ecological data often this is the case because in ecology we rarely replicate the exact same experiment. Even if we are looking at the same natural enemy with the same toxin, people do the experiment in different ways, with different toxin concentrations, for example. They'll look at different responses and will measure responses differently, so you can't assume that they are measuring one thing. You are actually measuring many different things. But we still combine all the data. Where study one finds a significant positive effect and study two finds a significant negative effect then the final conclusion from this approach is that there is a mixture of significant effects—a very different conclusion to come to. If there are multiple real response values and all of them are zero, in other words all of them are no effects, then all of evidence will be distributed as standard normal around zero. It provides a nice prediction. Significantly, we cannot conclude that if it's not standard around zero then there are multiple real responses because that is part of the assumption; we have to conclude that there are some non-zero effects. This is a way of determining whether effects are hidden within data.

GM-Bt Crops

We pulled together laboratory data on the effects of *Bt* toxins on natural enemies of insect pests. We looked both at direct and indirect effects; we found fifty-five studies with 273 responses measured. Figure 2 shows the fraction of observations plotted against the degree of effect on predators and parasites, with a standard normal distribution showing the zero-effects prediction. There was a slight skewing towards a negative effect, but it turned out to be non-significant; we can't say that *Bt* crops had significantly more negative effects than positive effects, but we can say that the *Bt* crops had both negative and positive effects. We don't know what those positive and negative effects were, nor do we know how big they were, but, again, the main conclusion was the presence of non-zero effects. We broke the data down for the following finer analyses:

- Separate common species
- Separate toxin types
- Separate direct and indirect effects
- Separate response types (survival, development, growth, reproduction, behavior, enzyme activity)
- Controls for non-independence among response types.

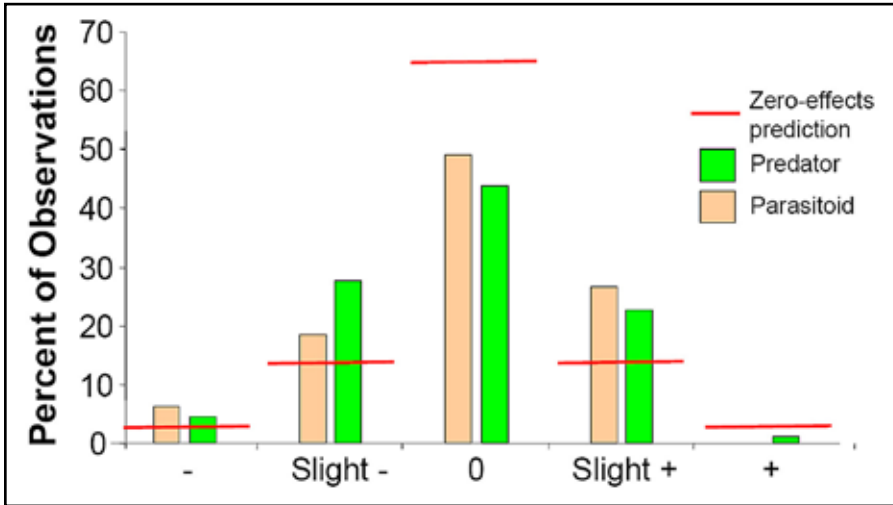


Figure 2. *Bt* toxins: non-zero direct effects (Lövei *et al.*, 2009)

Figure 3 shows that 70% of sixty-six comparisons had p values of <0.05 , whereas twenty comparisons, with relatively low sample sizes, showed non-significant differences. In all of the original papers the authors reported no direct effects, and in all of the review papers the authors reported no direct effects. Yet meta-analysis suggests that there were many undetected effects. We suggest the need to improve this ERA methodology so as to reduce the number of false negatives and to assess sustainability more accurately.

This is just one case. The general issue of how we assess sustainability within a risk-assessment context is a challenge that we have not yet grappled with in its full complexity.

THE LAST ORGANISM WILL BE AN INSECT

Mirid Bugs in China

Last year, Lu *et al.* (2010) reported positive effects of *Bt* cotton on populations of mirid bugs (Heteroptera: Miridae) in China. Figure 4 shows that numbers of these insects have increased since 2002 on *Bt* and non-*Bt* cotton alike. In other words where *Bt* cotton is planted in large areas, mirid bugs have become a pest. As it's a secondary pest, nobody intended to control mirid bugs with *Bt* cotton, so the technology cannot be faulted. However, in an ecological context, a new plant pest has emerged with attendant crop losses and increased applications of insecticides. Consequently, the financial and environmental benefits from *Bt* cotton have been degraded.

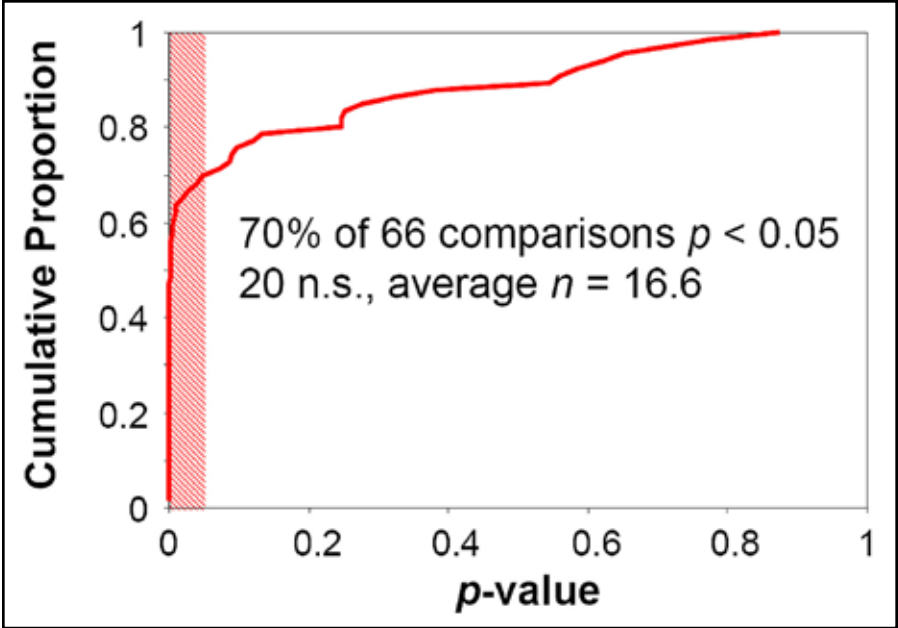


Figure 3. Test for non-random responses.

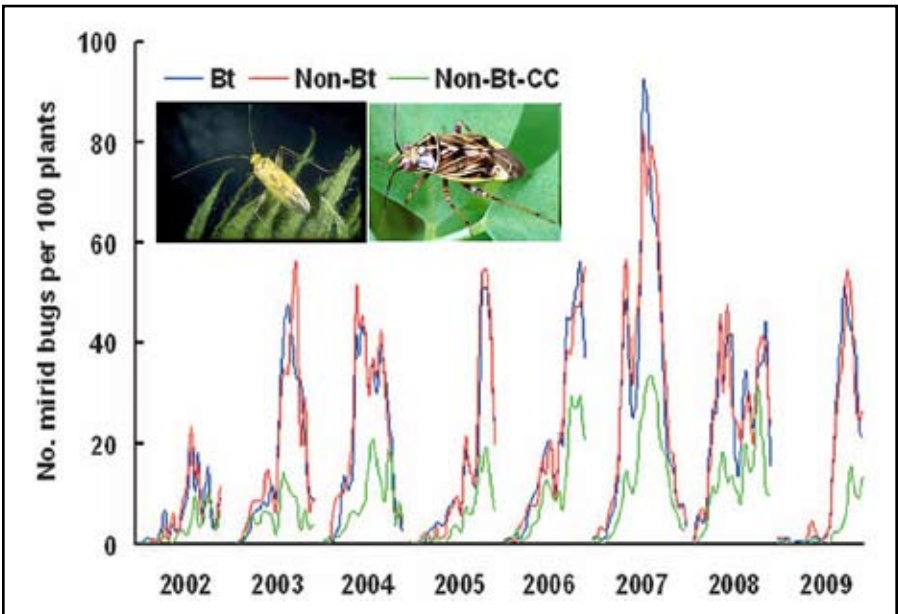


Figure 4. Mirid bug populations on cotton in China (Lu *et al.*, 2010).

We have a similar situation in the southeast of the United States that's less well known (Zeilinger *et al.*, 2011). Planting of *Bt* cotton has resulted in increased incidence of stink bugs such that they are now the most important pest of cotton in the southeast and most of the insecticide applied to cotton in that region is for control of stink bug. Attendant crop losses have not been huge. There has been an increase in insecticide application, but not to the level applied to non-*Bt* cotton; in other words there are still some net benefits from *Bt* cotton, which I consider to be a consequence of our excellent extension system which monitors these things and figures out solutions to problems so that we can actually respond and the financial benefits can be retained by farmers. Thus, sustained benefits from GM-*Bt* crops may depend on a vigorous public-extension service, and should not be attributed solely to the seeds.

Secondary Infestations

Another question we addressed was, "What are the causes of these secondary infestations?" One of the major explanations is that reduced insecticide application is a contributing factor. However, a contributing factor is not the whole story. We have mirid bugs in southeastern United States, but they have not become a problem.

Currently, we have several research projects to try to determine underlying factors. Three other possible explanations are as follows:

- Enhanced colonization and/or reproduction on *Bt* cotton
- A more likely factor is competitive release, *i.e.* killing the Lepidoptera that attack the bolls releases stink bugs from competition
- Also likely, on the basis of two years of data, is a build-up in the landscape within and/or between years.

Figure 5 shows data generated with two species of stink bug (*Nezara viridula* and *Euschistus servus*) when challenged by caterpillars of cotton bollworm (*Helicoverpa zea*) or tobacco budworm (*Heliothis virescens*) on cotton bolls. Unchallenged stink-bug controls are on the far left. The data reveal an interaction; in some cases, the stink bugs grew significantly more slowly when challenged with the higher numbers of caterpillars.

A stronger effect has been demonstrated in terms of where stink bugs lay their eggs. We did a simple study where we damaged the lower leaves of cotton plants with caterpillars and gave stink bugs the choice of undamaged leaves on damaged plants and undamaged leaves on undamaged plants (Figure 6). We found that, by a large margin, the stink bugs preferred to lay their eggs on the leaves of undamaged plants. We know that caterpillar damage causes both aerial signaling and induced signaling within the plant, but we don't know which applied here. However, this may play a role in the competitive release of stink bugs as discussed above.

CONCLUSION

Why are opinions on GM crops so polarized? As scientists, we know that most technologies have attendant risk and we tend to frame problems in terms of balancing benefits and risks in a way that is positive for society. I think that this is too narrow a framing of the problem. The core problem really is that some people see mostly benefits and some

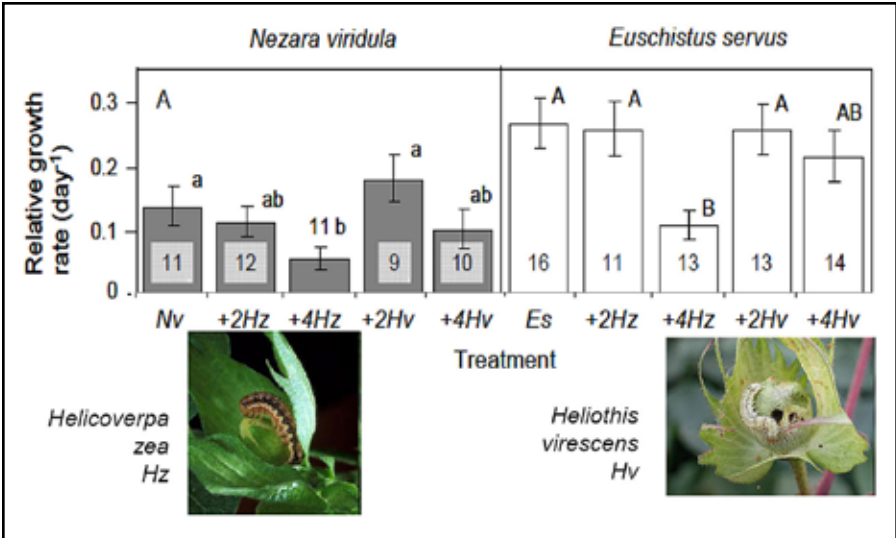


Figure 5. Resource competition when combined on the same boll (from Zeilinger *et al.*, 2011).

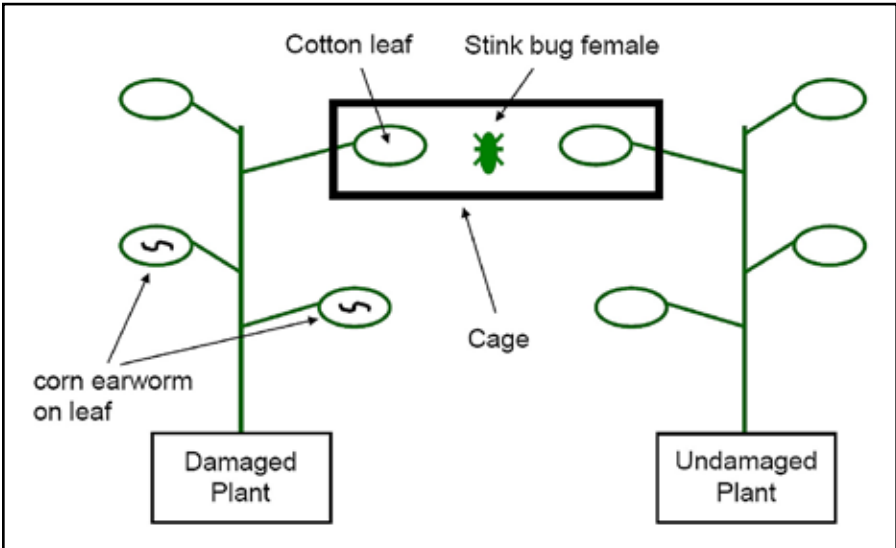


Figure 6. Stink-bug oviposition choice: experimental set-up

people see mostly risks and everyone balances the risks and benefits differently, including scientists. It's a social phenomenon with no agreement about how we weight the factors involved and, therefore, it is impossible to reach a consensus. Vulnerability arises from these socio-cultural factors and biological factors that need to be evaluated.

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