FIFRA SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

OCTOBER 13 - 15, 2004

ISSUES ASSOCIATED WITH DEPLOYMENT OF A TYPE OF PLANT-INCORPORATED PROTECTANT (PIP), SPECIFICALLY THOSE BASED ON PLANT VIRAL COAT PROTEINS (PVCP-PIPS)

THURSDAY, OCTOBER 14, 2004

VOLUME II OF IV

(Morning session)

Located at: Holiday Inn - National Airport
2650 Jefferson Davis Highway
Arlington, VA 22202

Reported by: Frances M. Freeman, Stenographer

C O N T E N T S

DR. ROBERTS: Good morning and welcome to the second day of our session on issues associated with deployment of a type of plant incorporated protectant. Specifically those based on plant viral coat proteins

We need to take care of a little bit of business before we resume our discussions. And the first thing we need to do in case we have any new members to the audience joining us today is to reintroduce the panel.

So let's take a few minutes to do as we did yesterday. Go around the table and ask each individual to state their name affiliation and expertise. As yesterday, let's start with Dr. Melcher.

DR. MELCHER: I'm Ulrich Melcher from Oklahoma State University in biochemistry and molecular biology. I'm a plant virologist interested in recombination, evolution, bioinformatics.

DR. SHERWOOD: John Sherwood Department of plant pathology, University of Georgia, interested in viral cross protection and epidemiology.

DR. ZAITLIN: I'm Milton Zaitlin, professor, emeritus plant pathology, Cornell University.

My specialty is virus replication.

DR. FALK: Bryce Falk, University of California at Davis. I'm a plant virologist. Interested in all aspects of viruses.

DR. ALLISON: My name is Richard Allison. I'm in the plant biology department at Michigan State University where I'm a plant virologist. I'm particularly interested in plant virus recombination.

DR. TEPFER: Mark Tepfer, I'm a research scientist at INRA which is a French government research agency. I worked on virus resistant transgenic plants for many years, bio safety questions, in particular, recombination as a special issue.

I also want to mention that I'm editor of a journal devoted entirely to gemo bio safety research. If you are interested, here are a few blurbs and you can find more about the journal.

DR. COOPER: Ian Cooper, Natural Environment Research Council of the United Kingdom. Research scientist concerned with virus ecology and the risks of genetically modified plants.

DR. HAMMOND: John Hammond, U.S. Department of Agriculture, Agricultural Research Service. I work with ornamental plants primarily, virus identification, characterization and virus resistance in transgenic plants and risk assessment

DR. STEWART: Neal Stewart, University of Tennessee. I work with transgenic plants, study their introgression and the ecological consequences of introgression.

DR. NAGY: My name is Peter Nagy from University of Kentucky. My specialty is mechanism of virus replication and recombination.

DR. BUJARSKI: I'm Jozef Bujarski from Northern Illinois University. I'm a molecular virologist interested in virus evolution, RNA recombination and replication. DR. STARK: I'm John Stark from Washington University. I work in ecotoxicology and risk assessment.

DR. GENDEL: Steve Gendel from the U.S. FDA at the National Center for Food Safety and Technology, interested in biotechnology, food safety and

bioinformatics.

DR. ISOM: I'm Gary Isom from Purdue University. I'm a neuro toxicologist with research interest in neuro degeneration. DR. PORTIER: Ken Portier, a statistician at the University of Florida, Institute of Food and Agricultural Sciences, with interests in statistical issues of risk assessment.

DR. HEERINGA: Steve Heeringa, University of Michigan. I'm a research scientist and director of the Statistical Design Group at the Institute for Social Research. I'm here as a permanent member of the FIFRA Science Advisory Panel.

DR. ROBERTS: I'm Steve Roberts. I am a toxicologist, Director of the Center for Environmental and Human Toxicology at the University of Florida.

We also need to cover a few announcements from our designated federal official, Mr. Paul Lewis.

MR. LEWIS: Thank you, Dr. Roberts. And, again, everyone welcome back to our second day of our FIFRA scientific advisory panel meeting and members of the audience for participating again, being observers for our

discussion over the course of today.

As I mentioned yesterday, the FIFRA scientific advisory panel operates under the guidance of the Federal Advisory Committee Act. This is an open meeting.

All materials that were shared with the panel are available in the docket. In addition following this meeting the panel will write its report summarizing its position serving as meeting minutes based on the discussion that occurred over yesterday and today and any discussion we have tomorrow in the public meeting.

The report will be available in approximately six weeks after the meeting. It will be available in the OPP docket. In addition, will be published on the EPA SAP web site. Thank you.

DR. ROBERTS: Thank you, Paul. I think we would like to start today by sort of recapping a little bit from some of our discussions yesterday. I'm going to turn it over to Dr. Kramer to maybe cover a few things.

DR. KRAMER: Thank you. I think we just wanted to start again by reviewing the charge to the SAP. That's to provide scientific advice to assist EPA in its

evaluation of several technical issues associated with PVCP-PIPs. Specifically to respond to a series of technical questions related to exposure and hazard considerations for PVCP-PIPs.

If we can switch to the slide of the role of the SAP.

I wanted to pull this slide up again because I think this was something as we were going through the questions yesterday that we were trying to make sure we got this information from you for each question.

But just to make sure that you understand that this is really the type of information we're looking for as we go through the questions today.

We would like for each question to understand the degree of risk for each issue, also the degree of certainty of the estimates that you are giving us.

Then the relevance of data versus hypothetically supported information and then also your opinion of the direction that the science is taking for the specific issues that are raised.

DR. ROBERTS: Thank you, Dr. Kramer. I think

this is going to come up in the context of revisiting question 10. We have been asked to revisit question 10 a little bit to clarify some things and I would like to do that.

Before we do that, first thing I want to do is we had a very useful discussion, I think, in terms of terminology yesterday.

And I think it became clear in that discussion of terminology that in terms of resistance and immunity and so forth what the agency was using and the questions as they were posed to us was resistance and had a definition I think that was different in the minds of some of the members of the panel. And I want to be sure to take just a little bit of time this morning and be sure that our responses to the agency's questions when they talk about resistance and tolerance, which they do in many of the questions, that we are using their definition of resistance as it was articulated in the documentation provided to us and not another definition of resistance that we might have as a panel.

I'm not sure about how to do that, but I want to

be sure. Can you sort of --

DR. KRAMER: As you are answering the questions, if you are uncomfortable using definition that we have and you would rather use the term immunity, that would be fine, but please don't change the definition of resistance and use that term while you are answering the questions.

DR. ROBERTS: Is that clear to everyone on the panel? The agency wants us to use their definition.

DR. KRAMER: The definition is more important than the term. We want to stick with the definition that we have which ever term is used.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: I was thinking about this also. I have, I suppose, answers to questions 6, 7, 8 and maybe even 9 that maybe somewhat are different than what we heard yesterday.

I'm not sure. Should I go through each of those?

DR. ROBERTS: Sure.

DR. MELCHER: Question 6 regarded the prevalence of immunity in wild relatives of crops. We heard from Dr.

Cooper about experiments indicating that undetectable levels of viruses are found in some such plants.

I think they were relatives of the brassica. It became clear in the discussion that the possibility that the assays used were not sensitive enough to distinguish between subliminal infection and immunity does exist.

Further, I believe that it is not common practice to attempt to make this distinction between immunity and some other level of resistance.

So unless others know that that's not true, I would conclude that the prevalence of immunity as opposed to other levels of resistance in wild relatives of crops is fairly unknown.

But I would suspect that it exists. But I don't think we have any degree of certainty on that.

So that's on 6. Should I go on to others?

DR. ROBERTS: Let's see if other members of the panel have a differing take on that. Dr. Falk.

DR. FALK: I'm sorry, Ulrich. I was reading while you were talking, but according to the definition in your appendix, we don't need to use the term immunity.

Basically, we just use resistance and tolerances. Is that right?

DR. KRAMER: We want to stick with a term, whichever term you would like, that means the plant is not infected by or is a nonhost of the virus concerned.

DR. ROBERTS: Dr. Cooper.

DR. COOPER: All I would say if you are trying to achieve an absolute limit on the detectable level of virus to prove immunity, it becomes technically very difficult to apply.

You have to apply a realistic level of detection using the most sensitive available tools on that particular occasion. And having regarded the fact that I think the viruses I was talking about were very high concentration, readily detectable viruses, the concept of immunity as an absolute is difficult to apply in practice.

Because there is a possibility mentioned by Dr. Zaitlin that single cells might be infected and the rest of the plant escapes.

Some aspect of that could be recognized by using the word field immunity which I think plant pathologists

and plant breeders use to describe the fact that a plant can be infected under rigorous inoculation challenge.

But in the field, remains free. And that's a practical use of that term with that qualification. But with immunity being an absolute term as one definition, it becomes impossible to measure, because there are always changes in technology which might move the barrier a little bit more.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I think that to all practical purposes a subliminal infection is irrelevant because it is unlikely to serve as a source of infection for another plant. And so a plant which is subliminally infected is effectively resistant and not of an epidemiological concern.

DR. ROBERTS: Mr. Zaitlin.

DR. ZAITLIN: I'm still thrashing my dead horse. I still think that we're worried about disease, not whether or not there is a subliminal infection or a very low level of infection that doesn't result in symptoms. I mean the real issue here is why do you want to protect the

plant. To protect it against disease.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: Dr. Zaitlin, the question is about the infection in wild relatives of the crop plants. And I think the reason for asking it is whether the virus could survive in the wild relatives whether or not the wild relatives are sick or not. It really doesn't matter.

DR. ZAITLIN: I would agree with that exception, but if we're talking about the crop plant itself.

DR. KRAMER: We're talking about the wild relatives here.

DR. ROBERTS: Dr. Falk. You didn't even have to signal. Your body language told me you wanted to speak.

DR. FALK: Because I'm so confused. Here is what I wrote for number 6 in trying to put together what people said. Most plants are resistant to most pathogens and viruses, in parentheses. Resistance is the norm and susceptibility is the exception. We had that in our discussion.

Tolerance resistance and immunity to indigenous pathogens, viruses are all present in wild populations in

many plants. The phenotypes of these range from supporting virus replication with no symptoms, being tolerance, to limited infection foci, being subliminal infections in a single cell, that is in parentheses, to no infection, immunity.

So that was --

DR. KRAMER: I think that gives us the information that we need with the understanding from your -- the latter half of that answer.

At the beginning you were using resistance with a different definition that we had, which was clarified later by essentially defining it as immunity.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: I am still confused now what level you would like us to look at. Would you include now the subliminal infections as something that you are interested in?

DR. KRAMER: Well, actually I --

DR. MELCHER: Or field immunity that Dr. Cooper mentioned?

DR. KRAMER: If what you are telling us is that

that is relevant for us to look at as well, then that is useful information for us to know. Our initial questions were not based on that.

DR. ROBERTS: Dr. Nagy.

DR. NAGY: I don't know whether this is actually the subliminal infection is not irrelevant because recombination can take place in single cells. So one other possibility is that use Bocom's (ph) definition, which instead of using immunity he likes to use extreme resistance.

In this case it just would mean that we have different levels of resistance and that might be helpful.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: Again, we're talking here about the wild relatives and in the wild relatives -- well, maybe the recombination is important. I'm sorry. It probably is.

DR. ROBERTS: I think that it is clearly in the minds of some of us on the panel or some on the panel that may have been thinking again about a different definition of resistance in crafting our answers than the definition

that the agency has.

We can make as a separate comment that in addition to what I think in the minds of the panel members is immunity, in addition to immunity they ought to also be thinking about resistance or other forms, other levels.

But nevertheless, their question was asked with resistance as some of us would define as immunity.

We need to be able to answer that question with that definition and we need to maybe look back at our response and make sure that our response is based on mentally substituting the word immunity for resistance in our response.

Dr. Sherwood.

DR. SHERWOOD: I have to reiterate what has been said earlier, that most plants are not host of most viruses. So I guess in answer to the question, the prevalence, I would say the prevalence is probably high.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: Maybe we're talking -- with wild plants there are sort of two types of phenomena that maybe we're mixing a little bit.

One is indeed most plants are non host for most viruses and the other is that, in fact, expression of symptoms in wild plants seems to be a rare fact.

So one can if you simply go and analyze wild plants you can find viruses. Most often you had not seen any symptoms. So that's a different type of behavior completely. The plants are infectable, but simply they are tolerant in the usual virologist terminology.

DR. ROBERTS: Let me leave it to the lead discussants on the questions where tolerances and resistance are mentioned in the question to think about the panel's response and whether or not the panel response makes sense if the word immunity were substituted for resistance.

And if it doesn't or if you think the discussion really was based on some other understanding of the question, to let me know and we will reopen that question. Does that sound reasonable?

Please let me do so at some point today before we get to the end.

The next thing is I would like to revisit

question number 10.

In responding to question 10 toward the end of our discussion, some members of the panel expressed their opinion as to whether gene flow or introgression was really something that we needed to be worried about.

I think what the agency needs from us to extend that discussion is whether or not that is based on, those opinions are based on speculation or whether there is some sort of data or objective evidence to support that.

Because in order to make regulatory decisions, they have to be able to point to some sort of scientific finding as opposed to merely expert opinion.

So I would like to revisit this question. And I'm going to give Dr. Kramer the opportunity also to sort of articulate and perhaps Dr. Milewski to give us the EPA perspective in terms of asking the questions about these conditions and sort of what they need to use these conditions in the way of support. Dr. Kramer.

DR. KRAMER: So yesterday I felt that the panel was really answering whether these conditions are sufficient and I pushed back a little bit to ask whether

they are actually necessary. And we got the answer from a few people that made me think they are not necessary.

I wanted to push back on that answer yet again. And just to say from our perspective, in order to conclude that they are not necessary, we would like to have you believe that gene flow is not a concern for any virus in any plant under any circumstances.

Short of that, what are the conditions. And we listed three possibilities that would help one to differentiate between those PVCPs that might be a concern and those that might not.

DR. ROBERTS: So with that clarification, Dr. Stewart?

DR. STEWART: I would say that I would not even go so far as to say that introgression is not a -- we can't completely rule it out.

In my review, I brought up the case of sorghum and Johnson grass as an example where I simply don't think we should produce transgenic sorghum because of how nasty weed johnson grass is.

Yesterday we heard about oat and wild oat.

That's another example where the wild host is a noxious weed and it can -- viruses do play on it. So that's another example. In my review in Nature Reviews Genetics, I only looked at the top 10, I think, top 10 or 12 crops.

Oat is one of those crops that is being cultivated less and less every year in the U.S., but still a case that we wouldn't want to go there.

So the new plant list that we'll show expands that top 12 or so many crops another notch. But you could imagine all types of situations where gene flow or introgression would be something that we would definitely want to avoid. So I prefer to think about your conditions as being sufficient or not.

DR. ROBERTS: Other opinions?

DR. KRAMER: Could I also interject again. If there is any way to stimulate some discussion on those exact conditions, maybe we got it exactly right the first try, but there hasn't been a lot of discussion on how those conditions might be edited. If there is any thoughts on that, we would appreciate hearing them.

DR. ROBERTS: Sure. Could you go ahead and project the conditions?

DR. KRAMER: I think that's the next slide. Should I read them again.

DR. ROBERTS: Yes, just to give people a little chance to think about them a little bit more while you are reading them

DR. KRAMER: The conditions again.

Number 1, 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 2, genetic exchange between the plant into which the PVCP-PIP has been inserted and any existing wild or weedy relatives is substantially reduced by modifying the plant with a scientifically documented method, for example, through male sterility.

Or number 3, it has been empirically demonstrated that all existing wild or weedy relatives in the United States with which the plant can produce a viable hybrid are tolerant or resistant to the virus from

which the coat protein is derived.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: In rereading the three conditions, it seems to me that the weak link is the second of the three. I think that the other two are relatively solid because present technologies for preventing gene flow or reducing gene flow are not 100 percent effective.

So there is a point of where you have to make some sort of judgment as to whether the -- if the gene flow does occur at a low level, what is the likelihood of this having a severe impact. So your question in the end of the day in asking what is acceptable risk, which is a very difficult one that sort of escapes from the scientific domain to some extent. But definitely the second one is a leaky one.

DR. ROBERTS: Is there a way to fix that?

DR. TEPFER: There is a lot of research going -- this is Mark Tepfer again -going on to increase the effectiveness of gene containment strategies. But as Neal Stewart mentioned yesterday, these truly, you know -- really resistant and proven strategies are several years

down the pipeline probably.

So that is the weak point, but it is being worked on.

DR. KRAMER: Do you think that Number 3 can stand alone? Is there a way to maybe combine Number 2 with some other condition that would enable it to stand?

DR. TEPFER: Well, again, it becomes -- it's not as absolute, because the third one is -- again, you know, you are talking about tolerant or resistant.

Sometimes you can have a small degree of additional resistance that can be added that could at least in theory have an additional fitness effect. But again, are we hair splitting.

Again, it becomes a question of well is this worth talking about. And that's the kind of terrain I don't feel happy on. Resistances may not be absolute.

If resistance in the wild relative is truly immunity, to get back into that more as definitions, in which the virus simply does not replicate and nothing happens ever, then, I would feel fairly comfortable.

But that remains to be demonstrated in nearly all

the weeds I know of.

DR. ROBERTS: Dr. Stewart and then Dr. Sherwood.

DR. STEWART: I think Dr. Tepfer has -- that's a great point and that we need to know more about what the virus is actually doing in the wild relative before you can make that call.

That's my only problem with Number 3. But just to illustrate the bit of problem in Number 2 and why it is really the weak link, and so if we're talking about a 1 percent introgression rate, which would be pretty high, but a 1 percent introgression rate from sorghum to Johnson grass or oat to wild oat and we can decrease that by 1,000 fold, well, your typical field in an acre is probably going to have 60 thousand Johnson grass plants.

So in your 1 acre, you are still going to have probably six hybridization introgression effects per acre.

If the virus is important for the wild host, if virus resistance is important, with those types of numbers you have to be more leak proof than what our current

technology can give us, and probably what the technology will be able to give us in five years. I say I think 10, 20 years we'll be able to do much better by combining mitigation strategies.

So to say that a terminator technology is going to solve everything I think is a bit misleading for the riskiest crops.

For crops that are less risky, that might be enough just to put you over the top as far as on a regulatory side.

DR. SHERWOOD: I think, though, that one can empirically conclude that if the virus has not been recently introduced or the weed has not been introduced, and those two have shared an ecosystem for a while, that that weed is either tolerant or resistant to the virus. Because it is there.

And as viruses are obligate parasites, they have to have a living host.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: One of the problems that I continue to have with this is that we seem to be

differentiating between resistance that is derived from a transgene and any resistance that is naturally present in the plant in either in the weed or in the crop and has the potential to introgress from the crop to the weed.

Disease resistance have been used in crop plants for a very long time. And have the opportunity to introgress into the wild species.

And there is no good evidence that I'm aware of that anybody has even looked for the introgression of natural resistance genes from the crop to the host or been concerned about possibility of that occurring.

But it is out there in many crops, and many of them do have related wild and weedy species and there has not appeared to be any concern in the past.

Why is there a concern now over resistance induced by a transgene? I don't see the difference.

DR. ROBERTS: Dr. Cooper.

DR. COOPER: Could I make a suggestion. Most of the movement of transgenes, it would be from the crop into the wild plant, the wild plant potentially has a very long life in the field.

The movement of genes from the wild plant into the crop is likely to be hard to find because that crop is very often an annual and you wouldn't need to think about subsequent generations.

Any transfer would be hard to find. Of course you could find it using modern techniques. And I proposed yesterday that the brassica system allows that surrogate system to be annualized.

We have specific genes in brassicas that have been defined in molecular terms as to their chromosomal positions, and those could be easily be monitored.

And they bring in another point which is relevant to 3 here. The concept of a pathotype, where they are usually pathotype specific, so they only work for pathotype 3 or pathotype 1 of turnip mosaic.

And that, therefore, means that you have to have regard not to the virus, but to a subset of that virus which has particular pathological attributes, in particular, genotype of hosts.

DR. ROBERTS: Dr. Stewart.

DR. STEWART: I think Dr. Hammond makes a really

good point on the difference between the transgene and natural resistance. And I think there are a couple fine points. I'm not sure that I agree with Dr. Cooper on the wild relative being longer lived than the crop.

They are probably going to be both annuals in that case. The difference is that hybrids must -- if you have hybrids, they must mate with the wild relatives every year or every cycle in order for the transgene to persist.

Now, there is a lot of -- well, go back to what is the difference. Well, much, I think, natural host resistance is multigenic. And this is unigenic in many cases.

It could be easier for the single gene to be transferred rather than a number of genes in a number of different loci. I think there has been a lot of work on introgression.

But mainly on the other side from introgressing natural plant resistance to crops. And that's very difficult that takes an intensive breeding effort. And it

is usually regarded that going that way is easier than going from crop to wild.

I don't know that I really answered anything at all. But just as some background there. A lot of work has been done on introgression.

DR. ROBERTS: Dr. Tepfer, but first let me before we get too far, I think in order to answer this question we just have to preface it by given that there is interest in controlling gene movement and regulating that for transgenic crops, to what extent do these conditions allow the agency to exempt a particular material, particular product?

What they are asking is: On a scientific basis, how strong are these conditions. Are these conditions going to be useful for them. And if not -- or if they could be improved, how would we improve them.

DR. KRAMER: It is really to the point where we can conclude that the product or the PVCP is of extremely low risk.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I guess I was going a bit farther

down the same trail that Dr. Stewart was. So maybe I should sort of defer those sorts of comments as being not necessary for the present questions.

DR. ROBERTS: Are there any additional comments on these conditions or suggestions on these conditions?

DR. KRAMER: Can I try to reiterate what I think we have heard, not just today, but yesterday too.

DR. ROBERTS: Please.

DR. KRAMER: And that is for condition number 1, that list can be expanded quite considerably. I think we're going to talk later on today about how exactly to expand that list.

But that condition as it stands would be a necessary condition to have.

For condition number 2, that's perhaps not a condition to have at this point at all. Perhaps something anticipating future techniques that might be closer to 100 percent would be a possibility.

And the third condition would be necessary to have. However, in light of the conversation yesterday, probably very few things would actually qualify for this

given that from yesterday the way that this would have to be demonstrated most likely would be through a manual inoculation in which you are setting essentially a very high bar for a product to be shown to be resistant.

DR. ROBERTS: Dr. Sherwood. Does that sound correct?

DR. SHERWOOD: To add to the last one, that known host range of all viruses is not known, so you would be putting a person in a situation that they would have to go and inoculate every plant in that environment to find out whether it is or is not a host.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I think that the third condition is too extreme because there is variability within populations and susceptibility in some individuals and resistance in others.

And you cannot prove that every individual or every population has resistance. If resistance is prevalent, that should be sufficient to meet condition 3 rather than every individual or every population of the weedy or wild species having resistance.

DR. ROBERTS: Dr. Allison.

DR. ALLISON: May I just add on point number 2, that while it sounds like a very good idea and we can look forward to theoretically this barrier can be established, that each one of the new techniques under consideration has its own regulatory hurdles to pass.

So to be able to speculate that 5 years from now or 10 years from now we'll be able to use these to curtail any gene flow is not necessarily -- that time span is not necessarily real.

DR. ROBERTS: Let's get back to the third condition. Is the third condition impractical or is there suggested, consensus suggestion that it should be redefined or maybe both?

I was following up on Dr. Hammond's suggestion.

DR. HAMMOND: I would suggest that rather saying all existing wild or weedy relatives to that resistance is prevalent in the wild and weedy relatives. Because there is never going to be a case where every individual or every population has a high level of resistance.

Dr. Cooper talked yesterday about variability in the populations of brassica with some individuals being immune and others having tolerance and others being susceptible. That is the case in most populations.

DR. ROBERTS: Then as a practical matter, do you have to define an acceptable level of prevalence?

PANEL MEMBER: No, you can just delete the word all from that sentence.

DR. HAMMOND: I'm not an ecologist of a population geneticist. I don't know what would be a reasonable level to define as prevalent. A population geneticist could probably come up with a reasonable definition of prevalent for that.

DR. STEWART: I guess I would add that this is going to be pretty easy to define empirically because the population genetics would favor tolerance or resistance if it were available. Right?

Because this would be selected on. And furthermore, if a species population, whatever taxon you want to look at does have tolerance, you will also have a good idea about the ecological niche that it is occupying.

So if you had nine plants out of 10 that were tolerant, making that one out of 10 also tolerant by the PVCP-PIP, it wouldn't have an effect on the plants, on the plant population.

I think that's a great idea, just deleting the all. I think that would be pretty easy to define. Any company that wants to actually look at this is going to do the field experiments anyway.

DR. ROBERTS: Are you interpreting all as meaning all species or all individuals?

DR. STEWART: The way it is written now it is all individuals.

What you are really talking about is the species. Whether the species or the populations within the species are mostly tolerant or not. Do they have it or don't they.

DR. ROBERTS: Actually, I thought it was a little ambiguous on that point.

DR. STEWART: Well, it is. I'm saying the way I read it. You could include all existing wild or weedy

species, wild relatives or weedy species.

DR. KRAMER: If I could clarify. When we wrote this we meant that the trait existed within the population. Not that every individual within every population.

Maybe you could tell us how we might clarify that in the language there.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I would rewrite that to say that tolerance or resistance to the virus is prevalent in the populations of all wild and weedy relatives.

DR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: I would like to change prevalent to just found. That way you don't have to worry about getting into the population genetics and percentage.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I suggest adding sexually compatible wild or weedy relatives. Because you will have -- the others you are not concerned about, the more distant ones.

DR. KRAMER: We have, for which the plant can

produce a viable hybrid. Is that something different from what you are saying? Okay.

Do I understand is there agreement to use to the term found and would that -- can that be then defined? Is that one instance?

DR. ROBERTS: They are sort of nodding. But let's see if we can get some verbal responses to your questions.

DR. STEWART: Found or how about observed?

DR. SHERWOOD: Observed is quite acceptable.

DR. ROBERTS: Dr. Sherwood says that's acceptable.

DR. KRAMER: In this case we are talking about immunity.

DR. SHERWOOD: We're talking about tolerance.

DR. KRAMER: Tolerance and/or immunity. Right.

DR. ROBERTS: Is there a different opinion among panel members on that?

DR. KRAMER: Just to clarify. If we're talking about finding tolerance and/or immunity within a

population, is that within all populations of the species or anywhere within the species?

DR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: Anywhere within the species. I thought the kind of consensus was that we're looking now at -- you are going to have to look at individual plants. And if you see an individual plant that has tolerance or immunity, it would then pass this bar.

DR. ROBERTS: Dr. Cooper.

DR. COOPER: Could I contribute the fact that if you have tolerance or immunity detected in one of the southern islands on the extremes of the continental USA, it may be very geographically and practically isolated in a genetic sense from the mainland of the USA. I think that is a real constraint on decisionmaking.

DR. ROBERTS: Not to answer for the agency, but presumably they would take that into consideration in making a management decision on that, but Dr. Hammond and then Dr. Tepfer.

DR. HAMMOND: I think that a single population wherever it is would not be sufficient. But it is not

realistic to examine all populations because for one thing you would have to do an extensive survey to find out where all the populations are.

It would be reasonable to survey populations scattered in the areas where the crop is expected to be grown.

The populations at the extremes are by themselves unlikely to be informative or useful. It is where the crop will be grown. But it needs to be more than one population. DR. STEWART: And fewer than all.

DR. HAMMOND: Many fewer than all.

DR. ROBERTS: That certainly narrows it down. Dr. Tepfer.

DR. TEPFER: So we are going someplace where it is more than a single observation of tolerance or immunity.

So we are having to observe it in several populations. Is that where we seem to be going -- in the area where the crop is going to be cultivated. It sounds like that's the sort of direction we're going in.

DR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: I have a real problem with a pragmatic implementation of such a guideline. How far in terms of miles or kilometers are you going to find putting a crop in a new area. There is constant shifting of what crops are grown in the United States and parts of the United States. Certainly one thing we encourage our producers to do is rotate crops around different portions of their farms which may be scattered over several different miles.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I would think a practical solution would be to suggest within the state multiple populations from within any state where the crop is expected to be grown. But certainly not to go down to the county level or the farm level.

That would be ridiculous.

DR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: Again, I would have problems with that. Just you have shifting agricultural practices. For me, for example, it's north Florida and south Georgia.

If they are a farmer producing in north Florida one season and going to their farm in south Georgia the next season, they are going to have to go through and do a survey in order to produce the same crop that they just produced across the state line the last year.

DR. ROBERTS: Dr. Stark.

DR. STARK: I have another problem with this statement, number 3. It says, it has been empirically demonstrated. Who does this demonstration? Where is the onus? Who is responsible for demonstrating that wild or weedy species have this tolerance?

DR. ROBERTS: Dr. Kramer.

DR. KRAMER: It would be the product developer.

DR. STARK: Again, you get to the point how do you define wild and weedy populations and their extent and range and all this.

In other words, if I was producing a genetically engineered crop and I had to come to the EPA and demonstrate that wild and weedy relatives have this tolerance, what are the limitations of this?

If I came and said, well, we went into Alabama and we are in a specific area we plan to grow this crop and we have sampled around the crop borders, is that sufficient? The way this is worded to me is is an impossibility.

I don't see how anyone could ever come to you with the right amount of data to meet that statement, empirically demonstrated tolerance in these species.

DR. KRAMER: Are you disagreeing then with Dr. Hammond's suggestion of how to sample a population of few populations within each state where the crop is grown?

DR. STARK: I'm not saying that. I'm saying the way it is written doesn't define anything. It is very open ended.

DR. KRAMER: I think it is helpful to have the suggestions from the panel on how it might be defined in a practical and meaningful way.

I actually think that we have gotten an answer from Dr. Hammond if everyone is in agreement with that suggestion.

DR. ROBERTS: I'm not sure that's the case.

DR. SHERWOOD: I'm not in agreement with the statement.

DR. KRAMER: And your suggestion would be that a single occurrence in any population would be sufficient?

DR. SHERWOOD: Yes.

DR. ROBERTS: Dr. Allison.

DR. ALLISON: With the variations in geography of different states, I think to be able to pick a number or something for a particular state is unreasonable.

Some states are -- the populations may be quite uniform, while in others the population is going to be tremendously variable.

And to make a decision on tolerance or immunity or resistance, it is going to be different -- the sampling would be different in different states.

DR. ROBERTS: Dr. Stewart.

DR. STEWART: You could take it away from the state distinction to a more of a regional distinction and leave that with the agency to define further. I would hate to define something as it has been said so stringently that no one can meet even though it is

reasonable.

DR. ROBERTS: Dr. Hammond --

DR. STEWART: I would also say that a situation in Virgin Islands might not be indicative of south Georgia also as far as population biology.

So I think a regional definition might get around that.

DR. ROBERTS: Presumably, the product can be registered in different regions. Is that right? You can specify or is it when it is registered it is registered everywhere?

DR. KRAMER: There are certain restrictions that can be placed on a product registration and exemption, but likely not have such restrictions.

DR. ROBERTS: Good point. Dr. Hammond.

DR. HAMMOND: I think a regionally representative population is probably the most practical way to go. For many of these species, just getting hold of seed from different populations is going to be a major effort.

There are not seed banks for weed populations

that I'm aware of that are held in many places. I think that John Sherwood is right and it does have to be restricted to what is reasonable and not to have to send somebody out at various times of year to collect seed populations from different places. That poses an unreasonable burden.

I think it would have to be a limited number of populations from a limited number of regions to be actually a practical solution to be able to achieve.

The burden could otherwise be easily pushed way too high to allow anybody to reach it.

DR. ROBERTS: Dr. Kramer, I think all the concept is clear, crafting the language for this condition is challenging.

DR. KRAMER: I think we have gotten a lot of helpful information from the panel for this question.

DR. ROBERTS: Thank you. Let's then move -- Dr. Sherwood.

DR. SHERWOOD: Something you might want to look into is find out how weed populations differ with the USDA climate zones for plants. That might be a way to going

about finding regions, is use of climate zones.

DR. ROBERTS: Thank you, Dr. Sherwood, for that suggestion. Let's go to question 11. Before we start that, I just want to point out that Dr. Gendel mentioned yesterday about previous SAP deliberation on issues associated with a similar issue.

And what has been distributed to you is the report from the previous SAP. So that may provide some helpful foundation for our discussions today.

Dr. KRAMER: So question number 11 --

DR. ROBERTS: Let's go to question number 11.

DR. KRAMER: To what extent are novel viral interactions, for example, recombination, heterologous encapsidation involving a viral transgene an environmental concern.

I would like here to also read the definition of novel viral interaction.

This is found in the appendix. Novel viral interaction means an interaction between portions of two or more different viruses. For example, through recombination or heterologous encapsidation, not expected

to occur in a mixed viral infection found in nature.

DR. ROBERTS: Dr. Tepfer, could you lead off our discussion on this.

DR. TEPFER: I have written a little bit of text. I will read it into the record to start. It doesn't really cover all the questions that are raised, but I wanted to start with this at least.

The degree of potential risks associated with heterologous encapsidation are quite different from those associated with recombination. The former being of less concern for several reasons.

First, in many cases, viruses that are closely enough related for heterologous encapsidation to occur are transmitted by the same vectors and thus the phenomenon would have no effect on vector transmission.

Second, if unexpected unwanted effects due to heterologous encapsidation were observed, damage could be limited by simply ceasing to grow the plant variety in question. The problem is reversible.

Third, there are excellent means of mitigation which make it possible to simply render the question moot.

For these reasons, the comparison or the aptitude for heterologous encapsidation to occur in transgenic and nontransgenic plants is of little importance.

In contrast, at least in theory, impact of recombination could be much greater since there is no abundant bioinformatic evidence that recombination has indeed as had been long suspected played a key role in the emergence of new viruses.

In the world of human health, for analogous reasons, there is great concern about the potential outcome of recombination between human and avian influenza viruses.

However, it can be argued that the important point is not the relative likelihood for recombination to occur, but rather whether recombinants in transgenic plants are different from those in nontransgenic plants.

In order to determine if novel events could occur, the situation in nontransgenic plants must be studied carefully, which has not been done very often.

We are currently addressing this by comparing recombinants in transgenic and nontransgenic plants. This

is actually getting redundant.

One part of this has involved mapping several hundred recombination sites between CMV and relative TAV across the viral RNA 3 which includes the coat protein gene in nontransgenic plants. And we have an article in press on this subject.

As expected, there were hot spots for recombination. At the majority of sites, precise homologous recombination was observed. However, hot spots for other types of recombination, homologous, imprecise, and aberrant recombination were also observed.

Similar experiments are underway using transgenic plants, but is too soon to present the results.

However, it is striking to note when the same templates and same primers were used with the risk transcriptase invitro, very similar assortment of homologous precise recombinants was observed, but none of the imprecise or aberrant sort. And here also there is an article in press.

Considering the great biochemical differences between the RNA viral replicase and a risk transcriptase,

this suggests that the determinance for homologous precise recombination would apply to a very wide range of situations.

And that at least for this type of recombination nothing novel would be expected in transgenic plants. The probable outcome of these experiments is likely to be that the recombinants in transgenic plants would be similar to those in non transgenic plants. Nonetheless, it has been noted that at least in the biological system that we are using, which is the cucumo viruses, recombinants that have a selective advantage relative to the parental strains have been observed.

Can be obtained. This is article by Fernandez-Quartero, et al., and there is also one from, I think, Ding's group. In addition in unpublished work from Zak Mol's (ph) lab, it was shown that one could obtain cucumo viral RNA through recombinants that induced worse symptoms than the parental viruses.

If you look at those two sorts of features, it would seem in this particular case -- these recombinants, however, were outcompeted by the parentals in

coinoculation experiments. So they would not be expected to persist.

If you look at sort of these two type of results, one, that cucumo virus with recombinants with selected advantage can be produced and that we can also observe viruses with worse symptoms, this does sort of suggest there is an evolutionary space for recombinant cucumo viruses with properties at least somewhat worse than those presently known.

DR. ROBERTS: Thank you, Dr. Tepfer. Dr. Melcher, what do you have to add?

DR. MELCHER: Probably not much. But I could underscore the comment of Dr. Tepfer that recombination is not likely to be a novel event in the case of a transgenic plant being infected by a virus, that recombination events have happened throughout the evolution of viral history.

You just have to consider what the structure of viral genomes are. They are a assemblage of different modules.

A protein -- a gene that would coat, for example, a movement protein could be next to a variety of

different kinds of proteins involved in DNA replication.

Could be involved next to a gene flow reverse transcription for RNA polimeraeces (ph) from different super families of RNA polimaraeces, next to a replication protein case of the gemini viruses. This movement protein could be on the other hand also associated with a variety of different types of coat proteins. Coat proteins for isometric viruses and coat proteins for helical viruses, filamentous and rod shaped viruses. There has been scrambling through evolution of these genes all the time.

And anything that could happen in a transgenic plant is probably very unusual and unlikely. Of course, there are time scales here involved.

The evolution that I have just been talking about is probably over many, many years. And you may be worried about more limited time scales.

The only case where this might be a problem, I think, and Dr. Kramer introduced to us yesterday already is i fa transgene containing plant is planted in an area where it encounters a virus that the virus that is part of

the transgene never encounters in nature, in different region or if that transgene is expressed in a part of a plant that the virus from which it comes is not normally expressed.

So those are possibilities. If they are there, then I'm sure recombination will happen with some frequency. It can happen frequently even without selection. So that's relative to the recombination issue.

And trans encapsidation is pretty much a dead end like you said. So I guess maybe I should quit there.

DR. ROBERTS: Thanks, Dr. Melcher. Dr. Sherwood.

DR. SHERWOOD: Really not much to add. Just to reemphasize Rick's comment about recombination occurring on a regular basis. And the viruses we detect today are probably the most fittest. That's why we detect those particular species.

And they do exist. As was said earlier, most viruses exist as a quasi species, so there is always a certain amount of variation in the population.

DR. ROBERTS: Comments from other panel members. Dr. Hammond.

DR. HAMMOND: Neither recombination, heterologous encapsidation or synergy are novel virus interactions unless viral genes are deployed in plant species which are not susceptible to the virus.

There is little justification for the use of virus constructs in crops in which the virus does not infect. Constructs from the cognate virus should be used instead. Viruses do and will evolve by recombination most typically between isolates of the same virus. However, recombination between viruses and different taxonomic groups also occurs and plays a role in virus evolution.

Recombination has been demonstrated between viruses and related transgenes, and most recombinants will be similar to the parental viruses whether resulting from recombination between two viruses in a mixed infection or between a virus and a transgene.

There is little a priori reason to believe that recombinants between viruses and transgenes will be more

of a problem than recombinants between two viruses infecting the same plant unless transgenes are derived from severe or exotic isolates.

As the general recommendation has been to use mild endemic isolates as the source of the transgene, this should minimize any potential for creation of novel isolates that would not equally or easily arise from naturally mixed infections. And mixed infections are common in both wild plants and in agricultural situations.

Heterologous encapsidation also occurs naturally in mixed virus infections as well as in coat protein expressing plants. Heterologous encapsidation in transgenic plants has very limited probability of course in interactions different from those that will occur naturally and will typically be limited to a single passage.

In contrast, when heterologous encapsidation results from a mixed infection, it is highly likely that both viral genomes will be transmitted to the new host allowing further interactions.

Indeed this is probably the only significant way in which potato virus C, an aphid non transmissible derivative of potato virus Y, can spread within a crop.

Use of viral genes that are responsible for synergy with other viruses is not recommended. But in any event, farmers will soon abandon any transgenic variety which was more susceptible to other varieties.

Synergy would also be restricted to the transgenic plant and is unlikely to have a significantly greater effect than in a mixed infection.

DR. ROBERTS: Dr. Zaitlin.

DR. ZAITLIN: In order for interactions to take place, the two viruses must be in the same cell. And it is known that in many cases of mixed infections the viruses exclude themselves. They are not in the same cell.

However, if you had a transgene and a super infecting virus, you then would have the possibility that they would, in fact, be in the same cell. But as enumerated here, that probably doesn't make this more of a concern.

DR. ROBERTS: Dr. Falk.

DR. FALK: In response to Professor Zaitlin. When viruses are excluding each other, are you talking about related viruses as opposed to unrelated viruses?

DR. ZAITLIN: The cases which have been demonstrated for mutual exclusion were done a long time ago in protoplast usually with related viruses. If they are put into the cell at the same time, they can both replicate.

But if you have one in there and then try to infect with another, these are protoplast experiments, you then cannot get virus -- you can get the virus in together. But not subsequent -- unrelated viruses it doesn't apply.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I wanted to say there is more recent evidence that a similar phenomenon exists between related viruses in whole plants. There have been two papers, one that came out last year with poty viruses, one that has just come out cucumo viruses showing that in non transgenic plants when you have these two related viruses

together they do tend to exclude each other. For instance, particularly with the poty virus, it very beautifully shown with flourescently marked, viruses that coat for GFP or DS red, that, in fact, the two viruses only existed in a single sort of layer of cells between the patches were singly infected.

So this phenomenon is apparently quite real in plants as well.

DR. ROBERTS: Other comments or areas of agreement or disagreement. Dr. Melcher.

DR. MELCHER: Just for historical record, I think this is not new information. The whole basis for the technology we're looking at is viral cross protection, which is inoculation of a plant with one isolate of the virus and that protects it against infection by related isolate of the virus that's usually more severe.

So that's old news, I think.

DR. ROBERTS: Any other comments on this question? Dr. Kramer. Do you want a clarification or a follow up question.

DR. KRAMER: I had a couple things. I wanted to

go back to Dr. Tepfer's original response in which you mention the idea that mitigating factors can be incorporated into the construction of a construct.

And I wanted to say that this question is really asking absent any type of mitigating factors. So I want to make sure that your answer for heterologous encapsidation would stand even given that.

DR. TEPFER: In the absence of mitigating factors which are commonly built into transgenes, then, of course, I think that there are a few cases where you wouldn't want to perhaps use full length coat proteins in certain luteo viruses that can be health risk for umber (ph) viruses, for instance.

Again, it is a case by case sort of a situation. There are a few situations where without mitigation I would feel a bit uncomfortable.

The reason I mentioned that usually this is not a problem is that often when you have heterologous encapsidation it is between closely related viruses that are transmitted by the same vectors anyhow.

Again, John Hammond mentioned the case of potato

virus c. It is another situation where the virus apparently requires assistance from a second virus. But those are rather rare circumstances. But those would be ones where mitigation would be required.

Whereas, the others, if you are just talking about two related viruses that are transmitted by the same aphids, then really there is no problem that is really perceived.

DR. FALK: I think that in the specific argument about heterologous encapsidation that you just asked Dr. Tepfer, I don't think that there are going to be novel interactions resulting from the transgene that would be an environmental concern.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: I have the same opinion. But I think I want to address what some people argue as a hazard of the trans encapsidation.

That is when this trans encapsidated virus gets into a host species that it normally doesn't, it may then be transmitted by another vector that has a specificity for that second plant and thus it could be propagated into

several different species that it normally would not have been. I think this is not a novel interaction because I think this has happened. There are some viruses that have very wide plant host ranges and probably the reason they do is they are transmitted by multiple vectors and it has happened before. I don't think it is a novel interaction, but it is something that has been brought up, I think, at least in some of the reading that I was given.

DR. ROBERTS: Dr. Kramer, do you have some follow up.

DR. KRAMER: I had another question. I have the sense that the panel isn't quite answering this question and that is that what I'm hearing from a number of people is saying that in most circumstances you are not going to get a novel viral interaction.

And therefore it isn't a concern except -- and then there is the list of a couple factors that I laid out yesterday that people are bringing up, if you plant the plant in an area where the virus doesn't exist.

And that's really where this question starts

given that you have that circumstance. To what extent do you have an environmental concern. If the answer is, but that's so rare, you have no environmental concern because novel interactions never arise even in those circumstances, I want that to be on the record.

But barring that, the question really is beginning, assuming that you do have a novel viral interaction through one of these routes that have been discussed, what concern do we have then?

DR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: I do not think you would have any more concern than deploying traditionally bred virus resistant plants in an area where the disease does not occur and you have a strain of virus that overcomes that resistance.

So any time you put resistance out there you begin selecting for the virus to overcome it.

So I don't see why that has any more environmental impact than does the use of transgenes.

DR. KRAMER: I guess because there isn't the opportunity in natural bred virus resistance to have these

sort of interactions between two different viral genomes.

That's what this question is getting at. Not the opportunity to overcome resistance, but the opportunity for recombination and heterologous encapsidation. DR. ROBERTS: Dr. Falk.

DR. FALK: You mentioned specific examples, but I would say that those specific examples that have been mentioned still do not provide any novel possibilities.

And the specific examples you mentioned, I think -- the only one I remember is that the triple gene resistance in squash basically and then --

DR. KRAMER: I could go through the list again if that would be helpful.

DR. FALK: Okay.

DR. ROBERTS: Would you please.

DR. KRAMER: We can flip up the slide. It is one of the last ones in my discussion from yesterday.

DR. ROBERTS: Dr. Zaitlin.

DR. ZAITLIN: I think the one example that has been already mentioned here is the study of Thomas with

potatoes in which they took resistant potatoes, transgene resistant potatoes, tried to super infect with other types of viruses to see whether novel ones would arise.

And I think they found in that study, which is limited, that they couldn't detect any.

DR. KRAMER: Let me just go through them. The first example was transgenic multi resistances. This wouldn't necessarily lead to novel viral interaction, but the idea being if you had, for example, a virus resistance trait stacked with an herbicide tolerant trait, that crop could be planted in an area for the herbicide tolerance trait, but may not necessarily be actually infected by the virus.

The second example is if you had heterologous resistance, which I think Dr. Hammond mentioned again today, that you actually were conferring resistance to a virus by using the coat protein from a different virus and you may not actually have infection by that virus in the plant.

The third was the use of an exotic strains coat protein. Actually perhaps intentionally in order to try

to stave off a new infection from that virus from outside if you are concerned about it spreading to a Hawaiian Island, for example.

The fourth would be expression of new cells or tissues by the use of different promoter that could allow a virus to interact with viruses that don't normally infect the cells that that virus naturally infects.

And the final one would be through just simple alterations in the coat protein such that you have created something that actually doesn't exist in nature at all.

Really, the question is getting at, I mean, I guess there has to be two parts. If there is no consensus that these circumstances could lead to novel interactions or that these circumstances are so rare that they would actually never occur, then that should be put into the record.

But if there is any agreement that through any of these you could get a novel interaction, the question is really asking in those circumstances is there an environmental concern.

DR. ROBERTS: With that clarification, Dr. Falk.

DR. FALK: Then I stand by my statement, which, no, I don't think there is any opportunity for any real world opportunity for novel interactions to occur that would be of any significance.

DR. ROBERTS: Dr. Melcher, do you want to weigh in on this?

DR. MELCHER: I guess so. Yes. These situations will occur and there may be a recombination event that. That recombination event will very likely either produce a viral genome that does not function and will not go anywhere or it will produce a viral genome that has already been explored during evolution sometime, somewhere.

And therefore is not likely to be a novel product. The event may be somewhat novel that doesn't usually occur, but somewhere or other it probably has occurred.

DR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: I would like to agree with Dr. Melcher.

DR. MELCHER: I should mention that Dr. Sherwood

works on viruses that are a very good example of this. It is got one of these movement protein genes, but all the rest of the genes are genes that you find normally in animal viruses.

The bunya viruses.

DR. SHERWOOD: People have tried using cross protection with tospo viruses. And Dennis Gonzales had very little luck.

But there is an example where you look at the genome of tospo viruses. It is in bunya verity that are primarily animal or people infecting viruses.

But somewhere along the line it picked up a movement -- a protein that allows it to move from cell to cell while all the other bunya verity do not have an analogous protein.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I want to object a little bit. I think that I never feel very comfortable with the idea that all the possible combinations have been tested in nature.

It is based on the assumption that we're at some

sort of evolutionary equilibrium. I think human activity in terms of moving plants, moving viruses, moving vectors from one location to the next has been extremely important in bringing things in contact which had not occurred previously. I would suspect if we really were at evolutionary equilibrium there would be no emergent viruses in any case because they would all have been tested.

So I think we just need -- I would prefer to back off a little bit from this idea that everything has already occurred in nature.

DR. ROBERTS: Any other viewpoints? Dr. Falk.

DR. FALK: I would still argue that it doesn't present any novel risk.

DR. KRAMER: The question is, is there any environmental concern?

DR. ROBERTS: Dr. Falk

DR. FALK: Novel viral interactions that would present an environmental concern and I would say no.

DR. ROBERTS: Dr. Cooper.

DR. COOPER: At the risk of being provocative, I

would say it is premature to make that assessment. We really don't have any evidence on which to make it yet.

At least that would be my opinion. You may be right, but you may be wrong.

DR. ROBERTS: Dr. Kramer, it appears the panel is divided on this.

DR. KRAMER: It does. Maybe if Dr. Falk could expand a little bit on what he is basing that decision on. I would also like to push back on a couple of the people before who were caveating earlier statements that under certain circumstances as long as you plant the product in the area where the virus naturally infects.

It seems people have backed off of that. I want to clarify whether they have or not.

DR. ROBERTS: I think Dr. Falk you were asked to clarify.

DR. FALK: Of the examples you mentioned, I don't see that those represent novel situations where we will get significant new effects.

DR. ROBERTS: Dr. Zaitlin.

DR. ZAITLIN: I would like to support that.

Because once again it is this idea of fitness. Novel virus would have to be more fit than the one from which it is derived or ones from which it is derived.

There are many examples as pointed out from Dr. Sherwood that these viruses are really populations of viruses. There are many strains contained within them. They are lurking around, but they don't get to express themselves because the most fit strain is the one that predominates.

DR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: I hate to say this, but my colleagues on this side of the isle the major impact, I think, it's going to be on increasing the diversity of viruses is just continuation of modern agricultural practices and that this is not going to pose any greater risk than the deployment of any other management practice or resistance genes or cross protection or any other thing that we have done in trying to prevent disease in crops.

DR. ROBERTS: Dr. Falk.

DR. FALK: I agree with what Dr. Sherwood said. And I think that Dr. Tepfer also pointed out the biggest

problem, I think, we have in new viruses is the activity of human beings and what we have moved around.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I essentially concur with what has recently been said. I think that there is little probability of novel interactions. The interactions can occur readily in mixed infections and any possible novel interactions I think are as minimal significance.

DR. KRAMER: For the second part of my question that would be under all circumstances. There is no caveat to that statement anymore about planting the product in the area where the virus infects?

DR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: I would say no more greater risk to the general population as getting in a car and driving.

DR. ROBERTS: I'm not sure that's very reassuring, Dr. Sherwood.

Dr. Melcher.

DR. MELCHER: I guess I should compromise a little bit and say yes there should be some caveats that

can be very easily put in, which I guess we'll be talking about later.

That is if you are using a transgene from a virus that is limited to certain tissues, when you make this transgenic plant you should not use a promoter that will express it all over.

You should have it just in being expressed in the tissues in which it exists.

DR. KRAMER: I realize we're going to get to that a little bit later, but the question is why would we bother doing that if nobody is suggesting that there is any environmental concern at all. So when we get to that question, are we going to be jumping back to this question here to try to understand why any of those mitigating things might be suggested.

DR. MELCHER: We're finding that not everybody is in agreement with this. So -- alright --

DR. ROBERTS: Dr. Kramer, I think -- Dr. Hammond can speak in just a minute. But this is probably going to be one of those things where there is not a consensus among the panel and there are going to be some differences

of opinion.

DR. HAMMOND: I think we do those things that Dr. Melcher was just addressing to stave off perceived risks not because we consider that they are real risks.

DR. ROBERTS: Dr. Zaitlin.

DR. ZAITLIN: Putting an impossible burden to say there is no possibility at all, none of us here would say that that's -- that there is no possibility at all. But as enumerated here, the probability of this happening is very very low.

DR. ROBERTS: I think Dr. Kramer -- it wasn't so much no possibility, but in sort of -- that the answers seem to be conditional. Under certain sets of conditions we're not concerned. And I think what she was trying to elicit if you throw out all the conditions and just in principle would you be concerned about this.

We seem to be getting -- the panel seems to not have a consensus answer on that.

Let's then move to question 12.

DR. KRAMER: What conclusions can be drawn as to whether the likelihood of recombination and/or

heterologous encapsidation would be increased or decreased in a transgenic plant compared to its non bioengineered counterpart.

DR. ROBERTS: Dr. Allison.

DR. ALLISON: Thank you. If I may read this into the record, please.

Viruses have gone through years of evolutionary refinement and adaptation to their host. During this time they have maintained evolutionary flexibility by preserving their capacity for RNA recombination, high mutation rates through an error prone replicase and reassortment in the case of multi component viruses.

Comparisons of viral nucleotide sequences of different viruses have identified blocks of similar sequences. These blocks are considered evidence of previous recombination events. Such recombination events most likely occurred during mixed infections.

While mixed infections are frequently viewed as providing extensive recombination opportunities, viral reproductive isolation maybe greater than envisioned.

Viruses do not constantly replicate within an

infected plant cell. Rather, the infection spreads from cell to cell.

As replication in one cell is completed, some of the newly replicated RNA is warehoused in a form of virions and the active infection spreads to adjacent cells where the process is repeated and the infection spreads throughout the plant.

Trans encapsidation and recombination events recorded in nucleotide sequences of different viruses suggest that two or more viruses replicate within the same cell simultaneously.

However, this may not be the norm. Finding two or more viruses in a plant or plant cell does not mean that they were introduced to that cell and replicated simultaneously.

In fact, this may be the exception. Consequently, a mixed infection may represent a collection of different viruses that were introduced independently over a span of time and never replicated simultaneously in the same cell.

In my own lab and as indicated by others that

there are other evidence of this, inoculations leading to mixed infections of brome mosaic virus and cowpea chlorotic mottle bromo virus were most successful when the inoculations were separated by two weeks. Simultaneous inoculations led to the recovery of only one of the two viruses. Thus mixed infections may not provide the unlimited RNA recombination opportunities envisioned when mixed infections are sorted. In contrast, a constitutive promoter ensures that the transcript of the viral transgene is available to a replicating virus in each newly infected cell. Consequently, recombination opportunities are constantly available to a replicating virus.

And this distinguishes recombination opportunities in PVCP PIPs from those of mixed infections. Therefore the likelihood of recombination appears to be increased in transgenic plants as compared to their non bioengineered counterparts.

Now, for trans encapsidation, encapsidation of viral genome depends on specific protein RNA interactions. The constitutive promoter ensures the availability of

coat protein, but reports suggest that the quantity is limited as compared to the coat protein translated directly from the viral genome.

If the viral genome is capable of trans encapsidation in mixed infections, it should also occur in transgenic plants. Theoretically, trans encapsidation in non bioengineered plants is dependent on the availability of the coat protein of one virus to the genome of the other, a situation dependent on the simultaneous replication and translation of the two viruses in the same cell.

Trans encapsidation is now a recorded event in both PVCPs and non bioengineered plants. Situations would be different for different plant species and transgenic lines. Therefore I find it difficult to clearly predict which situation will provide the greater number of trans encapsidation events.

DR. ROBERTS: Thank you, Dr. Allison. Dr. Falk.

DR. FALK: I believe that heterologous encapsidation events almost certainly will be less or

decreased in transgenic or non transgenic plants -- Or in transgenic versus non transgenic plants. I also believe that the biological significance of those will be even greater reduced.

Now, and I think we'll talk later about ways that we could modify them if they were biologically relevant, I don't think that new or greater possibilities for trans encapsidation and resulting biological effects will result even though some have said that all of the plants now express the transgene in all tissue types.

I still don't think that that's of biological relevance. So I think that the results will be decreased.

I also believe that many of these arguments could also be applied to recombination events. I think that one of the things that Professor Allison did not mention in regards to recombination is that certainly tighter of RNA molecules in mixed infections will be much greater than say the level of the transgene MRNA that's available for recombination events.

Also, I think several people have mentioned

things like virus exclusion. That is not -- when unrelated viruses coinfect the cell, that they set up their own little replication factories and sort of exclude the other virus, well, I think if that is true and there is lot of evidence then, in fact, that is not completely true.

But if that is true, then they would just as likely exclude host MRNAs. In fact, in our lab we have done experiments with mixed infections of unrelated viruses and show that we can get recombinant RNAs from the unrelated and coinfecting viruses.

And it is not dependent upon the time of inoculation. It doesn't matter whether you co inoculate or inoculate one before the other. So opportunities for recombination or RNA interaction events to occur in mixed infections are very great.

DR. ROBERTS: Dr. Bujarski, do you want to resolve this?

DR. BUJARSKI: It would be difficult to resolve, but I would like to add what I wrote on that question as regarding to RNA recombination. Theoretically, there

should be no difference in recombination possibilities for the transgenic RNA as compared to the non bioengineered viral RNA. However, several factors can contribute to recombination activity and thus the differences among them may define the final figures.

They include first the expression in different tissues of transgenic RNA as compared to non transgenic environment. Second, going deeper to the subcellular level the expression of transgene versus non engineered RNA may occur within different intracellular compartments.

Three, different levels of expression might occur. Usually they are lower for transgenic RNA for transgene viral RNA.

Four, replication and encapsidation might also differ in transgenic and non transgenic situations.

And five, possible molecular differences may exist between transgene and non transgene RNA.

So to answer the question I would say that there might be -- that there is a possibility that the probability or likelihood of recombination in both

situations might be different.

DR. ROBERTS: If I heard correctly, we have increased, decreased and might be different.

I think we have covered the possibilities. Let's see how --

Dr. Nagy and then Dr. Tepfer.

DR. NAGY: Actually, I'm not going to simplify, but make it more complicated. We have one more, actually, scenario, which I would like to add that I don't think we are only discussing the possibility of recombination, but also that the newly emerging recombinants are going to be more a fit than the original y type virus here.

For this respect I would like to add indeed I would agree with Dr. Allison that probably the possibility of recombination is going to be increased in transgenic plants.

However, the newly made recombinants might need to fight against the same type of resistance, which is now making the original, the y type virus less fit into that transgenic plants.

This is an added factor that I think is very

difficult to predict whether the new recombinants would be -- I mean the plant would not be resistant against the new recombinant.

DR. ROBERTS: Thank you. Dr. Tepfer.

DR. TEPFER: I wanted to sort of in support to Richard Allison's proposal to provide a bit of data, part of which is not published yet, we're in the process of doing this.

As I mentioned previously we are in the process of studying recombination in transgenic and non transgenic plants using reverse transcription PCR.

RTPCR is a very nice protocol for doing this. It is extremely sensitive.

And in quite a lot of experiments in plants infected with two cucumo viruses, non transgenic plants, we routinely find are able to detect recombinants on the order of 5 to 10 percent of the plants.

Now when we do exactly the same type of experiment with transgenic plants expressing a coat protein gene, we infect with a related cucumo virus. Here we see a much higher frequency of detection recombinants.

It is on the order of 30 to 50 percent.

This is I think in support of what Richard was saying previously.

DR. ROBERTS: Yes, Dr. Nagy.

DR. NAGY: I would like to go back. The new recombinants have increased fitness? DR. TEPFER: Actually I don't think that's the question of this particular point that's being discussed here. The question is not the fitness, but -- if you read the question that's posed, it is not the fitness. We're just looking at whether the transgenic plants are going to be a greater or lesser site for recombination to occur. Is that correct.

DR. KRAMER: That's correct.

DR. TEPFER: I don't think that's part of the question at this precise moment. DR. ROBERTS: Yes, Dr. Bujarski.

DR. BUJARSKI: I would like to add one thing that has not been mentioned. Isn't it possible that the expression of viral MRNA can actually trigger RNAI pathway? And in this aspect then in a way the

recombination likelihood might actually decrease.

DR. ROBERTS: Dr. Allison.

DR. ALLISON: We have experiments that are intended to address that. Our hypothesis is that, in fact, in the situations where RNA is -- RNAI is invoked, then we anticipate and hypothesize that there will be much less recombination in which case, although this is not relevant to this particular question, there may be a superior technique to coat protein mediate a resistance.

DR. ROBERTS: Are there other panel members with experience in this that want to weigh in?

The panel is divided.

DR. KRAMER: Since we have no agreement, can I for clarification ask if Dr. Tepfer's comments were the only ones actually supported by data? He mentioned some unpublished studies.

Is anybody else referring to anything besides theory in answering this question?

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: I think Dr. Allison presented some data too.

DR. ROBERTS: Dr. Allison, would you clarify that?

DR. ALLISON: In our experiments we have been able to recover numerous recombinants, many of which are blind alleys (ph) in that the recombinations have led to pieces that are not truly going to be a virus because the open reading frame is not maintained.

But these were in transgenic plants that were expressing a part of the coat protein gene. But those experiments were not done in direct comparison with non transgenic plants.

DR. ROBERTS: Dr. Falk.

DR. FALK: And I also alluded to some data we published in PNS a few years ago where we did show that unrelated viruses do, in fact, recombine in cells and do make functional MRNAs. But we also did not then have the other control being transgenic plant in those experiments.

DR. KRAMER: Thank you.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I have no cause to query the data

that's been presented. However, one of the conclusions from the 1995 USDA APHIS and AIBS workshop is that the question that should be posed is at what level of use does the risk associated with recombination in transgenic plants outweigh the agricultural and environmental benefits of resistance.

I think that that question cannot yet be answered, but that there is definitely significant benefit from deploying resistance.

DR. ROBERTS: Dr. Allison.

DR. ALLISON: I definitely agree that the benefits outweigh the risks. The worst thing we can get is a new plant virus. And new plant viruses emerge by natural, more natural mechanisms all the time.

DR. ROBERTS: Again, these are sort of big picture comments. They are important, but I would like to -- we can certainly tackle those at the end. I would like to sort of get through the more technical questions that are being posed first, though. Are there any other comments on question 12? Dr. Sherwood.

DR. SHERWOOD: I just wondered if EPA is

interested in changing that question to what conclusions can be drawn as to whether the likelihood of viable recombination and/or heterologous encapsidation occurs.

Insert the word viable since that's really what would be of interest. If it is non viable, it really doesn't matter if recombination events occur. It only occurs if you are going to get an infectious virus out of it.

DR. KRAMER: I guess the thought would be that production of a viable recombinant would be an extremely rare event, which would be even more difficult to study directly.

If we can show just an increase in overall recombination rates, we would then assume that you would get an increase in overall production of viable recombinants.

If that's an incorrect assumption, then please comment on that.

DR. ROBERTS: Dr. Tepfer will.

DR. TEPFER: In the experiments that we have done at least with the non transgenic plants with 2 wild

type viruses, these are not mutants, these are perfectly viable viruses, we haven't -- I think we have done about 2 dozen of the recombinants. We have looked at the biological properties afterwards.

About half of them -- I don't have numbers in mind. But about half of them are non viable. But there are quite a few of them that are viable. Some of them have other sort of more minor defects.

They affect their ability to move within the plant, things like that, but the truly dead viruses are not by any means a majority.

DR. ROBERTS: What is the time frame for publishing those results, Dr. Tepfer?

DR. TEPFER: It's in press -- we're in the process of writing that up. There are 2 articles that will be appearing in the next couple months.

DR. ROBERTS: Thank you. Dr. Zaitlin.

DR. ZAITLIN: As you can see, there is disagreement here, but the question doesn't give us the opportunity to say that the possibility is unchanged. It only gives us increased or decreased.

DR. KRAMER: That would certainly be an option.

DR. ROBERTS: Yes, I think if someone wants to articulate that -- I believe the beginning Dr. Nagy's comments indicated that's a possibility as well or I'm sorry Dr. Bujarski's comments.

Dr. Tepfer.

DR. TEPFER: I want to say that from my personal point of view, this is not the important question either. I think that if you consider that there is going to be a vast array of recombinants created in transgenic and non transgenic plants, the question is fitness and biological properties.

Just the fact that we may be seeing a higher rate of appearance recombinants in transgenics is not necessarily a factor of risk.

We do see it. That's what we have seen, but I don't believe that that's a problem.

DR. ROBERTS: Dr. Allison.

DR. ALLISON: In terms of viable recombinants, of the recombination events that we have recorded, by far

the majority are non viable recombinants. It is only through means of selecting for viable recombinants that we have been able to recover them successfully.

In our experience competing those that were viable with the wild type virus, the wild type virus was always a superior virus and outcompeted in a period of 2 to 3 passages the recombinant virus, which was then excluded from the plant.

DR. ROBERTS: Dr. Nagy.

DR. NAGY: A question for Richard Allison. Did you test the viability in transgenic plants, resistance against the Y type virus?

DR. ALLISON: No. It was not done in resistant plants. Is that what you mean? DR. NAGY: Yes.

DR. ROBERTS: Dr. Bujarski, do you want to add something?

DR. BUJARSKI: I would like maybe try to recapitulate somehow what we are saying. That no matter if the rate of recombinants is low or higher, later on there is some more important biological bottlenecks, bottlenecks that can simply somehow define the final

outcome of the viral RNA population.

In this aspect, indeed, I would agree that really it doesn't matter if there is a decrease or increase likelihood of recombination, but what is important is this more downstream final outcome.

DR. ROBERTS: Dr. Kramer, I think that probably our response will include the point made by the panel, that the fitness of the recombinants is an important factor ultimately in determining risk.

Dr. Allison.

DR. ALLISON: I have a way of interjecting things at inappropriate times in this meeting. However, I think I would like to add one more thing to the record at this point, because it ties together something that Dr. Zaitlin has brought up, subliminal virus infections and the possible use of those in recombination events.

So viruses infecting the transgenic plant are well adapted to the plant species. Therefore, the useful incorporation of genetic information through recombination may be a rare event and a recombinant would be rapidly excluded by the wild type virus.

In contrast, subliminal virus infections, those viruses that replicate in the initially infected cell, but lack the necessary mechanism for systemic infections, may find all or part of the viral transgene useful.

While the coat protein structural role is consistent among viruses, various additional functions, including systemic movement, have been identified in different viruses.

Laboratory experiments investigating the availability of the transgenic transcript for recombination with a replicating virus have been criticized as providing an unreasonably high degree of selection pressure.

However, subliminal viruses undergo a similar selection pressure as the genome that obtains the key to open the door to the cell next door will find an expanded reproductive environment.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: I'm trying to recall the literature on coat protein involvement in movement through the plant. And I may not recall it correctly. But my

impression is that the coat protein is required for movement beyond the initial clump of cells, that the initial movement is really just what the movement protein function is.

So in that case, the subliminal infection would not result -- being aided by a coat protein that's transgenic would not result in further movement. But I may be wrong there because my memory is not so good.

DR. ROBERTS: Dr. Falk.

DR. FALK: I was going to make a similar comment to Dr. Melcher's and say that, yes, it is unlikely, then, in my interpretation of what Professor Allison said, that the virus would ever get out of that initially infected cell and if it doesn't it is not going to move on to another plant.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I think it depends on what viral group you are talking about. There are some where the coat protein is absolutely essential for both local and long distance movement, in which case at least theoretically this kind of thing could occur.

But it is not the case of all plant viruses, obviously.

DR. ROBERTS: Everybody is nodding. That might mean it is a good time for a break.

We'll take advantage of this moment. It is 10:25. Let's take a 15 minute break. Come back. We'll decide whether or not we're finished with question 12. If we aren't, we'll finish it. If we are, we'll move to the next one.

(Thereupon, a brief recess was taken.)

DR. ROBERTS: Let me be sure that we have actually finished off 12 before we go to 13. Let me ask the panel members if they have anything they would like to add on 12.

I don't see anything. Let me ask Dr. Kramer if there is any clarification.

DR. KRAMER: Could I ask directly once again an issue that came up at the end. That is, would the panel say it is an incorrect assumption that an overall increase in the frequency of either heterologous encapsidation or recombination would also increase the frequency of a significant event of heterologous encapsidation or viable

recombinant?

MEMBER: Could you repeat that? I'm sorry.

DR. ROBERTS: Yes, you may have to repeat the question.

DR. KRAMER: I guess I sensed from the panel before that there was some discomfort with the premise of this question. That is that there would be any overall significance in an increase in the rate of these events.

And the assumption behind this question is that an overall increase in recombination per se would lead to an increase in the production of a viable recombinant. Is that an incorrect assumption?

DR. ROBERTS: Dr. Allison.

DR. ALLISON: I believe that just looking at the odds, the more recombinants you make, the more likely you are to come up with a viable recombinant. You interpret that correctly.

DR. ROBERTS: Dr. Tepfer and then Dr. Falk.

DR. TEPFER: I think my point of view on this is that if you have the same types of recombinants that are present under the sort of circumstances, simply increasing

the frequency doesn't change the impact.

The real question is the nature of recombinants that are created and not their frequency. If it is the same ones, then it is not going to have a different impact.

DR. ROBERTS: Dr. Falk, then Dr. Bujarski.

DR. FALK: I agree with what Mark just said about the nature of the recombinant. Richard used the term viable. But I think it is really the nature or the potential or the significance of that recombinant.

And I would argue that lumping heterologous encapsidation and recombination and sort of -- and considering them similarly here, which it sounds like from the question, that I think they are different.

I would say for heterologous encapsidation it is no.

DR. ROBERTS: Dr. Bujarski and then Dr. Melcher.

DR. BUJARSKI: As regarding recombination, I would like to agree with Mark and Bryce Falk.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: I detected disagreement, I think, between Mark and Bryce. No, I'm sorry. Dr. Allison spoke first. Right?

DR. ALLISON: Yes, I did.

DR. MELCHER: I agree with the assumption and I don't see any way that the mechanisms that generate recombinants have a way of detecting whether the recombinants are going to be viable or not viable.

I think that if there is a small proportion of viable recombinants that could be generated, increasing the general frequency of recombination will increase their general frequency also.

DR. KRAMER: So there is disagreement on this issue as well?

DR. MELCHER: Apparently.

DR. ROBERTS: Dr. Nagy, then Dr. Tepfer, then Dr. Hammond.

DR. NAGY: I would like to make sure this is a theoretical assumption. We don't really have exact data about the significance of the recombinants generated in transgenic plants.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I guess what I was trying to say is the -- of course if you have -- you are going to generate more recombinants if you have a higher frequency of recombination. I don't think that that's anything that anyone would like to question. It is just a question of whether this is significant.

I guess there is a difference between whether you think the abundance of the recombinants is limiting or not in terms of their emergence.

I think that what I'm trying to argue is that it is quite likely that the abundance of the recombinants is not the eliminating (ph) factor, but their ability to be viable and to outcompete other types of molecules in the plant and in the ecosystem.

In which case, if you increase their absolute numbers, you haven't really changed anything if you are talking about the same populations of molecules that are being produced.

DR. HAMMOND: I think we have -- I don't think any of us are questioning that an increased frequency of

recombination will lead to an increased frequency of viable molecules. The question as Mark Tepfer has just said is whether there is any significance.

And I believe that there is no a priori reason to believe that recombination between a virus and a transgene will lead to any more significant a recombinant than a recombination between 2 viruses and a mixed infection.

DR. ROBERTS: Dr. Allison.

DR. ALLISON: I would just agree with that.

DR. ROBERTS: Anyone else want to weigh in? Dr. Kramer?

DR. KRAMER: I think we can move on to the next question.

DR. ROBERTS: I was going to say I think our response is as clear as it is going to be at this point. Let's move on to question 13.

DR. KRAMER: How effective is deleting the 3 prime untranslated region of the PVCP gene as a method for reducing the frequency of recombination in the region of the PVCP gene.

Is this method universally applicable to all potential PVCP PIP constructs. Would any other method work as well or better. What methods are sufficiently effective and reproducible such that actual measurement of rates to verify rate reduction would be unnecessary?

DR. ROBERTS: Is there any background on this question you want to give us that might help us?

DR. KRAMER: This is really looking at what the practicality might be in the usefulness of having a factor that's similar to what is listed in I think question 16 such that a product could be shown to have reduced risk essentially because it had employed such a method.

DR. ROBERTS: Dr. Bujarski, could you lead off our discussion on this one?

DR. BUJARSKI: Yes. In terms of recombination rate, the ability of viral RNA to undergo replication enhances the chances for recombination not only because replication propagates and increases the level of recombination substrates, but also because of the mechanism of recombination involved.

Template switching by the replicase enzyme

during replication, RNA replication is a commonly accepted mechanism for viral RNA recombination. Therefore, the removal of 3 prime UTR is expected to reduce a combination rate due to the bar (ph presence.

However, to be effective, to remove 3 prime UTR must carry to the signals of RNA replication that are recognizable to the viral replicase. Thus, in some cases, the replication signals other than the 3 prime UTRs must be removed from the transgene MRNA to reduce its replicate ability.

Other methods of alleviating the risk of recombination would involve the reduction in sequence homology, for instance, by changing the third base in the cordon triplet between PVCP PIP and the viral RNA or reduce the sequence or modify the sequence composition GC versus AU content.

Also, it must be said that the transgene MRNA by itself might induce RNA silencing events that could reduce the levels of recombining RNA substrates.

It is, however, questionable whether all these methods are sufficiently effective to warrant the rate

reduction beyond the safe level. And this remains to be established experimentally.

Several general aspects must be considered here. First, it is likely that each particular viral system responds differently to the bav (ph preventive approaches because each value has its own set of tools securing molecular interactions during recombination.

Second, although not sufficiently proven, recombination may depend upon the host genome environment. In other words, the same values might recombine with a different rate because of host -- different host factors involved.

Third, there might be different mechanisms other than copy choice (ph) supporting viral RNA recombination. For instance, breakage and 3 ligation (ph) and then the removal of replication signals might not completely prevent to avoid crossovers.

In reality, however, the Naston (ph recombinants might be overpowered by selection pressure with best fit virus that outcompete the recombinants especially in field situations where viruses replicate under so-called low

selection pressure.

Overall, there is nothing universal approach in eliminating the risk of viral RNA recombination. And although we might reduce its probability, the measurement of recombination rates might be necessary.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: I agree with Jozef's original comment on the 3 prime untranslated region.

But I would like to go into it a little bit more because I think it is just a matter of words and so forth, because Dr. Kramer yesterday did present in her slide that besides removing the 3 prime untranslated region one would exclude replicase recognition sites or other hot spots.

So the method of removing the three prime untranslated region is not universally applicable to all potential PVCP PIP constructs. Deleting the 3 prime untranslated region will only be important for those viruses in which this region contains the promoter for minus strand synthesis, which is not all plant viruses.

A more important statement would be that all viral promoters minus strand plus strand and subgenomic

ought to be avoided in such constructs.

I have 2 other ways that have already been mentioned, but would like to make sure that they occur here, that one could reduce -- what are we trying to do, we're trying to reduce the frequency of recombination.

One is limiting expression of transgenes to those tissues in which the virus from which the transgene comes. That's a desirable method for reducing the frequency.

And the other is making sure that the level of transcription is the minimum required for the protective effect, so that the frequency of recombination is reduced.

As far as whether this is effective and reproducible enough to preclude measurement of rates, I really do not have any knowledge and I would defer to my colleagues that do have that knowledge.

DR. ROBERTS: Thank you. Dr. Allison.

DR. ALLISON: If I can again read into the record, I just want to state that I agree with Dr. Melcher about the non universal placement of replication signals

in the 3 prime UTR, untranslated region. And that's not addressed in the statement here.

The 3 prime untranslated region has been maintained in genetic constructs partly because it is believed to provide stability to the RNA transcript within the cytoplasm due to their typical secondary structure and partly because they are associated with the original clone.

As numerous CDNA clones used to establish coat protein mediated resistance used primers that hybridized to the 3 prime terminus of the virus. This is especially true of poly idinolated (ph) viral RNA such as poty viruses.

Due to the biochemical rules of DNA and RNA replication, the replication processes initiated at the 3 prime end of the virus utilizing a sequence/structure maintained in this 3 prime UTR. As it was stated this is not 100 percent, but it is frequently found there.

Thus by including the 3 prime UTR in the construct, a related virus may recognize the viral transcript as a template and initiate replication on the

transgene transcripts.

Two things may happen. The replication complex may use this initiation point to begin replication and switch back to the viral RNA as a template during recombination.

Or the replication complex may make a complimentary copy of the viral transgene which may also be available for RNA recombination.

Since all viral RNA viruses must use the 3 prime UTRs as replication initiation sites, it makes good sense to withhold these sites from the viral transgene as it would appear that they enhance recombination activity of the transgenic transcript.

In laboratory experiments where transgene constructs have been involved in recombination events, removal or destruction of the 3 prime UTR, untranslated region, have reduced recombination to below experimental detection limits.

If transcript stability becomes an issue, other nonviral RNA stabilizing sequences could be added.

Now, in addition to removal or destruction of

the 3 prime UTR, several additional methods have been suggested for reduction of recombination events involving the transgene.

These include using the smallest resistance generating viral fragment possible to generate resistance. Disruption as Jozef has said disrupting a potential AU rich recombination hot spots. Providing point mutations and or deletions in the transgene that would enable -- that would disable a functional aspect of the coat protein when identified.

In addition, Dr. Melcher has indicated using the smallest amount of transcript as possible.

These methods do not have to be used independently. They can be combined to help ensure that either recombination does not occur or that the incorporated segment is useless to the recombinant.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: This question is based around reducing the frequency of recombination. But as we have already discussed previously, there is a difference between the frequency of recombination per se and the

frequency of viable recombinants.

Removing the 3 prime end certainly reduces a lot of the possibility of recombination as does the deletion or avoidance of incorporation of other replicase recognition sequences such as subgenomic RNA promoters.

Similarly, minimizing the transgene expression levels by deliberate induction of RNA silencing, but then this little point of using the coat protein gene itself, as it is not necessary to have the coat protein gene expressing coat protein to induce RNA silencing, there are many other ways by which one can make any potential recombinant less viable by introducing mutations.

But I think those are dealt with more appropriately and in one of the succeeding questions. I'll leave it at that. DR. ROBERTS: Thank you. Any other comments? Dr. Kramer.

DR. KRAMER: Can I reiterate what I heard then? You've listed a number of different strategies that could be employed to reduce recombination. It's sounds like there is not a single strategy that would be universally applicable to all constructs. That, rather, it would have

to be looked at on a case-by-care basis.

It sounds like -- actually, I think there was only one person that directly addressed the last part of the question as to whether any of these methods are sufficiently effective and reproducible such that you would not need to actually measure rates to verify that they had worked.

Is there agreement -- I think what we heard from one person was that these methods were not -- you are not willing to commit to say that these methods were sufficiently effective and reproducible.

Is there any other discussion on that?

DR. ROBERTS: Any disagreement with that statement? Dr. Allison.

DR. ALLISON: I agree with the statement. That at this point none of these methods are going to be 100 percent efficient. While the easiest recombinants to recover are those that are viable and have resulted from homologous recombination events, there are numerous, at least in our laboratory we have recorded numerous heterologous recombination events leading to nonviable

viruses.

In each one of those cases it would appear that these types of approaches would not have inhibited those recombination events.

DR. KRAMER: But would the overall rate be decreased if one of these methods were employed?

DR. ALLISON: Yes.

DR. KRAMER: Without measuring it? You can be confident of that?

DR. ALLISON: I feel confident.

DR. ROBERTS: People can weigh in verbally on that. Dr. Tepfer.

DR. TEPFER: I wanted to agree with Richard Allison on that. I think that when you remove the 3 prime non coating region, the only recombinants that can occur that would be viable are double recombinants.

If the frequency of crossing over at any given site is x, then you are increasingly enormously the -- you have to multiply the two frequencies in order to be able to recover the double recombinant.

Just mathematically, physically, I think we can

all feel rather confident that doing this, forcing double recombinants to be the only viable ones is indeed a way of decreasing the frequency.

I would be surprised if that were in itself controversial. Whether that is sufficient or not, of course, is going to depend.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: I guess when I said I would defer to my colleagues, I thought that Dr. Allison would make it clear that his conviction is based on experiments from his laboratory, which I think is correct.

DR. ALLISON: That's correct.

DR. ROBERTS: Any other follow ups?

DR. KRAMER: No. Thank you.

DR. ROBERTS: Let's move on to the next question.

DR. KRAMER: Are any methods for inhibiting heterologous encapsidation or transmission by insect vectors universally applicable to all PVCP PIPs? Which methods are sufficiently effective and reproducible such that actual measurement of rates to verify rate reduction

would be unnecessary?

Just as a clarification where this question is coming from, we're looking at a factor -- to judge qualification of a product we would want something -- we want to know how specific we can be in laying out that factor. Is there something that is going to be known to work in all cases and also looking at the practicality of it.

If rates needed to be measured, that's important to know.

DR. ROBERTS: Dr. Falk.

DR. FALK: I would say in answer to what you just said, can anything be applied in all cases, I would think that at this time, no. But I think that the more we learn about factors that are important in these two events, yes, things could be done. But I think it would vary on a case-by-case basis.

And I think it would only be important to do that if these were determined to impose a real risk.

I think the first question here is asking two different things. And I think it is important to realize

that heterologous encapsidation and transmission by insect vectors is not synonymous. That I think, yes, you do need to have heterologous encapsidation to get transmission.

But because you have heterologous encapsidation does not necessarily mean that you will have insect transmission. So it is important to remember that.

If we were talking about affecting heterologous encapsidation, obviously, it would be important to just delete or mutate amino acids -- the cordons in coating (ph) for the amino acids that are important in virion assembly.

In many cases, we don't know this. But we do in many cases. Certainly all filamentous rod shape viruses have 3 amino acids conserved spread out among the protein itself.

I'm not positive that those are important in virion assembly. But since they are conserved among all filamentous plant viruses, and this would be serine, arginine and aspartic acid that something like that could be considered if heterologous encapsidation was important.

Certainly, in regards to insect transmission, we do know for some viruses we do know important features that are important in some cucumo virus transmission, poty virus transmission and luteo virus transmission.

This was given in the background information. It has been brought up in discussion yesterday. Those domains or amino acids also could be considered if heterologous encapsidation and subsequent transmission were deemed to be a risk.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: Just in following up on Bryce Falk's comments, with which I agree quite entirely, I think there is only one case that's been described where it has been possible to interfere with the virus assembly process which is to say with heterologous encapsidation and to conserve the resistance phenotype.

This is from Edgar Mize's lab (ph). So I think that would be a rather difficult test to try to generalize. I think there is also evidence from tobacco mosaic virus in Roger Beach's lab(ph) that the assembly process itself is important in the resistance that's

conferred.

So that might be a counter example where it may not be possible to interfere with potential heterologous encapsidation without losing the resistance.

I'm sort of maybe more positive than Bryce is about interfering with the vector transmission itself.

Admittedly, there are these three cases that are rather well known groups of viruses where we know how to interfere with the vector interaction in a way that does not prevent the resistance phenotype.

I think what is important is sort of the principle involved that where necessary this kind of strategy can be developed so that there may be other virus groups in which it may have not been studied yet which may be desirable to prevent heterologous vector transmission to occur.

And then this can be explored using the same sorts of principles as has been developed with these three virus groups that are very clearly understood.

So I think that in a sense from my thinking you are getting close to at least what could be a considered a

potential universal principle in the case of preventing vector transmission. Where as I would say that preventing the encapsidation itself from occurring is not likely to be universally possible.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: I guess I should first address the question of interfering with assembly that was brought up. The residues that Dr. Falk mentioned, the serine, the arginine, the aspartic acid, at least two of those are directly involved in the assembly of particle.

As Dr. Tepfer mentioned that's probably important for the protection effect. So the two go hand in hand.

On the other hand, there are other residues in these coat proteins that are not as universally conserved, but play roles in forming the structure, and it might be possible to engineer a coat protein gene so that it has one of three residues altered so that it would not co-assemble with the invading virus.

The hazard of doing that is that it might make it possible to co-assemble with still another virus. So I

think that's a very speculative risky sort of thing.

However, there are other things that one can think about. There are two kinds of viruses, the isometric ones and the elongated ones. Each of them have components on their surface that are mostly the ones that are involved in interaction with other organisms. For example, the vector.

So restricting the portion of the PVCP transgene to that encoding the structurally essential core may be a somewhat universal strategy for limiting adverse effect of trans encapsidation, but not for limiting the trans encapsidation itself.

I'm saying the vector transmission part may be reduced by concentrating on the core particles. For isometric viruses, that would mean using only the s domains, omitting the p domains that interact with those other organisms.

For helical viruses, maintaining the four helix bundle core and the internal nucleic acid binding loops while dispensing with the surface displayed n terminal and c terminal ends would reduce the chances of nonwanted

vector transmission, but still likely retain the effectiveness as a plant protectant.

Trans encapsidation could be prevented or inhibited in another way if the plant contains resistance genes for all the viruses that are capable of infecting that plant.

Such a plant would not allow infection by an invading virus, thus there would not be a genome of an infecting virus to be trans encapsidated. The difficulty of this approach of course is manyfold, I suppose.

But two that I can think of; one is identifying all the viruses capable of infecting the plant, it may be not possible, not only its major pathogens, but other viruses too. And there is of course the technical challenge of creating such plants. Another universal strategy and one that I think is well recognized is to limit the amount of transgene protein produced. The lowest concentration consistent with protection should be used to reduce the frequencies of trans encapsidation just as for recombination.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I believe that there is no universal approach. Different virus groups assemble differently and are transmitted differently. And I think that we know enough about most virus groups to be able to suggest approaches that work for specific virus groups such as removing the DAG triplet from most poty viruses, removing the read through domain from luteo viruses or ablating the RNA binding sites in some instances so that particles cannot be assembled.

However, there are other virus particles that are stabilized rather than by protein RNA interactions, solely by protein protein interactions. And in that case, you have no RNA binding site to ablate.

So that requires a different approach and would need to make modifications to the surface motifs that are involved in vector transmission.

Closest thing to a universal approach is to get away from using coat protein and go to RNA silencing, which is kind of a different approach, and then it is not relevant to coat protein, which we're discussing here.

With reference to assembly mutants, there is a

potential problem with modifying subunit subunit interactions that would prevent assembly. Because in some instances, those may induce a hypersensitive response that would be deleterious to the host plant. I think that's been shown with Jim Colver's lab (ph) that some of the TMV coat protein subunit subunit interaction sites when mutated induced the hypersensitive response as well as inhibiting particle assembly.

To do that does not necessarily solve the problem.

DR. ROBERTS: Other comments. Dr. Falk.

DR. FALK: I think the last thing did solve the problem because you are probably not going to be able to regenerate that plant.

DR. ROBERTS: Dr. Kramer.

DR. KRAMER: I'm going to follow up with given that there is not a single method that would be universally applicable, given that we might have methods that would be known to work in a particular virus group, could you answer affirmatively for the second part of the question that for certain situations, certain virus

groups, certain modifications within those groups you may not need to actually verify rate reduction because those methods are known to occur?

DR. ROBERTS: Panel response. Dr. Hammond.

DR. HAMMOND: I would believe that to be true.

DR. ROBERTS: Dr. Tepfer is nodding for the record. Dr. Falk.

DR. FALK: I'm not aware, though, that those are done in transgenic plants. Are you?

DR. HAMMOND: Which?

DR. FALK: The methods that are sufficiently effective and reproducible such that actual measurements to verify rate reduction would be unnecessary. I think we have done these things with virus mutants and things and now we're suggesting that they should work in transgenic plans, but I don't know that they have.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: Isn't there work published on deleting the DAG motif in poty viral --

DR. FALK: Yes.

DR. TEPFER: I think there is. I think also

that some of the transgenic CMV CP plants are using a nonaphid transmissible strain, and I think that that was verified that --

DR. FALK: I know you are right.

DR. TEPFER: For the poty, I'm sure. In any case, the CMV I know was used with a nonaphid transmissible strain. Whether that was actually verified after the fact, I'm not absolutely certain.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: As far as I'm aware, removal of the read through domain of luteo viruses has been demonstrated to ablate insect transmission. Removal of the immuno (ph) terminus of poty viruses ablates aphid transmission.

Mutation of the RNA binding site in poty viruses prevents encapsidation and still allows effective resistance. That's work from Edgar Mize (ph).

There are certainly aphid nontransmissible isolates of cucumber mosaic virus that have been identified and many other instances.

There is good evidence for some virus groups. I

would not say that there is good evidence for all virus groups, but for a significant number of virus groups.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: I guess to be specific, I would say that if there is good evidence that the virus without these groups -- whatever the modification is, are not insect transmissible, then there should not be any need to require further documentation for that particular gene in a transgenic plant.

DR. ROBERTS: Is there agreement with that among the panel members? Let the record show lots of heads nodding.

Any other follow ups, Dr. Kramer.

DR. KRAMER: No, thank you.

DR. ROBERTS: Let's go ahead and take the next question, then.

DR. KRAMER: How technically feasible would it be to measure rates of recombination, heterologous encapsidation and vector transmission in PVCP PIP transgenic plants in order to show that rates are reduced?

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: I guess I quibble with words sometimes. So bear with me. Recombination rate is defined as the number of recombination events occurring per unit of time. The virologically meaningful unit of time is a replication cycle.

Unfortunately, we do not have accurate numbers for how many replication cycles occur between the initial infection of a plant and the harvest of the virus.

The number depends on how many cells are on the pathway that the infection takes, whether a new replication cycle must occur in every cell, in the pathway and whether multiple cycles occur in a single cell.

These factors are likely different for different viruses. Fortunately, there is another quantity that is easier to measure and directly related to recombination rate, and that's the frequency of recombinants.

The rate of recombination is equal to the frequency of recombinants divided by the number of replication cycles or some other measure of time.

Since the question really implies an interest in

whether recombination rates are reduced, it can be answered confidently if the frequency of recombinants is reduced. I assume the comparison is between an unmodified PVCP PIP transgene and a PVCP PIP transgene modified to address some of the concerns that have been dealt with elsewhere in the discussion.

So with that proviso, then, going on, it is technically feasible to measure the frequency of recombinants in a population of viruses.

One popular approach is to create multiple molecular clones of a region of the viral genome in which the combination may have happened and determine their nucleotide sequences scoring whether or not they are recombinants.

A large number of clones is of course required to obtain statistically valid results, but with current costs of nucleotide sequencing, such an undertaking is not prohibitive.

A second approach is allele specific real time prolimiraese (ph) chain reaction.

In this approach, two pairs of primers are

designed, one pair in which each primer will only prime from the genome of the infecting virus and the other pair in which each primer will only prime from the transgene.

Three or four PCR reactions are carried out. Those with each pairs I just described. They serve as controls to measure the amounts of nonrecombinant sequences and those in which the pairs exchange partners allowing amplification of the reciprocal recombinants.

When the PCRs are performed in real time mode, reliable estimates of the amounts of each template present are obtained. The frequency of recombinants is the quotient of the sum of the exchange values over the sum of all values.

A third approach is possible if the sequences differ in multiple restriction and nucleus recognition sites. Restriction will generate unique recombinant DNA fragments whose quantity can be determined by densatomety of gel laphritic (ph)
separation of the digestion products, and comparisons with quantities of the corresponding non recombinant fragments will result in a value that is the frequency of

recombinants, which is relevant.

Those are the methods I know about as far as recombination goes. The question includes I think also trans encapsidation and vector transmission.

So trans encapsidation relative to this, the rate concept has even less relevance than for recombination. For trans encapsidation to be a problem, the rate needs to be very close to that persistent encapsidation otherwise there will not be any trans encapsidated variance.

But, again, as with recombination, the frequency of trans encapsidated genomes can be a useful measure for the potential of trans encapsidation.

The frequency of these trans encapsidated genomes can be measured reliably by at least one of two methods, and probably my colleagues will add some more. Immuno capture PCR or RTPCR in real time format should reveal how much of the viral nucleic acid target of the reactions is encapsidated in particles that contain the PVCP that's the target of the antibody that's used.

An antibody against the transgene PVCP is used

to separate virions that bear sub units from the transgenes from those that do not. The nucleic acid in them is released and quantified by a quantitative PCR or quantitative RTPCR, RT being reverse transcriptase, as appropriate to the virus.

Concurrently, the total virion population is also subjected to release in quantitative PCR or RTPCR, and that value serves as the denominator for the quotient, but gives the frequency of trans encapsidated genomes.

The second technique for trans encapsidation is amino electron microscopy in the plant bearing PVCP transgene. The only genome encapsidated can be assumed to be that of the invading virus.

The question then becomes what proportion of the virions formed have a capsid that is composed in part of sub units provided by the transgene.

Staining of virions with tagged, for example, by peritin (ph) antibodies against the transgene PVCP should allow distinction of stained virions from unstained ones. A difficulty with this approach is only a small percentage of capsid sub units usually react in such

studies.

When only a small percentage of the sub units derived from the transgene, many virion particles may escape scoring as being trans encapsidated.

However, since the aim of the assay is to test for reduction of the frequency of trans encapsidated genomes, the underestimation resulting from this problem is minimized as long as there is an appreciable evidence of trans encapsidation in the case of the non modified PVCP transgene.

That's the second part. That's the trans encapsidation. Then there is the vector transmission.

Vector transmission plays a role in two scenarios. When PVCP transgenes are used as plant induced protectants, plant incorporated protectants, it is often the case that the protection afforded the plant is not absolute.

So low levels of the virus may result or the plant may even eventually escape from the protection.

The second scenario occurs when infection is by a non targeted virus and trans encapsidation has occurred.

So the assays for vector transmission are diverse because the diversity of organisms that vector plant viruses are diverse.

They are arthropods. They are fungi. They are nematodes. They are mammals. Mechanical transmission through contact.

If the virus vector is well understood, there usually are assays available for measuring the efficiency of vector transmission. For example, in the case of arthropods, the experiments involved caging arthropods with disease plants for a specified period of time, the acquisition access period, and then transferring them to healthy test plants for a second period of time, that's the inoculation access period of time.

For viruses whose transmission mode is circulative and or propagative, an additional period of feeding on healthy plants intervenes between those two to allow the virus to circulate and replicate in the arthropod before it's delivered.

Scored are percentages of test plants that become infected. These tests are pretty standard in

vector entomology. So in summary, for all three aspects of the question, it is technically feasible to test whether modification of the PVCP gene reduces recombination, trans encapsidation and vector transmission.

That's my opinion.

DR. ROBERTS: Thank you. Dr. Falk.

DR. FALK: I agree mostly with Ulrich and I think such analyses are technically feasible. However, I must again say that I think the important question is the biological relevance if such interactions are even occurring.

And if they are, if they are of no relevance, I don't see why you would want to go ahead and lower them. And if they are important, these interactions, I think it would be important then to determine not just can you lower them, but how low do you need to lower these.

Ulrich talked about measuring rates of trans encapsidation and I agree largely with the approaches that he suggested. I do think that it is not simple. And even techniques like amino capture RTPCR, it is important to have care and appropriate controls to make sure that we're

detecting biologically active heterologous encapsidated RNAs.

And such approaches are a little bit difficult.

I think measuring vector transmissibility as Ulrich said can be done, but then again the significance of transmissibility is important in terms of whether it is transmissible or whether it is not transmissible because the results here only really reflect the experimental conditions.

But yes, you could compare them relative to a control, but I'm not sure about the biological significance.

I defer to my two colleagues in terms of talking about the recombination.

DR. ROBERTS: Dr. Nagy.

DR. NAGY: Actually, I agree with both Ulrich and Bryce that it is technically feasible to measure both the recombination frequencies and heterologous encapsidation and vector transmission.

The next thing I would like to add is that I think it is feasible and relatively easy to do is to

integrate plans with the two known viable transcripts to see the recombination frequency with those.

That's I think a very good way to measure.

DR. ROBERTS: Let me get to Dr. Bujarski and then we'll take Dr. Tepfer. DR. BUJARSKI: I do not have much to add besides some other possible techniques including for instance quantitative western blott when a specific epitope is for instance recovered due to recombination when you use a specific monoclonal antibody. Thank you.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I just want to add some sort of bench level reality to this. We have been working a lot on detecting recombinants in non transgenic plants. And the techniques that we have finally developed are extremely sensitive.

And even so that we're very close to detection limits. So the recombinant molecules in these plants are extremely rare. And what we have been able to do with cucumo virus so far at least has not been able to be applied to other viral groups for a variety of reasons

which could be due to an even lesser abundance of the molecules in question, differences in sensitivity of the techniques used, but it is a very difficult kind of experiment to carry out. We have had to work very hard on this.

I'm a little bit concerned about trying to go from sort of hypothetical ways of doing this and to the actual realities of carrying it out. So I think it is much, much harder than it looks.

So I would be very uncomfortable with mandating something that was not feasible. So I think that doing that type of experiment would only be feasible for perhaps a very small number of viral groups where it has been demonstrated it can be done.

In the area of vector transmission, I have more of a question for my colleagues. I know that one can measure this rather nicely with aphids and perhaps other insect vectors. What about fungi and nematodes?

Can you do also do feeding experiments? It seems rather more difficult by it. It is not my area of expertise. I'm just asking the question whether this can

be done with non insect vector systems.

DR. ROBERTS: Let's see if there is any response from the panel then we will go to Dr. Hammond for a comment. Does anyone have experience with other vector systems? Dr. Melcher.

DR. MELCHER: No, I don't have experience. If someone has experience, they should go ahead. I have some knowledge.

DR. SHERWOOD: I have some experience with polymyxin and wheat soil born mosaic virus. It is very difficult to get a virus free and maintain a colony of that fungus.

DR. ROBERTS: Dr. Cooper.

DR. COOPER: With the nematodes, certainly, tricadorits (ph) it is certainly possible and the other nematodes too. Technically exacting, but possible

DR. ROBERTS: Dr. Hammond. You had another comment.

DR. HAMMOND: I essentially agree with the methods that have been proposed for dealing with recombination and with vector transmission. Vector

transmission is difficult to do and hard to quantify, but it can be done.

I have a correction or suggestion compared to the detection of heterologous encapsidation in that amino specific electron microscopy is very difficult to use as a measure of the degree of encapsidation. There is an enormous variability within the viral population from transgenic plants in the proportion of transgene coat protein that is incorporated.

We have attempted to do this and have published on it using a monoclonal antibody that is specific for the transgene coat protein. We are readily able to detect the transgene coat protein in particles of viruses able to infect these transgenic plants, but it is extremely difficult to quantify.

Some particles have no transgene coat protein incorporated. Some particles appear to have a few molecules of the trans coat protein and some particles are almost entirely encapsidated in the transgene coat protein.

You cannot quantify on that basis because the

number of particles you would need to look at and count and try to quantify is just too large to get meaningful results. However, if you have a transgene coat protein specific monoclonal antibody or a suitably specific polyclonal antibody, then it is possible to use various forms of ELISA or western blotting to determine the proportion of the transgene coat protein that is present in a virus preparation.

I would suggest it is much easier to do that in a purified preparation than to do it in extract because of the relative difficulty of quantifying and determining relative amounts out of the same extract if you are using crude sap rather than a purified preparation.

But you can then use RTPCR, amino (ph) capture RTPCR using antibody specific for the transgene coat protein or you can use a polyclonal and RTPCR or the Western and direct ELISA methods. But ISCM is a very difficult method to use for quantification.

DR. ROBERTS: Other comments from panel members?

Dr. Tepfer.

DR. TEPFER: I said it previously I just want to reiterate the extreme difficulty of a lot of these things.

I'm sort of in the same way that we were talking previously about the difficulties of reducing the impact of gene flow in terms of how many populations and how many states you needed to study and so on in the wild populations.

Here I think we're in a sort of similar situation. What you are trying to do is extraordinarily difficult. Particularly in the case of heterologous encapsidation in most situations, probably not worth the effort.

So that needs to be taken into account.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: I would like to ask Dr. Tepfer relative to the recombination what method he did use that was successful and which methods he tried that were not successful.

DR. TEPFER: In our hands what we have done is reverse transcription PCR. It is very easy on paper. But

since the molecules we're detecting are extremely rare, it turned out very difficult to do. This is obviously non quantitative.

This is sort of a plus minus sort of situation. So what we can see is what are the proportional of plants in which we see recombinants, but we can't quantify them.

Colleagues at the Tobacco Institute in Bejerak (ph) have used real time PCR using some of the same primer pairs and they have been able to do that to detect recombinants with cucumo viruses. So that has worked.

The things I was referring to that did not work were basically in fact using the same kind of reverse transcription PCR type protocol to other virus groups.

It is colleagues within a EC funded project. Edgar Mize working with poty viruses in particular tried very, very hard to get the same strategy that we use to work on potys and he never could get it to work.

So it is just a warning that even what looks easy on paper can be difficult if not impossible.

DR. ROBERTS: Dr. Bujarski.

DR. BUJARSKI: I would like to add following what Mark says that PCR, these RTPCR techniques require numerous controls.

For example, we have to make sure that RTPCR by itself does not generate recombinants.

DR. ROBERTS: Any other comments or suggestions in response to this question?

Dr. Kramer. Any follow ups.

DR. KRAMER: I think the answer is fine. Thank you.

We have one more question on this general topic area. Let me suggest that we go ahead and take this question number 16 and then break for lunch.

DR. KRAMER: Please comment on how necessary and/or sufficient each of these conditions is to minimize the potential for novel viral interactions.

Please address specifically what combination would be most effective or what conditions could be modified, added or deleted to ensure that potential consequences of novel viral interactions in PVCP PIP transgenic plants are minimized.

Number one, the genetic material of the PVCP PIP is translated and/or transcribed in the same cells, tissues and developmental stages naturally infected by every virus from which any segment of a coat protein gene used in the PVCP PIP was derived.

Number 2. The genetic material of the PVCP PIP contains coat protein genes or segments of coat protein genes from viruses established throughout the regions where the crop is planted in the United States and that naturally infect the crop into which the genes have been inserted.

Number 3. The PVCP PIP has been modified by a method scientifically documented to minimize recombination. For example, deletion of the 3 prime untranslated region of the coat protein gene.

Number 4. The PVCP PIP has been modified by a method scientifically documented to minimize heterologous encapsidation or vector transmission or there is minimum potential for heterologous encapsidation because no protein from the introduced PVCP PIP is produced in the transgenic plant or this virus does not participate in

heterologous encapsidation in nature.

DR. ROBERTS: Dr. Kramer, is there any background you want to give on this question?

DR. KRAMER: I think the background is the same for the question about gene flow that we were considering before. That is that we're really looking at whether we can come up with a set of factors that the agency could use to determine products that are extremely safe.

And that is that in order to -- when we're asking whether these conditions are necessary, in order to conclude that they are not necessary, one would have to say that under no circumstances with any virus in any plant planted in any region in the United States would there be a concern for the condition that the factor is attempting to address.

DR. ROBERTS: Thank you. Dr. Tepfer.

DR. TEPFER: I must confess that I find this a particularly difficult question. What I'm going to do is go through the 4 sort of suggested, the questions, the sub questions, the points that are raised briefly. And then sort of make a few comments at the end.

The first one has to do with having the gene expressed in the same tissues as the virus is usually present in normally infected plants.

Admittedly, this is perhaps helpful to prevent the virus in interacting with distantly related viruses that would have different cell tropiziums. But this is a rather marginal sort of circumstance.

There are of course plant viruses which are phloem restricted. But most plant viruses tend to go pretty much through a wide range of cell tissues.

So this doesn't strike me as an extraordinarily useful thing to insist upon. The second thing, the second point has to do with only using coat protein genes or segments thereof, which I thought was an interesting thing to have added in from viruses that are prevalent in the area where the genes -- where plants would be deployed. Here again, this doesn't seem to me to really be very discriminatory. It seems to me that this is going to be the case in nearly all circumstances.

I think that the types of plant viruses that we

see tend to be rather broadly distributed. For instance, with cucumber mosaic virus you tend to see the same types of strains almost worldwide with a few exceptions -- with group 1 b, which is more prevalent in Asia than elsewhere, but that's changing very rapidly.

Here, again, this strikes me as a rather poorly discriminant sort of a suggestion to make.

We have talked already in the third point previously. The third point has to do with deleting the 3 prime untranslated region. This I think we all agree will decrease the frequency of recombination in transgenic plants.

Whether that remains adequate or not is something also that we discussed again previously. It has to do with to my sense again value judgments as to what is acceptable in terms of frequency recombination and whether recombination does lead to molecules that we would not like to see being created.

The fourth point has to do with preventing heterologous encapsidation or vector transmission.

And I thought one thing in here again we have

talked about this can be done. The question is whether this is necessary. Whether there really are cases where we need to do something about this or whether we can simply say, well, heterologous encapsidation is not a big problem except perhaps for one or two cases that would stand out from this.

And then one thing that struck me in the formulation of this question is that it then goes on to suggest what about transgenes from which no protein is synthesized.

I would simply like to suggest that this last possible idea is probably the only really good suggestion among them.

I think if you are really concerned about heterologous encapsidation, if you really want to absolutely minimize the degree of recombination, probably the best thing to do is to simply abandon coat protein protection to start with and to focus on RNA mediated group protection that is related to post transcriptional gene silencing also known as RNAI.

It seems to me that -- my take on this question

is really, well, you have all these sorts of things that will sort of attenuate possibly a little bit or maybe a little bit more some of these things, questions that are being raised. You are talking about sort of small adjustments.

But if you are really concerned, why not simply head for bigger adjustments and say, well, if it is really a concern, we should do RNAI and not protein mediated protection at all.

That's my provocative conclusion to this. I don't think most of these are extremely useful, but I don't think most of them really address questions that are particularly all that critical.

DR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: I would have to agree considerably with Dr. Tepfer's well-stated answer and he dissected the question I think quite well.

Again, number 1, not particularly useful. I think it would be difficult to put the proof on the person that was proposing that they are able to demonstrate that considerably.

Secondly, in regards to distribution in the United States, that only putting the transgenic plant in an area where the virus is naturally found prevents, say, for example, if you see an epidemic in South Carolina one year and have a good idea that it might be in Georgia the next, if it prevents the next growing season not being able to deploy the control measure without first having gone through a disease year.

We have kind of beat number 3 to death, I think, in terms of the untranslated region or the adequacy and frequency of that.

Certainly, I think in number 4 would agree that -- whether or not the trans encapsidation is really an issue that is of any significant long term ecological or environmental impact.

Again, coming to this meeting I would have to agree with him that, and it has been said earlier yesterday, that whether or not this technology that we were talking about in terms of expression of coat protein is kind of something that's the initial model T on how we're going to bring about transgenic resistance and

whether it is really even going to be relevant to how we go about doing transgenic virus disease control in the future with things that are now being understood about the RNI mechanism.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I agree essentially with what Mark and John have said before me. I do not believe that there is significant risk. However, I believe that there are ways to reduce the perceived risks.

I have already stated that it is better to use endemic isolates than exotic isolates as a source of transgenes.

I have also expressed that I think it is better to use constructs that have been engineered to minimize accumulation to reduce perceived risks rather than real risks. And that RNAI is probably the way to go.

Having said that, the second part of the question here about viruses that are established throughout the regions where the crop is planted, I would like to point out that there is considerable variability of the isolates present within the crop and that sometimes

this is very highly variable and that certainly for some viruses the variation present within a region is equivalent to the variation between regions.

So there may be variation in some instances between the isolates that are present in different geographic areas of the world. However, with some viruses it has been demonstrated that the variability within one region is equal to the variability that has been observed between geographic regions. And one example of that that I can point out to is some recent work that was done on hosta virus x where variability within the United States is equivalent to the variability within isolates in Korea. And that there is essentially no difference in the variability between the two regions.

So that isolates variations that occur in Korea are very similar to variance that occur in America.

Examples of the variability within isolates have been presented for papaya ring spot virus and turnip mosaic virus and for other poty viruses that have been discussed extensively in the book, The Poty Verity put together by Schuplar, Ward and Brandt (ph).

To oblate perceived risks, there are many things you can do. You can modify the coat protein to prevent RNA binding, to interfere with subunit, subunit interactions, to eliminate the APHID transmission regions that read (ph) through domains.

To use defective copies of genes that are known to interact with other viruses, avoidance of replicase recognition sequences, use of untranslatable or anti (ph) sense sequences, defective interfering RNAs, permitting genes that are effective through different mechanisms to increase the level of resistance.

So there are many things that can be done. But I believe that these would all be to reduce perceived risk rather than actual risk.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I must confess I'm extremely uncomfortable with the idea of requesting that people do things to deal with perceived risk. I think that this is something that we have suffered enormously from in Europe.

I think if biotech is in the pitiful state that it is in Europe presently, one of the great problems has

been trying to address perceived risk. It is an endless process. You cannot get to the end of it.

And I think that some of the things that John has been talking about could perhaps better be stated as trying to deal with what is a very difficult question. We may need to get at the end of this is what are the levels of acceptable risk.

And I think that couched in those terms I would be more comfortable with some of these suggestions that he was making, but I think that talking about perceived risk is just opening an absolutely horrendous Pandora's box. Let's not do that, I would suggest strongly.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: I guess I'm confused about Dr. Tepfer's attitude from the discussion we had earlier on recombination. The attitude with respect to number 2, the avoidance of putting the plants out in regions that viruses don't naturally exist.

I ask you to think about maybe 5 or 10 years ago in the United States where we did not have plumb pox virus. We were awfully concerned that plumb pox virus

would come into the United States.

If at that time we had put out orchards which had a VCP PIP for plumb pox virus and perhaps there would have been a recombinant and then we would have the equivalent of plumb pox virus if they would have recombinant with some other poty virus that was in that vicinity.

I would have thought he would have had that kind of a scenario in mind. But maybe he doesn't think that that recombination could have happened. I'm not quite sure.

DR. TEPFER: Thank you very much for bringing that up. I think you are in fact absolutely right. I was thinking of more like strain differences and things like that. But it is true that if you are talking about the rare situations where a virus is absent from a whole region or continent, then, of course, the question is one that needs to be addressed.

And obviously, I thank you very much for bringing this up. It was not an exaggeration, but an overgeneralization on my part. Thank you.

DR. ROBERTS: Dr. Nagy.

DR. NAGY: I have comments regarding the first point. Actually, I think this is a relevant comment because if you take the examples of barley yellow dwarf virus, which is the coat proteins in -- natural infection is being expressed only in the phloem.

If you have transgenic plants which express coat protein everywhere, all tissues, I think that incredibly increases the transfer combination. Not with barley yellow dwarf, but other viruses which infect those tissues.

I think this is unnecessary things to do. I would actually recommend this first point to reduce the chance for recombination between different viruses.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: Here, again, I did say -- I was talking about the most general cases. I did mention there were exceptions, in particular, the phloem limited viruses. I was suggesting this is not a universal problem, but certainly in the cases you are talking about. And I think I did mention that.

This is something that should be taken into account.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: Two points of clarification. In the particular case of plumb pox virus, as far as I'm aware there is actually no other poty virus that is known to infect pronus (ph), other than plumb pox virus.

So the risk of recombination with another poty virus infecting pronus is absent.

With respect to barley yellow dwarf, barley yellow dwarf virus can actually replicate in mesafilled (ph) protoplasts of cereals. I'm not sure about the question with barley yellow dwarf itself, but with other luteo viruses in mixed infections they can escape from the phloem and multiply in other tissues.

This has been shown with potato leaf roll virus in Scotland. I'm trying to think -- was it PVY?

DR. COOPER: Yes.

DR. HAMMOND: So in mixed infections, luteo viruses can escape from the phloem and replicate in other tissues. Again, the possibility of something happening in

transgenic plants as a result of expression of the transgene in other tissues is not so very different from what can occur in mixed infections.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: Responding to the plumb pox virus. This was meant to be a hypothetical example and not necessarily that specific.

But imagine the case where the virus does not exist in the United States at all, but we have a desire to protect our crop against that virus. We put in a transgene. There may be some possibility for recombination. That was the point.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: If I may come back to that again. If we had had pronus resistant to plumb pox virus, there would have been many fewer orchards that were bulldozed and destroyed to prevent the spread of plumb pox virus.

The cost of the introduction of plumb pox virus is in the millions of dollars. And even beyond the attempts to eradicate the virus locally, those orchards cannot be replanted for another 7 years after the last

case of plumb pox infection is found in that area.

These costs for the growers in those areas are enormous and the benefits of having had resistance to plumb pox virus would have been tremendous.

DR. ROBERTS: Dr. Kramer.

DR. KRAMER: I have a couple follow-up questions.

DR. KRAMER: I hear that there is some disagreement over conditions 1 and 2, whether there would be any use for them at all. If we just let that, correct me if I'm wrong, but if we just let that disagreement stand, could I ask whether conditions 1 or 2 -- could you give me a sense of how onerous they would be?

How many products might not fit under these conditions, or is it just the case that the people who would think they are unnecessary just find them not useful?

DR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: I guess in terms of condition 2, I would view that as someone having essentially write a section 18 for the use of coat protein mediated

protection.

Be an emergency label per se being able to use that crop in a state. I think it would be a case of -- it would be too little too late.

Essentially, you would have to go to, like I said, go through a growing season and show that the disease was now present in your state or area or whatever that we're going to define that before you would be able to use the technology.

DR. ROBERTS: Dr. Hammond.

DR. HAMMOND: I think that both really are unnecessary. And with respect to question 1, going back to the luteo viruses, the study by Thomas, et al., with the plants that were transgenic for both potato leaf roll virus and potato virus Y and field tested over, was it, six seasons, and there was no evidence of any untoward, any different viral activity, any difference of disease symptoms present over that time.

That is the best evidence that we have at this time and there is no evidence for any change in virus or disease as a result of that even though potato leaf roll

is normally restricted to the phloem and the transgene was expressed in essentially all tissues in that case.

DR. ROBERTS: Dr. Tepfer and then Dr. Allison.

DR. TEPFER: To answer, try to address more directly the question of the cost of implementing something like this, I think generally speaking, again, there are exceptions for these, these are not costly questions.

For instance, using preferably coat protein genes from local or not too distantly geographically isolates is a sort of thing that is relatively easy to do. It is not a complex problem.

It is the sort of thing that one is likely to anyhow since one tends to have better protection using homologous viral sequences than using distantly related ones. Although, there, again, there are exceptions to that.

To the tendency is to try to use local strains, local coat proteins. So it is sort of already more or less general practice. That's all I wanted to say.

DR. ROBERTS: Dr. Allison.

DR. ALLISON: In reference to number 1, while theoretically it might be practical, that, in reality, most of these promoters that are tissue specific are very leaky or leaky to some degree. There is really no concrete insurance that you are not going to get expression in these other cells.

DR. KRAMER: How many viruses are there that would not be expressed in all cells anyway?

DR. ALLISON: I don't know if I can tackle that one. In terms of a number, I can't give you a hard core number. But there are certainly cell types that --

DR. KRAMER: I guess I could say are most viruses infecting all cells in the first place? Is that true?

DR. ALLISON: I would tend to go on that side of the dividing line. Yes. There are, as we talked about phloem limited viruses, there are parts of the plant -- there are viruses that don't appear to be in the seeds because they are not seed transmitted. So they may not be in that replicating tissue.

Probably my colleagues can add to that.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: Most viruses I think are poty viruses and poty viruses are all over. They are the most abundant kind of plant virus, a poty virus. Is that not correct?

DR. ALLISON: More than 200 different poty viruses.

DR. ROBERTS: I guess I didn't -- the last part of it you turned away and talked to your colleagues. Most of them are poty viruses and?

DR. MELCHER: Therefore -- poty viruses are infecting all cells. So, therefore, most of the viruses are infecting all cells.

DR. ROBERTS: Thank you. I just wanted to make sure we got that heard and on the record.

Dr. Tepfer.

DR. TEPFER: Even going beyond poty viruses, of course, most other virus groups are also rather general. It is more I think the exceptions which are tissue specific.

DR. FALK: A couple of very minor points here.

To say that most viruses are poty viruses I would rephrase that a little bit and say that the virus family with the most members would be the poty verity (ph). So yes.

It is not all cells. Because within the plant there are many, many, many cells. They have the potential to infect all living cell types, I would say, but they are not infecting all cells for sure.

DR. ROBERTS: Dr. Kramer.

DR. KRAMER: Then I have another question. There is still some disagreement, but we'll just let that stand over whether there is any value in having conditions 1 and 2. But there does seem to be agreement that if conditions 1 or 2 were met there would be no need for conditions 3 or 4.

Correct me if that's wrong, but the real question is if conditions 1 and 2 were not met for those that would find some value in them, would satisfying conditions 3 and 4 be sufficient, 3 alone, 4 alone?

Could you maybe expand on how these might be used in the cases when 1 and 2 are not met?

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I don't agree with your starting premise. I don't think that meeting conditions 1 or 2 in any way is a guaranty.

These are ways of minimizing a little bit certain potential risks. I don't think it is going to have a major effect. To my way of thinking, these are things which may or may not be done and the difference is not going to be great.

It was more in that sense that I was saying that these are perhaps not particularly important except for a few exceptional cases.

So I don't think that to my thinking they would in any way exempt consideration of the other factors that are being raised.

DR. KRAMER: But I understood -- I didn't hear any support for the idea that conditions 3 and 4 would be necessary.

DR. HAMMOND: I would agree that that I don't think that conditions 3 and 4 are necessary. They are things that would reduce perceived risk. Potential risk.

But I do not believe that they -- I do not believe that the risks are significant that these conditions need to be imposed.

DR. ROBERTS: Other viewpoints. Dr. Tepfer.

DR. TEPFER: I want to go back to -- among the 4 points that we have raised, the one that seems to be potentially the more interesting to my thinking at least has to do with point 3 as a means of reducing the frequency recombination because I think that the potential impact recombination is relatively great.

On the other hand, I think that again it is in a way a weak measure. So that, in fact, my -- the reason why I don't think that it is important to impose this entirely is I don't think that it is, in fact, entirely effective.

So instead of saying that it is -- we don't need to impose any of these 4 conditions because this is merely a question of perception, I would say we don't need to impose them because I don't think they would be all that effective to make it worthwhile. DR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: I would have to agree with that. Because I don't know -- particularly, 1 and 2, going back to those, how you would ever establish the bar to measure those.

As we have heard, there is a lot of variation how viruses are distributed in plants and how mixed infections impact that distribution. And certainly there is a wax and waning of distribution of viruses in the United States as epidemics come and go.

I don't know how you would over time -- what would you use in order to establish the bar for meeting number 2.

DR. KRAMER: I think I heard a suggestion from Dr. Hammond earlier. What if number 2 were rephrased to say that the virus -- the coat protein is from an endemic isolate of the virus?

DR. SHERWOOD: I don't know if that is directed to me.

DR. KRAMER: To anybody.

DR. SHERWOOD: You are going to have to define endemic. The American Phytopathological Society in

conjunction with APHIS has been trying to develop a list of endemic viruses for nearly 10 years.

We cannot come to agreement with APHIS on the definition of endemic.

DR. HAMMOND: I would have to say that the use of endemic isolates as I understand endemic isolates is by far the most common and makes the most sense.

You do not have to go and get a permit to use an -- to have in your possession an endemic isolate if it comes from your own state.

It is in most cases the position of choice. However, as I stated before, in many viruses there is a great degree of variability and there is no significant difference between the variability that is present in local isolates and isolates that are present across the world.

In some instances there is, but there are also many isolates that have not been characterized, so the degree of variability is probably greater than we're currently aware.

DR. ROBERTS: Other viewpoints?

DR. FALK: What are you thinking now?

DR. KRAMER: Well, I heard that there was some support for the idea that conditions 1 and 2 --

DR. FALK: I'm just talking about the last point here.

DR. KRAMER: Whether we would be able to identify endemic?

DR. FALK: Yes. Does endemic mean United States?

DR. KRAMER: Yes.

Elizabeth was saying there is potential that we could define endemic based on the region where the crop might be planted, so that a virus isolate from the Virgin Islands would not necessarily be acceptable for planting in another area of the United States.

DR. FALK: The only reason I brought that up, certainly the triple gene resistance squash is grown all over the United States. And that's a -- I assume those must be New York isolates of the virus that were used. That's very effective everywhere.

United States is good, I think.

DR. ROBERTS: Dr. Kramer -- let's get Dr. Sherwood. We had interrupted you. You were about to say something about points 1 and 2.

DR. SHERWOOD: If United States is going to be used as definition of endemic, I would certainly endorse that. But if we use a state by state definition of endemic that APHIS is using, then I see this very difficult for any company to be able to bring a product to market.

DR. ROBERTS: Dr. Kramer, you were about to sort of give your impressions back on 1 and 2 and sort of get some closure on where we stand on those.

DR. KRAMER: Right.

As I understand it, there is some support for the idea that there would be some value in having 1 and 2 to minimize risk with the caveat that we would amend 2 to define just that the coat protein was from an isolate endemic to the United States.

There is not agreement on that, I understand, but there are some people who feel that those would be valuable. And that there is no agreement -- no one

supports the idea of conditions 3 and 4 under any circumstances including when conditions 1 and 2 are not met.

DR. ROBERTS: Let's see. Does everyone agree with that assessment?

Does anyone disagree with that assessment? Dr. Tepfer, Dr. Sherwood.

DR. TEPFER: I just -- I don't think that it should be useful to impose condition 1. It seems that it is only going to be a restraint for a small number of virus groups. I'm still not convinced that that's absolutely useful to do so.

DR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: That would be my question on how would the agency evaluate that as a criteria. Is it possible to evaluate that as a criteria considering the discussion about the impact of mixed infections and other things that we have talked about.

DR. KRAMER: I guess I would throw that question back on you. Are you saying, then, that you don't think that's possible to evaluate?

DR. SHERWOOD: No, I don't.

DR. ROBERTS: I have heard a lot of reservations about the value of 1 and 2. Let me see. Are there panel members that feel that 1 and 2 have value? Dr. Falk?

DR. FALK: I was going to say I agree with what Mark, the way he rephrased the statement. I don't see any reason for one.

DR. ROBERTS: Dr. Allison.

DR. ALLISON: I believe it is worthless.

DR. KRAMER: Could I ask Dr. Tepfer if he is suggesting that there might be certain cases that could be identified now in which number 1 would be important?

DR. TEPFER: The only cases we talked about were the phloem restricted viruses where perhaps in a risk -- minimizing risk to some extent, potential risk, perceived risk below a level of -- the reason I object particularly to basically all 4 of these is that it is I think very dangerous to think that applying any of these would confer any real protection against potential risk.

I think that that's why I think that it is

dangerous to mandate things that are not effective.

Considering the flexibility even of at least some of the phloem restricted viruses such as the luteos, if you have an infection by other type of viruses, this can lead to the luteo being released from the phloem into the other cell types.

Again, you are just going through more -- in a sense you're creating possible sort of hoops that people will need to be hopping through giving a false sense of assurance that this does something. It also distracts people from trying to think about what might in fact be the real critical issues if there are any.

Even if you could sort of draw a very hypothetical sort of proposal to say, well, yes, for luteos let's try to do it with just phloem promoters, which are more or less phloem specific, I think you are still sort of fooling yourself into thinking this would really be effective in preventing something.

DR. ROBERTS: Dr. Kramer, we can continue to poll the panel. I wasn't sure I heard a lot of support for conditions 1 or 2. I'm not sure how divided the panel

really is on that.

Dr. Melcher.

DR. MELCHER: I think the reasons for conditions 1 and 2 are that they will minimize recombination, minimize trans encapsidation and minimize vector transmission. So they are really covered by items 3 and 4. Given the hesitation, perhaps they are not necessary, then.

If the company can convince that the PVCP PIP has been modified to minimize recombination, maybe that would be grounds for an exemption, regardless of how they have done it. They might have done it by using 1 and 2 and convince you that that's the reason that they have -- that the risk is minimal. DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I guess what we're seeing is two different reasons for objecting to these 4 proposals. One is that some people are objecting because they think that -- because they don't think that the potential risks are important and that reducing them is significant.

And another way of object is to say that they

aren't effective. I think we're seeing two different ways of objecting to these 4 types of mitigating proposals.

DR. KRAMER: I guess you are one of the people who says they are not effective. Maybe I'm actually jumping back to a question from earlier today. Would you suggest that the risks are significant enough that some type of strategy should be employed, if not these, then something else?

DR. TEPFER: That's a difficult one. I think that -- we don't have any -- you are really getting to the question what is acceptable risk. And that's an extraordinarily difficult question to deal with.

I'm not in a regulatory sort of mindset myself. I sort of avoid asking myself that question. I understand for you that's the critical one.

DR. KRAMER: I guess what I feel that we haven't gotten was when I was pushing so hard back on I think question 9 was what the level of risk is.

And then the question of acceptable can come later. But I don't really feel like we got a handle on.

I understand the panel seems to be saying that

in the vast majority of cases there is no real appreciable risk at all, but people keep pointing out specific examples in which there might be some appreciable risk.

I want to understand in those conditions, in those rare cases what is the level of risk that we're talking about.

DR. TEPFER: But it is something that we can't quantify. What units are you talking about in risk?

There are no values that we can apply to any of these potential risks that we have been talking to. You can't quantify it in dollars. You can't quantify it in --

I think that you are asking us to do something that is pretty difficult to do, if not impossible.

DR. ROBERTS: Dr. Melcher.

DR. MELCHER: Maybe the risk people should say this. But risk is the product of frequency times hazard. So hazard is probably measurable in dollars. Frequency is something that we -- perhaps they are asking us to come up with. I think we really don't have a good number for frequency, but it certainly -- if we did, it could be quantified.

Is that not correct?

DR. KRAMER: I think we're also asking for your opinion on what the hazard is. In these rare cases what really is the concern.

DR. ROBERTS: This is certainly -- I know this is the bottom line question. And maybe what we ought to do is return to this as the sort of one of the last questions and we can try again. I mean, I know that's what you are trying to get from the panel. But many of the questions are worded fairly specifically.

And the panel is sort of focused on answering those. And we can pose that broader question to the panel and through some dialogue probably give you as good as we can.

Let me propose that we do that kind of at the end. I thought one of the things you were going to hit on this one was if --let's assume for the moment that -- if you are of the position that there is not an appreciable risk, then these really don't matter.

If assuming for a moment that there is a risk that you want to avoid, do these conditions help identify

those situations. And if these conditions don't, what conditions -- and there seems to be some opinion these conditions really aren't very useful, are there other conditions that would be more useful for that purpose.

DR. KRAMER: Yes.

DR. ROBERTS: Let me pose that to the panel. Dr. Hammond and then Dr. Tepfer. DR. HAMMOND: I would like to quote a couple things from the OECD consensus document on general information concerning biosafety of crop plants. Specifically viral resistant through coat protein gene mediated protection.

With or without the use of transgenic plants, new plant virus diseases will develop that will require attention.

And a quote from Dan Roshum (ph) of Agriculture and Agri Food Canada, it is likely that current means of detecting and controlling new diseases in this country would be adequate to control any new virus resulting from recombination between a transgene and another virus.

New viruses will arise whether from mixed infections, from natural evolution and selection from as a

result of the quasi species nature of plant viruses or from recombination between viruses and transgenes.

I don't see any difference in the quality of the viruses that are likely to arise by recombination from mixed infections or from a virus infecting a transgenic plant. If there is no difference in the quality of the virus that would result from the 2 cases, why are we overly concerned with the possibility, and it is a hypothetical of a different virus arising from recombination within a transgenic plant.

DR. ROBERTS: Dr. Tepfer.

DR. TEPFER: I guess I'm going to start by responding to some of that. I think that indeed now viruses will appear. And that's one of the facts of life. I think that one of the sort of the question we will need to ask maybe at the end of this is to go into more detail about is this a complete nonissue in which case we have been wasting 3 days.

In any case, we're quite used to dealing with plant viruses. There is no reason to be worried about new viruses that will appear because we simply know how to

deal with it.

This has been proposed for many years, that we should simply stop worrying about all this, because, after all, plant pathologists and agronomists have been dealing with viruses for decades and they know what they are doing.

I think that's an interesting point of view. I think we should put that off to the end. I think that's one of the big picture questions we need to deal with.

Concerning whether new viruses will occur in transgenics that are different from non transgenic plants, I think that everyone believes there will be no difference. I would just like to wait until we have a little bit of data. That's what we're trying to do.

There is no reason to suspect otherwise. Everyone's working hypothesis, everyone's I would say in many cases conviction is that there will be no differences. Nonetheless, I think it would be very reassuring if we had some solid data showing that indeed the same viruses are occurring by recombination in the transgenics as in the non transgenics. But I think that's

where we're headed.

DR. ROBERTS: Back to my question, then. If you accept that the possibility exists that there may be some new viruses but don't see value in these particular set of conditions, are there other conditions that would be more useful. Dr. Tepfer.

DR. TEPFER: That's why I suggested at the end of my first remarks on this question was that if that was where we were headed, then we should simply abandon coat protein expressing plants.

Because in the face -- if you decide that the risks are significant, the 4 possible mitigation techniques are relatively weak, then you should look for something that is going to be quite different.

I think, for instance, RNAI type, PTGS resistance is a good alternative. Either we decide that they are important or they are not important and then in accordance with that afterwards either contending (ph) with the coat protein or we don't.

DR. ROBERTS: Let me ask for other viewpoints.

DR. FALK: Certainly there are some examples now

of effective coat protein. So I don't think -- in papaya and in squash. We don't want to eliminate those. But I think the future I think will be moving the direction that you are suggesting.

I think we don't want to eliminate what we have because it is working and it is good. It is showing definite benefits.

DR. ROBERTS: Dr. Kramer, I think the panel sees limited value in these conditions. But does not come up with alternative conditions.

DR. KRAMER: Okay.

DR. ROBERTS: Dr. Sherwood.

DR. SHERWOOD: I don't have conditions, but I would have to conclude after sitting here for 2 days that the risk of using coat protein mediated resistance is no greater than the risk of emerging viruses. And one example in Georgia in the late 80s is tomato spotted wilt mated from the West Coast with franklinia accidentalis (ph). We were suffering losses of 40 million dollars in our peanut crop and significant losses in tobacco crop and also significant losses in our vegetable

crops due to that disease.

We know that transgenic resistance works very well in tobacco. It also works very well in peanut. But that technology is not going to be used because of lack of consumer acceptance and other issues.

I don't perceive this as having any greater risk than the normal risk that we face during the continued either new strains or new viruses coming into our production areas or viruses occurring there overcoming resistance that has been deployed.

DR. ROBERTS: Dr. Kramer.

DR. KRAMER: Unless I can think of another way to rephrase this question over lunch, I think I'm done for now.

DR. ROBERTS: I will give you that opportunity. Let's break for lunch.

If you come up with another way to rephrase it, we will entertain it first thing right after lunch. Otherwise, we'll move on to the next question. Let's break for an hour. Come back at 1:30.

(Thereupon, a luncheon recess was taken.)

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