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FIFRA SCIENTIFIC ADVISORY PANEL (SAP)
OPEN MEETING
OCTOBER 13 - 15, 2004
ISSUES ASSOCIATED WITH DEPLOYMENT OF A TYPE OF PLANT-INCORPORATD PROTECTANT (PIP), SPECIFICALLY THOSE BASED ON PLANT VIRAL COAT PROTEINS (PVCP-PIPS)

WEDNESDAY, OCTOBER 13, 2004

VOLUME II OF IV

(Afternoon session)

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A F T E R N O O N S E S S I O N

MR. ROBERTS: We decided that we were going to finish up question 3 after lunch and get a change to maybe think about the list a little more, and I see that the question has now been I wouldn't say amended but let's just say it's been completed by showing the footnote that it was inadvertently dropped of on the copy distributed indicating how the United States is

defined, and there's also a tentative list we'll call it of species based on discussion prior to lunch.

So let's then -- and I don't know what Dr. Stewart, do you want to sort of lead off the after-lunch discussion on this or maybe Dr. Tepfer might be a logical person. No, he's pointing to Dr. Stewart, Dr. Stewart can you lead off the discussion to finish off question 3.

DR. STEWART: Well I guess during lunch we discussed sugar cane as possibly having or that was before lunch, you know we discussed sugar cane as being more of a tropical crop, anyway there's minimal acreage in the U.S. that has sugar cane. Most of our sugar comes from sugar cane in the tropics, so shatter

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cane is a wild relative that could potentially be there.

The one thing that I would like to say about this list of plants is that it should be open for revision during the next few weeks as we really dig into it, so we'll add some things here, perhaps take some things away, I mean because I don't -- no one here has an encyclopedic knowledge of all plants, all crops, all wild relatives, and I would especially say in the trust territory of pacific islands.

MR. ROBERTS: We're deciding what we can do about that under fagu (ph).

MR. LEWIS: Thank you, Dr. Stewart. This is Paul Lewis again, designated Federal official for the SAP and again the intent of the meeting we have here is to have an open discussion of dialogue on all of the issues (inaudible) once the meeting is over the panel is writing its report basically summarizing the points that occurred here, so if we have any new ideas or suggestions after this meeting in terms of reflecting it as a panel consensus it's after the fact, so if you want to use the time today and

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tomorrow or the rest of the meeting time to look at this list and digest it and revise it you're welcome to do that, but once the meeting is over we want to come to a conclusion on the panel's position. Thank you.

MR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I just want to suggest that we all continue to think about this seriously because if some of these minor crop plants could be exempted because they are no wild relatives this could make a huge difference in terms of what can be done from a biotech point of view because the burden of going through the regulatory hoops is extremely heavy particularly for minor crops, so I would suggest that all of us continue to think about this over the next day or two and we can try to toward the end of the three days try to make a more complete list of -- not that it would take very long, just to try to get as much on as we can.

DR. GENDEL: Actually having dealt with similar kinds of issues in other contexts I would like to suggest that maybe what the people who know this

subject should think about is perhaps not making the list complete but developing a criteria or mechanism by which the Agency can look in the future to make these decisions because I don't think anybody's ever going to have a complete list that answers all these questions, so what I think the Agency probably needs are a set of guidelines to how to go about making these decisions in the future.

MR. ROBERTS: For the record that was Dr. Gendel. Dr. Cooper.

DR. COOPER: Could I make a suggestion that circulars go out to the plant breeders of these crops who might well have unpublished experiences which will be relevant to this and they could be collected together. At the present moment these are scattered and very often unpublished experiences which would be relevant to know answers to these questions and we don't have very many if any plant breeding represented here.

MR. ROBERTS: Other comments or suggestions, Dr. Hammond.

DR. HAMMOND: This list has a very small

number of ornamentals on but there are a very large number of ornamentals for which various problems are significant for which genetic engineering is under investigation and could reasonably be exempted by virtue of lack of wild relatives certainly I think in, certainly within the continental U.S., and I think probably many of them not in the territory of the south pacific, so there are a large number of ornamentals that could usefully be appended to that list.

I could add a few now.

MR. ROBERTS: Okay. Go for it.

DR. HAMMOND: Ornothogolum (ph) is a crop that is currently under consideration in our own lab, is gladiolus on the list, we have engineered gladiolas. There are wild relatives of geranium --

DR. STEWART: Actually the geranium is not a geranium, the genus is not geranium, the wild geraniums are geraniums.

DR. HAMMOND: But the new guinea impatients --

DR. STEWART: They have wild relatives.

MR. ROBERTS: For the record the discussion was between Dr. Hammond and Dr. Stewart. In the interest of time, and I'll get to Dr. Sherwood in just a second, maybe we can, individuals around the panel can sort of brainstorm this evening over dinner or lunch or something or during the course of the meeting and we can give the Agency our best impressions at

this meeting of what the species are and then perhaps can make some recommendations about how to construct a more formalized list, obviously people are sort of reacting off their knowledge, but would that be satisfactory to the Agency and we can work on that during the course of this meeting. Dr. Hammond.

DR. HAMMOND: There is certainly a relatively recent reference and review of which ornamentals have been engineered or people are trying to engineer and from that it would certainly be possibly look at which of them have known wild relatives and which don't.

MR. ROBERTS: Perhaps we can get a copy of that reference while offline or something here and that might assist us in constructing our list.

Dr. Sherwood did you want to add something.

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DR. SHERWOOD: Yes. In trying to address this question in regards to what was suggested rather than make a list the question poses with the stipulation of that which they can produce viable hybrids in nature and maybe that should be the criteria that in addition to the crop plants listed those plants including ornamentals and others should be considered exempt if they are known not to produce viable hybrids in nature, that might be something added as an addendum to expand the list.

DR. STEWART: This is Neil Stewart, for many of these plants, especially once we get into the minor crops I'm not sure the data available.

DR. TEPFER: I just want to point out that as we get into more and more minor plant species some of them could also be invasive in themselves, so we need to not go toward exempting species that are potentially invasive.

MR. ROBERTS: Dr. Kramer, it seems that we need a little more time to work on this one, so we will do that and before we close out we will revisit this question and give you our updated view on the

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species.

DR. KRAMER: Should we go onto question 4 then?

MR. ROBERTS: Let's go ahead and go onto question 4.

DR. KRAMER: What laboratory techniques used to achieve genetic exchange between species, for example, embryo rescue, use of intermediate bridging crosses, protoplast fusion, are not indicative of possible genetic exchange between these species in the field? Conversely, what techniques, if any, used in laboratory or greenhouse experiments provide the most reliable indication of ability to hybridize in the field?

MR. ROBERTS: Dr. Stewart, you're up again.

DR. STEWART: Lab-intensive methods to combine germ plasm such as embryo rescue and

protoplast fusion are not predictive of gene flow in the field. Hand crosses in growth chambers in greenhouses are marginally useful. Hand crosses can show if species are sexually compatible and if a

transgene at a particular locus is transmittable and at what frequencies it provides a best-case scenario for transmission.

In the field there are factors that could prevent hybridization and integration including non-overlapping flowering, pollen competition, non selection linkage to sequilibrium (ph), genetic exclusion, and competition within plant communities. So the short answer is that the lab techniques are not very useful in predicting gene flow in the field.

MR. ROBERTS: Okay. Dr. Cooper.

DR. COOPER: I would argue that they give you a worst-case scenario which is probably what would be very useful in a risk assessment, at least the hand crossing would even though the (inaudible) means a delivery of the pollen in that species would be an animal like a bee. Clearly the use of bees in glass houses in many hives and other things is a perfectly convenient technology, it takes a little time, it doesn't produce necessarily very high rates of transfer but they may be more realistic, but the rates I would argue of transfer is possibly not so relevant

as the absolute occurrence of it.

So clearly wind and insect pollination is going to require different approaches towards the suitable delivery of the technology, but I would disagree with what seminus (ph) said, seminus said hand crossing is of little concern, I personally think it's a reasonably convenient way of measuring what can happen in the field albeit perhaps not at the rate level, but the fact that it can occur.

MR. ROBERTS: Dr. Hammond.

DR. HAMMOND: In general I agree with that, certainly wind pollination and bee pollination can be managed under contained conditions with some care and modifications, there are a number of other techniques that can be used to demonstrate crossability that have very little relevance in the field and I would add to those chromosome injection, application of pollen to cut styles or pre germination of pollen, cases where emasculation is necessary to achieve pollination because some species are normally self-fertilized and exclude foreign pollen and especially there are some species that are cryptogomist, that fertilize within

their closed flower before the flower is even open, and of course there are cases where pollen storage is used to, collected from spring flowering species and used to pollenate fall-flowering species or vice-versa in that, that certainly has been used in our group with some tree species that where some flower in the

fall and others in the spring, so pollen storage for long periods is definitely not relevant to what happens in the field.

I think that's about all I had to add.

MR. ROBERTS: Dr. Melcher and then to Dr. Tepfer.

DR. MELCHER: I would agree with Dr. Cooper that the laboratory-type crossings are probably very useful because some of the impediments that Dr. Stewart mentioned are not really solid impediments, for example, the times of flowering, flowering times in plants are often determined by just a few genes and a mutation in one of those genes could very easily reverse the situation and thus make the cross pollination possible.

MR. ROBERTS: Dr. Tepfer.

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DR. TEPFER: If we're going toward the idea that pollination tests in the greenhouse -- our reviews, I just want to mention it's also a bit dependent on the genotype of the plants that you're working with so with certain brassicas you can get good crossing with one genotype and not another genotype even within the same species, so you have to be a little bit careful about that.

MR. ROBERTS: Over comments on this question, yes, Dr. Cooper.

DR. COOPER: Could I make a comment in relation to what John Hammond said, the concept of mentor (ph) pollen is sometimes brought up, it may be related to what you were talking about. So you use pollen to go to a different species perhaps even put it at the same time as your test species on the stigmatic surface and this does facilitate fertilization, this could be a realistic assessment in nature because it certainly could happen, but storing the pollen before you did that for long periods of time is obviously less relevant, but one of the ways in which is applied is of course to irradiate the

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pollen that you're using to help the process, that is clearly just removing a problem from your technology rather than anything else it doesn't actually say it doesn't happen in nature.

MR. ROBERTS: Other comments? Dr. Kramer is our response reasonably clear or would you like some clarification?

DR. KRAMER: I think that's fine, thank you.

MR. ROBERTS: If there are no other comments on 4 let's move onto number 5.

DR. KRAMER: Given that current bioconfinement techniques are not 100% effective, what would the environmental implications be of extremely low transfer rates of virus-resistance genes over time?

MR. ROBERTS: Dr. Cooper, could you lead our discussion on this one?

DR. COOPER: Yes, I would say potentially yes, a slow-burn impact that might escape recognitation (ph) early enough to allow eradication of a problem, that's certainly a possibility. There are of course many uncertainties because of the

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variability of crop to wild gene-type flow, but the biggest gap concerns the actual magnitude of the resulting harm, not the fact of the movement.

If the transgenic gene was linked to agronomic traits including large seed size and other factors like that it's quite likely linkage drag would hold the process up, but there would be no stable introgression in that circumstance probably, however that benign outcome cannot necessarily be assumed to be overriding, for a variety of reasons the experience has been that hybrids survive poorly, but very rare and complex genetic exchanges do get perpecuid (ph) the brassica genome provides examples of a very complex series of apparent relationship changes that have taken place over a long period of time perhaps, but nevertheless the unlikely events do happen.

There is no reliable baseline to fall back on and as a need I would suggest for more specific hybrids between crops and wild relatives to be created and their fitnesses tested in the field, and that is obviously an area which was touched upon earlier this morning. We have little direct evidence of fitness

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over a whole lifetime in anything, it seems to me that our experiences with viruses and wild brassicas in the UK has revealed such complex in directions involving different vital genotypes and genetic diversity in the plants that prediction of the outcomes can certainly not be generic. In the UK the necessary planned releases would not necessarily be authorized and it is now appropriate to investigate the traditional -- in our particular case a specific virus that happened to be turnip mozaic virus resistance -- those that naturally occur in the species like brassica rapa and brassica negru (ph) and to use those as surrogates of the transgenic which would not be so difficult to get permission to do, we need the information concerning the trait and its outcome and that would be one way of getting it.

Providing hybridization and stable introgression are possible genes from crops may increase infrequency when the gene confers greater lifetime fitness, that is the theoretical assumption behind this. This doesn't mean that hybrid plants would necessarily become more persistent or invasive,

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simple they could be an affect on biodiversity including some description of genetic integrity of local ecotypes, the hybrid-derived wild species may become more genetically uniform and never do, but these biodiversity changes may be important in certain

circumstances.
There may be fixation of the new genotype in a scattered subpopulation and if this was known that a wild species was sufficiently compatible for gene flow to occur the assumption should be made that the probability of hybridization overtime will be one, and if harm is anticipated it's clearly one of those things one wouldn't wish to authorize. Thank you.
MR. ROBERTS: Thank you, Dr. Cooper. Dr. Bujarski.
DR. BUJARSKI: I basically agree with Ian Cooper and since this is not within the specialty of my research I have no other comments to add.
MR. ROBERTS: Dr. Allison.
DR. ALLISON: Richard Allison. The rate at which a gene may escape has little to do with its establishment, rather it's integration into the

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population is dependent on its selective advantage in its recipient. The virus pressure in this PVY test the selective advantage, low transfer rates will influence the time required for integration, but low or high transfer rates should be treated similarly as they have little difference over time.
While low transfer rates decrease the likelihood of gene transfer within a given season we must evaluate the long term, if you know it's going to happen it is just a matter of time. We can look forward to better bioconfinement methods in the future, however at this point we should assume that given a sexually compatible recipient we should plan for gene escape.
MR. ROBERTS: Other comments. Dr. Falk.
DR. FALK: In terms of genoscape and its relevance to viruses I think I'm going to say what I said earlier is that we don't really know that plant viruses have a role in weed or natural plant survival or invasiveness. I think there are examples where virus resistance does not contribute to invasiveness. For example in California a serious virus disease,

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beet curly top (ph), and it is transmitted by the beet leaf hopper and both of these are introduced species that were introduced in the late 1800s and both have very wide host ranges.
The natural California vegetation which included perennial grasses and sage brush are resistant or immune to beet curly top, however many dicot species are susceptible. Through time these native species actually have been affected severely by cattle overgrazing and by other farming practices and the dicots that are susceptible to these viruses have spread now that these destructive practices that have led to elimination of the perennial grasses in sage brush have been stopped, these plants that are resistant have not at all moved back in or showed any sort of advantage to re colonize their original areas.

I think another point in thinking about gene flow is that gene flow and to related species and virus susceptibility are not also synonymous, so not all plants that are going to be related will be susceptible to the same virus.

MR. ROBERTS: Dr. Hammond.

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DR. HAMMOND: To follow-up some discussion that we had earlier, disease resistance genes have been deployed in crops through tradition grading methods for years and to date there has not been significant research done to look at the consequences of the introgression of these genes from the crops into wild species, but that does not appear to have been any obvious increase in weediness as a result of that potential integration. The methods exist now from genomics to go looking for those genes and to determine whether they have introgressed in crops and to determine whether those genes do have any influence in the persistence or weediness of the wild species and that should be done, but it seems to me that there is very little difference between transgenes that confer virus resistance and naturally occurring genes that confer disease resistance.

The tools are there, we have the option to go and look at it and I see no reason why virus resistance from transgenes should be of more concern than any other natural gene that has had the opportunity to introgress from crops to weeds.

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MR. ROBERTS: Other viewpoints, Dr. Tepfer.

DR. TEPFER: I'm just a bit concerned about generalization. I think we need to keep this in a case-by-case sort of perspective. I think that what this sort of emerging consensus that there seems to be no evidence for the ecological release having occurred in the past that we know of is one thing, but there are also cases where virus resistance could perhaps provide a sufficient booth to a wilder weedy species, I mean the obvious sort of hypothetical case here is the wild oat which is a terrific weed already, it is susceptible to BYOV, Bailey Yellow Dwarf Virus, I just would think be we need to be a little bit careful and think about individual cases rather than trying to generalize.

MR. ROBERTS: Dr. Sherwood and then Dr. Falk.

DR. SHERWOOD: I think another thing to be considered is the plasticity of the viral genome and that although there would be no reason to think that virus resistance genes either by transgenes ones that were developed through convention breeding would be anymore stable in weeds than they would in the crop

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plant, and it's fairly evident that virus resistance is overcome on a fairly regular basis in crop plants, and so why would that not occur in weed species as well.

DR. FALK: I agree exactly with what John Sherwood just stated, I also think that the example of bailey yellow dwarf virus in wild oats. I don't think that the data do show in fact that bailey yellow dwarf viruses do have any effect on the natural incidents and colonization of wild oats.

MR. ROBERTS: Dr. Melcher.

DR. MELCHER: Relative to the wild oats and bailey yellow dwarf virus it occurs to me that maybe I should read to you part of the letter that was submitted, written by Roger Bechie (ph) where he says from the aspect of control of an epidemic disease control in weedy species is a positive thing since weed species are the source of most epidemics of plant viral disease worldwide, indeed one method to control such diseases is to remove alternative hosts, so he's saying that if the wild oats were to acquire resistance to the virus, the virus level would

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decrease in the reservoir in a reservoir for growth of the crop plants. It's probably not relevant to the question, but I thought it was worth reading.

MR. ROBERTS: Thank you. Other comments? It seems we have some differences of opinion on this which is fine, but I just wondered if there is anymore dialogue that we need to sort of clarify panel's position on this. I don't see any, so let me ask Dr. Kramer.

DR. KRAMER: I guess I would just asked if you could possibly clarify when you talk about a case-by-case evaluation is there any additional guidance you might provide about the criteria we would use in such an evaluation or is that something that might require more thought?

MR. ROBERTS: I assume your question is for Dr. Tepfer?

DR. KRAMER: Yes.

DR. TEPFER: I mean I want to come back to what Falk just said about the wild oats, of course this hasn't been demonstrated but I think this is a case I would be a little bit more concerned about than

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certain others because of the already weedy nature of the potential recipient species, so I would suggest that potential recipients that are already potentially weedy should be of particular concern. I think there are also cases where this is much less likely to occur in which the recipient is in an extremely limited sort of a ecosystem not very invasive, and that I would suggest would be reason to be a little bit less worried about this, I think it might be one criterium that should be considered.

MR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: I think this morning we had a very good presentation by AFIS (ph) about the criteria that could be considered in addressing this question and I believe those are reached by consensus of the

scientific community as important in making these case-by-case evaluations.

MR. ROBERTS: Dr. Stewart.

DR. STEWART: I would just add on the case-by-case basis, a lot of times there are several different categories of plants that you might be worried about and not worried about, so the ones with

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no wild relatives well of course that's when you don't worry about gene flow or increasing competitiveness or weediness of any wild relatives, on the other end are the wild oats the Johnson grass which is closely related to the sorghum are probably -- the Agency or any company will probably never go there as far as transgenics simply because the gene flow issue is going to be a done deal and nobody really wants to have liability over what would almost certainly be a transgenic weed that would persist in the environment for a long time, so I think that's one thing that Dr. Tepfer is kind of getting at on a case-by-case basis.

MR. ROBERTS: Yes, Dr. Nagy.

DR. NAGY: One additional comment I would like to make is that it's possible that if transgenic weeds can be widespread that it can change the selection pressure for a combination, so it is possible that in -- phonetically (ph) possible although I don't have any published information on that, that in those weed species no new viruses would have much better chance to emerge than currently (inaudible) or others, so this is an important issue I

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think for the record, now the selection (inaudible) can be changed in that situation.

MR. ROBERTS: Dr. Kramer, are there any other follow-ups on this question?

DR. KRAMER: No, thank you.

MR. ROBERTS: Let's go ahead then and take question number 6.

DR. KRAMER: Please comment on the prevalence of tolerance and/or resistance to viruses in wild relatives of crops..

MR. ROBERTS: A little bit open question, but we'll let Dr. Falk lead off our discussion on this.

DR. FALK: Tolerance resistance immunity to indigenous pathogens and viruses is present in wild population of many plants and this was an area of significance that particularly in early days virus biology and plant breeders of course have searched for germ plasm sources to use in breeding programs for resistance. One example is a book published in 1993, Resistance to Virus Diseases of Vegetables by Kyle -- Kyle is the editor, in chapter-by-chapter going through that book they list sources of virus

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resistance found for cucurbets, for lettuce, for peppers, for tomatoes, for peas, for beans, and they document the sources as being various land races and

plant introduction lines or wild species basically, lettuce, resistance in lettuce like tukusitiva (ph) to lettuce mozaic virus comes from related species like tucavirosa (ph) , like ticasuligna (ph) , like tucasariola (ph), and in some cases these are single gene types of resistance.

In the last chapter of that book Sorenson states that most of the sources of resistance that we utilize in breeding programs come from foreign germ plasm and we are increasingly relying on wild relatives of cultivated species for virus resistance genes. In barley there's a very good example of single gene resistance, barely yellow dwarf virus from Ethiopian wild barley -- let me see where I'm going because I thought we were doing this tomorrow -- I think also instead of just documenting resistance we can say that there must be resistance tolerance in weedy species when we introduce new crops to areas and viruses suddenly appear in those crops and there are

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many, many examples in the literature of that one is when cucow (ph) was planted in regions of Africa there was no indication ahead of time that cucow swollen shoot was an endemic virus in that area but now that an introduced crop species was planted there it was unable to be grown commercially and was basically eliminated, so the virus obvious was indigenous in native plants and was not causing detectable effects in the wild population.

Another think to think about I think in terms of resistance in wild populations and this is from a review article written by Jim Duffus (ph) in 1971 where he talks about the role of weeds in plant virus incidents and epidemiology and he says that viruses can become pathogens of wild plants when susceptible crops are grown in their vicinity, that we grow susceptible crop plants that do become infected and now you have a uniform widespread source of inoculum that is a source of inoculum for the weeds and not vice-versa and we often think of the alternative. That's all I have.

MR. ROBERTS: Thank you, Dr. Falk. Dr.

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Zaitlin.

DR. ZAITLIN: I would like to point out that most plants and we're including the wild relatives we're talking about disease is the exception, resistance is the norm. I mean that pertains to all kinds of pathogens of plants and animals. If we became diseased in response to every pathogen we came in contact with we would be in a bad way.

Now on thing and I will talk about this a little later in my response to question 8, we I looked at some of these types of resistances they are not conventional resistance gene induced as Dr. Falk talked about, so they're so-called subliminal infections, that the virus is able actually to infect

the initial cell into which it is placed but it can't move from there, so there's a restriction and a capacity of this virus to move out of the infection court.

MR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: I would just like to build on that a little bit. When we think of these terms of virus resistance and virus tolerance they are I think

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defined differently by different groups of people and the direction of this discussion that I'm hearing is that resistant is an absolute, either the virus is not there or the plant's susceptible, and that's generally not the case, particularly in transgenic plants and most of the studies that have been done in the field there's been a delay of symptom development which is what you're really looking for from a plant production side is an absence of disease phenotype even though the virus may be there, and so probably the same thing is going to happen in natural populations if there are quote unquote "resistance" genes there that there's going to perhaps be less virus replication or delay in symptom development and not in absence completely of virus there's probably going to be very little impact that occurs.

In some of the work that I did when I was at Oklahoma State on virus resistance to weight soil born you know there's delay of movement out of the roots of the plant and in a resistant cultivar that's modulated by temperature and so building on what Dr. Zaitlin said you had these subliminal or limited infections

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that occur in a tolerant or resistant plant, but they are not resistant in the absolute sense that we thing that virus is not replicating there.

MR. ROBERTS: Dr. Zaitlin, follow up?

DR. ZAITLIN: Yeah, I think it goes back to the definition as John talked about as to what plant virologists consider resistance and what say plant breeders consider resistance. I remember some years ago we have a well-known plant breeder, Cornell Henry Munger, the resistance of various vegetables, he said come into the greenhouse I want to show you my resistance squash, well they sure look sick to me, but then he showed me the plants, their parents, and they were less sick than their parents, that was the criteria he used.

MR. ROBERTS: Yes, Dr. Melcher.

DR. MELCHER: Speaking of definitions I have a feeling listening to the comments that perhaps my definition of tolerance not quite the same as the others, the way I think of tolerance is that the plant is loaded with virus but it has absolutely no symptoms, and this is definitely something that is

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known for a number of plant and virus combinations and I'm not sure whether that's what the other people were

thinking, that's my thought.
DR. SHERWOOD: I would certainly agree with Ulrich when it comes to weeds, that you will have weeds that are just loaded with virus but have no phenotypic symptomatic expression of being diseased.
MR. ROBERTS: Dr. Hammond and then Dr. Cooper.
DR. HAMMOND: I think just to go back to that we've already talked about the fact that many times wild weed species have multiple infections with no or insignificant apparent symptoms, so they are obviously tolerant to a significant degree and there is little affect of many viruses on many wild species. Plantagolancilatra (ph) is the weed with which I'm most acquainted, that is naturally infected by at least 26 different viruses and the three phytoplasmas and experimentally known to be infected by at least 13 other viruses and at least one other phytoplasma and most with minimal symptoms, a great deal of tolerance in wild species.

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MR. ROBERTS: Dr. Cooper.
DR. COOPER: I just put in a new work which is immunities, specific virus immunity occurs in wild plants in plastoanegra (ph) seedlings were collected from a span of a few kilometers and manually challenged with turnip yellow mozaic virus which is a readily transmittal virus of that sort. The proportions of immune seedlings and that were 21 out of 31 so that you can actually put real numbers which we have done for manually inoculated plants and for a virus like turnip yellow mozaic that's not a probably wholly unreasonable thing, you've got an easy virus to detect, the means of detecting it's illogically disease is normal, except in that population where there was not disease from turnip yellow mozaic and no turnip yellow mozaic occurred.
Another thing I would say that no brassica negru systemically invaded but beat western yellow's (inaudible) virus and never showed symptoms in any of the plants that we looked at, so that we can put numbers and we have indeed gotten numbers for proportions of those categories which I'm defining

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immunity as absolute exclusion of virus and detectable amounts.
The other terms where we did make an attempt tivengernsinine (ph) a couple of papers in phytopathology a few years ago to define the terms and reconcile the usage by plant breeders and plant biologists of the terms in resistant, susceptible and immune, and they have been accepted by many, but perhaps not by all.
MR. ROBERTS: Other comments by panel members on this question? Dr. Kramer have we confused the things with regard to terminology?
DR. KRAMER: I think so. I would like to

draw everyone's attention to the appendix that we, the Agency provided for resistant and tolerant and just ask for maybe some direct comment on whether these definitions are acceptable or if what I'm understanding from Dr. Cooper perhaps immune is really the word we're looking for to go with the definition for resistant.

MR. ROBERTS: Could you read these for us because I think I see people started scrambling around

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to try and find those, but let's take them one at a time and maybe if you could read them and get some feedback.

DR. KRAMER: Let me start with the definition of tolerant which I didn't actually hear any disagreement about and see if we can maybe agree on that first. That definition of tolerant means the plant is able to sustain the effects of a virus infection with negligible or mild symptom expression and negligible or mild effects on fitness or growth despite the presence of the virus within the host.

MR. ROBERTS: Sound good panel. They're nodding for the record.

DR. KRAMER: The definition we have for resistant is -- means the plant is not infected by or is a non host of the virus concerned. And I guess I would ask a two-part question here, one, what term would you use for that definition is really the main question we have, and then the second part would be if you would use another term for example immunity for that definition then could you also provide a definition for resistant.

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MR. ROBERTS: Dr. Melcher.

DR. MELCHER: Well I can start with the -- I guess there are different levels of resistance in my view. Immunity is when the virus enters the cell and nothing happens, it does not replicate in that initial cell. The next level of resistance is what Dr. Zaitlin talked about, the subliminal infection. It's able to enter a cell and replicate in that cell but it does not spread any further than that one cell. And then there's another level where the virus is limited to a small area which is typical of the hypersensitive response and that's what I think a lot of people think of as resistance, although probably resistance refers to all three levels of those levels in my opinion.

MR. ROBERTS: Dr. Zaitlin.

DR. ZAITLIN: This brings me back to my comment of this morning about the definition of PVCP-PIP and you were talking about it refers to virus infection, here we're talking about disease, we're having situations here where a plant actually is infected but there's no disease, I think you ought to go back and look at that definition you gave us.

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MR. ROBERTS: Let's go ahead and give Dr.

Kramer some feedback an resistance, make sure we give a clear response on that. Dr. Sherwood.

DR. SHERWOOD: What I want to do is ask the other panel members if they knew of cases of PVCP-PIPS in which there is an absolute non host resistance conferred, because all of the literature I'm familiar with is that there is a delay in replication of the virus, the amount of virus or a delay in symptoms that's produced or in case of gene silence and recovery.

MR. ROBERTS: Dr. Tepfer.

DR. ZAITLIN: I don't know whether that's been really investigated like the situation that Dr. Cooper gave earlier did they actually look to see if whether there was anything like a subliminal infection?

DR. COOPER: Can say that they were tested rigorously, serialogically and in other ways to detect a virus which is generally a very abundant and easily detectable agent.

DR. ZAITLIN: But what I'm talking is did it

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fact replicate in the initially infected cell.

DR. COOPER: The initially infected cell might have been so rare as to have the consequences diluted by the proportion of cells surrounding it that were not infected, however the net result was that you could not detect by normal means the presence of the virus, but the absolute presence of the virus in a single cell somewhere in the plant was not rigorously sought.

DR. ZAITLIN: I remember years ago there was a study by a fellow who was at the Los Angeles Arboretum and he had a wealth of plants at his disposal so he tried to infect them with the back (ph) mozaic virus and then to see whether in fact he could recover virus from them. Now the tools that he had available to him are very different from those we have today, he essentially tried to extract it and test it biologically, but interestingly enough he found in many, many species and I can't enumerate but I remember it included things like ferns, he showed that they were in fact hosting those viruses but to a very, very limited degree.

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MR. ROBERTS: I think Dr. Tepfer was going to respond to Dr. Sherwood's question.

DR. TEPFER: I think I was. Just wanted to mention that there are cases in the literature of the PTGS mediated resistance in which there is no apparent infection to start with. Now I would presume that there was at least at the early stages something that would have to be at least a subliminal sort of infection, but there are cases where it's not a recovery-type behavior, but where you simply see no virus and no infection.

MR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I can address that in one particular instance where we have plants that express antisensaronate (ph) to being yellow mozaic virus and we had some lines that went through the typical recovery infection and recovery state, but we had one line which we were never able to detect infection even in the inoculated leaves even using a hundred micrograms per mil of viral inoculum violizer (ph), we did not go to the level of PCR so we cannot guarantee that there was no subliminal infection but using the

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ilizo (ph) which is pretty sensitive using monofinal (ph) antibodies we were not able to detect virus in the inoculated leaves, so in that case that appeared -- I described that as immunity.

DR. SHERWOOD: So would it be safe to say then that the expression of a transgene it would be a rare event that would make a plant immune to a plant virus.

DR. COOPER: I think that would be true and the cases of plant expressing coat protein we never observed lines in which we could not detect infection. There were some lines in which a high proportion of plants escaped infection, but there were no coat protein expressing lines that I have worked with that were not infected at some level or other.

MR. ROBERTS: I don't mean to interpret but we are working towards answering Dr. Kramer's question about a definition about resistance.

DR. KRAMER: If I understand correctly I think we got consensus that the term for absolute exclusion of the virus would be immunity --

MR. ROBERTS: The panel is nodding. So

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you're right then to the second part, how would you define resistance.

MR. ROBERTS: Dr. Allison, you were not nodding.

DR. ALLISON: I just want to contribute something that may help to define subliminal infections from resistance, and that is it seems that in the subliminal infection the virus lacks the ability to move from the originally infected cell, that is it can't open the door, it can't get through the plasma desmina (ph), however it still maintains the ability to replicate so it has that sort of machinery available to it, and we've done experiments where we've taken animal viruses and put them into barley protoplast and watched the replicate, however there is no way that that animal virus, flock house virus in this case, was able to move within a barley plant, so if that helps the definition of subliminal infection.

MR. ROBERTS: Dr. Kramer, did you get your definition of resistance to?

DR. MELCHER: I think she was asking whether

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we had definitions for resistance and it seems if I'm interpreting Dr. Sherwood's comments there's two different uses of resistance, there is resistance to infection and there is resistance to disease and they may not be the same, is that right?

MR. SPEAKER: That's correct.

DR. COOPER: Well the susceptibility and sensitivity are two parts of a see-saw but go into the disease expression scenario, but the resistance is very often one that might additionally involve nonacceptance by a vector or even deterrence by a vector at some distance remote from the plant so that the vector component and resistance should also be considered that the plant could be resistant to the deliver of a virus into it by a vector which didn't like it, but it is relative amounts of infection in proportion to inoculum would seem to be something like the definition of resistance, but it's a variable always in my eyes anyway.

MR. ROBERTS: Well this seems to be an important sidebar for our discussion because we're not using terminology consistently we're going to have a

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lot of problems providing clear feedback, so please feel free to use as much time as you need for it, so not only for the benefit of the Agency understanding what's said but among the panel so we're all using consistent terminology to the extent possible. Dr. Tepfer.

DR. TEPFER: I think that some of the difficulty we're facing has to do with a tradition plant pathologist or division of susceptible resistance and the definition that is proposed in the appendix which specifically focuses on transgenic plants which is a bit of a different situation. We have lots of cases that don't seem to exist in transgenic plants and things that may exist in transgenics that don't exist elsewhere, so that's part of the source of the confusion I think.

DR. KRAMER: I think we have the information that we need, but could I also ask, I'm not sure if this is the correct place, but I wanted to go back to something Dr. Zaitlin said about the definition of a PVCP-PIP.

MR. ROBERTS: Sure.

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DR. KRAMER: I just wanted to read into the record again the definition that we're using because I'm not quite clear on what point you're trying to make. PVCP-PIP means a plan incorporated protectant created from the gene or a segment of the gene that coats where a coat protein of a virus that naturally infects crop plants. So within --

DR. ZAITLIN: I was taking -- a slide that talked about controlling virus infection, that's the words I saw on one of those slides.

DR. KRAMER: Looking at the slides here this

is the definition that we're using for a PVCP-PIP. I think probably what you're referring to may be the taking that and using that to how we define it as a pesticide, but the definition of a PVCP-PIP in and of itself is simply the plan incorporated protectant created from the gene or a segment of a gene that coats with the coat protein of a virus that naturally infects crop plants and that's -- if you disagree with that definition then please comment.

MR. ROBERTS: I don't hear a disagreement. Have we handled at least for now the

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terminology issues?

DR. KRAMER: I think so.

MR. ROBERTS: Then let me ask you, this came up as I had asked you about our response to this question 6 are there other follow-up questions are related to this, is our response reasonably clear on 6 the one we're just finishing up?

DR. KRAMER: Yes.

MR. ROBERTS: Let's go ahead and do question 7.

DR. KRAMER: Please specify techniques that do or do not provide measures of tolerance and/or resistance that are relevant to field conditions. And I guess in light of our prior discussion I would change this to say please specify techniques that do or do not provide measurements of tolerance and/or immunity that are relevant to field conditions.

MR. ROBERTS: Dr. Stewart.

DR. STEWART: Well since I'm not a virologist per se I'm probably not the best guy to lead off with, but I will go ahead and take a stab on the basis of other transgenic plants that provide some type of

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difference in fitness, but this will be short and I certainly hope my associate discussants will come to the fore here.

Greenhouse and growth chamber experiments are marginally predictive of selection pressure in the field as the result of environmental effects and local viral load that very spacially and temporally or I would say that one would expect a very spacially and temporally in the field, that said there seems to be merit in using greenhouse and growth chamber experiments in assessing viral tolerance if for a number of viral strains and plant genotypes is used.

It is preferable to use specific transgenic events into wide range of viral strains and challenge experiments. Viral load can be assessed by alisa (ph) or other molecular slash biochemical techniques, disease can be assessed by visual asas (ph) in many cases, crop yield however integrates among resistance, tolerance, and other variables as a rough index for fitness.

MR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I looked at this

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mechanistically and thought about things that you could measure that would reflect tolerance or resistance and I would go to resistance in this case rather than immunity, immunity is immunity, there is not much you can do to it to measure it except say it exists.

There are degrees of resistance that may be useful or may not be, but tolerance can be measured as high viral tighter without symptoms or with minimal symptoms and resistance can be measured as reduced tighter compared to a susceptible plant. Measures of resistance can be utilized through production of total biomass, yield of specific components, height of the plant, leaf number, effects on shooting, flower number, seed number, seed size, lack of symptoms, and persistence of the plant in the environment.

And any of those components can be affected by the environment under which the plant and the virus are growing, so some things will have little effect at high temperature and have much more pronounced effect at low temperature or vice-versa depending on the virus and the plant concern, for example I have one

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virus that I have been working with recently and using a house plant and a cotiomabenthamiona (ph) and under most conditions it produces a mozaic or model, but if we grow the plants in a growth chamber under low temperature conditions we get first localized necrosis and then systemic necrosis, so you can have something that normally does very little to the size of the plant but produces from visible symptoms and under low temperature conditions produces necrosis starting out localized and ending up with terminal necrosis which essentially ends the productive life of the plan, so it is growth chamber experiments under a variety of conditions necessary for a full assessment and there are many measures by which you can determine the degree of resistance or tolerance.

MR. ROBERTS: Dr. Falk.

DR. FALK: I will further confuse this, so I think in terms of this question I would add provide measures of tolerance or resistance or susceptibility that are relevant to field conditions, and I think that screening -- it is very difficult when we are doing any sort of screens in greenhouse or growth

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chambers or whatever because typically if the virus we're using is mechanically transmissible that's what we do, it is much easier, this is really quite artificial, where typically in mechanical inoculations we have a much greater inoculum load than is encountered naturally than would be encountered in the field and susceptibility from mechanically inoculation must be interpreted in that light I believe.

I think that under natural conditions the great, great, great majority of plant viruses are

going to be spread by specific vectors that transmit them, and things like pubescence or the hairs on leaves will affect natural infection and susceptibility in nature whereas the plant itself may be fully capable of supporting virus replication if you mechanically inoculate it, but if it is transmitted by aphids in the non circulative, nonpersistent manner the plant can show effective resistance under natural conditions.

Similarly if the aphid or other insect has to feed in the flowum (ph) the plant can be perfectly susceptible to virus replication and transport, but if

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the vector cannot find the flowum where the virus has to be delivered the plant will issue effective resistance, so I think inoculation protocols do not give good measures of tolerance or resistance and must be considered. Even contemporary inoculation procedures using Agrobacterium to deliver clone DNA's, the Agrobacterium has its own defined host range and we can make mistakes as we have where we can say the plant is resistant to the virus when in fact it was resistant to the Agrobacterium that was used to deliver the virus.

In regards to susceptibility, I think when we measure phenotypic or biological effects after experimental inoculations those also give a false interpretation of the significance and natural effects due to virus infection as both the co-discussants here have mentioned, environmental conditions and et cetera, time of infection, all of these effect the severity of the symptoms and how we might measure susceptibility resistance or tolerance.

MR. ROBERTS: I would like to ask other members of the panel to contribute to this, Dr.

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Tepfer.

DR. TEPFER: I want to ask for a bit of a clarification, could we consider perhaps a mechanical inoculation as sort of a worst-case technique for inoculation. Are there cases where you have better resistance with using a mechanical inoculation as compared to vector mediated infection because in our hands it usually seems to correlate fairly well with the few viruses that we've worked with, are there cases where it doesn't work.

MR. ROBERTS: Dr. Falk.

DR. FALK: I'm not sure about how answer that, in what I was talking about here it says relevant to field conditions and that was my point of bringing things up like leaf pubescence because we know those plants are perfectly susceptible and we could inoculate them mechanically and we could inoculate them if we forced aphids on there, but under natural conditions they do show effective field resistance or tolerance, so I'm sure there are examples both ways in terms of what you're saying, but

I'm just trying to say -- what I was trying to say and what I represented earlier is that I think we have to take care in interpreting the significance from what we've done under these controlled conditions and how those really relate to what's happening under natural conditions.

DR. COOPER: I would just say that resistance is very difficult to measure except you can make a model of the field in a plant pot in a glasshouse and you can take soil containing nematods (ph) of fungi carrying the viruses and put your plant under test in it and that's another way to get an estimate of ability infect relative to another species of cultivar or whatever -- we routinely do that.

There was viruses that we study that way of course you transmit mechanically but very often inefficiently and therefore you don't chose to use that, it's really where the viruses have a particular close relationship with the vectors you can't neglect that reality in the assessment of resistance and that's the best you can do with some of the systems.

Others of these vectors, the mites and the other animals are sometimes very difficult to handle and they can't easily be mimicked.

MR. ROBERTS: Other comments from panel members, Dr. Nagy.

DR. NAGY: I would like to add one thing to Dr. Tepfer's comments is that I think it's lots of times the tolerance and how we measure it is also, there is a delay in a symptom induction in this kind of thing, so I would like to add that the time of measurements is a major factor in estimation.

DR. KRAMER: I'm just wondering, we heard from Dr. Falk's examples of techniques, specifically manual inoculation would perhaps not provide an accurate measure of tolerance or resistance relevant to fields condition but could you maybe suggest something that would, would field experiments for example be necessary?

DR. FALK: I think that mechanical inoculation is what we do because we can do it, I was only trying to suggest that it's not perfect and that we remember that. I think in terms of any then to interpret what we see from our experimental inoculations in the greenhouse to field conditions we will have to do field experiments of course, I'm not trying to argue against mechanical inoculation, we're all going to do that, right, but it's just not to assume that because things work that way that they're going to apply to the field.

MR. ROBERTS: Dr. Sherwood and then Dr. Melcher.

DR. SHERWOOD: Just to add to that in a plant improvement program I was involved in resistance was

just a component of that overall program and so when you began working with plant breeders they're looking at another agronomic features, not just resistance that have to go hand-in-hand and basically they rely on the disease nursees (ph) which are conducted out in the field so the two go hand-in-hand to bring something along as a variety and as Dr. Zaitlin said you know you're looking for perhaps one that's less sicker than the parents were.

MR. ROBERTS: Dr. Melcher.

DR. MELCHER: I'm not sure I am going to get the words right but I would like to ask Dr. Falk if on the laboratory inoculations is the problem that one is

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seeing false positives but not false negatives, in other words it over estimates the resistance of the tolerance but if there was a resistance or a tolerance that -- there's not going to be a resistance or tolerance that shows up in the field that does not also show up in the laboratory, I guess that's the crux of it.

DR. FALK: I'm not sure I understand what you said. I think what I was trying to say was I think there is effective natural resistance tolerance to infection that can be missed by experimental severe inoculation in the greenhouse.

DR. MELCHER: I'm confused, I withdraw the question.

MR. ROBERTS: Let me pose a follow-up questions, are there any techniques that are not relevant to field conditions such that you would say that it really has no value, I don't mean to put words in Dr. Kramer's mouth, but as I read this you know I think they're trying to get information from us in terms of which of these techniques have value and which don't and it sort of has a tendency to kind of

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dichotomize them and I realize that there are shades of gray and they have value, but to the extent that which we can identify techniques and that just really have no relevance and shouldn't reply on them from making decisions about what's going on in the field are there any that the panel can identify?

DR. FALK: I didn't' mean to say that non of them are useless because they are all useful, but I just thought that all of the things that we do must be interpreted in the context of how we've done them is all I'm trying to say.

MR. ROBERTS: Dr. Hammond.

DR. HAMMOND: May I ask what the relevance of this question is to the charge of the panel because farmers aren't going to grow something that does not have relevant tolerance or resistance.

DR. KRAMER: We're really looking at whether it would be possible to identify wild or weedy relatives having already tolerance or resistance to the virus whose coat protein was inserted into the

transgenic plant.
MR. ROBERTS: Dr. Tepfer.

DR. TEPFER: That puts a very different light on it because I think we know of several cases of plants that when you take them into the greenhouse, wild plants you can infect them using mechanical inoculation which are simply never infected in the field, so I think that doing the experiment we were all thinking in the other sense, you have possibly resistant crop plant, what happens when you take it to the field, but going in the other direction I think that we are in a complete, a rather severe black box in fact, I don't think we have very much knowledge about how easy it is and what are the predictable ways of doing it in the greenhouse, that type of experiment maybe other people...

DR. HAMMOND: I would like to follow-up on that because we find especially with some houseplant virus combinations that you get nothing most of the year but a few weeks in the fall and a few weeks in the spring you can do some useful work, and there are a lot of environmental variables, the growth stage of the plant, the quality of the day length, the physiological state of the plant whether it's in

active growth, whether it's entering a reproductive phase that have enormous influence on how easily you can infect it, you can put plants in the dark for a day before you inoculate them and then succeed in infecting something that you cannot ordinarily succeed in infecting, and that is a very artificial means and is not relevant to what's going on with weeds in the field so you have to be much more careful in drawing inferences about weed plants which is why I asked what the relevance of this question was because I was looking at it from the crop end.

If you're looking at it from the weed end you do have to be very careful with the environmental conditions under which you look at it and look at the natural vectors rather than mechanical inoculation and look at it with low vector populations and high vector populations. In that case it is very relevant and if you do it mechanically and under ideal plant growing conditions you will see things that will be of no relevance at all in the field.

MR. ROBERTS: Dr. Kramer if it sounds all right with you perhaps in our response to this we will

clarify at the beginning of the response that you clarified that we were really talking about this from the weed and that way it will by easier for our response to be understood, so let's just as a note to us when we're writing our response we just indicate that we clarified that and with that clarification our response is and then -- Dr. Stewart.

DR. STEWART: And so this really does pertain

to wild plants, not weeds per se.
DR. KRAMER: I would say both.
DR. STEWART: Because weeds are -- most weeds that weed scientists would call weeds are recently evolved entities are very different from wild plants, that's just a note of clarification. We can take a look at all of things I suppose.
MR. ROBERTS: Dr. Sherwood.
DR. SHERWOOD: I would just like to add that one just has to look back to the beginning days of plan virology where one would go out and collect native species and use those as a range of indicator host to look at what the reaction of various viruses, what their reaction of various viruses was and

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certainly they would not be ones that you would find in them in nature but could artificially inoculate them to either systemic infection to the virus purification or local lesions in order to isolate individual isolets, so all the work through the 20s and 30s was done with wild species or native species to differentiate plant viruses before you had the tools of today.
MR. ROBERTS: With the clarification then we've had, Dr. Hammond and Dr. Tepfer had certainly responded and I want to just ask the panel that with that clarification does anyone else want to respond differently than what they responded for? Okay. Then back to Dr. Kramer, with that clarification is the response from the panel reasonably clear or do you want to ask some follow-ups?
DR. KRAMER: I think it is reasonably clear that we don't know a whole lot about how to do this relevant to field conditions if I understand correctly.
MR. ROBERTS: Is there any disagreement among the panel on that statement? I don't see any.

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Let's go ahead then and take question number 8.
DR. KRAMER: How do environmental or other factors for example temporal variations effect tolerance and/or resistance given the expected variability what measures of tolerance and/or resistance would be reliable. And again I would say I think we mean to use the term immunity as we've just discussed here rather than resistance.
MR. ROBERTS: Dr. Zaitlin.
DR. ZAITLIN: I just knocked my name tag over the edge of the desk.
First of all my response is a repeat of something I've already said, anyway it says that most plants are resistant to most viruses, disease is the exception, in many cases the disease resistant plants exhibit no symptoms but it is also apparent in the relatively few cases that have been investigated the virus can infect the resistant plant, the initial cell

of entry, but the virus cannot spread from that site so no disease results, thus much resistance is affected by an inhibition of cell-to-cell movement

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rather than a restriction on virus replication per se, environmental factors can affect this mode of resistance, but it has not been studied extensively.

Principally elevated temperatures can encourage virus movement and such movement may break conventional resistance, this is particularly evident when resistant results in a necrotic local lesion. This phenomenon has been well studied and can result in a systemic movement of the virus, furthermore when the ambient temperature is reduced subsequently the whole plant then can become necrotic.

Resistance to plant viruses generated by plant breeding involving incorporation of resistance intecrops from other cultivars or species is often not stable because viruses can replicate in such plants, and as we discussed before resistance is often scored as a reduction of symptoms and thus there is a selection of variant virus isolets (ph) that can overcome the resistance. Fewer than 10 percent of the 54 host virus resistant gene combinations enumerated by phrase and gurwitz (ph) in review in 1987 remained effective over a long period.

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The effect of the environment on this process has not been investigated although it is probable that environmental conditions that enhance virus replication would increase the probability that resistant breaking virus isolets could be induced or selected for.

On the other hand, coat protein induced resistance has proven remarkably stable in the most prominent case papaya ring spot virus in Hawaii. The resistance is viral strain specific or isolet specific, but ring spot isolets from either regions of the world could overcome the resistance in laboratory tests, it does not happen in the field. And I have recently inquired at my friends at the University of Hawaii who confirmed that. They are concerned however that there may be some isolets being generated at papayas other than the big island where the papaya ring spot virus resistant plants are grown may be evolving.

They are trying to actually overcome this by pyramiding virus resistance sequences to these other isolets. The other commercial application of coat

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protein made of resistance out of viruses in squash is more complicated in that the plants have resistance to three viruses, thus the probability breakdown of resistance would be enhanced, but I know of no reports of that happening and perhaps Keith Reddenbaugh (ph) who is here from Siminus (ph) could confirm or refute that charge.

The conclusion is that resistance breaking is a function, is most probably a function of changes in the virus not the plant in both conventional resistance and coat protein (inaudible) resistance. And ask my colleagues if they really know of instances where the plant gene itself, resistant gene itself is modified to breakdown the resistance. I think comparing the two types of resistance, the natural and the coat protein mediated resistance, it seems to be the most stable and most reliable.

MR. ROBERTS: Dr. Melcher.

DR. MELCHER: Regarding the first example that Dr. Zaitlin mentioned this is I believe the case of tobacco mozaic virus interacting with the N gene of tobacco and in that case it is an indirection between

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the virus and the plant that is breaking down, so I don't think that we can really attribute it to either the virus or the plant, it's the two working together.

There is another case where temperature has in effect on the breakdown of resistance and that is in an effect that is well known for all organisms and that's heat shock, when there's a sudden increase in temperature the organism shuts down the synthesis of most of its proteins and turns on another set of proteins called the heat shock proteins, the ones that are turned down should include probably the proteins that are involved in resistance, so as far as temperature goes that's definitely a environmental factor that would affect tolerance and/or resistance and I still want to call it resistance and I will get to why in a second.

I don't know about other factors, perhaps I can rely on my colleagues, other factors might be plant water status, light intensity, light durations, solenity and so forth, they may have effects on a breakdown of resistance, but I am not sufficiently an expert to say anything about them.

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Tolerance is important for the survival of the tolerant species, but it allows the creation of virus reservoirs for transmission to other species that are neither tolerant nor resistant and I really don't know anything about environmental factors relative to tolerance but they are probably important there. What I may know something about is the measures used for measuring these things and they were discussed in response to the previous question to some extent but not completely, so maybe it's worth my going into that a little bit now.

The lowest level of resistance which is what I call immunity and I think some of my colleagues agreed with me on that, there is not replication. To test for that the test is either to take isolated cells from the plant and try to infect that and see that there is no replication in those isolated cells or to do some kind of a detection where you can look

at single cells and say the leaf, one way would be to use a virus that is tagged that will express for example gene fluorescent protein and then after the appropriate incubation time look at the leaf and see

if you can find single cells that are fluorescent green, if you cannot find any then there was no infection of even a single cell.

The same assertive technique can be used for the next level, subliminal section, subliminal infection, if you find just a single cell without a cluster of cells being fluorescent then that is a reflection of the inability of this virus to move out of a single cell and would be a subliminal infection. The further levels I think then you begin to get into things that have been mentioned before, eliza (ph) various nuclaic (ph) acid detection techniques like hybridization and RTPCR.

I think we're supposed to say how these would be reliable as far as looking at environmental factors, I think the extrapolation is obvious that if you're interested in how the environmental factors affect resistance or tolerance with these methods you have to do the experiments under a variety of conditions, variety of temperatures, light intensities, and so forth, and I believe that's all I can offer, not very much I'm afraid.

DR. KRAMER: I just wanted to make a point of clarification, I've not sure if it's necessary, but given the misunderstanding of the last question I thought I might go ahead and do that.

This question number 8 directly follows from question number 7 where we're really looking at whether it would be possible to identify tolerant resistant immune plants that were relatives of any BCP transgenic plant and therefore you might be less concerned about the transfer of any type of resistance to that population and so when we're looking at how well environmental factors may impact those measures are really considering whether it's possible at all to measure those given the types of variations that you would expect under natural conditions.

MR. ROBERTS: Dr. Cooper.

DR. COOPER: A lot of would argue the least you can do is you can collect seeds from wild populations, bring them in the glasshouse, you challenge them with a virus that's in measure which you are going to then follow-up as we described earlier. It is not an absolute measure and it doesn't

easily relate to viruses obligated transmitted by pollen or in some sophisticated way, but at least it gives you one measure and then you look at the plants, you test the plants and then you come into the area of considerable debate as to whether you use this word to describe what you found as some other word.

MR. ROBERTS: Dr. Sherwood.
DR. SHERWOOD: And I would add that tolerance is probably very easily measured out in the field because most of the weeds that are seen are nonsymptomatic for virus disease yet will test positive for viruses under conditions and so that plan I guess in the terms that we're using today will be tolerant to that virus infection since it was nonsymptomatic yet positive in some test for the virus.
DR. STEWART: I have a question to the virologist about some other environmental effects that I haven't heard about, whether these could be important or not such as increased UVA or UVB, increased ozone or soil contaminants, say heavy metals or whatever, could that affect tolerance or infection

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or disease for that matter, and I'm thinking especially now if we bring wild plants or other plants into the greenhouse or growth chamber where these things are probably not going to be factors does that make any difference.
DR. ZAITLIN: I think light has been investigated in this case and is it has to be light of photosynthetic quality, it's a common practice if you're conducting an essay, you put the plants in the dark beforehand and then you can then infect them better, but you put them in the dark in the absence of CO2 I think it won't work well.
DR. TEPFER: I will just sort of answer rebounding from what Dr. Cooper said that you can still use I think the test by mechanical inoculation in the greenhouse as distinguishing at least between susceptibility and non susceptibility, so that if you admittedly all of these things like ozone or water stress or UV in a particular way the plants are generally more susceptible and if you can then still not infect them that probably means that that might be rather difficult to infect, but if they are infectible

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in the greenhouse in these rather soft conditions then you have a harder question to answer.
DR. ZAITLIN: I would like to ask my colleagues if they know of any case where the actual plant gene itself has broken down. I know as Dr. Melcher pointed out I mean these resistances are an interaction between a virus and the plant gene whether it be a transgene or a natural gene, but I don't know of any cases numerated where shown that a mutation in the plant gene has in and of itself caused the resistance to break down, does anyone know?
MR. ROBERTS: For the record there was no positive response.
DR. STARK: Just a comment and maybe a point of clarification for me because I'm not a virologist as well. It would seem to me, I agree with what's been said over here by Mark, you want to start with

the worst-case scenario to see if you can infect a plan but then ultimately what would really be good would be to have some standardized techniques. We've only done this in toxicology quite a bit as well where different labs do things very differently, they expose

organisms in a different manner, at different times of the day, at different life stages, things like this, and it can all have great influence in susceptibility on toxicants and I assume the same thing would hold true with disease, so it would be nice to have a series of standardized approaches when trying to investigate whether or not you're going to have problems like gene flow and other disease transmission.

MR. ROBERTS: I get the sense that the Department is asking us for advise about how you might construct those tests or what they should look for in those tests, especially with your clarification, Dr. Kramer, on 7 and 8 is ability to do tests other than field tests that would have some sort of predictive value and what can you do, what can be reasonably done, what sort of techniques can be used, and given the fact that environmental factors can vary what needs to be paid attention to, what would you need to vary or sort of work into you -- what would be the key things to work into your studies to be sure that you produced results that might have value in the field or

predictive value for the field, is that where you're headed?

DR. KRAMER: Yes.

MR. ROBERTS: Okay. Dr. Tepfer.

DR. TEPFER: In just response to John Stark's precise point, I'm afraid that's a very sort of utopian idea, if you you've been working on a small number of plant species, each single plant species has a different sort of optimum situation for infection and indeed there are lots and lots of factors that intervene in terms of just growing the plants in the greenhouse, the age of the plants, the quality of the inoculum, the list is extremely long, so I don't think that for going from one plant species to the next or even to one virus to the next on a given plant species you can come to some sort of standardized sort of protocol, all you can do is say well things like viral plants will be often more susceptible when they're younger or well if the leaves are sort of softer it would seem to be easier to infect, and so can sort of make a catalogue of generally how to increase susceptibility, for there to go to standard conditions

I don't see how we can do it given the diversity of the plants the viruses.

DR. STARK: Good point, even within the animal realm you run into the same kinds of issues, good point.

DR. STEWART: So speaking of things in the utopian realm, since viruses aren't fairly simply as far as things go, could there ever be any proteomic type approaches where you look at protein interactions to be able to predict susceptibility, if you could take the whole gumish from the plant and probe that on some type of viral platform, since we are running ahead of time.

MR. ROBERTS: We can have some of this side bar discussion, but my concern is I don't think we're really giving the information or the advise they're looking for on frankly 7 or 8, and I think we may need to work a little more to try and get back to that, but we can entertain your discussion, your question a little bit loosely. Dr. Melcher, were you going to respond to that?

DR. MELCHER: Well, yes, with proteomics as I

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understand it you need to know something about the proteins that that organism makes, and if we're talking about of these wild and weedy relatives I doubt that there would be very much information about the proteiums of those to do any comparisons with.

DR. COOPER: I would also say that from experience with brassica proteomics and genomics they environmental conditions are very influential on the outcome of what you see, and therefore the environment is definitely a parameter that you have to build into your experiments at great expense.

MR. ROBERTS: Back to questions 7 and 8. DR. Sherwood.

DR. SHERWOOD: I will give a shot at that, I think the use of mechanical inoculation and or appropriate vector inoculation will help in determining whether a weed species is a host or not a host, but in terms of hooking at the specific parameters in regards to virus replication, how it is going to act in the field, that that is not going to be -- there's not going to be an approach to do that in the growth chamber or the greenhouse, that would

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have to be done under field conditions.

MR. ROBERTS: In doing those studies looking at question 8, are there some key variables that you need to address in your study which I think is what question 8 -- with your understanding about environmental or other relevant factors, what kinds of things would you need to look at or incorporate into those studies? Dr. Sherwood.

DR. SHERWOOD: I think they have all been mentioned and generally as a virologist you are trying to put the host in the most susceptible condition, so inoculating young vigorous growing plants, preparing your inoculum so it has the highest degree of infectivity, darkening the plants before inoculating them, we used to put wet paper towels over the plants after they were inoculated so the leaves didn't dry

out, whether you're going to use carborundum (ph) or carbide, or whatever else you're going to use, all of those things are kind of in-house, I don't know, witches brew or each lab differs a little bit differently and how they go about inoculating plants.

DR. KRAMER: So can I just try to reiterate

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MR. ROBERTS: Yes, please do, and if we are still not getting this right please let us know.

DR. KRAMER: From what I'm understanding is that it may be a fairly simple task to identify a plant that is the is tolerant or resistant, if you able to show that through manual inoculation or other such techniques that you aren't getting a virus infection that's a reasonable conclusion to make.

The converse isn't necessarily true and then if you are able through laboratory techniques to show that you are able to get infection can't necessarily apply that directly to a field scenario and in that that circumstance you're just faced with a much more daunting task to try to demonstrate that there would be true tolerance or immunity under natural conditions given the type of variation that we can expect under environmental conditions.

DR. COOPER: I will just make one final comment on this, Rothomstead (ph) a well know virological center at one time, the Scottish Crop Research Institute another one, had kinopoteium

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quinoris (ph) test plants. The kinopoteium in each of those two glasshouses reacted very differently to a whole range of viruses, they were as far as one could judge the same species but for unknown reasons they were very different and would not help your approach.

MR. ROBERTS: Despite that comment is -- I want to be sure it is, do we agree with Dr. Kramer's sort of summary back of what she heard us say on this point, yes, yes, all right, good. Dr. Hammond.

DR. HAMMOND: I essentially was going to back up what Dr. Cooper said and the people -- the (inaudible) in the Netherlands had collected ketopoteium seed from various sources and inoculated it with a number of viruses and found considerable variation in the susceptibility.

MR. ROBERTS: All right. Dr. Kramer, do you think we have done this?

DR. KRAMER: I think so.

MR. ROBERTS: As good as we're going to do. Let's do one more before we go to break.

DR. KRAMER: Question 9, what would be the ecological significance if a plant population acquired

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a small increase in viral tolerance and/or resistance above a naturally-occurring level. And perhaps I can just start off with a verification of this question and that will be we're really considering again if we

can identify that there is natural tolerance or immunity within a plant population that's a wild or weedy relative of a VCP transgenic plant is there any ecological significance of conferring upon that plant additional tolerance and/or immunity from the incorporation of the PCP transgene.

MR. ROBERTS: Dr. Stewart, you're a popular guy as a lead discussant.

DR. STEWART: Well, so this is going to touch a little bit on, this question and the next question, and I'm going to through in some stuff on this last clarification so if someone wanted to be provocative earlier this will be as provocative as I can get.

So gene flow is defined by the formation of hybrids and back cross hybrids is not a risk per se, gene flow is not a risk, the consequences of gene flow may be, theoretically a small boost in viral tolerance or resistance under constant and viral pressure could

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cause an increase in relative fitness, an increase in fitness would theoretically cause an increase in transgene frequency that would eventually be fixed in a population.

This scenario would not necessarily confer increased competitiveness in plant communities however and this scenario pertains to a directly transformed plant of an isogenic line that is a crop not a wild plant. There are many generations from a transgenic crop to introgress near isogenic transgenic wild plants. In F1 hybrids the host genome will contain proportional genomic constituents of the two parents.

In BC1 hybrids with back crossing onto the wild plant and selection for the transgene and assuming equal size parental genomes an average of 25 percent crop genome would be in the BC1's along with the transgene, and BC 2's 12.5 percent, the BC 3's will have 6.25 percent crop genome and 93.75 percent wild genome on average. And this is as far as back crossing usually gets for testing of wild relatives, so while most BC3 plants will appear to be very similar to the wild host, they're expected to contain

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around 2,000 crop genes along with a single transgene or two or three transgenes affecting, altogether affecting the fitness landscape, that's on average in an average in advance transgenic background back cross plant such as BC3 the transgenic effect can be expected to be swamp by the hitchhiking crop genome effect, that's ecologically insignificant. With apologies to John Dunn no gene is an island.

So if you are not back crossing onto a tolerant wild relative you could look at putting in a little bit of gasoline onto a raging fire, therefore once again it would have an even less effect then if you were back crossing onto a susceptible wild relative host. If there's already tolerance you know what's a little more tolerance, and so if you add to

the natural barriers to introgression, physical containment, genous restriction technology, transgene mitigation technologies, and male sterility, there would be almost complete barriers to introgression.

MR. ROBERTS: Dr. Stark, do you have anything to add to this?

DR. STARK: No, I'm going to pass on that, I

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think we covered it very well.

DR. COOPER: Probably not much effect, the breeding system might be crucial, out crosses would be less affected than inbreeding types and importantly I think in any increase in the magnitude of the virus would have a potential to create more viruses available for evolution to take place if you had a tolerance sort of situation in that circumstance, so if that simply addresses the question probably not much.

DR. SHERWOOD: I just take and using the words as we are, with immunity I don't see if the plant isn't immune already I don't see how we could increase immunity and so that then gets us to viral tolerance, and if we're using tolerance as we are what would occur perhaps is an increasing amount of virus in the plant, but let's take us out of ecological consideration in that the rate limiting step in all of this is going to be the movement of the virus by the appropriate vector and that's going to be the gateway as to whether or not it's going to have an ecological impact, and the question would then be increase if you

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increase the amount virus in a plant it's more tolerant is that necessarily going to lead to more transmission within the crop and within the weed and I don't think if we really know that in terms of whether a high virus content plant is a much better source of virus leading the epidemics than a plant that has you know maybe half as much virus, where does that break point occur.

DR. ALLISON: I belive that my comment is basically the same as yours, in terms of resistance if there's a lesser amount of virus within the wild population then there's a less inoculum for the crops species itself and this is what Dr. Beechie was referring to in his comments.

DR. MELCHER: I guess I can try to go one step further and consider population dynamics over a longer period of time, if the transgene gets into the wild species does provide some sort of advantage even though I agree with Dr. Stewart is not very likely, that means that there is a gradual reduction in the virus population that will be effecting that species in that particular region and with the reduction of

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the virus population there is a reduction in the selective pressure to keep the transgene, so even though the transgene may be established temporarily,

in the long run it will be reduced probably to low levels. I'm not a population geneticists, but that seems reasonable to me, maybe others can correct me.

DR. STEWART: There's several things swimming in my brain here as far as viruses and wild plants and crops and weeds, so virus evolution is faster than plant evolution, I guess that's a fair statement. So if we're talking about crops and I'm thinking about a monoculture here, monoculture of a crop where you have a big target for the virus, that's going to be a different situation than a wild plant community which would be fairly diffuse, that is the number of wild plants of a particular species that could be a wild relative would be of a much less dense than the crop, so I'm trying to figure out why wild plants really matter very much at all.

I can understand why weeds might matter because the weed density in crop fields can often be as high as the crop density, so weeds and wild plants

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are two different things, and I'm not sure where I'm going with this, but I'm hoping one of you can tell me.

MR. ROBERTS: Dr. Cooper.

DR. COOPER: Could I just make a comment on the context of biodiversity conventions that the U.S. may not subscribe to, but in other countries the diversity of the plant population whenever it grows could be relevant to a risk assessment and therefore wild plant numbers, diversity, performance, and such like could become an issue.

DR. STEWART: So on the risk assessment we're usually predisposed to think about creating increased weediness, increased invasiveness, if we're talking about decreasing the competitive ability or fitness of a wild plant species population whatever or creating hybrids that will place another species in jeopardy and I think that's a totally different thing.

Something that's often not appreciated, I don't know how many examples there are in real live where a single transgene might actually place a plant population or species in jeopardy, it's worth

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considering anyway, I think it is especially worth considering by the EPA, I will say that.

MR. ROBERTS: Any other points on this question? Dr. Kramer.

DR. KRAMER: I think that's fine, thank you.

MR. ROBERTS: Let's take a 15-minute break, let's try to reconvene at 3:50.

(Break.)

MR. ROBERTS: As we begin our discussion I just want to give everyone notice that it's my intent to just take up one more today, number 10, because we will have a change in topic to viral interactions, we'll begin with that one first thing in the morning. I would like to go ahead and take 10 then I will offer

a brief opportunity for go backs, we don't do a lot of those, but if there's a comment that you in the discussion of the first ten questions that we've covered today that you forgot to make and we moved on since we're moving kind of quickly I'll give you the opportunity to go ahead and address that now and then I would like for the panel to meet in closed session just to discuss planning for the write-up for the

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minutes for today's first session.

So let's go ahead and take question 10.

DR. KRAMER: Please comment on how necessary and/or sufficient these conditioners are to minimize the potential for the PVCP-PIP to harm the environment through gene flow from the plant containing the PVCP-PIP to wild or weedy relatives. Would any other conditions work as well or better? If we go to the next slide then we actually have the conditions up there and I would like to read through those, number one, the plant into which the PVCP-PIP has been inserted has no wild or weedy relatives in the United States with which it can produce viable hybrids in nature, for example, corn, tomato, potato, or soybean.

Number two, genetic exchange between the plant into which the PVCP-PIP has been inserted and any existing or wild or weedy relatives is substantially reduced by modifying the plant with a scientifically documented method, for example, through male sterility. Or number three, it has been in periodically demonstrated that all existing wild or weedy relatives in the United States with which the

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plant can produce a viable hybrid are tolerant or resistant to the virus from which the coat protein is derived.

And it would just like to clarify this question to make sure people understand how the Agency is envisioning these three factors here, and that is the work group came together before getting the panel's advice and tried to come up with factors that the Agency could potentially use to evaluate when a product would be of such low risk, that it might not be necessary to undergo all regulatory requirements at the Agency. And these are the factors that we were able to come up with and now we're asking for the panel to comment on these particular factors.

MR. ROBERTS: Thank you, Dr. Kramer. Dr. Cooper, could you lead off for a discussion on this one.

DR. COOPER: Well I would say that we addressed question 1 to this morning to some degree and I have little to add. In question 2 it's important I think to recognize at least one mechanism, male sterility has the potential to impact on wild

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life because wild life eats seeds and even pollen, and therefore that aspect should be considered if not

given a lot of weight. And they have at least the potential to prove a risk of harm on wild life diversity I would suggest in the environment.

I generally subscribe to the if it can happen it will happen school and the evolutionary time scales therefore are rather more important than whatever have been implied in some of these statements earlier, but question 3 is certainly an area that I have greatest uncertainty with, perhaps even the least useful, it should be perhaps replaced with some reliance on the specific virus isolets that's being considered co evolving with the crop, viruses change and get selected for in local conditions.

Pathotype, the concept of the fact that a virus that infects one sort of plant may be a different virus in the genetic sense to the same virus that it doesn't infect the same plant, so pathotype is one of the terms used in circumstances like that where the host range is important.

Furthermore the virus from which the coat

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protein was derived may be very different from the one that it's protecting against, so lettuce mozaic virus provided by my colleague here on my right was used genetically engineered brassica in hybridization experiments because there was a benefit against turnip mozaic virus, both related in the sense of being potty (ph) viruses but different viruses and I have no evidence that lettuce mozaic virus will be detectable under that name in brassica's, but I have to say I haven't personally looked for it.

There may be no effect for all the variety of reasons we talked about, linkage drag in particular this morning, but all I would say is that it is very difficult when you have the diversity of plants, the diversity of the viruses, and they should all be considered in your case-by-case risk assessment.

MR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I have little to add to that. I think that in general if the coat protein is being deployed against the virus from which it came there is very little reason for concern and these conditions should therefore be suitable and appropriate.

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DR. STEWART: I would agree with Dr. Hammond these seem to be fairly a conservative set of conditions to give something a free pass.

DR. TEPFER: Well since Neil is not being provocative I guess I should instead.

DR. STEWART: I was provocative on the last question.

DR. TEPFER: This is my turn. Just in regarding the second point in all seriousness I think a lot of the sort of confinement techniques trying to reduce the gene flow are not a hundred percent effective and therefore if we go along with what Ian was just saying if it can't happen it will, then we

need to really carefully consider other sorts of strategies. And I would simply like to suggest that it is time for us to overcome the tabu of talking clearly about the usefulness of gurt strategies as gene flow preventive mechanisms essentially.

I think it is extremely important, we could have a very, very, very valuable resistance genes put into plants that cross readily with terrible weeds, if we have a good girt strategy behind it that really

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will keep it from being transmitted we should be able to go ahead and do it. And I think that we're at a point where we need to come out and say it and so that's I'm doing so.

And another point I would like to say in this regard is that if there were a girt strategy that was made freely available so it's not a question of industrial strategies to try to take over the world of the seed markets and things like that which is a lot of the opponents in Europe are using is a way of knocking people over the head, trying to prevent girt strategies from being implemented is just the big companies trying to take over and so on, if there were gurts that were available to small companies to people in academic labs, particularly to work on some of the less important crop lands once for developing countries, this could really turn around an enormous perceptual issues about using gurts, what they're for because people completely forgot these are gene confinement strategies and not just ways of trying to monopolize germ plasm.

MR. ROBERTS: Dr. Cooper.

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DR. COOPER: I was intending to say something in this context in another part of the program, but recombination mediated by safety measures like girt, especially cre lux indegrays and G jix cleavage (ph), have the potential to release something that might be living in the genome of the plant and I think it would be prudent for an experimenter to have diligently investigated the genome of the host to look for occult virus genome segments that might be triggered as a result of this technology, so although the technology is perfectly appropriate and should be available I think it is prudent in this area where there is more and more information albeit not always public information, but at least the experimenter should have realistically have access to the genomic information pertaining the crop they're concerned with, and to investigate that as a prelude to or in parallel with doing the experiments would be useful in case genie gets let out of the box.

At the moment the only sorts of viruses known in these occult forms are I supposed DNA contained viruses that are rather a rare phenomena, but at the

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moment we have no clear measure of how prevalent they

are and it is a concern.
MR. ROBERTS: Other points?
DR. STEWART: Well I just might add so girt is for the record keepers gene used restriction technologies, g-u-r-t. There is always the tandem mitigation technologies where you combine transgene of interest that might confer fitness with another transgene either in a transgene fusion or at least in the tandem pair which would tend to decrease fitness or increase domestication, and those are reasonable things to look for in the future.
I think what the EPA would like though is to have certain conditions where if a company just brought them a product and I don't think we're going to see any gurts within the next five years coming up for commercialization, I hope I'm wrong, I hope it's sooner than that, but are these things reasonable, are these conditions reasonable, and I think they probably are reasonable.
And I would also add if something can happen it will but I'm not sure that, I'm still not convinced

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that introgression is going to happen even in something like the brassicas where there's lots of wild relatives and when we're talking about transgene introgression.
DR. COOPER: Well the truth of the matter is we don't yet have the atlas cooper (ph) making an interjection. The truth of the matter is we don't have the information which would give us assuratives in that matter. On balance at the moment it does seem possible that stable introgression will occur in some (inaudible) species and at least in those we should be a little more careful perhaps.
MR. ROBERTS: Dr. Kramer.
DR. KRAMER: I just wanted to ask Dr. Cooper a question, would you then disagree with the other respondents in saying that you think that factors two and three would be inappropriate or I just wasn't clear if you were agreeing or not.
DR. COOPER: I think they are appropriate with care is about I would say, they are minor issues, I suspect they are minor issues associated with male sterility, there is a potential for an impact upon the

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wildlife which might not have been here or to considered, not all things eat pollen and not many things eat seed but clearly some do and they are potentially significant.
Especially I would have to say in England where the impact of transgenics on the bird population was an unintended and unexpected consequence of the current debates that are going on, so perhaps we're more sensitive in England to that sort of impact on wildlife.
As to question 3 it was really just to highlight the fact that not all viruses are the same I

suppose and that when you're looking for whatever you're looking for bear in mind that the virus that was used as a transgene may have no relationship to the one that you're protecting against, generally it's going to be similar, but at least it's a possibility that might not be.

MR. ROBERTS: Dr. Melcher, I think you have a comment.

DR. MELCHER: This would be changing the subject a little bit, this item 3 includes the

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phraseology are tolerant or resistant which I objected to earlier, I would like to at this point withdraw my objection, I had a discussion with Dr. Kramer on the break and I now understand what she means and what the Agency wants and I feel like I need to explain that to the rest of the panel because I think some of my panel members had similar opinions as I did.

I guess it is a matter of black and white, either the plant is tolerant and there is a lot of virus that replicates in the plant or I forget which black or white, but the other direction there is absolutely no virus in the plant, everything else in between is gray, and the grays are very difficult to handle in a regulatory sense because they are conditional and it is very difficult to establish all of the conditions that might be necessary to keep the gray from being important, is that I think a fair assessment of what we said?

DR. KRAMER: Yes.

MR. ROBERTS: Dr. Kramer, did you have something else you wanted to add?

DR. KRAMER: I did want to ask another

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question particularly in response to Dr. Hammond's comments, it seemed to me that you were really address the question of whether these criteria were sufficient, but I would also ask it from the other perspective do you think they're necessary at all, that is it necessary to have any criteria with which to judge a product on the basis of gene flow concerns.

DR. HAMMOND: Coat protein I'm not sure there is, I don't have any significant concerns about coat protein genes even if they do introgress.

DR. KRAMER: And could the other panel comment on whether they agree with that or not?

MR. ROBERTS: We can ask.

DR. STEWART: I think we have seen some written comments to that effect, and I would agree with them that I don't really see gene flow as being a big issue with coat proteins.

MR. ROBERTS: Any other panel members want to express an opinion one way or the other with that? Dr. Tepfer.

DR. TEPFER: For me we're at the situation where it's a matter of value judgments in a sense. If

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we can agree that a gene flow may occur, that this can lead to introgression, that this may confer -- this fitness advantage to the wilder weedy species conceivably all of this have never been demonstrated, this could lead to ecological release.

The question is what is the degree of uncertainty, what is the extent of imagined, because we're imagining now a damage that is acceptable, that's what we're talking about a this precise moment as I understand it, I don't particular care to engage in that kind of discussion, it seems like that's really getting quite a bit past what my scientific in this case allows me to pronounce on.

DR. ZAITLIN: I too think that we may be in some sense overreacting here to the transgene phenomena because we haven't really applied these standards to resistances which have been generated by more conventional means, so we're applying a different standard here. If we can demonstrate that there really were poor consequences of the natural resistance escaping maybe then we should be more concerned about this, but so far I haven't seen it.

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MR. ROBERTS: Anyone else?

DR. KRAMER: So I haven't heard anybody disagree with the statements of Drs. Hammond and Stewart that no criteria are necessary other than Dr. Tepfer who thought that really addressing this question at all is moving beyond the scientific issues as I understand it that are answerable with the data that we have.

MR. ROBERTS: Silence is ascent.

DR. MELCHER: I guess it is due to the lack of expertise in the gene flow field, so I am neutral.

MR. ROBERTS: I think that some members of the panel just may not feel comfortable expressing an opinion because it's not sufficiently within their area of expertise, really all we can do is ask those who feel comfortable enough to express an opinion to do so and I think that's where we are right now.

Any other follow-ups on 10? Follow-up comments from panel members or any questions from Dr. Kramer or the Agency related to number 10?

DR. STARK: Along the lines of Dr. Tepfer, this bothers me when we say I don't worry about

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introgression in gene flow with protein coats, we really don't know what might happen ultimately in the long term with these types of things granted the evidence of these are not a problem and the risk of a problem is very low, but I would be very hesitant to just say don't worry about it, you just don't know, there are too many unknowns.

MR. ROBERTS: All right. I think we have probably covered as much from 10 as we're going to get. Let me then ask the panel, we have covered ten questions today which is good progress, but we moved

through some of them very quickly and I want to give the panel an opportunity at the end of the session today for a go-back on 1 through 10, number 3 of course is still open and we're going to be working on that, but on the other ones is there any other comments that you know and now in thinking about them that you didn't make during our discussion that you did like to put into record or make now?

DR. STEWART: I would like to see clarification from the EPA, when they seek to regulate or cease to regulate something what is the time scale

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that is used, because we've heard things from ecological time scale to evolutionary time scale and I'm pretty sure we're going to get a some point the geological time scale and I'm sure the EPA's not worried about something that's ten thousand years out, but I mean what is the time frame that we should really be considering here, because we've had virus resistant plants with viral coat proteins out on the market for you know going on ten years now, eight to ten years and you know eventually it seems to me that we would see something if there's something to be seen, and if not at some point we need to make the determination that their equivalent to the conventional, so this is just -- since we have a little bit of extra time once again, what are we thinking about when we think about these regulations?

DR. KRAMER: I think I maybe look at Charlene Matten (ph) over there because this is an issue that obviously the Agency has had to deal with resistance management before, maybe Charlene you would have something to say here?

MS. MATTEN: This is Charlene Matten, I work

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in the biopesticides and pollution prevention division and I'm I guess more than just the Power Point mover today, but when we talked about insect resistance, management and that question was asked we truthfully did not define our time frame because our division director gave us a good example and I will share that with you, she said if we were looking at the murder rate would we say what a minimum murder rate would be, do we want three murders, five murders, ten murders, so she had said that it's best for us to say we want the least amount of murder as possible, and so in this case we want the longest time possible and for resistance management we said the longest time possible, but we also know that the models we were using were 15 year time frames and we also know the patent lifetimes are -- what are they 19 years, so we know that in terms of long term, it was at least 15 but anything else, it was 15 to infinity, but we knew less than ten was not reasonable so that's the best answer I can give you and in all honesty it was not defined but it was anything below ten was not good, fifteen and above was good but what the outside was

not defined.
MR. ROBERTS: Dr. Hammond, did you have something that you wanted to add on a previous question?
DR. HAMMOND: In response to that the average lifetime of an agricultural variety is less than ten years, I think it is six or seven years of wide use for most varieties, there are few that last longer than that.
DR. STEWART: But these transgenes really go beyond conventional variety since they get introgressed into various plant varieties, so I think it is worth while to take a long view, I'm just not sure what that long view really is.
MR. ROBERTS: Dr. McClintok do you want to respond?
DR. MCCLINTOK: I've worked in the pesticides program and also the toxic program which is OPPT and we have never openly discussed a time frame about products that we have regulated in terms of what we would look for. I would add though that as science changes and/or as the data comes in we would surely consider that information with the products that we do regulate, so in terms of a time frame I never had those discussions.
DR. ALLISON: If I understand this correctly that your decision is being made in order to allow for continued breeding with the transgene present, so in terms of a particular crop variety being available and useful for six years that's really not what we're talking about because we can use this, a company or individual breeders can continue to move this particular gene into other and better varieties as time goes on, is that correct?
So at what point, is there any point at which you revisit this as other techniques come along that may be superior. I always view this as kind of the model T of biotechnology, we're sitting around right now but certainly there's going to be sports car versions ten years from now or maybe less, at what point do you revisit these issues and say all right this technology is no longer appropriate because there is so much better technology available and companies should therefore use that is crossed out, is there any provision for that or thoughts along those lines?
MR. ROBERTS: Let me interject because my intent was to use this time to sort of get more comments on the record regarding the first ten questions. I mean I think it's a valid question you're asking, I'm not sure that it pertains directly to our answering these questions and it may be a question that's best addressed as a side bar to Agency folks during a break.
DR. ALLISON: It was actually intended to be

at the end of the questions.
MR. ROBERTS: Then let me ask you one more time, are there any go-back comments on 1 through 10? Dr. Kramer.
DR. KRAMER: I guess I will just comment partially on follow-up to the prior discussion that really especially when you are considering your answer to number 10 we would like to make sure that there is an inclusion there of your relative certainty of the estimate, remember that's one of the things that we want to try to understand for all of these questions, and part of the reason relates to this question of
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time frames and that is as the Agency is considering how to regulate these produces there are certain things that the Agency can do that are not going to be -- that are -- our time frame is not necessarily the same as the products that are on the market right there, right now.
MR. ROBERTS: Okay, were there any follow-up clarification questions on 1 through 10, I will give you the opportunity at the end of each one, but sort of in retrospect looking back is there lingering questions, it may be best to take those now while the questions are still fresh on the minds of the discussants. If there aren't any that's fine.
DR. KRAMER: I don't have any.
MR. ROBERTS: Then let's go ahead and close today's session, I think we have made excellent progress in getting through the list of questions. We will -- let me ask Paul Lewis if he has any closing statements or announcements to make before we close the session. We're going to reconvene at 8:30 tomorrow morning to begin with question number 11, and then I would ask immediately after the close of the
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session if the panel members would meet in closed session to discuss writing the minutes for today's session, but before we adjourn let me ask Paul if he's got anything he needs to say.
MR. LEWIS: Thank you, Mr. Roberts, and thank all the panel members for being engaged and contributing a great deal for the discussion we had in the course of today and I will be working with you this evening in terms of at least of trying to bring together some of your thoughts as a right to gather meeting minutes and looking forward to discussion tomorrow and for the public to again to be invited to hear our deliberations over the course of our meeting tomorrow morning and afternoon, thank you.
MR. ROBERTS: Then if there's no other business for today's session this session is closed, again we will reconvene tomorrow morning at 8:30 and again taking up the rest of the questions and again I would ask the panel members to meet immediately or now in the meeting room to talk about write-up of the minutes. Thank you.

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I, Monica Knight Weiss, Stenotype Reporter, do hereby certify that the foregoing proceedings were reported by me in stenotypy, transcribed under my direction and are a verbatim record of the proceedings had.

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MONICA KNIGHT WEISS