# RESPONSE TO PEER REVIEW REPORT
# OF
# BIOTECHNOLOGY REGULATORY SERVICES
# DRAFT PROGRAMMATIC ENVIRONMENTAL IMPACT STATEMENT

**USDA Animal and Plant Health Inspection Service**

**Biotechnology Regulatory Services**

**Riverdale, MD**

**July, 2007**

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# APHIS Response

## Introduction

**Nature of the Review Document**

APHIS Biotechnology Regulatory Services (BRS) has undertaken a review of the scientific portions of its draft programmatic Environmental Impact Statement (EIS). This scientific dissemination was determined to be influential scientific information based on the guidelines issued by the Office of Management and Budget (OMB) in their peer review bulletin of December, 2004.

The scientific section of the draft EIS, consisting primarily of Chapter 4.1, Impacts of Genetically Engineered Organisms, along with some supplementary information in the appendices and introductory information from Chapter 3, "Affected Environment, is intended to provide the reader with an informative overview of the aspects of the human environment that will be impacted by BRS regulations. As such, the document is not an exhaustive treatment of any particular science, but is intended to present generalizable examples of the kinds of impacts that must be considered.

**Nature of the Review**

Through an intermediary (contractor), the Review Document was provided to four expert reviewers who independently assessed the document offering suggestions for improvement. Each reviewer was asked by the contractor to provide a reviewer report and reviewers were encouraged to submit “track changes” edits on the document. Reviewers were asked to review the document in a very short amount of time and APHIS greatly appreciates the flexibility and cooperation of the reviewers under this constraint.

Because of this expedited process, the Reviewer Reports returned to APHIS are not uniform, with some reviewers making an exhaustive report and others relying on more extensive comments in their “track changes” documents to elaborate on the general comments in their reports. In an attempt to present the most accurate picture of the reviewers’ comments, APHIS has appended select comments from reviewers’ “track changes” edits to this response document in order to directly address the issues presented by the reviewers. The reviewers track changes documents are made available for public viewing along with the Reviewer Report.

**Result of the Review**

In conjunction with their reviewer reports, reviewers were asked to answer the following question, with one of the three presented answers:

> Does this scientific information presented accurately and objectively provide readers with a broad base of knowledge to understand the aspect of the biological and physical environment that is likely to be affected by the regulations currently administered by APHIS BRS?
>
> Please select one of the following responses:
> (1) Yes, subject to minor editorial changes (if any).
> (2) Yes, but only after revisions have been made to address specific weaknesses.

(3)  No, this scientific document has significant shortcomings in its treatment of the natural and physical environment affected by APHIS BRS regulations.

Three of the four reviewers chose option (2) *Yes, but only after revisions have been made to address specific weaknesses*.  One reviewer chose option (3) *No, this scientific document has significant shortcomings in its treatment of the natural and physical environment*.

In response to the reviewers who chose option (2), APHIS has made substantial edits to the document that largely address the concerns expressed by the reviewers.  A detailed response to each reviewer's comments is included below.  For the reviewer who has chosen (3), APHIS has similarly affected changes within the document in an attempt to address the reviewer's concern, and a detailed response follows.  As a result of the review, and improvements to the document made by the incorporation of reviewers' suggestions, APHIS has determined that the revised document will be published as part of the draft programmatic EIS.

## Response to Reviewer Report 1

APHIS has copied the text of the Reviewer's Report and added a response where appropriate.

### Introduction

Following the USDA/APHIS 'Charge to Peer Reviewers of Influential Scientific Information Contained in the APHIS BRS Draft Programmatic Environmental Impact Statement, this review report addresses the following parameters: Completeness, Currency, Accuracy, Uncertainty, Objectivity, and Clarity and ends with an overall conclusion. Detailed comments and suggestions have been provided to USDA-APHIS in a 'track changes' commented version of chapter 4.1.   This 'track changes' version is available as an addendum to Reviewer Report 1

**Reviewe**r—
**Completeness:** This draft EIS identifies the potential factors and relevant issues raised with the development and commercial use of GE organisms relevant to human health and the environment.

**Currency:** For the most part, the information presented reflects current scientific thinking on the subject.  Additional information has been presented in the draft document using track changes and comments.  The main short-coming **of** this draft EIS is in meeting its intent "to provide non-expert readers with sufficient context and background with which to understand the biological and environmental science used to by the APHIS to evaluate potential impacts on the human environment arising from possible changes to APHIS regulations for GE organisms." Two examples are offered below in addition to examples offered as edits and comments in the text.

**APHIS—**APHIS has tried to address the reviewer's issues individually as they appear in the report and in the 'track changes' text.  In many instances, suggestions were incorporated into the text.  In cases where the suggestions were not incorporated, it was determined that the information was not appropriate for an overview document such as this one.  All of the reviewers made suggestions for including additional information, and APHIS has been forced to make difficult choices about what information must be left out due to constraints on the size and complexity of the document.

**Reviewe**r—
**Accuracy:** The information presented is scientifically accurate; or comments and edits have been offered in the text where there is reason to question accuracy.  Some of the conclusion or summary sections do not read like conclusions or summaries; rather, they seem to bring out more information that could more appropriately been covered under a different heading or folded into the text.  Suggestions for where this might be done are made as edits and comments in the text.

**APHIS—**APHIS has addressed the reviewer's comments, where appropriate, by editing the text. The track changes version referred to by the reviewer is available for comparison to the final text.

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**Reviewe**r—
**Uncertainty:** This draft EIS could offer more context and background in dealing with the "scientific uncertainty" questions raised by GE organisms. The many examples from the extensive experiences with conventional plant breeding and cultural practices already provided are very helpful, but more examples are needed to meet the stated intent of this document, namely "provide non-expert readers with sufficient context and background with which to understand the biological and environmental science used to by the APHIS to evaluate potential impacts on the human environment arising from possible changes to APHIS regulations for GE organisms." Additional suggestions are made as edits and comments in the text.

**APHIS—**APHIS has incorporated many of the suggestions made by the reviewer. Others have been considered but not included based on the need to limit the size and scope of the section. All of the reviewer's suggestions can be seen in the track changes version of the document.

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**Reviewe**r—
**Objectivity:** As far as it goes, the presentation of the body of scientific knowledge is balanced and objective. There are sections that seem to focus selectively on plant-insect interactions where examples from plant-microbe interactions would seem more appropriate, but there is no evidence that references are selectively cited or discussed in such a way as to introduce bias into the document.

**APHIS—**APHIS has considered the comment, and made corrections in specific instances in the text. One reason that plant-insect interactions are frequently considered is the current use and potential for development of insecticidal GE crops. In some cases, therefore, these interactions deserve special attention.

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**Reviewe**r—
**Clarity:** Suggestions have been made in the text where the information as written might not be understandable for a non-expert audience with a modest understanding of biological and environmental sciences have been, or where the information struck this reviewer as vague or ambiguous.

**APHIS—**APHIS has made these corrections, where appropriate, or addressed the concerns **of** the reviewer with alternative text. The reviewer's track changes comments can be seen here.

**Conclusion**

The Charge to Peer Reviewers referred to above, requests the peer reviewers to explicitly answer the following question: **Does this scientific information presented accurately and objectively provide non-expert readers with a broad base of knowledge to**

**understand the aspect of the biological and physical environment that is likely to be affected by the regulations currently administered by APHIS BRS and by possible changes of those regulations?**

**Reviewer—**My response/recommendation with regard to this question is that this chapter is acceptable after revisions have been made to address some weaknesses, specified in the above report and illustrated in the 'track changes' version of the report.

As indicated in the comments above, the scientific information presented in this document is, for the most part, accurate and objective. However, it should offer more information to help the reader with context and background, i.e., to "provide non-expert readers with a broad base of knowledge to understand the aspect of the biological and physical environment that is likely to be affected by the regulations currently administered by APHIS BRS and by possible changes of those regulations."

Without changing the focus of this document on GE organisms, it would seem particularly helpful for the non-expert reader to be made more aware of historic as well as recent advances in crop improvement using other methods of genetic modification, or using gene splicing methods to move genes between varieties of the same crop species which could well be the main use of GE in the future.

Examples of ways to provide this additional context and background information are offered as edits and comments in the text. However, here I offer two particularly relevant examples that I realize might be considered inappropriately addressing regulatory and policy implications but which I believe are relevant to context and background. The examples are derived from my own familiarity with wheat and barley in the U.S. Pacific Northwest.

The first example is the tolerance to the herbicide imazamox, developed in wheat using BASF patented technology and marketed under the trade name Clearfield®. Unlike Roundup tolerance, which involves introduction by plant transformation of a gene coding for a glyphosate-insensitive variation of the enzyme 5-endopyruvylshikimate-3-phosphate (EPSP) synthase, tolerance to the acetolactate synthase (ALS)-inhibiting family of herbicides involves the use of mutagenesis of the plant's gene for production of a variation on ALS. Four Clearfield®-type varieties of winter wheat have been developed thus far by PNW wheat breeders. Of these, three are herbicide-tolerant selections of existing varieties, i.e., 'Madsen', 'Stephens', and 'Coda', produced by exposing mutagenized seed of these varieties to lethal doses of the ALS-inhibiting herbicide imazamox and picking the survivors. The fourth, developed by Oregon State University by conventional plant breeding and released as 'ORCF-101,' is the product of a three-way cross involving the herbicide-tolerant CV9884 as the donor, produced in France by mutagenesis of the French variety 'Fidel,' and the PNW winter wheat varieties 'Madsen'and a 'Malcom'/'Stephens' hybrid (see Peterson, C.J. 2003. Release of the new OSU Clearfield wheat variety. http://cropandsoil.oregonstate.edu/wheat/reports/OWC_CF_1.

Other examples of crop varieties produced by mutagenesis could just as easily be presented, in the context of what we have learned or assume with these varieties with respect to health and environmental safety questions that should now help guide or predict with more certainty what can be expected with GE varieties developed for herbicide tolerance.

**APHIS—**APHIS appreciates the example being cited, however, broad considerations about the nature of GE crops compared to other varieties include discussions of policy that are beyond the scope of this document. This section of the draft EIS is an attempt to lay out the potential environmental concerns of APHIS regulation of GE crops. In many places in the text, the reviewer's comments have been addressed through the addition or modification of material.

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**Reviewer—**The second example is the recent report based on a collaborative project between Washington State University and the University of Minnesota, namely the transformation of barley for resistance to stem rust using a gene for stem rust resistance from barley (Hovarth, H., Rostoks, N., Brueggeman, R., Steffenson, B. von Wettstein, D., and Klienhofs, A. 2003. Genetically engineered stem rust resistance in barley using the Rpg1 gene. Proc. Nat. Acad. Sci. 100:364-369.) The widely held belief among non-experts and somewhat reinforced by information presented in this document is that genetic engineering is about moving genes between unrelated organisms, even between kingdoms. However, the greater use of this technology in the future will be expediting the breeding process by splicing genes from one variety into another variety of the same crop plant species. In the case of disease resistance genes, it will be possible to update popular varieties by splicing in or stacking genes for resistance as needed in response to changing virulence in the pathogen population. This can also now be done such that the new phenotype contains only the gene of interest, without the genetic marker. Here is where context and background are especially important, since implications for human health or the environment of varieties modified with a single gene from another variety introduced by GE should be no different than that of a line isogenic for the same gene but derived using backcrossing.

**APHIS—**APHIS appreciates the example provided. The topic of using GE techniques to move genes within a species (between varieties) is discussed in another section of the draft EIS where the appropriate policy considerations can be addressed.

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**Reviewer—**In addition to these two examples relevant to the development and commercial use of GE crops, I offer the following brief comment on the draft document **Biological Control of Pests.**

This document is well written but is focused on traditional and historic use of biological control of insect pests and weeds. The title of the document would more accurately be **Biological Control of Insect Pests and Weeds** since there is no reference to biological control of plant pathogens. The document could easily set biological control of plant pathogens aside in the introduction by clarifying that plant pathogens, being microorganisms, require the use of other microorganisms (antagonists) for their biological control, and that any use of a microorganism introduced as a product for pest

or pathogen control requires registration by the EPA as a "microbial pesticide" under authority of the U.S. Federal Insecticide, Fungicide and Rodenticide Act
For more information on the science and practice of biological control of plant pathogens the readers could be referred to Baker and Cook, 1974 and Cook and Baker, 1983[1]. For a comprehensive discussion and scientific framework on the use and evaluation of the safety of microorganisms intended for pest and plant disease control, the reader could be referred to Cook et al., 1996.[2]

Hopefully these comments and suggestions will be helpful. This is a very comprehensive chapter already, and needs only the additional examples from traditional and modern breeding and management practices as context and background to make it an excellent document.

**APHIS—**APHIS appreciates the comments on the section on biological control. This section has been deleted in the draft EIS and biological control is treated in other sections of the draft EIS.

**Additional Concerns highlighted in "Track Changes" Document**
Although the above represents the reviewer's report in its entirety, APHIS has decided to include excerpts from the reviewer's "Track Changes" edits that represent significant comments so as to make the measures taken in response to these comments explicit. It should be clear that the entire track changes document is available for the public to view and that the comments below have been pulled from the "Track Changes" document by APHIS, without additional input from the reviewer. Because of discrepancies in the numbering of different versions of the document, the comments are here identified by the section title and subtitle closest to their appearance in the "track changes" document.

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Commercial Seed
ProductionTrade and Value

**Reviewer—**"The increased sophistication of plant breeding to produce crops meeting very specialized needs and market niches has in recent decades increased the need for **high** standards of seed genetic purity in order to assure identity preservation in increasingly diversified markets." Equally or more importantly, many plant diseases that

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[1] These two books provide the scientific framework for biological control of plant pathogens and take the concepts into quite different directions from that of insects and weeds. For example, lowering the pathogen population would be nice but is not necessary for successful biological control of plant pathogens, if the antagonist keeps the plant healthy. These books are: Baker, K. F. and Cook, R. J. 1974. Biological Control of Plant Pathogens, W. H. Freeman and Co., San Francisco, 433 pp. (Book, reprinted in 1982, American Phytopathological Society, St. Paul, MN. Cook, R. J., and Baker, K. F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN. 539 pp.

2 This journal article is written by a team of insect pathologists, weed scientists, nematologists, and plant pathologists that worked on this for more than two years. It lays a common framework for the safe use or microorganisms intended for pest control. I led this team motivated by using the experienced I gained from production of the OECD document on scale up of crop plants developed by biotechnology. The article is: Cook, R. J., et al. Safety of microorganisms intended for pest and plant disease control: framework for scientific evaluation. Biol. Control 7:333-351. 1996.

depend on seed **transmission** of the pathogen have been controlled by use of seed produced under conditions and with practices to certify it **as** pathogen free. Control of fungal, bacterial,, and viral pathogens dependent on seed transmission to produce disease is considered one of the major accomplishments in plant health management in the 20th century (Cook, R. J. 2000. Advances in Plant Health Management in the Twentieth Century. Annu. Rev Phytopathol. 38:95-116.)

**APHIS—**APHIS agrees that this is appropriate to consider when addressing commercial seed production and has edited the section to include this information.

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The Future of Plant Agricultural Biotechnology

**Reviewer—**With respect to input traits, two issues account for why adoption of biotech-derived varieties have slowed in recent times: market saturation for the two traits that account for 99+% of the 220 million acres, namely glyphosate resistance and Bt (as mentioned below but which should be mentioned first); and failure to obtain approvals in the export markets, namely Japan and Europe for other crops, e.g., wheat, with these same traits or even other events of the currently approved crops (corn and soybean) with variations on use of Bt for example. The failure of the New Leaf and New Leaf Plus Russet Burbank potato in the market place was due to fast food industry led by McDonalds refusing these potatoes for making fries because of non acceptance in their European market. RR wheat is on the shelf because of no approval in the Japanese market. This has nothing to do with "a number of health issues" and to imply such would be blatantly false!

**APHIS—**APHIS has received multiple comments from reviewers with differing views on the cause of the slowdown in adoption of GE varieties. Recognizing that this is a complex issue, involving complex interactions of multiple forces, APHIS has re-worded the paragraph to reflect this.

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**Reviewer—**"It has been suggested that certain traits introduced through genetic engineering of crop plants might confer weedy characteristics to the plants, thereby creating new weeds in managed areas." For context and background, also mention of the use of gene transfers from weeds into crop plants, e.g., by chromosome insertions and manipulations. Alien genes from wild relatives of crop plants have been used extensively and safely in crop variety development for more than 50 years. E.g., see Jones, S.S., Murray, T. D., and Allan, R. E. (1995) Use of alien genes for the development of disease resistance in wheat, Annu. Rev. Phytopathol. 33:429-443. The genes transferred to wheat are from diploid and tetraploid weedy relatives of wheat and have not caused the new varieties of wheat to be weedy.

**APHIS—**Although APHIS appreciates that this is a complex topic, in the context of this overview it has been determined that it is appropriate to mention that there are concerns about the potential of GE crops to become weeds. Later in the text, the document notes that no novel weeds have been generated from GE crops. There is lively scientific debate about whether, or under what circumstances, GE crops might pose a risk of increased weediness, and it is not appropriate to explore all of the potential considerations in this overview.

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Potential Changes in Weediness and Invasiveness
GE crop plants and weediness

**Reviewer—**"Crop plants can in some cases be considered weeds, and some crops have more weedy characteristics than others." The volunteer corn plants in the soybean field that killed the Texas biotech company (name escapes me) was a weed in that context. The most important weed for no-till wheat and barley is volunteer wheat and barley that serve as hosts (green bridge) for root pathogens between harvest of one crop and planting the next crop (Smiley, R. W., Ogg, Jr., A. G. and Cook, R. J. Influence of glyphosate on severity of Rhizoctonia root rot and growth and yield of barley. Plant Disease 76: 937-942. 1992.)

**APHIS—**APHIS has added an explicit mention of the fact that crop plants that volunteer after the field has been harvested are commonly considered weeds.

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Wild Relatives with Acquired GE Traits as Weeds

**Reviewer—**"Most participants agreed that gene stacking (i.e., insertion of multiple transgenes) to confer a broad spectrum of pest resistance would be less predictable with respect to ecological consequences than single-trait resistance."

There is also extensive experience with multiple disease resistances bred into crop plants, eg. Wheat in the PNW with resistance to two rust diseases, eyespot foot rot, and common smut, or in Kansas with resistance to three rust diseases, soilborne mosaic virus, Hessian fly, and greenbug, with no issues related to weediness. I suggest that more be stated in this section on the long history of breeding crop plants for mainly disease but also insect resistance, all combined (stacked) into the same varieties.

**APHIS—**Although APHIS agrees that this is an important consideration, the purpose of the section is to point out the concerns, without going into all the complex arguments for and against. The specific quote is referring to the results of an expert consultation, and APHIS feels that it is most appropriate to present the results without additional editorial comment.

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**Reviewer—**"This suggests the possibility that the transgene, by conferring increased fitness, could have an ecological impact on wild sunflower populations, either increasing the number of modified plants within a population, creating more such populations, and/or creating more extensive seed banks of such plants." As is brought out near the end, in the section on GE resistance to viruses, but which also should be mentioned here, weeds are also a major source of pest infestations and pathogen inoculum and therefore any transfer of resistance to these crop relatives could also help reduce the potential for new epidemics or epizootics within the crop.

**APHIS—**The section is presenting a basic overview of the potential for GE crops to affect populations of weedy relatives. Although it is certainly true that resistance genes could reasonably be expected to decrease the prevalence of pathogens in weedy populations, APHIS does not believe it is appropriate to pursue all the myriad possible ramifications in this overview.

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Invasiveness Potential

**Reviewer—**"Very few crops have been shown to be persistent and invasive outside of cultivation (Hancock and Hokansen 2001)." If there are any, it would be helpful to name it (them); persistent would be easy, e.g., potatoes volunteering in the second and not just the first crop after potatoes in the rotation, but invasive??? Maybe cereal rye, but even the continued volunteering of cereal rye has been an issue of persistence and not invasiveness. This sentence needs to clarify the distinction as a minimum; examples would help even more.

**APHIS—**Hancock and Hoakanson list barley, rapeseed, rice, sorghum, sunflower, and wheat as persistent and invasive. APHIS recognizes that the characterization of invasive species is a difficult task, and there can be reasonable disagreement about this list of invasive crop species. Instead of debating the specifics, APHIS would prefer to offer the general conclusion as stated in the original text.

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Potential Affects of GE Crops on the Soil
Introduction

**Reviewer—**"Interactions also exist on a large scale: traditional agricultural practices, including tillage, irrigation and herbicide and pesticide use have significant and predominately detrimental environmental impacts. Both scales of interactions should be considered when evaluating potential effects of GE plants on soil and water environments." This is a very important point that needs more comment. Consider, for example, the impact of crop rotation on soil organisms. We know a great deal about the changes in both pathogens and beneficial rhizosphere microorganisms in response to crop monocultures versus crop rotations. Below is a quote from an article in the proceedings of NABC 15.

"…our understanding of environmental impacts [of new varieties] and the effort to reduce them through science, education, regulations, and innovations have been based on two principles.

- *It is the management used to grow the variety, i.e., the intensity of tillage, pesticide use, planting date, irrigation, etc., and not the variety itself that has impact on the environment; and*

- *Each new crop variety usually leads to changes in the management system used to grow that crop.*

A new high-yielding variety may require more fertilizer or pesticides to attain its full yield potential, as was the case with the IR-8 semi-dwarf variety of rice released by the International Rice Research Institute (IRRI) in the 1960s. In this example, *management*, especially fertilization and pesticide application, changed in response to needs of the variety. The breeding programs that followed up on this breakthrough in yield potential, and that continue to this day, have worked largely to develop replacement varieties that maintain the high yield potential but also have genes for resistance to the diseases and pests as well as improvements in other agronomic traits and quality measures. The replacement varieties may have no higher yield potential but rather, and often just as important, they allow the crop to be produced with management that is both more economical and has less impact on the environment. In the example of direct seeding to protect soil, water and air resources by preventing soil erosion, the change in management happened first, and is now being followed by the development of varieties that fit the management, such as soybeans with herbicide-tolerance."

**APHIS—**APHIS agrees that changes in management and agricultural practices associated with GE crops are considerations when conducting environmental reviews. However, in an overview document such as this one, it is impossible to go into detail about every conceivable interaction between the GE crop, the environment, and the agricultural practices associated with both. APHIS has tried to present broad and balanced examples of the types of interactions that are considered without undue emphasis on potentially negative or potentially positive outcomes.

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**Reviewer—**"Beyond the food safety aspects of GE crops, the nutritional quality of these crops should be analyzed to ensure that they are as nutritious as the same crop that was developed through traditional methods and grown under the same conditions (NRC 2004)." As <u>all</u> of the varieties of that crop developed by traditional breeding? The <u>most nutritious varieties</u> of that crop developed by traditional breeding? The <u>recipient variety not engineered</u>? This requirement seems absurd. Traditionally- bred varieties of any given crop are highly unlikely to be exactly the same as sources of nutrients, but so what? The range of variation has not been an issue. Citation of the NRC 2004 study does not validate the stance expressed in this statement. Cite experience with traits induced by mutagenesis? If ever there is a method to change the nutrient status of the variety, it would be mutagenesis. This paragraph needs major modification.

**APHIS—**APHIS agrees with the reviewer that this section is unclear, and is duplicative with the following section on plant composition. These two sections have been combined and edited for clarity and to address the reviewer's concerns.

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**Reviewer—**"The likelihood of those Lepidoptera insects being directly affected by the Bt depends on the scale of the crop, i.e., in cases of small scale field trials, any impact at the population level of affected Lepidoptera insects is very unlikely cases of large scale commercial use, the estimation of likelihood should consider the presence and feeding behavior of those Lepidoptera insects, which depends on those insects and on the crops involved." For what? Food? Habitat? Is this about the Monarch butterfly issue and corn pollen on leaves of milkweed? This issue seems like a real stretch. The implication of this argument reminds me of the British work published in *Science* 3 or so years ago reporting that a certain species of field birds (larks?) were negatively impacted by HT sugar beet because weed control was so good it left too few weed seeds in the field for the birds. Surely plant breeders will not need to answer these kinds of questions for APHIS. And since the great majority of U.S. crops are naturalized alien plant species, what did those insects do before that crop was introduced for commercial use? What would they do when fewer acres of the crop are planted because of economic considerations, e.g., as is happening in the U.S. of late with wheat.

**APHIS—**APHIS appreciates the reviewer's comments, but has chosen to leave this section of the document substantially unchanged. Other reviewers have commented that the treatment of GE crops with insecticide traits in this section minimizes the potential for negative impacts and should include more information on studies showing potential effects. In light of these opposing views, APHIS believes that the section, as written, presents the issue in an appropriate context to let the reader of this overview know that non-target effects are a consideration.

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Assessment of Environmental Effects
Potential unintended effects on the target organism

**Reviewer—**"One potential method to circumvent or delay cross-resistance is to plant two or more Bt crops, each of which produces a Bt protein with a mode of action different from the others. The theory behind spatial refuges is that it is very unlikely that a pest population would develop resistance to multiple unrelated proteins. However, for many pests, a single individual will only experience a single plant, and therefore a single Bt protein (mode of action), during its development. Because many pest larvae do not move from plant to plant, and would not be exposed to multiple Bt proteins, spatial refuges have not been implemented."
An even better method is to stack two Bt genes with different mechanisms in the same plant, making it necessary for the insect pest to defeat both genes to overcome this resistance. This approach has become standard practice for cotton in Australia using what is call "twin gene" control. However, it is necessary to use both genes stacked form the beginning; if done sequentially, i.e., first one gene and then the second after the pest has defeated the first, and that new biotype allowed to build up first, then the advantage of stacked genes is no different than using the two genes in separate plant genotypes.

**APHIS—**APHIS has added some language to the text to include mention of this resistance management strategy.

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Potential effects on plant-insect interactions

**Reviewer—**Many plant diseases are favored by water or other abiotic stresses that predispose the plant to more aggressive attack by the pathogen. Fusarium root and crown rot of wheat is one such disease, and is managed in the PNW by use of practices and varieties that avoid or tolerate plant water stress and not by use of a resistance trait directly inhibitory to the pathogen.

Cook, R. J. and Papendick, R. I. Influence of water potential of soils and plants on root disease. Annual Review of Phytopathology 10:349-372. 1972.

**APHIS—**In response to this, as well as comments from other reviewers, a brief introduction to plant stress and response, and the inter-relatedness to disease has been added to the text in the section "Examples of Assessing Potential Impacts of GE Plants on the Environment."

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**Reviewer—**"As plants are engineered to become tolerant of abiotic stress, there is a need to consider the complex interactions between genetically-engineered stress-tolerant plants and insect herbivores."
Insects are constantly foraging for just the right food source, but do not always succeed owing to the natural cycles of food supply. Is the concern that the physiological change(s) may result in greater susceptibility of the GE crop to the insect as a pest? This is not a new issue for plant breeding [It has happened many times that the release a variety improved for one trait revealed after scale up that it was vulnerable to some different hazard] but the issue has not previously been brought under APHIS regulatory authority, if that is the intent of this discussion. If the concern is that a new pest problem could arise, this needs to be made clear.

**APHIS—** APHIS has regulatory authority over plant pests, and analyzes GE crops for their potential to alter plant pest risk. In this context, insect herbivores are frequently plant pests and a GE crop's effect on these organisms would be important for APHIS regulatory mission. Additionally, in the context of APHIS requirements under NEPA, insect herbivores that are not plant pests and effects on them could be considered. This section has been included in the document to give the public an example of the kind of information APHIS may be requesting from applicants in the future in order to complete risk assessments.

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Genetically engineered crop plants tolerant to low levels of nutrient availability.

**Reviewer—**The repeated emphasis on plant-insect interactions in relation to changes in plant physiology is curious. I am not suggesting that the points made here be extended to plant-microbe interactions, but will point out that plant pathogens are just as responsive if not more responsive to these changes than are insects. Obligate parasites such as rust fungi are less active on stressed than non stressed plants while facultative parasites and saprophytes are more able to produce more disease on stressed than non stressed plants. This is outlined in the first chapter of Baker, K. F. and Cook, R. J. 1974. Biological Control of Plant Pathogens, W. H. Freeman and Co., San Francisco, 433 pp. (Book, reprinted in 1982, American Phytopathological Society, St. Paul, MN.
As mentioned in comment 44, Fusarium root and crown rot of wheat is managed by managing water stress, such that the predisposing effects of the stress on the disease are mitigated.

**APHIS—**APHIS agrees that effects on plant pathogens are considerations. However, the text in the document is included as examples of the kinds of considerations that APHIS may take into account for future regulations.  They are not meant to be entirely exhaustive.  Language has been added to the text to help clarify that these are examples, and that similar considerations would be taken into account for other plant pathogens and non-target organisms.

## Response to Reviewer Report 2

APHIS has copied the text of the Reviewer's Report and added a response where appropriate.

### Introduction

As directed in the USDA/APHIS 'Charge to Peer Reviewers of Influential Scientific Information Contained in the APHIS BRS Draft Programmatic Environmental Impact Statement, this review report addresses the issues of completeness, currency, accuracy, uncertainty, objectivity, and clarity and ends with an overall conclusion. Detailed comments and suggestions have been provided to USDA-APHIS in a 'track changes' edited version of Chapter 4.1. This 'track changes' document is available as an addendum to Reviewer Report 2

**Reviewe**r—

**Completeness:** In general, the chapter is comprehensive. Among several curious omissions, however, was any discussion of community diversity impacts of widespread use of GE (specifically herbicide-resistant) crops; there are several relevant papers from the multi-year British Farmscale Study that are not cited (Proc Roy Soc. B 272 (2001). Inasmuch as concerns have been raised, even a brief mention of impacts of transgenic plants on pollinators would also have been useful (the entire monarch/Bt corn controversy, more or less resolved in a series of six papers in PNAS 98 (2001), was effectively unmentioned as was the ongoing discussion about transgene product impacts on honey bees, reviewed in Malone et al. 2001 Apidologie, among other places.

**APHIS—**APHIS has tried to address the reviewer's concerns where they appear in the 'track changes' version of the text. In several places, the document makes clear that GE crops must be considered in the context of the agricultural practices associated with them. Although community diversity impacts can be important consideration in the review of individual GE plants, in the context of the British Farmscale study, the results were associated with the effectiveness of the agricultural practices (elimination of weedy plants in fields) rather than an inherent property of the GE herbicide tolerant crops. Although these results have implications for the larger consideration of agricultural practices, the details of the study and the context required to present it understandably are simply too large to be included in an overview document such as this one. Although the document does not explicitly discuss honeybees, much attention is paid to the effects of GE crops on non-target species. Again, this document is meant as an overview to make readers familiar with the range of environmental considerations that are relevant to APHIS regulations, not an exhaustive treatment of each of these considerations.

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**Reviewe**r—
**Currency**: Approximately three dozen references are less than two years old and the authors have made an effort to access a broad diversity of sources, including specialty journals, stringently peer-reviewed journals, government publications and web sources.

One place where currency is a severe problem is in the section on biological control of weeds; essentially a decade of literature documenting adverse impacts of biological control agents, including those introduced for weed control, on non-target species. There are two reviews in Annual Review of Entomology germane to this subject (Louda et al. 2003; van Lenteren et al. 2006) that are not included in the discussion or reference list. The failure to cite or acknowledge this literature has implications for accuracy and uncertainty.

**APHIS—**The biological control section has been extensively restructured based on comments from this and other reviewers. The references suggested here have been added along with text to reflect a more accurate representation of what is currently known about the effects of biological control organisms.

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**Reviewe**r—
**Accuracy:** For the most part, the report is accurate. Several errors or misspellings are indicated by "track change" edits on the document. Perhaps as a consequence of an effort to make the text accessible, some imprecision was introduced in places (e.g., use of "animal" to mean "vertebrate").

Again, the section on biological control of weeds is dogmatic in its assertion that there have been no adverse impacts of biocontrol agents on nontarget native plants; recent publications on *Rhinocyllus conicus* and Platte thistle provide just one glaring exception to these bald statements.

**APHIS—**Errors have been corrected both as a result of the peer review process and APHIS's normal editing procedures. As stated above, the biological control section has been extensively reworked in order to address reviewers' concerns. Text implying that biological control agents have not had adverse impacts has been removed or clarified to reflect a more accurate picture of current views on biological control.

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**Reviewe**r—
**Uncertainty:** There was little attention paid in the document to effects of human behavior on environmental impact and there is a literature to suggest that compliance (e.g., with providing refuges, Carriere et al. 2001), carelessness, or even deliberate flouting of regulations can change risk levels. As well, throughout the document, many outcomes were described as low-risk but no effort was made to quantify probabilities (which may have been more effective at conveying risk level) (e.g., "a consensus exists among scientists that the likelihood of small peptides to sensitize an individual is low"). There is also greater certainty stated in the section on biological control of weeds than is justified by current literature; moreover, there is no acknowledgment or recognition that the degree of contemporary attention to risk assessment did not necessarily apply in the past and agents may have been introduced in the past (which are now established) that would not pass muster by contemporary standards.

Among the dogmatic and less than adequately documented statements are (see Track Change text for reference suggestions):

– "The host range of the biological control organisms that is observed in the field is always smaller than the host range that is observed in the laboratory because environmental factors reduce the number of species that may be attacked due to unfavorable environmental conditions".
– "long-term, extensive damage to a non-target plant population, which has never been documented from a biological control agent"

**APHIS—**The reviewer raises three general points related to uncertainty that will be dealt with separately here.
1. Human behavior and the influence on risk: This portion of the document is an overview on the potential environmental consequences of APHIS regulations regarding the introduction of GE plants. In other parts of the draft EIS, APHIS presents information that deals with regulatory compliance. It bears repeating that no matter how well developed APHIS regulations are, they are only effective if they are being observed and followed. This is not different from other laws and regulations. The article cited is addressed in a comment below from the reviewer's "track changes" edits.
2. Quantification of risk: This document is not a risk assessment. It is intended to introduce key concepts to consider regarding the potential environmental impact of APHIS regulations. In some cases, events characterized by APHIS as "low risk" have never been observed to occur, but the agency presents the possibility to readers because it is not certain that the risk will never occur. In an overview document such as this, it is not possible to quantify all the potential risks, but APHIS believes the qualitative characterization of risk in this document is accurate and supported by current scientific knowledge.
3. Biological Control Appendix: This section of the document has been reworked to address the reviewer's concerns. The particular quotes addressed have been removed and language has been modified to reflect a more current understanding of the effects of biological control agents.

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**Reviewe**r—
**Objectivity**: There were places where qualifying language, justified by the literature, was missing, which created an impression of bias. In the definition of Cry proteins, e.g., the statement is made that these "are harmless to mammals and most beneficial insects" but these proteins are broadly toxic within an order, so what kills target lepidopterans is likely to kill nontarget lepidopterans as well.

**APHIS—**The reviewer's comments have been addressed where they appear throughout the text. In addition, APHIS has tried to reduce the appearance of bias by removing unnecessary qualifying language that is not justified. In the course of this review, different reviewers have pointed out places where the document shows a bias towards minimizing risk or shows a bias for exaggerating risk, sometimes in the same instance. Where appropriate, APHIS has tried to make it clear where expert opinion is not unanimous.

**Reviewer—**
**Clarity**: See text for tracked changes where meanings weren't clear and where alternate wording has been suggested. Generally, the text was clearly written (although the document reads as if there were multiple authors—the first few sections were much less well-constructed than subsequent sections).

**APHIS—**APHIS has tried to respond to the reviewer's comments where they appear in the track changes document. In addition, some editing and organizational changes have been made to improve the overall continuity of the document.

**Conclusion**

The Charge to Peer Reviewers requests the peer reviewers to answer the question, **Does this scientific information presented accurately and objectively provide non-expert readers with a broad base of knowledge to understand the aspect of the biological and physical environment that is likely to be affected by the regulations currently administered by APHIS BRS and by possible changes of those regulations?**

Referring to the review report and to the detailed comments and suggestions in the track-changes version, my answer to this question is **(2) Yes, but only after revisions have been made to address specific weaknesses.**

**Additional Concerns highlighted in "Track Changes" Document**
Although the above represents the reviewer's report in its entirety, APHIS has decided to include excerpts from the reviewer's "Track Changes" edits that represent significant comments so as to make the measures taken in response to these comments explicit. It should be clear that the entire track changes document is available for the public to view and that the comments below have been pulled from the "Track Changes" document by APHIS, without additional input from the reviewer. Because of discrepancies in the numbering of different versions of the document, the comments are here identified by the section title and subtitle closest to their appearance in the "track changes" document.

Seed Biology and Commercial Seed Production
The role of seeds in plant reproduction

**Reviewer—**"Crop plants with perfect (complete) flowers are largely self-pollinated, and the majority of crop plants are self-pollinating." I'm not convinced this statement is entirely true. It may be true for grasses, but it's not true for most nuts, fruits and vegetables.

**APHIS—**APHIS has made changes to the text, based on the comments of this reviewer and other reviewers to clarify the description of crop plant reproduction to more accurately reflect the range of reproductive strategies seen in crops.

Accumulation of storage materials

**Reviewer—**"Seeds store large amounts of chemical substances not stored in any other parts of the plant." As written, this isn't exactly correct. For example, carbohydrates are often stored in roots.

**APHIS—**APHIS has re-written this paragraph to more accurately represent that seeds are a major storage tissue for a variety of compounds, but they are not the only place that these compounds are found.

Wild Relatives with Acquired Genetically Engineered Traits as Weeds

**Reviewer—** Most crops are descended from early successional species with many characteristics of weeds aren't they? The "Dump-heap theory" of Anderson 1952?

**APHIS—**Although APHIS recognizes the scientific implications of the question, it is beyond the scope of this document to explore this issue. Throughout the document, in response to reviewers' comments APHIS has tried to remove instances of over-generalization and over-simplification. APHIS believes that the revised document adequately deals with the issues surrounding the transfer of GE traits to wild relatives without delving into a more rigorous scientific discussion of crop origin.

Potential affects of GE crops on Soil
Interactions with soil organisms

**Reviewer—**"For example, soil-dwelling organisms are likely to have had previous exposure to proteins from *Bacillus thuringiensis*, a naturally-occurring soil bacterium, which potentially mitigates the impact of Bt crops on the soil ecosystem." Are the proteins in the soil microbe identical in amino acid composition to transgenic Bt proteins?

**APHIS—**In some cases the proteins have identical amino acid composition, but in others, minor changes have been made to a few amino acids. APHIS recognizes that these changes could conceivably have an effect on the interaction of the protein in the soil. APHIS stands by the assertion that the origin of the transgene should be considered in future assessments, and considers amino acid sequence to be relevant to the origin of the protein.

Nutritional Quality of Genetically engineered Plants Compared to their Traditional Counterparts
Background

**Reviewer—**Changing the nutritional quality of a crop through GE might also render it more susceptible to herbivore damage (e.g., carotenoids, complete proteins, linolenic acid, sterol source); is this factor relevant?

**APHIS—**Although APHIS believes this may be a relevant consideration when dealing with evaluations of individual GE plants, it is not necessary to include this topic in an overview document. In part because of this comment, in conjunction with other reviewers' comments, a brief introduction has been added to the text at the beginning of the subsection "Examples of assessing potential impacts of GE plants on the environment" in order to introduce the idea that plant stress and response to different stresses are interrelated.

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GE Insect-Resistant crop plants
Potential effects on non-target organisms

**Reviewer:** "When those insects are not present in the area of planting or do not use the crop involved as main source of food, then an impact at the population level of those insects is very unlikely." But not impossible and in fact Bt corn has a demonstrable effect on a nontarget lepidopteran that does not in fact utilized the GE crop (Sears et al. 2001, Zangerl et al. 2001; Obrycki et al. 2001)

**APHIS—**This issue has been addressed in the text. APHIS does not believe that Bt crops have a significant effect on populations of non-target organisms that do not feed or utilize the crop itself, when compared to the use of insecticide sprays, which is the usual alternative to Bt crops. This does not dispute the fact that Bt plants, and in particular, pollen containing Bt can have lethal or sub-lethal effects on some Lepidoptera under experimental conditions, but this effect needs to be considered in the context of real-world field conditions. APHIS still believes that the literature, as a whole and taken in context, supports the conclusion as presented. This does not change the fact that the document highlights for the reader that effects of insecticidal plants on non-target insect populations is a consideration when analyzing GE crops.

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**Reviewer—**Refuges are treated as needed to control insect pests with non-Bt insecticides or other appropriate IPM practices and managed according to practices in the Bt field." Refuge compliance is a problem, isn't it? Carriere et al. 2005

**APHIS—**Refuges are discussed here to provide the reader with a contextual example of how GE insect-resistant crops are managed. Refuge compliance, specifically, does not fall under the purview of APHIS, but it is a consideration in how APHIS analyzes data and conducts risk assessment. In the cited article, overall compliance was >88% in five of six years. While there is clear room for improvement, APHIS still believes that this level of compliance does not preclude mention of this management strategy in an overview document. It is also worthwhile to note that, to date, resistance to Bt crops has not been observed in the field.

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**Reviewer—**"Although wildlife may be exposed to Bt protein, there is no evidence to date that shows toxicity to wild or domesticated mammals, fish, or avian species, and there are no reports of adverse effects from the commercial poultry industry after several years of using Bt corn in poultry feeds." Are there data from use in catfish aquaculture?

**APHIS—**Although studies have been conducted on the affects of Bt crop feed on catfish, APHIS is unaware of any studies conducted to look at the affect of Bt catfish feed on other organisms.

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**Reviewer:** "Accidental aquatic exposure from Bt crops is extremely small, and there is no evidence for sensitivity of aquatic species to Bt proteins (EPA-BPPD 2001)" (excluding Bti and Diptera; you mean only Btk and corn, right?)

**APHIS—**APHIS has adjusted the text to indicate that it is referring to Bt proteins from GE crops. Bti is used to control mosquitoes and is not relevant to the discussion of Bt proteins in GE crops.

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GE Crop plants tolerant to low levels of nutrient availability
Potential effects on plant-insect interactions

**Reviewer—**"Thus, genetic modifications that result in plants increasing in either nitrogen or phosphorus content may potentially affect insect herbivore populations on stress-tolerant plants because of the sensitivity of insect herbivores to nitrogen and phosphorus content." What about higher trophic-level effects? And where do pollinators fit in?

**APHIS—**This section of the text is included to make readers aware of the potential environmental affects of genetic modifications that affect nutrient uptake. Because this is an overview document, details of ecological interactions are not explored in this context.

---

GE virus resistant crop plants
Development of new virus diseases through Heterologous Encapsidation

**Reviewer—**Is there any difference in dynamics of interactions between insect vectors and persistent and nonpersistent plant viruses?

**APHIS—**Although these interactions do have different dynamics, these differences are not relevant to the discussion of "Heterologous Encapsidation".

---

Herbicide tolerant crop plants

**Reviewer—**There's no discussion here of the community diversity impacts of extensive use of herbicide-resistant GE crops, as documented in the British Farmscale studies; Haughton et al. 2003, e.g., documented reduction in abundance of butterflies in GE beet and oilseed rape and bees in GE beet fields (see also Roy et al. 2003)

**APHIS—**Because this document is an overview, rather than a comprehensive examination of effects of herbicide tolerant crop plants, APHIS does not feel it is appropriate to include this level of detail. It is pointed out in several places in the text that GE crops are planted in the context of a variety of agricultural practices and this data falls within that range.

---

Silviculture
Tree traits under development

**Reviewer—**Is there any consideration of the effects of transgene products in post-harvest wood and in wood waste? How about fire?

**APHIS—**APHIS has added a paragraph to this section regarding the disposition of post harvest products from trees. Because this is a general overview, APHIS does not believe that additional detail regarding fire and its effects on GE products is appropriate.

**Response to Reviewer Report 3**

APHIS has copied the text of the Reviewer's Report and added a response where appropriate.

**Introduction**

Following the USDA/APHIS 'Charge to Peer Reviewers of Influential Scientific Information Contained in the APHIS BRS Draft Programmatic Environmental Impact Statement, this review report addresses the following parameters: Completeness, Currency, Accuracy, Uncertainty, Objectivity, and Clarity and ends with a final recommendation.

**Reviewe**r—
**Completeness:** All potential factors and relevant issues known to me and of relevance to the statement have been considered in the document that I was provided for review.

**Currency:** Although the information presented in the document is in general current, the references to the introductory section (pages 1-5 inclusive) tend to be older textbooks and reviews (Allard, 1964; Bidwell, 1974; Brady, 1974; Esau, 1977, and others). While it might not be expected that such introductory information would have changed significantly in recent decades, the authors are advised to consult more recent texts and update the introductory information as necessary to reflect current thinking.

**APHIS—**While APHIS appreciates this comment, much of the cited information is very basic information about the biology of plants and their environment. APHIS has made efforts throughout the document to update the currency of references based on reviewers' comments. In the introduction, some effort has been made to update textbook references to more current texts. However, if the information is non-controversial or falls into the realm of generally accepted knowledge APHIS has not felt it necessary to update these references.

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**Reviewe**r—
**Accuracy:** Page 3, second paragraph under Physical Environment. Change from "roots release organic acids" to "roots release organic compounds." Roots are known to release many compounds that impact nutrient availability and soil development.

**APHIS—**Suggestion accepted

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**Reviewe**r—Page 4, first paragraph. Add a citation after the final sentence, which relates transpirational water loss over a season to several inches of rainfall.

**APHIS—**This sentence has been deleted because it is unnecessary for the conclusions contained in the surrounding text.

---

**Reviewe**r—Page 5, first paragraph, next to last sentence – Rather than stating that altered photosynthetic efficiency may require "additional" water and fertilizer and "increase" overall environmental fitness, consider emphasizing that these factors may be altered in either direction—increased or decreased. I know of no evidence that altered photosynthetic efficiency works in only one direction—to increase.

**APHIS—**This paragraph has been modified to reflect the multi-directional possibilities for changes to photosynthetic efficiency.

---

**Reviewe**r—Page 5, last paragraph – State that the purpose is either unknown or not apparently related to growth. In the absence of data, it is not advisable to state definitively that there is no relationship to growth.

**APHIS—**This paragraph has been modified to reflect the multi-directional possibilities for changes to photosynthetic efficiency.

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**Reviewe**r—Page 8, discussion of flower structure and pollination strategies. This section does not give an accurate overview of pollination as it relates to crop plants. There is discussion of self-pollinating crops with perfect flowers and cross-pollinating crops with imperfect flowers (corn). Missing is discussion of crop plants that have perfect flowers yet cross pollinate to a significant extent. Alfalfa is an example, as is canola (APHIS own website describes the latter as about 35% cross-pollinating). I suggest that attention be given to the second paragraph on this page. Indicate that some important crop plants have perfect flowers yet outcross. There is abundant information on this phenomenon in the plant genetics and breeding literature.

**APHIS—**The text has been modified to address this concern. The text now indicates that crops occupy a range between the extremes of self-pollination and cross-pollination

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**Reviewe**r—Page 9, third paragraph – Some seeds can be stored for more than 100 years and remain viable. The phrase “several years” vastly understates this fact.

**APHIS—**This fact has been incorporated into the paragraph.

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**Reviewe**r—Page 9, fourth paragraph – Proteins, carbohydrates, and lipids are indeed stored in parts of plants other than seeds. Witness the potato or the watermelon.

**APHIS—**This paragraph has been adjusted to no longer state or imply that seeds are the only storage site for these chemicals.

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**Reviewe**r—Page 13, third paragraph – Replace “work in a new place” with “work in progeny.” This keeps the focus on the scientific level.

**APHIS—**This paragraph has been modified to reflect this concern, although the suggested changes have not been introduced verbatim.

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**Reviewe**r—Page 21, second paragraph, first sentence – The crop could also become established in an unmanaged habitat if crop land is allowed to return to an unmanaged state.  Farms are abandoned quite often in some parts of the country, and when this happens, there is no need for the physical transport of seeds or other plant parts.

**APHIS—**APHIS has added a statement indicating that GE crops may be introduced to unmanaged ecosystems through the abandonment of farms or fields.

---

**Reviewe**r—Page 22, first paragraph.  It would be helpful if the authors would state whether or not standard growing conditions have been established by AOSCA for all crop seeds or just some of them.  This point is ambiguous in the text.

**APHIS—**This point has been clarified to show that AOSCA has standard growing conditions for many, but not all crop seeds.

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**Reviewe**r—Page 31, second paragraph.  All methods of genetic modification not only have the *potential* to alter a plant's genetic makeup.  They are designed to do so.  This should be stated explicitly.  This paragraph also confuses the issue of what can and cannot be detected with the issue of what is intentional and what is unintentional.  This confusion is unfortunate, because it implies (last sentence) that intentional changes are easier to identify and that unintentional changes are more difficult to identify.  In reality, these are unlinked phenomena.  Often the desired change is exceptionally difficult and costly to measure (elevated levels of some functional food chemical, for example), while unintentional changes are easy to spot as gross alterations in plant form.  The authors should make this point explicit.

**APHIS—**The text has been adjusted to address this comment by removing the word "unintentional" and indicating that some types of changes are more difficult to identify.

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**Reviewe**r—Page 33, second paragraph.  In context, the second sentence should state "the allergic potential of every protein."

**APHIS—**APHIS believes that the sentence communicates the desired information without this change.

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**Reviewe**r—Page 47 and 48, bulleted list of issues.  I believe it inaccurate to omit the potential effects of drought or saline tolerant crop plants on pathogens, including viruses.  In theory, these could be as significant as effects on insects and weediness.

**APHIS—**These bullets are intended to illustrate concerns that are specific, or especially important to the review of drought and saline tolerant GE plants.  The effect on pathogens is not highlighted at this point in the text because it is not more relevant to these crops than other GE crops.  Because of this, and other comments by reviewers, a brief introduction to plant stress has been added at the beginning of this section, in order to introduce the idea that the individual stresses that affect a plant are related to each other in view of the plant's overall health.

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**Reviewe**r—Page 50, first paragraph. Grafting is a form of mechanical transmission. It would be better to state "mechanical transmission such as grafting" than "mechanical transmission and grafting."

**APHIS—**This change has been made.

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**Reviewe**r—
**Uncertainty:** Page 1, paragraph 2, sentence as follows: "The agency recognizes, however, that in a scientific approach, it cannot make general conclusions about the safety of GE organisms." I find this sentence to be grammatically awkward and to be inadequate in dealing with the uncertainty surrounding GE organisms. The emphasis should be on the state of current scientific knowledge and the extent to which it allows specific and more generalized conclusions to be/not be made.

**APHIS—**The sentence has been modified to reflect the reviewer's concern.

---

**Reviewe**r—Page 26, fourth paragraph. In the absence of data on persistence of nonmodified DNA in soil, the data on persistence of GE DNA are, as the writer points out, largely meaningless—at least from the standpoint of whether or not GE DNA is any more persistent than unmodified DNA. On the other hand, if the question is whether or not plant DNA persists for up to two years in the soil, then the answer is yes. It might be helpful to explicitly separate these two issues as they are dealt with here.

**APHIS—**APHIS acknowledges that the data is not conclusive, but has opted to include the information in order to make the reader aware that the persistence of DNA residues in soil may be an issue of concern.

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**Reviewe**r—Page 27, fourth paragraph. To state that "soil- and plant-associated microbial communities and their interactions are not completely understood" is to paint a very bright picture indeed. Most microbial ecologists would write in terms of very poor understanding. We are nowhere close to complete in our understanding of these communities.

**APHIS—**APHIS has adjusted the text to imply a less complete understanding of microbial communities and their interaction.

---

**Reviewe**r—Page 29, third paragraph. It would be helpful if the writers would draw two conclusions from this paragraph. The first is that the potential for generalized HGT in soil is vanishingly low, and the second is that the potential for HGT of a specific, small segment of a genome is vanishingly smaller still. It would be logical to invert the final sentence of this paragraph (top, page 30) to indicate what is significantly smaller rather than what is significantly greater.

**APHIS—**Based on this reviewer's comments in conjunction with the comments of other reviewers, APHIS believes that the likelihood of HGT being addressed here are adequately considered in the text.

**Reviewer**—

**Objectivity:** Page 2, last paragraph. The text here should be revised to indicate that there are thousands of associations, many beneficial and many detrimental. As written, there is a subtle bias that most such interactions are detrimental (hundreds of negative), but only a few (certain) are positive. Also, please italicize the genus *Rhizobium* to be consistent with the style on page 5.

**APHIS—**This text has been modified to address the reviewer's concern

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**Reviewer**—Page 3, first paragraph. Emphasize that GE traits could impact both positive associations and negative associations. There is undue emphasis here (see comment above) on negative associations.

**APHIS—**This paragraph has been deleted for organizational/editorial reasons.

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**Reviewer**—Page 6, first paragraph – Altering secondary metabolite production may also have negative effects—it is not just an issue of more fit and higher value. These changes can work in either direction.

**APHIS—**The sentence has been altered to remove the implication that changes in secondary metabolite production may only lead to improved fitness.

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**Reviewer**—Page 9, second paragraph – In reality, no crop plant "perfectly" holds its seed until harvest. I suggest changing the last sentence of this paragraph as follows: "No crop does this perfectly, and thus some grain is inevitably lost prior to harvest."

**APHIS—**Although this text has not been adopted verbatim, the paragraph in question has been modified to indicate that seed loss is common and can be significant.

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**Reviewer**—Page 11, first paragraph, last sentence – It is unclear why the requirement for science based policies and regulations merits special emphasis (the word "especially") when GE varieties are traded. This sentence implies a kind of bias that allows decisions to drift away from science so long as the issue is unrelated to GE varieties—yet be strictly held to science when GE varieties are considered. I believe that these agencies have a commitment to science-based decisions, regardless of the details of the kind of seeds.

**APHIS—**The sentence has been removed to avoid implying that other decisions are less rigorously supported by science than those affecting GE varieties.

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**Reviewer**—Page 19, first paragraph, last sentence. Did Keeler imply that GE crops are as likely or no more likely to be a source, etc.? The construction of the sentence leads me to believe that the intent was one of no more likely, but the words state otherwise. This is an issue of accurate representation of a citation.

**APHIS—**The text was modified to read "no more likely."

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**Reviewe**r—Page 48, second paragraph. The second sentence should indicate that nitrogen is not the only nutrient with this potentially beneficial effect.

**APHIS—**Text has been added to the document to make this clear.

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**Reviewe**r—Page 48, third paragraph. The statement that "such significant sensitivity toward even minute changes" seems overly dramatic relative to the much more general and tempered verbiage earlier in this section. If even tiny changes in nutrients content have such dramatic effects, the authors might want to insert a series of citations documenting the effect.

**APHIS—**The text has been modified to incorporate this suggestion, and the language of the sentence has been altered to more accurately reflect the conclusions of the paragraph.

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**Reviewe**r—Page 61, third paragraph. Consider beginning the first sentence as follows: "Extensive reliance….". I believe that this qualifier is valid—the concern is more with vast acreages treated with the same herbicide than with small spots.

**APHIS—**This change has been made.

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**Reviewe**r—Page 62, second paragraph. Transfer of herbicide resistance genes into related weed species would be of concern not only to farmers, but to many others—homeowners, natural resource managers, municipalities, Etc.

**APHIS—**Text has been added to reflect this concern.

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**Reviewe**r—
**Clarity:** Throughout the manuscript – The authors should adopt a standard convention for citing references. The current version of the manuscript sends mixed signals—sometimes multiauthored works are cited as first author et al, other times multiple names are listed. References sometimes are cited as "Jones (1999) showed," and other times in the form "Jones (Jones, 1999) showed." These are issues of careful editing so as to convey the impression of careful content.

**APHIS—**These concerns have been addressed during the internal editing process that will occur according to standard APHIS procedures.

---

**Reviewe**r—Page 1, paragraph 5: "these features control." Consider replacing the word control with a phrase such as "take into account." I am not sure that regulations ever are able to truly control environmental interactions.

Page 2, paragraph 2: "compete so effectively with crop plants to cause economic harm." Clarify by changing to "compete so effectively with crop plants that they cause economic harm."

**APHIS—**This paragraph has been deleted for organizational/editorial reasons

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**Reviewe**r—Page 3, first paragraph under Physical Environment: "A wild plant which is more able to exploit limited resources may be more likely to survive and reproduce, while a highly efficient crop plant may be more likely to produce large yields." The reason for including this sentence is unclear. Don't large yields more or less equate with survival and reproduction of all plant species?

**APHIS—**This sentence has been removed to avoid confusion.

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**Reviewe**r—Page 4, last paragraph, next to last sentence, typo: "sunlight may grow (not grown) successfully. See also page 5, second paragraph, third line, typo: "absorbed by plant (not plants) roots."

**APHIS—**This correction has been incorporated into the text.

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**Reviewe**r—Page 7, third paragraph: "When seed is used to reproduce plants, it has genetic and other quality characteristics that differentiate it from grain." I suggest that this sentence be rewritten to emphasize that the characteristics are constant. The issue is not that these characteristics sometimes are absent; rather, it is that depending on the use, these characteristics can be important or not important.

**APHIS—**This paragraph has been deleted for organizational/editorial reasons

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**Reviewe**r—Page 7, last paragraph – replace "man" with people. Ditto for page 9, paragraph 5.

**APHIS—**These concerns have been addressed in the APHIS editing process.

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**Reviewe**r—Page 9, sixth paragraph – Include a citation to support the statement that starch and hemicellulose are the two main forms of carbohydrates stored in seeds.

**APHIS—**A citation has been added for this statement.

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**Reviewe**r—Page 10, third paragraph – I do not believe that transforming seeds for these purposes always involves (as is implied here) genes not currently present in plants. This may be the case, but there are other instances of gene transfer from one plant species to another.

**APHIS—**The text has been edited for clarity, and to no longer imply that transformation only involves DNA that is not present in plants.

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**Reviewe**r—Page 12, fourth paragraph: "Their use is supported by decades of industrial experience." Consider changing this sentence to: "Their use is supported by decades of experience by plant breeders and the seed industry."

**APHIS—**The statement has been modified as suggested.

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**Reviewe**r—Page 14, second paragraph. Replace "giants" with "wonders." The former word emphasizes size, while the issue is one of flexibility and adaptability. Delete the word "anti-disease" from the sentence at the very end of this paragraph. The phrase is clear without this cumbersome modifier.

**APHIS—**The paragraph has been altered to address these suggestions.

---

**Reviewe**r—Page 17, second paragraph – replace the phrase "wild free-living" with "noncultivated." This enhances clarity and follows parallel construction.

**APHIS—**This change has been made.

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**Reviewe**r—Page 17, third paragraph: Also several definitions (insert the word of) "invasive….

Page 17, third paragraph – "Transformative." Avoid this term if possible in discussing environments—many readers could confuse the common definition with the scientific definition involving DNA transfer. If the word must be retained, then define the context explicitly in this paragraph.

**APHIS—**APHIS believes that the common use of the word transformative is adequate in this context.

---

**Reviewe**r—Page 17, fourth paragraph – Consider replacing "will be considered a weed in some particular instance" with "could be considered a weed under some particular conditions." This adds clarity.

**APHIS—**This paragraph was modified to address the reviewer's concern, although the suggested text was not adopted verbatim.

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**Reviewe**r—Page 19, second paragraph: Should be Williamson (1993) at the beginning of the sentence with no separate citation at the end of the sentence.

**APHIS—**The document has been edited for consistency according to APHIS internal guidelines.

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**Reviewe**r—Page 19, second paragraph – 10:10 rule. I am not sure if this rule should be presented as a model and predictor. The connotation here is one of a scientific "rule of thumb," yet words such as model and predictor imply a more detailed mathematical modeling exercise. The authors should clearly differentiate between these two possibilities.

**APHIS—**The paragraph has been changed, and the word "rule" was substituted for "model."

---

**Reviewe**r—Page 20, second paragraph: – Pea lectin, not pea leptin. This is an important difference.

**APHIS—**This correction has been made.

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**Reviewe**r—Page 20, third paragraph – Replace "bred in conventionally" with "introduced by conventional breeding." The former is a jargonish phrase.

**APHIS—**This change has been made.

---

**Reviewe**r—Page 21, heading in middle of the page should read: "Gene Flow via Hybridization with Wild Relatives." There also seems to be information missing (or some sort of garbling) in the fifth line of this section (and rapeseed….).

**APHIS—**This section of the text has been adjusted to eliminate the jumbled text.

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**Reviewe**r—Page 23, first paragraph . Consider replacing "years of cultivation" with the more relevant "years of experience."

**APHIS—**The text was adapted to incorporate this suggestion.

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**Reviewe**r—Page 24, third paragraph, typo: It should read, "If the transgene in question….."

**APHIS—**This change has been made

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**Reviewe**r—Page 25, last sentence of narrative on page, typo: Should be rate of the product's entry. Also page 26, last two lines, typos: Should read "In cases where biomagnifications and bioaccumulation are likely."

**APHIS—**This change has been made

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**Reviewe**r—Page 27, third paragraph: Should read "GE glufonisate- and glyphosate-tolerant crops." This missing hyphen is essential for clarity.

**APHIS—**This change has been made.

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**Reviewe**r—Page 28, second paragraph. I am not sure of the need for the last clause in the final sentence of this paragraph. Other than microbial and invertebrate communities in soil, what other kinds of organisms could be impacted? Could this clause simply be deleted?

**APHIS—**There are soil dwelling vertebrates that might be affected i.e. moles. APHIS believes the sentence is appropriate as is.

---

**Reviewe**r—Page 29, first paragraph: Insert the word conditions after the word natural in the fourth sentence from the end of this paragraph.

**APHIS—**This change has been made

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**Reviewe**r—Page 30, fourth paragraph. There is unnecessary ambiguity in this paragraph and also a section that is garbled ("and it is thus far unclear that introducing"). An objective reading of the science as summarized in this manuscript would, I believe, allow for a stronger statement to be made—the potential for impact of GE technology on soil and its components is vanishingly small compared to the impact of traditional agriculture on these components.

**APHIS—**The text has been modified for clarity.

---

**Reviewe**r—Page 31, fourth paragraph, grammatical error that obscures the meaning of the sentence: More the phrase "into crop plants" from the end of the first sentence to a position right after "to move genes." Otherwise, this phrase gets lost in a whole lot of intervening verbiage.

**APHIS—**This change has been made.

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**Reviewe**r—Page 31, third paragraph – It is unclear if "new varieties of whole foods" means foods from new crop varieties (lettuce from a new variety, for example) or new kinds of whole foods (a fresh salad containing a new mixture of different greens). I think the former is meant, but the wording leads to ambiguity.

**APHIS—**APHIS believes the phrase "whole foods" to be of common usage.

---

**Reviewe**r—Page 32, second paragraph, last sentence. To more accurately reflect the activities of EPA and FDA, consider: "that help ensure that potential adverse effects of GE crop plants are adequately assessed."

**APHIS—**APHIS believes that the section adequately describes FDA and EPA's role in food safety. The purpose of including this text is simply to give the reader a broad overview of the involvement of other federal agencies.

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**Reviewe**r—Page 33, third paragraph. This paragraph is exceptionally convoluted and difficult to understand, especially the first sentence—which is filled with references and abbreviations. It would be helpful if it could be broken down into several simpler sentences. Consider modifying the second sentence as follows: "The scientists participating in this effort created a decision tree, termed ILSI/IFBC, for the assessment of potential allergenicity." This should make it clear that the ILSI/IFBC report referenced in the next paragraph is the same as the report discussed in this paragraph.

**APHIS—**The organization of this paragraph has been addressed by APHIS in the editing process

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**Reviewe**r—Page 34, section (d). Insert a comma between processing and should for clarity.

**APHIS—**This concern has been addressed by APHIS during the editing process.

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**Reviewe**r—Page 35, first paragraph. It would be helpful if the authors indicated whether or not this new decision tree has been accepted. I believe that this may be the intent of the last sentence in paragraph 3 on this page, but the reader tends to get lost in the verbiage related to the various reports and recommendations. If there is any question about understandability, I suggest that wording be revised to be explicit.

**APHIS—**The decision tree is presented as an example of how scientific and regulatory bodies are looking at allergenicity. The paragraph describes the origin of the tree and where it has been published, and subsequently modified.

---

**Reviewe**r—Page 36, second line in first paragraph, typo: "heat and other food processing conditions, is." Also, third paragraph: "individuals who have previously"

**APHIS—**These issues have been addressed by APHIS during the editing process.

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**Reviewe**r—Page 39, first paragraph. For the sake of clarity, please insert "the primary" between "in" and "published literature" in the second sentence.

**APHIS—**This change has been made.

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**Reviewe**r—Page 41, second paragraph, next to last sentence, typo: should be "whether there have been any unintended changes."

Page 42, second paragraph. The authors should consider using headings or some other mechanism to let the reader know that the issue of vegetative insecticidal proteins will be considered later (much later, actually—on page 46). Because there is so much verbiage on crystal proteins, I was quite sure that the authors had forgotten about the other type of proteins. It would help if the reader would be informed that the second type of proteins will be dealt with, even if much later in the manuscript.

**APHIS—**APHIS believes that, for the purposes of this overview, the level of subheadings is adequate. Although the gap between the initial mention of vegetative insecticidal proteins and further discussion is large, the points being discussed in the interim are intended to be used as examples of the typical considerations associated with environmental reviews, rather than a detailed description of either class of protein in particular.

---

**Reviewer**—Page 43, second paragraph. Consider qualifying the first sentence by stating that continuous production would have to be on extensive acreages. This is an important qualification from a practical viewpoint.

**APHIS—**Text has been added to indicate the scale at which this becomes a concern.

---

**Reviewer**—Page 43, third paragraph. After the word refuge, it might be helpful to insert a parenthetical statement alerting the reader to the fact that this term will be defined in context and dealt with later in the manuscript.

**APHIS—**APHIS believes the meaning of refuge can be taken from the context of the paragraph.

---

Page 44, fourth paragraph. Consider stating that "Structured refuges are areas containing non-Bt host plants."

**APHIS—**This change has been made.

---

**Reviewer**—Page 45, last paragraph, typo: "the grower's responsibility."

Page 46, third paragraph. Consider inserting "the potential of" before "accidental aquatic exposure." In addition, the final sentence in this paragraph leaves the reader wondering at the significance of APHIS assessment under the Migratory Bird Act and the Threatened and Endangered Species Act. Why is this information important for the reader of the present document? Also at the very end of the page, it should be "The objectives of much….".

**APHIS—**The sentence is included as an illustration of what APHIS takes in to consideration during its reviews. Editorial and typographical changes have been made based on the reviewer's suggestion.

---

**Reviewer**—Page 48, first sentence. Replace jargony phrase with "A primary rationale for development of stress tolerance…." Towards the middle of this paragraph: It is unclear to me what is meant by "receiving environment." Please make this explicit.

**APHIS—**The text has been adjusted in response to these comments.

---

**Reviewer**—Page 49, Conclusion. Indicate the date as of which the statistics on permits are valid. There is also a typo in this paragraph (should be "continues to move from").

Page 50, fourth paragraph. Plant expression of viral genes has another advantage—it overcomes the time and labor problem identified in the paragraph above this one.

**APHIS—**APHIS does not believe that modification of the text is necessary in this case.

---

**Reviewe**r—Page 51, first paragraph. Does 900 virus resistant plants literally mean 900 individuals or does it mean 900 different tests?

**APHIS—**The statistic refers to 900 field trials of virus resistant plants. The text has been adjusted to make this clear.

---

**Reviewe**r—Pages 52 and 53. Heterologous encapsidation can be very difficult to understand and complicated to describe. I believe that there are several ways in which this section could be made more understandable. One is to rearrange the three scenarios and orient the narrative towards the insect. Scenario 1 (Scenario 2 in the narrative under review) would thus read: Virus does not move because no insect is available. Scenario 2 (Scenario 1 in the narrative under review) would thus read: Insect carries the heterologously encapsidated virus to a nonhost plant. Scenario 3 would read: Insect carries the heterologously encapsidated virus to a host plant. To me, at least, it is easier to think forward from insect to plant than backward from plant to insect. This structure also simplifies the narrative at the top of page 54, which is Byzantine as currently written.

In this general section, also consider using phrases such as "virus is transmitted to" rather than "virus is inoculated onto." Great attention needs to be given to this section if the reader is to truly comprehend heterologous encapsidation and its potential significance.

**APHIS—**This section has been edited taking into account the reviewer's suggestions. Although all of the suggestions were not adopted, APHIS believes that the revisions should satisfy the reviewer's concerns.

---

**Reviewe**r—Page 54, last paragraph. Should be carrot mottled dwarf.

**APHIS—**The correct name of the disease (believe it or not) is carrot motley dwarf.

---

**Reviewe**r—Page 55, second paragraph, typo: Period missing at end of final sentence. Also, third from last sentence, bottom of this page, consider "sequence similarity between the two viruses." Also, last sentence this page, consider "structural similarity between the polynucleotides."

**APHIS—**These suggestions have been considered, and corrections have been made where appropriate.

---

**Reviewe**r—Page 56, second paragraph. Who is the "their" referred to by Rubio and colleagues? Are the levels of recombination from Rubio's own experiments or from the experiments of Green and Allison and/or Borja et al.? The sentence structure does not make this point clear.

**APHIS—**This paragraph has been re-worded to make the identity of the pronouns clear.

---

**Reviewe**r—Page 56, third heading—"more viruses infect the same plant." And page 57, fourth paragraph: "given what is known about their ability."

Page 60, third paragraph, sentence beginning "Although with concern…." is garbled.

**APHIS—**This sentence has been re-worded during the APHIS editing process

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**Reviewe**r—Page 62, the two footnotes in the middle of the page appear to be orphaned—antecedents not clear.

Page 67, second paragraph. I suggest that the authors point out here that life span is another major difference (this topic is amply covered in previous pages—but it is not emphasized here in the summary.)

**APHIS—**The text in this paragraph has been modified to indicate that lifespan is also a major difference between crop plants and trees.

---

**Reviewe**r—Biological Control addendum, page 1, paragraph 1, sentence reading: "As practiced by trained professionals, biological control is a highly developed process." The purpose of this sentence within the context of this section is unclear to me. I recommend that the authors include a statement to the effect that specialized knowledge and training are needed by those actually implementing biological control. Words about "a highly developed process" can, I believe, be deleted.

Biological Control addendum, Conclusions. I do not understand the rationale behind the description of biological control as an "ethical" pest management tactic. This adjective does not appear elsewhere in the document, and its inclusion here seems awkward. I cannot imagine circumstances under which unethical pest management would be practiced.

**APHIS—**The biological control section has been extensively modified to reflect the concerns of this and other reviewers. The statements that the reviewer is quoting in this instance have been removed.

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**Conclusion**

The Charge to Peer Reviewers referred to above, requests the peer reviewers to explicitly answer the following question: **Does this scientific information presented accurately and objectively provide non-expert readers with a broad base of knowledge to understand the aspect of the biological and physical environment that is likely to be affected by the regulations currently administered by APHIS BRS and by possible changes of those regulations?**

After careful review of the document and after having used the criteria outlined above, I believe that the scientific information is presented accurately and objectively for non-expert readers, provided that revisions are made (as detailed above) to address a small number of specific weaknesses. Thus my response to the question in the charge to peer reviewers is: "(2)  Yes, but only after revisions have been made to address specific weaknesses.

## Response to Reviewer Report 4

APHIS has copied the text of the Reviewer's Report and added a response where appropriate.

I followed the instructions and format provided to me in the Charge to Peer Reviewers. I start off with an analysis according to the six criteria of the review, Completeness, Currency, Accuracy, Uncertainty, Objectivity, and Clarity (Sections 1-6 of this report). I make a brief comment on a supplemental text received (Section 7). I end with an overall assessment of Chapter 4.1 (Section 8).

**Reviewer—**
**Completeness :** Much of the discussion on gene flow centers on gene flow between GE crops and their wild relatives. In reality, in the U.S. at least, this is a minor concern, except if one deals with crops such as sunflower, strawberries, tobacco, blueberries, and a few others. The major issue of gene flow affecting GE crops in the U.S. is between GE varieties, on the one hand, and classically bred varieties, whether in conventional agricultural systems or other systems, such as organic agriculture. This is not dealt with in this text.

**APHIS—**APHIS recognizes that gene flow from GE crops to conventional crops is an important issue, but this issue is addressed elsewhere in the draft EIS (i.e. Chapter 4.2: Impact of APHIS' Current System). In terms of scientific and environmental issues, this type of gene flow does not pose any substantially different risks than environmental release of the GE plant. For example, if an herbicide tolerance trait from a GE corn field crosses into a conventional corn field, the resulting crop would not be substantially different than the original GE corn plant, in terms of its potential impact on the environment. The confinement of experimental field trials, to prevent gene flow from experimental GE varieties with uncertain environmental impacts is discussed in other sections of the draft EIS, including Chapter 4.2. Concerns of organic farmers and other farmers with an interest in ensuring their crops do not contain GE materials are marketing concerns rather than environmental impact concerns and are also dealt with elsewhere in the draft EIS. In the special case of seed purity, a discussion of seed purity standards and growing practices is included.

---

**Reviewer—**"GE plants engineered for stress tolerance show an increase in nutrient content, either nitrogen or phosphorus, through either an accumulation of nitrogen-based osmoprotectants during drought and salinity or an increased nutrient use efficiency."

I am surprised that the topic of nutrient status and disease susceptibility is not discussed here. This is a well-known phenomenon: e.g.,

Huber DM, Watson RD. 1974. Nitrogen form and plant disease. Annual Review of Phytopathology 12: 139-155

**APHIS—**Because of this, and other comments, a brief description of plant stress and the interactions between plant stress and response to pathogens, disease and herbivory has been added to the beginning of this section.

---

**Reviewer—**Page 53: “Therefore, the focus of this section will be GE plants expressing VCPs or VCP genes from RNA viruses. “

One of the issues with the current regulatory system is that it seems to be running behind technological developments. Therefore, I would suggest that this document be more proactive and discuss potential issues with alternative GE mechanisms for virus resistance.

**APHIS—**Although the text deals specifically with VCP genes, the consideration will be broadly applicable to other types of virus resistance.  Text has been added to make this clear.

---

**Reviewer—**Page 62-63: “Where crop rotation is practiced, HRCs can become weeds in a crop rotation system if the second crop is an HRC engineered to be resistant to the same herbicide to which the original crop was resistant.”

Further information is available at:

http://www.gmsciencedebate.org.uk/topics/forum/0051.htm

http://ipm.missouri.edu/ipcm/archives/v16n7/ipmltr1.htm

**APHIS—**APHIS appreciates the additional sources.

---

**Reviewer—**Page 63: “GE crop plants producing pharmaceuticals and vaccines”

This statement is insufficient especially for a document that should be forward-looking and address future applications of genetic engineering. It does not take into account the different steps prior to commercial production, not the steps following harvest of the commercial production up until processing, all of which represent potential for commingling of the PMP and food or feed production chains.

**APHIS—**APHIS believes that the section deals with the issues surrounding PMPs appropriately.  The purpose of the section is to make the reader aware that there are challenges with regard to segregation of these products from food production.  Because each crop/product combination is different, it is not appropriate to discuss specific examples at length.  APHIS believes the potential for mixing of PMPs in food production has been made clear to the reader without a detailed outline of the commercialization process.

---

**Reviewer—**

**Currency:** This text shows a tendency of not using the latest information, especially from the peer-reviewed literature. I have listed references in the relevant sections. I would add here one general reference:

Snow, A.A., D.A. Andow, P. Gepts, E.M. Hallerman, A. Power, J.M. Tiedje, and L.L.

Wolfenbarger. 2004. Genetically engineered organisms and the environment: Current status and recommendations. Ecological Society of America Position Paper. [Online]. Available from Ecological Society of America http://www.esa.org/pao/esaPositions/Papers/geo_position.htm. And Ecol. Applic. 15: 377404 (2005).

**APHIS—**APHIS has attempted to address the reviewer's concerns throughout the text. In many cases, the references were not published at the time the document was originally written and they have subsequently been added to the reference list. In some instances, the text was adapted to reflect the findings of the literature.

---

**Reviewer—**
**Accuracy:** Page 1: "The system of safeguards and controls imposed by APHIS on all GE organisms within the scope of authority has been highly effective in ensuring that releases are conducted in accordance with the conditions set by APHIS..."

This is an overstatement. Located in the introduction of this chapter, it sets the tone for everything that comes after it. If regulations have worked, then why should they be updated? Instead, APHIS should quantify this assertion. The Prodigene case is the most prominent case that made it into the public eye where regulations were not followed. Granted, the USDA was able to address the problem. However, how many other, perhaps minor cases, have not been publicized? To quantify this statement, APHIS should provide information on incidents that put into question the regulations, their nature and frequency.

**APHIS—**While APHIS maintains the validity of the statement, it has been removed from the section, as inappropriate in this context. For the reviewer's additional comments, this text is an overview of the scientific issues related to environmental release of GE plants, and as such, it is not the forum for a detailed listing of compliance infractions. The draft EIS does contain a section that deals with compliance issues.

---

**Reviewer—**Page 2: Paragraph on plants

This paragraph misses a statement about plant reproduction, not only one of the most important if not THE most important function in any organism but also a trait which can also be modified by genetic engineering and underlies one of the environmental concerns, namely gene flow by pollen and seed. Although discussed later, it seems strange not to even mention plant reproduction in this introduction.

The last sentence of this paragraph, by its omission of plant breeding, suggests that only genetic engineering can accomplish the goals mentioned, namely "can alter the value of a plant to humans and may also affect one or more of the physical and biological interactions between plants and their environment." Breeding for altered composition (e.g., canola) or disease and pest resistance have been successfully accomplished by classical breeding.

**APHIS—**This paragraph has been moved to an earlier chapter, for reasons of organization. In the earlier section, it serves a very basic introductory purpose, and details on specific activities (i.e. reproduction) are not warranted. APHIS has added a sentence that mentions plant reproduction as having environmental effects. In light of the reviewer's comment, the last sentence has also been modified to indicate that, classical breeding can also be used for altering composition, disease resistance etc.

---

**Reviewer—**Page 5: Physiological environment
"Although breeders have been trying to alter photosynthetic efficiency for many years, the complexity and number of genes that must be expressed to coordinate efficient photosynthesis has limited breeders' ability to significantly improve photosynthetic efficiency."

This is an incomplete assessment. Plant breeders have been able to improve yield, which, by definition, is genetically more complex than photosynthetic efficiency! There are other reasons why progress has not been achieved: a) the inherent inefficiency of ribulose-bisphosphate carboxylase/oxygenase; and b) phenomena associated with source-sink partitioning. See, for example:

Richards, R.A. 2000. Selectable traits to increase crop photosynthesis and yield of grain crops. Journal of Experimental Botany 51:447-458.

**APHIS—**The statement has been modified to reflect the complex reality of photosynthetic efficiency and yield as agronomic traits and the citation has been added.

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**Reviewer—**Page 8: "Crop plants with perfect (complete) flowers are largely self-pollinated, and the majority of crop plants are self-pollinating. It is possible for pollen from another plant to cause fertilization in these self-pollinating plants, but the probability is very low because pollination typically occurs even before the flower petals open. "

It is true that some of the major crops are self-pollinating: e.g., rice, wheat, soybean, etc. There are, however, major crops that are either cross-pollinating: e.g., maize, rapeseed, or vegetatively reproduced: e.g., sugarcane, potato. Furthermore, the text creates the impression of a simple situation where crops are either cross-pollinated or self-pollinated, with few intermediates. This is far from being the case. Self-pollinated crops do outcross, although not at the same frequency as outcrossing species. This lower level of outcrossing is nevertheless sufficient to lead to the appearance of noxious weeds: e.g., rice (see below for references), or modify the distribution of genetic diversity between populations and within a crop species' genome.

References for rice:

Arrieta-Espinoza, G., E. Sanchez, S. Vargas, J. Lobo, T. Quesada, and A.M. Espinoza. 2005. The weedy rice complex in Costa Rica. I. Morphological study of relationships between commercial rice varieties, wild Oryza relatives and weedy types. Genetic Resources and Crop Evolution 52:575-587.

Gealy, D.R., T.H. Tai, and C.H. Sneller. 2002. Identification of red rice, rice, and hybrid populations using microsatellite markers. Weed Science 50:333-339.

Gealy, D.R., D.H. Mitten, and J.N. Rutger. 2003. Gene flow between red rice (Oryza sativa) and herbicide-resistant rice (O-sativa): Implications for weed management. Weed Technology 17:627-645.

Suh, H.S., Y.I. Sato, and H. Morishima. 1997. Genetic characterization of weedy rice (Oryza sativa L.) based on morpho-physiology, isozymes and RAPD markers. TAG Theoretical and Applied Genetics 94:316-321.

Zhang, N.Y., S. Linscombe, and J. Oard. 2003. Out-crossing frequency and genetic analysis of hybrids between transgenic glufosinate herbicide-resistant rice and the weed, red rice. Euphytica 130:35-45.

References for bean:

Papa, R., and P. Gepts. 2003. Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (Phaseolus vulgaris L.) from Mesoamerica. Theor. Appl. Genet. 106:239-250.

Papa, R., J. Acosta, A. Delgado-Salinas, and P. Gepts. 2005. A genome-wide analysis of differentiation between wild and domesticated Phaseolus vulgaris from Mesoamerica. Theor. Appl. Genet. 111:1147-1158.

**APHIS—**The text has been modified to address the concerns of the reviewer, without delving into the technical detail suggested by the inclusion of these references. Language has been added to indicate that crops exist on a continuum of reproductive strategies, between completely self crossing and entirely out crossing.

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**Reviewer—**Page 9: top of the page: The cross-pollination of maize is not due only to the separation of male and female flowers. It is also due to the fact that male flowers mature before female flowers on the same plant (called protandry). Thus, the mating system of maize of more complex than portrayed here and the statement that “pollen is released in close synchrony with maturation of female flowers” is inaccurate.

Page 9: “Fortunately, the predicable (sic) nature of flowering dynamics and improved methods for modeling pollen dispersal are making it possible to quantify loss of genetic purity under field conditions.”

There are limits to flowering predictability. There are variables that influence its characteristics, including the weather during flower development and after flower opening, the level and type of cross-pollinators, etc., that diminish the predictability of flowering and seed set, including the level of cross-pollination. This provides a false image of technical control over a phenomenon with inherent variability beyond genetic determinism.

**APHIS—**APHIS has edited the sentence to remove the implication that flowering dynamics are predictable, while still acknowledging that advances in our knowledge are allowing for greater ability to predict loss of genetic purity under field conditions.

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**Reviewer—**Page 9: "Some crops do not do this perfectly and some grain is lost prior to harvest."

I agree with this statement. It is important to note here that not all crops have been domesticated to the fullest (such as maize). I have provided examples with Track changes. Note that one of the crops in question is transgenic crop, oilseed rape. The lack of seed retention at maturity, combined with seed dormancy, causes herbicide-resistant, transgenic oilseed rape to become its own worst weed and can account for uncontrolled dispersal of herbicide-resistant oilseed rape in the agricultural landscape. See also comment on p. 20. Crawley et al. (2001) may not have noted an increase in weediness for rapeseed, but it should be noted that this crop is already weedy in itself!

**APHIS—**The paragraph has been re-worded to address the reviewer's concerns, and now indicates that seed dispersal mechanisms are not entirely absent in many crop species, and seed loss can be significant.

---

**Reviewer—**Page 11: "When a crop is exclusively self pollinated, there is no need for pollen control in seed production, because little pollen is released into the air around the flower."

This situation is a rarity; it is actually more appropriate to consider that most self-pollinated species have a low, but variable and non-negligible level of outcrossing, especially those whose flowers are visited by insects.

This is an awkward statement. I would say: "The ease in producing hybrid seeds makes hybrid varieties a more practical approach in cross-pollinated crops than in self-pollinated crops.

**APHIS—**The first sentence has been deleted, in order to avoid the over-simplification, and the second sentence has been modified to address the reviewer's concern.

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**Reviewer—**Page 11: "it is less likely that hybrids will show strong hybrid vigor."
The authors should cite their sources on this statement, which is an over-generalization. There is actually hybrid vigor in selfing species as well. The main obstacle to commercial

hybrids in selfing species is not the lack of hybrid vigor but the development of an economically feasible system for the production of hybrid seeds.

**APHIS—**This statement has been deleted, and the text now indicates that self crossing plants are more amenable to inbreeding programs.

---

**Reviewer—**Page 13: "For outcrossing crops, unintended crossing during open pollination is the major source of unintended off-type pollinations in the field. "

Again, this is also the case for selfing species, although to a lesser extent. Furthermore, the text oversimplifies in that it only considers two categories: selfing vs. outcrossing. In reality, even selfing species can in some years and locations exhibit higher levels of outcrossing.

**APHIS—**APHIS believes this has been adequately addressed earlier in the text. For an overview document such as this one, it is unnecessary to delve into every possible contingency or rare event in every instance of discussion.

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**Reviewer—**Page 13: "The vast majority of unintended outcrosses and of off-type plants come from adjacent fields."

However, occasional long-distance dissemination events can assure the dispersal of a gene in spite of its relative rarity because the most important factor influencing the persistence of a transgene will be the selective advantage it confers. The migration does not have to be high ($m > 1$) to reduce differentiation between populations. In addition, repeated gene flow will assure establishment of a transgene even if it does not confer a strong fitness advantage.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97-159.
Rieseberg, L.H., and J.M. Burke. 2001. The biological reality of species: gene flow, selection, and collective evolution. Taxon 50:47-67.

Neubert, M.G. and Caswell, H., 2000. Demography and dispersal: calculation and sensitivity analysis of invasion speed for structured populations. Ecology 81, 1613-1628.

**APHIS—**APHIS acknowledges the truth of the information provided by the reviewer, but does not feel it is appropriate to include this much detail in this section of the document. The text referred to relates to sources of off-types in seed production. The statement that "the vast majority of out-crosses… come from adjacent fields," is accurate, and conveys that there are other, minor sources of out-crossing events. The selective advantage of the transgene is not of interest in the context of seed production. The text has been adjusted to explicitly state that long-range pollination remains a possibility.

---

**Reviewer—**Page 14: "This rapid growth has more recently slowed due to a number of issues of health and environmental concern and lack of an existing commercial marketing mechanism for plants with unique non-traditional traits and uses."

There are a number of other reasons for this slowdown:
a) Lack of additional, economically viable transgenic traits besides Bt and herbicide resistance; for example, even new products may only be grown on small scales.
b) The inherently slow nature of new cultivar development (independent of regulatory procedures)
c) The negative economics of technology fees vs. additional genes.

**APHIS—**Multiple reviewers have offered a variety of different explanations for this observation. Instead of trying to choose between them, or explain them all, APHIS has decided to adjust the text to be more explicit about stating that there are a variety of convergent factors that explain the observation.

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**Reviewer—**Page 20: "Many participants felt that the types of pest resistance traits being tested or released commercially were not fundamentally different from those bred in conventionally and, as such, would present similar ecological risks. However, some participants disagreed and contended that some transgenes could have a much greater impact on weediness."

One of the major justifications for genetic engineering is that it can bring in new traits not present in the native genetic diversity of the crops species. In this case, it would be difficult to compare resistances obtained by conventional breeding and genetic engineering. An example is Bt-maize with resistance to European corn borer. To my knowledge, there is no comparable level of resistance in conventionally bred maize lines. Therefore, you cannot talk about "similar ecological risk."

**APHIS—**The concept that is being discussed in this part of the chapter is whether or not GE traits present fundamentally different ecological risks than traits introduced through conventional breeding. Although it may be true that resistance to European corn borer has not been found in maize that is comparable to GE maize with Bt traits, there is a wealth of knowledge available about the introduction of resistance to pests and diseases, including insect resistance, in agriculture and the management of crops with these traits. Another reviewer has suggested APHIS include more information about "extensive experience with multiple disease resistances bred into crop plants… with no issues related to weediness." In light of this, APHIS still feels that the conclusions of the workshop participants are appropriate to include in this section. It is also noted in the paragraph that some participants did not agree with this assessment. In short APHIS feels that the treatment of this issue in the text is adequate for a document of this nature.

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**Reviewer—**Page 21: "the escape of a GE crop is not inherently more likely than the escape of any other crop."

I suggest adding: “although it could be less although it could be of more concern than with conventionally bred crops depending on the genetically engineered trait.”

**APHIS—**This suggestion has been incorporated in text at the end of the paragraph, although the text was not adopted verbatim, the concept has been included.

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**Reviewer—**Page 22: “the Association of Official Seed Certifying Agencies has established standard growing conditions for crop seed production which result in very low levels of outcrossing (AOSCA 2003).”

But the criteria for seed purity have evolved. In pre-GE days, overall genetic purity was important and a tolerance of several % was in place. Since the introduction of GE crops, the tolerance has been reduced considerably to less than 1%. You should quantify what you mean with "very low levels".

**APHIS—**APHIS feels that the included text is appropriate for dealing with this issue in an overview document. The AOSCA standards vary based on crop and it is not appropriate to detail what quantitative data is available on a crop by crop basis in this document. In terms of environmental concerns, APHIS has addressed this in an earlier response to this reviewer.

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**Reviewer—**Page 25: “Multiple introductions via repeated instances of gene flow may be necessary before a potentially invasive species can become established (Ellstrand and Schierenbeck 2000; “)

The same can be said for individual genes. Repeated gene flow can establish a (trans)gene, even it if it does not confer a strong fitness advantage or no advantage at all:

Lenormand, T. 2002. Gene flow and the limits to natural selection. Trend Ecol Evol 17:183-189.

Haygood, R., A.R. Ives, and D.A. Andow. 2003. Consequences of recurrent gene flow from crops to wild relatives. Proceedings of the Royal Society of London - Series B: Biological Sciences 270:1879-1886.

**APHIS—**The context of the discussion in this paragraph is the persistence of GE plants with respect to invasiveness. The establishment of a transgene in wild populations would only be a concern, in this context, if the gene was likely to increase the invasiveness potential of the population.

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**Reviewer—**Page 31: “Each method of genetic modification, including the two most common methods currently employed, traditional breeding and genetic engineering, ...”

Genetic engineering is not a method to develop new cultivars; it is a method to introduce new genetic diversity in a crop. Even when using transgenes, plant breeding remains the only comprehensive way to develop an improved variety.

Gepts, P. 2002. A comparison between crop domestication, classical plant breeding, and genetic engineering. Crop Sci. 42:1780-1790.

**APHIS—**APHIS appreciates this perspective, but the context of the text is not a discussion of cultivar development. The text in the surrounding paragraph is an attempt to introduce the concepts that are relevant for evaluation of GE crops potential effect on human health, and the quoted excerpt is part of an effort to put GE crops into perspective with other genetic modification techniques.

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**Reviewer—**Page 43: "Genetic engineering of plants that contain Bt proteins in all tissues continuously throughout the growing season has overcome many of the limitations of Bt microbial insecticides."

All tissues: This is also a problem if Bt is expressed in tissues in which its expression is not necessary, e.g., pollen

**APHIS—**The effects of Bt expression on non-target organisms is addressed later in the section.

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**Reviewer—**Page 43: "Commercialization of Bt crops has resulted in fewer insecticide applications and thus lower management costs (Fitt 2000; Schnepf et al. 1998). "

Is there any more recent data on this important topic, e.g., from 2005 or 2006? In addition, this should be discussed in more detail depending on the crop, particularly maize and cotton. My sense is that insecticide applications have been reduced in cotton but not in maize. Therefore, such a sweeping statement is inappropriate.

**APHIS—**APHIS has added a citation to more recent literature (Sankula S. *et al.* 2005). Because the citation agrees with APHIS's previous information, that insecticide applications have been reduced in both cotton and corn (maize) the text has not been substantially modified.

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**Reviewer—**Page 44: "In the cases of the GE crops with Bt genes to date, the gene products are well known to specifically target a small group of Lepidoptera."

What about Coleoptera, Diptera, etc. This seems to be an almost meaningless paragraph. In the case of large-scale field trials or commercial fields, on what basis is additional testing then required? What testing should be conducted? For how many year?

**APHIS—**APHIS has significantly modified this paragraph and now includes reference to Coleoptera targeting Bt crops. Diptera are targeted by Bti which is used to kill mosquitoes and is not considered here by APHIS because it is not relevant to the discussion of GE crops. The sentences about additional tests have been removed because of comments by this reviewer and others indicating that the comments were inappropriate or not useful. The purpose of this section is to describe a general overview of concerns related to Bt or other potentially insecticidal crops, rather than to describe in detail data requirements or prescriptive remedies that APHIS would consider in the event of potential adverse effects.

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**Reviewer—**Page 58: “Additionally, it is not likely that the potential for synergy occurring in GE plants expressing virus genes would be greater than in natural mixed infections (Hull 2004). Except that with current technology, the transgenic virus resistance would be expressed constitutively throughout the plant. This might not be the case with natural infections.

**APHIS—**The expression of virus genes in GE virus resistant plants must be examined on a case by case basis. Although it is true that constitutive expression of viral genes could lead to increased exposure of a protein to viruses, many virus resistant transgenes show little or no protein expression. When taken together, these facts support the conclusion found in the citation.

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**Reviewer—**Page 59: “This is somewhat different than the relationship between crops and plant viruses. Most of the major crop species used in today's agriculture (e.g. soybean, rice, wheat, beans) have been subjected to intensive artificial selection over centuries and only have low survival under most natural conditions. The vast majority of the crops used in agriculture are much less fit, under natural conditions, than wild or weedy plants. Because of this, the impact of virus infection on crop plants is potentially more severe than on many wild or weedy plants.”

This paragraph needs to be re-written. The lack of fitness of domesticated plants in the wild is due to traits such as reduced seed shedding, seed dormancy, change in photoperiod sensitivity, etc. These traits do not necessarily have a relationship with disease resistance. Therefore, assuming that reduced fitness of domesticated plants in the wild leads to higher disease susceptibility is a non sequitur.

**APHIS—**APHIS believes that the text adequately addresses the uncertainty of crop disease susceptibility compared to wild relatives by suggesting that the affects are “potentially” more severe. In this case, the reviewer’s statements are accurate with regards to certain fitness traits, but incomplete. Because crop plants have been bred over generations with little or no selection for disease resistance, they are much more likely to have lost their ability to resist disease than wild plants that are subject to natural selection based on disease challenge. APHIS believes that the conclusions stated in this section of the text are accurate, in a general sense, and that these conclusions are not overstated.

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**Reviewer—**Page 59: “There have been a relatively low number of confirmed cases of introgression (Stewart, Halfhill, and Warwick 2003).”

There is a different analysis of the situation by Ellstrand et al. (1999). Even assuming that one considers only the cases of introgression and not just the cases of gene flow, hybridizations between wild and domesticated plants are widespread and exist even in predominantly self-pollinated crops. The estimates of the frequency are probably underestimates because of the lack of appropriate markers to detect introgression. See Papa and Gepts (2003) and Papa et al. (2005).

**APHIS—**APHIS has added text to the subsequent paragraph in order to provide clarity. APHIS does not suggest that introgression is not a possibility, only that it is not more likely in GE plants than in conventionally bred, virus resistant plants.

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**Reviewer—**Page 71: "Breeding. The process of sexual reproduction and production of offspring."

This definition is deficient in the context of this presentation, which seeks to compare genetic engineering and (plant) breeding. A more circumstantiated definition is necessary.

I suggest the following:
"Plant breeding is an applied, multidisciplinary science, which represents the application of genetic principles and practices associated with the development of cultivars more suited to the needs of humans than the ability to survive in the wild. It uses knowledge from agronomy, botany, genetics, cytogenetics, molecular genetics, physiology, pathology, entomology, biochemistry, and statistics.

from Schlegel, R.H.J. 2003. Dictionary of plant breeding. Food Products Press/The Haworth Reference Press, New York.

**APHIS—**The definition of breeding has been expanded to be more appropriate for this context, although the language suggested by the reviewer was not adopted verbatim in order to keep the definition short and understandable to a non-technical audience.

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**Reviewer—**Page 73: "Fecundity. The capacity for producing offspring."

As defined here, this is fertility. Fecundity refers to the number of gametes or progeny of an individual.

**APHIS—**APHIS has added language to clarify this definition.

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**Reviewer—**Page 73: "Gene flow. The spread of genes from one population to another, by the movement of individuals, gametes, or spores."

Should add seeds! "Gene flow. The spread of genes from one population to another, by the movement of individuals, gametes, seeds, or spores."

**APHIS—**The word *seeds* has been added to the definition for clarity.

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**Reviewer—**Page 74: "Gene stacking. The use of plant breeding to combine two or more genetic traits into a single plant variety."

Should be: “Gene stacking. The use of genetic engineering to combine two or more genetic traits into a single transformant.” In plant breeding, the practice of accumulating several genes governing a trait such as disease resistance is called pyramiding.

**APHIS—**This definition has been altered for clarity. Gene stacking can refer to two separate engineering events that are then combined into one variety using conventional plant breeding methods.

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**Reviewer—**Page 75: “Marker gene. A gene of known function or known location that is inherited in Mendelian fashion and facilitates the study of inheritance of a trait or a linked gene. “

One should add to that: “The gene also has high heritability, i.e., its expression is not influenced by the environment.”

**APHIS—**Marker genes are sometimes linked to environmental, or variable promoters which may make their expression influenced by the environment. APHIS believes the definition is appropriate as written.

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**Reviewer—**Page 75: Mutagenesis is, by definition, artificial. This should be added to the definition.

**APHIS—**Mutagenesis can be performed deliberately by humans, or it can occur under natural conditions. It is not clear what the reviewer means by “artificial,” and APHIS believes the definition is appropriate without change.

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**Reviewer—**Page 77: It is pleiotropic and not pleiotrophic!

**APHIS—**This typo has been corrected

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**Reviewer—**Page 78: “Rotation. The number of years required to establish and grow trees to a specified size, product, or condition of maturity. A pine rotation may range from as short as 20 years for pulpwood to more than 60 years for sawtimber. Full rotation is the total time from planting to harvest. Half rotation would be approximately half the time to reach maturity or harvest. “

I suggest a more complete definition: “Rotation. A) In forestry, the number of years required to establish and grow trees to a specified size, product, or condition of maturity. A pine rotation may range from as short as 20 years for pulpwood to more than 60 years for sawtimber. Full rotation is the total time from planting to harvest. Half rotation would be approximately half the time to reach maturity or harvest. B) In crop production, the cycle of crops grown in successive years in the same field. Rotations are instituted to limit the spread and accumulation of diseases (especially soil-borne diseases) and pests and to manage plant nutrients.”

**APHIS—**APHIS has added the reviewer’s text to the definition of rotation.

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**Reviewer—**
**Uncertainty:** Page 26, bottom: “Obviously, soil and natural bodies of water are not sterile environments and there are a number of abiotic and biotic factors that will affect accumulation and persistence, such as soil type, aeration, water movement, and soil biota activity.”

This statement suggests that long-term studies are lacking that follow the fate of the Bt toxin over several years, especially in areas in which Bt crops (maize, cotton) are grown in short, recurring rotations.

**APHIS—**The reviewer is identifying areas of uncertainty with this comment. Although there are studies following the fate of Bt toxin, and importantly, studying the effects on non-target populations over several years, there is always the potential to collect more information. APHIS has not modified the text in response to this comment.

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**Reviewer—**Page 30, bottom: Horizontal gene transfer:
There is a need to cite references reporting on studies that have analyzed the presence/absence of HGT in “Bt crops ...currently grown in many countries, in many diverse environments, covering millions of acres.” If this is not possible, then this should be listed as an uncertainty.

**APHIS—**APHIS has removed the reference to the lack of data to suggest HGT has occurred involving Bt crops.

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**Reviewer—**Page 47: “Genetically engineered drought and saline tolerant crop plants “

This whole section is very vague and probably reflects the lack of information on the consequences of drought- and salinity tolerance.

**APHIS—**The reviewer is again identifying areas where there is a perceived uncertainty. While it is true that drought and saline tolerant crops are not common, and thus experience is limited, APHIS feels that there is enough information to anticipate the kinds of environmental impacts that might result from these crops and provide examples of the type of data required to review them. The text has not been modified to reflect this comment.

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**Reviewer—**Page 57: “Based upon currently available information, it appears that the potential for recombination in virus resistant GE plants (i.e., virus-virus & plant-virus) would be similar to the natural occurrence of recombination in virus infected, non-GE plants (Falk and Bruening 1994; OECD 1996; Rubio et al. 1999).

But what about the results of Greene and Allison? My sense is that this issue is still up in the air.

Zaitlin M, Palukaitis P ANNUAL REVIEW OF PHYTOPATHOLOGY 38: 117-143 2000

**APHIS—**APHIS believes that experience with GE plants and more recent publications suggest that Green and Allison's results are a laboratory phenomenon that are not directly applicable to virus resistant plants in the field.  It remains true that recombination is a possibility, but APHIS believes this to be no more likely than the natural occurrence of recombination in infected plants.

**Reviewer—**Page 58: "Given the knowledge of the roles that different virus genes play in synergy, developers can also select only those genes that likely would not contribute to synergism, or include mutations in such genes so that their potential impact is limited. "

But this knowledge is limited at this stage. The HC-Pro gene is an exception to this pattern.

**APHIS—**It is always possible to learn more about a particular phenomenon.  APHIS stands by the assertion that developers will be able to decrease the likelihood of viral synergy with GE virus resistant crops by careful gene selection and design.

**Reviewer—**Page 60: "Current knowledge and data suggests that gene flow from a GE virus resistant plant to a wild or weedy plant is not likely to provide different exposure from that which occurs under natural agricultural and environmental settings."

I would agree with this statement except that there is actually little data to go by because most transgenic crops have been planted outside areas of domestication, thus, in areas where wild and domesticated types do not co-exist within pollinating distance.

**APHIS—**APHIS acknowledges that there is always room for improvement in the available data.  The text presents an appropriate level of background information in order to familiarize the reader with this issue, based on the best available scientific knowledge.

**Reviewer—**Page 68: "For some of the traits that are being engineered into trees, it may not be possible to gather data on the effect of the trait on the environment over many years. A good example would be genes for lignin modification. It will take years to produce such data. Therefore for some traits, APHIS may need to consider whether particular assumptions can be made without hard data when petitioned for deregulation."

I question whether this is the right approach to regulation. I guess it is "Let us keep our fingers crossed."

**APHIS—**This statement has been deleted from the text, to prevent misunderstanding. The intent of the paragraph is to address potential issues and challenges associated with APHIS regulation of GE trees.  It is not intended that APHIS will make individual or categorical determinations in this document, and the implication that APHIS will either base decisions on assumptions or make determinations on anything other than a case-by-case basis is unintentional.  The rest of the paragraph, detailing the consequences of longevity in trees and the difficulty of data collection remains germane to the overview.

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**Reviewer—**
**Objectivity:** As detailed in my comments above and in the "track changes" version, the assessment in this chapter minimizes effects on the environment and human health. In a number of places, it also selectively cites both among and within articles the information that tends to minimize actual or potential impacts on the environment or human health.

**APHIS—**The reviewer's comments in the track changes document have been taken into account where appropriate, and in general language has been adjusted to reflect areas of uncertainty and remove instances where effects are minimized.  However, other reviewers have pointed out that APHIS has not appropriately put the effects of GE crops on the environment in the appropriate context compared to other crops.  In light of these conflicting views, one suggesting APHIS is minimizing impacts, the other suggesting that APHIS is exaggerating potential impacts, APHIS has believes the document, as a whole, presents an appropriate overview.

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**Reviewer—**
**Clarity:** Page 3: "A wild plant that is more able to exploit limited resources may be more likely to survive and reproduce, while a highly efficient crop plant may be more likely to produce large yields."

This sentence sets up a false duality between wild and domesticated plants. Exploitation of limited resources (wild) and efficiency at producing a higher yield are not mutually exclusive traits. One of the most distinguishing traits is the higher harvest index in domesticated plants, i.e., a larger proportion of the photosynthates end up in the harvested part of the plant, e.g., grains. Wild plants may produce the same amount of biomass but a larger part remains in the vegetative organs. Furthermore, wild plants may not be better able to withstand stresses, whether biological or physical. The environment of wild plants is quite different from that of domesticated plants. For example, wild plants grow often at low densities where disease epidemics have more difficulty becoming established. Likewise, insect pests are kept under control not only by the lower density, but also by a suite of parasites and predators. Thus, there may be less selection pressure from diseases and pests in wild populations.

**APHIS—**This sentence was deleted based on this comment and comments from other reviewers in order to avoid confusion.

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**Reviewer—**Page 8: "flowering forms"

Please make this more explicit.

**APHIS—**This phrase has been changed to "flower morphology" for clarity.

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**Reviewer—**Page 12: "Adaptation of outcrossing crops to the hybrid condition"

This is an awkward statement. I would say: "The ease in producing hybrid seeds makes hybrid varieties a more practical approach in cross-pollinated crops than in self-pollinated crops."

**APHIS—**This statement has been re-worded for clarity and readability, although the suggested text was not used verbatim.

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**Reviewer—**Page 12: "Hybrid seed production involves well defined production steps, and much of the structure of the commercial hybrid seed industry is influenced by the difficulty and cost of pollen control, which control and limit, but do not eliminate, outcrossing and mixtures. In the production field, the sequence of seed increases generally follows a pattern where:"

There is a confusion here between "hybrid seed production" and "the commercial hybrid seed industry". The former refers to the production of seed for F1 hybrid varieties (e.g., maize); the latter refers to seed companies that produce new varieties through hybridization, whether these are F1 hybrid varieties, pure line varieties, or any other type of variety. I suggest you just delete the word "Hybrid" at the beginning of the paragraph: "Seed production involves well-defined …."

All of these varieties go through a certification program, whether public or private, that includes the steps in the bulleted list. There is an unnecessary split in this text between hybrid seed production and the next section (Crop Improvement …) leading to text duplication.

**APHIS—**This section has been deleted, due to the duplication of information found in the following section on seed production in traditional breeding.

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**Reviewer—**Page 12: "Conventional breeding, at its most basic, is a process in which differences in plants are observed in small plots, the differences are compared with the needs of the person doing the selection, and the plots that most fit the selector's needs are saved and perpetuated. Other variants are eliminated from the selected gene pool."

This is an antiquated view of plant breeding going back to the 19th century (e.g., Luther Burbank). If I could suggest another definition from Schlegel (2003) and Gepts and Hancock (2006): Plant breeding is an applied, multidisciplinary science. It is the application of genetic principles and practices associated with the development of cultivars more suited to the needs of humans than the ability to survive in the wild; it uses knowledge from agronomy, botany, genetics, cytogenetics, molecular genetics, physiology, pathology, entomology, biochemistry, and statistics (Schlegel, 2003). Of particular importance is the ability to transfer, in addition to major genes, large suites of genes conditioning quantitative traits such as productivity and other traits of interest to humans. The ultimate outcome of plant breeding is mainly improved cultivars. Therefore, plant breeding is primarily an organismal science even though it is eminently suited to translate information at the molecular level (DNA sequences, protein products) into economically important phenotypes.

Schlegel, R.H.J. 2003. Dictionary of plant breeding. Food Products Press/The Haworth Reference Press, New York.

Gepts, P., and J. Hancock. 2006. The future of plant breeding 10.2135/cropsci2005-12-0497op. Crop Sci 46:1630-1634.

**APHIS—**APHIS believes that the reviewer has provided a fine definition of plant breeding, but that it does not meet the requirements of the chapter for ease of understanding by a non-technical audience. The definition of plant breeding included in the chapter has been modified to reflect more modern practices to address the reviewer's concern.

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**Reviewer—**Page 13: "of corn"

Clearly, corn is one of the major commercial targets for genetic engineering. However, the text would be strengthened if the perspective was broadened to address a broader range of crops: e.g., agronomic and horticultural crops.

**APHIS—**APHIS has added text to indicate that corn is mentioned as an example of an outcrossing crop. Although an exploration of the details regarding a broad range of crops would be informative, it is not appropriate for an overview document, which must be limited in size and complexity.

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**Reviewer—**Page 13: "In the U.S. both varieties and genes can be patented. Varieties can also be protected under plant breeder rights. The International Union for the Protection of New Varieties of Plants coordinates a simplified plant breeder's rights system with standardized claims."

I suggest to make this text more explicit:
"In the U.S. both varieties and genes can be covered by a utility patent. Furthermore, vegetatively propagated varieties (except those of potatoes) can received a plant patent. Crop varieties can also be protected under plant breeder's rights (Plant Variety Protection). The International Union for the Protection of New Varieties of Plants (UPOV) coordinates the plant breeder's rights system with standardized claims at the international level."

**APHIS—**APHIS has determined that the level of detail included in this suggestion, while accurate, is not appropriate for an overview document of this type.

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**Reviewer—**Page 14: "The first decade of commercial plant agricultural biotechnology has seen remarkable growth from a mere 6 million acres in GE crops in 1996 to over 220 million acres in 21 countries in the 2005 growing season. The year 2005 also marked the point where cumulatively over one billion commercial acres of GE crops had been grown world wide."

These seemingly high numbers (taken from ISAAA, I suppose) need to be put in perspective to provide a more objective image to the public on the part of a public administration like USDA. Is 220 million acres a lot? It represents only 6.3% or 4.5% of total arable land in the world depending on FAO or CIA data, respectively.

http://faostat.fao.org/faostat/form?collection=LandUse&Domain=Land&servlet=1&hasbulk=0& version=ext&language=EN
https://www.cia.gov/cia/publications/factbook/fields/2097.html

**APHIS—**APHIS has included this information to illustrate the growth in the cultivation of GE crops over time, rather than an absolute metric of their prevalence.  The reviewer's estimate of what percent of the world's arable land this represents has been added to the text.

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**Reviewer—**Page 15: "Risk assessments for GE organisms:..."

This is an important paragraph. I would be more explicit in defining terms here: transparency, explicit in addressing uncertainties,....

**APHIS—**Some effort was made to address this suggestion, but APHIS intends this section to provide a brief overview of risk assessment characteristics rather than a complete outline of the risk assessment process.

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**Reviewer—**Same question for next page: "receiving environment": what features of this environment are considered?

**APHIS—**APHIS reviews the relevant features of the receiving environment on a case by case basis, consistent with our regulations and compliant with all applicable statues. Because this is not a detailed presentation or analysis of APHIS risk assessment model or procedures, the level of detail is appropriate for providing brief overview of what is taken into account in APHIS risk assessments.

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**Reviewer—**Page 17: Replace cultivated by domesticated: not every cultivated plant is domesticated, but every domesticated plant is cultivated. It is important to distinguish cultivated and domesticated plants in this context because fully domesticated plants are unlikely to escape the cultivated environment. See also comment on next page!

**APHIS—**APHIS believes that in the section referred to, cultivated is a more appropriate term.  The section is not addressing the escape of crops from the field, but is concerned with the introduction of genes from crops to relatives in unmanaged environments. Cultivated crops that may not be completely domesticated can still transfer genes into unmanaged populations.

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**Reviewer—**Page 18: “crop plants often are themselves not very weedy and have a low propensity for persistence when not managed in an agricultural context, whereas wild relatives by their nature may have weedy characteristics and an ability to persist in the environment.”

See text with track changes. It is important not to oversimplify by considering only two categories: crop vs. wild. Among crop plants, the degree of domestication varies. Fully domesticated crops, such as maize, will not survive on their own in the wild; partially domesticated crops, such as oilseed rape, can survive because they shed their seeds, which have a certainly level of dormancy. Both seed shedding and dormancy are characteristics of wild plants, which increase the weediness of the crop.

**APHIS—**The text has been adjusted to reflect the complexity of the range of weediness characteristics in crop plants.

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**Reviewer—**On the same page, at the bottom: To this list, should be added traits such as: seed dormancy, seed shedding, photoperiod insensitivity, etc. Same comment on p. 20, top.

**APHIS—**The list included in the chapter is taken directly from a citation, and therefore cannot be modified by APHIS as such. However, some of the characteristics mentioned by the reviewer are encompassed within the list, even if not stated directly. For instance, “seeds remain viable for a long time” is synonymous with “seed dormancy,” and “Plants are adapted for both long distance and short distance dispersal” encompasses the idea of seed shedding.

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**Reviewer—**Page 31: “Genetic modification”
The use of the expression "genetic modification" should be avoided. This is a misnomer best replace by the expression "genetic engineering". All organisms, even viruses, are genetically modified in one way or the other as they are subject to mutation, recombination, … Without "genetic modification" we would not have biodiversity on this planet. Clearly this is not what we are discussing in this document.

**APHIS—**In this particular section, APHIS is trying to put genetic engineering into the context of other genetic modifications. The point is to illustrate that some aspects of genetic engineering are similar to methods that have been employed in traditional breeding, producing similar results. APHIS believes the text accomplishes this goal.

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**Reviewer—**Page 31: “Traditionally, new varieties of whole foods have not been subjected to extensive chemical, toxicological or nutritional evaluation prior to marketing.”

There is a good reason for this. Consumption of some crops dates back more than 10,000 years, from before the beginning of agriculture during the hunter-gatherer era. Even hunter-gatherers had developed technologies to detoxify plants before consumption documented by anthropologists. Thus, on this basis alone, humans have received plenty

of exposure to compounds contained in these crops, including modern varieties. The difference with transgenes is that they can introduce new compounds to which human beings have not been exposed unlike the situation with naturally occurring compounds.
Johns, T., and I. Kubo. 1988. A survey of traditional methods employed for the detoxification of plant foods. Journal of Ethnobiology 8:81-129.

**APHIS—**The potential for genetic engineering to introduce novel proteins is discussed in the following paragraph.

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**Reviewer—**Page 32: It should be clarified that FDA evaluations are voluntary only.

**APHIS—**In the context of the paragraph, the roles of FDA and EPA in evaluating GE crops are briefly described.  Although it is true that FDA pre-market assessments are voluntary, APHIS is not aware of any makers of GE food crops that have not participated.  Including this information in the text would necessitate the inclusion of contextual information, in order to avoid giving the false impression that FDA only has "voluntary" authority over food safety of GE foods.  Because APHIS is not inclined to offer an exhaustive account of the regulatory approaches of other agencies, the text has not been changed.

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**Reviewer—**Page 43: "Agricultural biotechnology has increased the number of ways in which crops can made resistant to pests>"

Apart from Bt, what other ways of dealing with insects pests have been commercialized?

**APHIS—**Bt is one additional way in which crops can be made resistant to pests. Research is active on creating GE plants with other forms of pest resistance.  It is also true that GE (in general) can now be used to make plants resistant to pests in addition to conventional breeding, thus an increase in the number of ways.

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**Reviewer—**Page 45: "Structured refuges are non-Bt host plants that are managed to provide sufficient Bt-susceptible adult insects to mate with potentially Bt resistant adult insects. These matings result in Bt-susceptible offspring."

It should be clarified that the progeny will be susceptible to Bt only "(assuming that Bt-susceptibility is a genetically recessive trait)" Does the evidence support this? Reference(s)?

**APHIS—**A citation has been added to the text referring to an article that makes the case for the presented strategy for reducing the likelihood of insect resistance: Tabashnik *et al.* 2004 (see references in the final document).

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**Reviewer—**
**7. Additional text on Biological Control provided**: I was asked to comment on a separate document describing Biological Control. It would not fit in Chapter 4.1 as there

is no relationship to genetic engineering other than providing a valuable alternative to disease and pest control.

**APHIS—**APHIS has extensively modified the Biological Control Appendix based on comments from this and other reviewers. The section is included because APHIS may regulate GE biological control agents in the future.

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**8. Final conclusion: Does this scientific information presented accurately and objectively provide non-expert readers with a broad base of knowledge to understand the aspect of the biological and physical environment that is likely to be affected by the regulations currently administered by possible changes of those regulations?**

**Reviewer—**No, this Chapter 4.1 document has significant shortcomings in its treatment of the natural and physical environment affected by APHIS BRS regulations.
There are shortcomings on the substance as well as the form as detailed in my comments above and in the Track changes version. The text: 1) in certain parts ends up minimizing risk (see my Track changes; 2) while I acknowledge that this text is intended for non-experts, the current text presents in certain sections overly simplified information (e.g., wild vs. domesticated, self- vs. cross-pollinated) without considering intermediate situations (see my Track changes); and 3) is a combination of different sections written presumably by different individuals, with the ensuing differences in style and unnecessary duplications. In this regard, I would like to congratulate whoever wrote the section on Sylviculture, which is by far the most-balanced and best-written section of the chapter! It sets a standard for a necessary re-write of Chapter 4.1.

**APHIS—**APHIS acknowledges the reviewer's concerns and has attempted to address many of them in the text of the document. In many cases, text was added to eliminate over-simplified explanations and to present a more accurate presentation of the nature of crops. Although most of the reviewer's substantial comments are addressed above, APHIS has attached the "Track Changes" version of the document in order to allow the public the opportunity to see the reviewer's comments in the context of the original document.

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**Document Changes Subsequent to Completion of the Peer Review Process**

The changes to the draft EIS, described above, were made directly after the peer review had been completed. However, as is common with documents as complex as this one, the draft EIS continued to undergo a process of substantive and editorial changes. These changes were sometimes made to portions of the EIS which had been subject to peer review. In addition, almost a year has passed since the peer review had been completed and, inevitably, new research relevant to the topics discussed in the EIS has been published, and this research has been incorporated into the EIS where appropriate.

Although these changes were made after the peer review had been completed, they were made in keeping with the original goals of the peer review process: completeness, currency, accuracy, certainty, objectivity, and clarity. For the sake of transparency, changes implemented after the peer review was completed are summarized below.

**New references added supporting existing assertions**–References to scientific or other publications that were published after the peer review or that were brought to APHIS' attention after the peer review were incorporated have been inserted throughout the document as appropriate. APHIS believes these additions contribute to the document's completeness and currency, without sacrificing its objectivity.

**New references added supporting new or altered assertions**–In several cases, newly published information was found that either presented an alternative or opposing view of an existing assertion in the document or supported new assertions. These references were inserted, and assertions were added or edited as appropriate. Specific instances where assertions were added or altered include discussions of decomposition of plant debris from Bt crops (See IV.A.3.b.(1)—Accumulation and Persistence), frequencies of spontaneous mutations in soil bacteria (See IV.A.3.b.(4)—Horizontal Gene Transfer), and the likelihood of a GE plant becoming a weed or invasive species (See IV.A.3.a.—Potential Changes in Weediness and Invasiveness). APHIS believes these additions contribute to the document's completeness, accuracy, and currency, without sacrificing its objectivity.

**New background information added**–It was determined that in several places in the EIS, additional background or explanatory material was necessary to assist readers in understanding complex scientific or regulatory issues. Specific instances were discussions of genetic engineering technology (See, generally, Chapter IV.) and descriptions of the Coordinated Framework (See I.C.—Interrelationships with Other Federal Agencies). The added information was taken generally from public sources such as the APHIS website and governmental and non-governmental publications, and citations were supplied as appropriate. APHIS believes these additions contribute to the document's clarity, without sacrificing its objectivity.

**Excessively technical language deleted**–It was decided that certain passages in the document were either too technical or were superfluous. These passages have been removed from the document. Specific examples are discussions of seed biology (See

IV.A.1.b.—Seed Biology and Commercial Seed Production) and plant virology (See IV.A.4.c.—Genetically Engineered Virus-Resistant Plants). APHIS believes these additions contribute to the document's clarity, without sacrificing its objectivity.