Workshop on Safeguards for Planned Introduction of Transgenic Potatoes

A report of a meeting held in St. Andrews, Scotland, August 16-17,1991

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Edited by

John P. Helgeson
Agricultural Research Service, United States Department of Agriculture
Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

and

Howard V. Davies
Scottish Crop Research Institute, Invergowrie
DD2 5DA, Dundee, Scotland

SUMMARY

On August 16 and 17,1993, a meeting was held in St. Andrews, Scotland, to consider the safety of releases of transgenic potatoes. Topics discussed at this meeting were the potential for outcrossing of potato lines, types of experiments being planned or carried out to test actual outcrossing of transgenic plants, and means by which "escapes" of the introduced genes into the environment may be minimized. Representatives of 9 countries were present to discuss these questions as well as to relate the experiences in their countries. Many of the attendees were governmental, university, or private company persons who am currently involved in research dealing with the molecular genetics or molecular biology of potato. Representatives of potato processing companies, governmental boards and a representative from the National Wildlife Foundation of the U. S. were also present. This report summarizes the proceedings and findings of this workshop.

Introduction

The potato, *Solanum tuberosum*, is a major source of food for many in the industrial world and it is essential for survival for many in the developing world. In the United States. the value of the crop may exceed $2 billion/year, making it 4th or 5th highest in value of all crops grown in this country. In spite of this high value, the potato varieties grown in the U. S. lack many important traits such as resistances to viruses, bacteria, fungi, and nematodes.

Economic factors such as d i d content, shape, skin color*and processing characteristics have generally dictated the types of potatoes that have b a n grown. New varieties are difficult to establish and old varieties generally predominate. For example, the variety Russet Burbank usually accounts for over 40% of the U. S. production. This variety is almost universally susceptible to pests and has been protected against disease by pesticides and by careful management of seed production. Now, however, growing pressure against the use of certain pesticides threatens this variety and other highly susceptible cultivars. Thus, the search for alternative means of protection is particularly desirable.

The improvement of biotechnological techniques for producing transgenic plants corresponds with this increased need for alternative means of protection against pests. Biotechnology promises new ways of improving crops without necessarily remixing all of the genetic material as with conventional crosses in standard breeding. Thus, in the case of potato, a gene for viral coat protein production or synthesis of *Bacillus thuringiensis* (*Bt*) toxin may be inserted to give resistances against viruses or insects. respectively.

Potato may be a particularly useful model for examining practical genetic engineering. Because of the fundamental research already done on tissue culture and transformation of potato. the means of inserting genes am now available. Because of the potato's ready asexual propagation by tubers, the transformed plants can be increased rapidly without genetic modifications. Thus, it appears quite possible that the practical releases of useful transgenic plants may come with potato much earlier than with some other crops. Within 5 years literally millions of transgenic plants may be growing on thousands of acres in our major potato producing areas.

Several groups have become concerned about whether these advances in biotechnology could have adverse effects. For example, what happens if purposely inserted gents "escape" into other plants? Is there a potential for an environmental problem ? Are there risks that have not been considered adequately? Are there key experiments that should be done before major releases of the transgenic plants on a commercial scale? What experiments have already been done that bear on these questions? TO examine these questions, a workshop was held August 16 and 17,1991 in St. Andrews, Scotland, immediately following the 2nd International Symposium on the Molecular Biology of Potato. The topics considered were:

I. What is the potential for transfer of genes from a transgenic potato into another species or line?
II. What would be the consequences of transfer of genes from transgenic plants to other plants?
III How can any adverse consequences be eliminated or minimized?

Thirty four persons from 9 different countries attended the workshop. A list of the attendees is attached as an appendix. Because of this international representation it was also possible to discuss differences between the rules on field tests of transgenics in the various nation's represented.

Three sessions were held; one on Friday, August 16 and two on Saturday, August 17. For each session, one speaker gave a keynote talk and 4 to 6 others contributed information on specific areas. This summary comes from notes prepared by Dr. Howard Davies of the Scottish Crop Research Institute and the chairperson, John P. Helgeson. In addition, various documents provided to the chairperson by participants after the meeting have been used to clarify specific points.

I. What is the potential for transfer of genes from a transgenic potato into another species or line?

A. Outcrossing in potato

The key way in which a gene can be transferred from one plant (or species) to another is by outcrossing. To address this potential in potato and related species, Prof. John Hermsen of the Agricultural University, Wageningen, the Netherlands, was invited to give the keynote lecture for the workshop. Dr. Hermsen has spent many years addressing the problems of crossing wild *Solanum* species with potato cultivars. Among the points presented by Professor Hermsen were:

1. In South America, the center of origin and diversity of potato, much intercrossing has occurred and still does occur between *Solanum* species. Many examples of natural hybridization are well documented and it appears that interspecific crosses have greatly contributed to the evolution of the potato as we know it today. Most tuber bearing *Solanum* species intercross readily and if such species are present or interplanted in experimental fields, gene flow is likely to occur. Conversely, when sexually compatible relatives of potato are not present, as is the case in the Northern United States and Canada, the opportunities for gene flow are obviously greatly reduced.

2. There are various barriers to interspecific hybridization even when two different *Solanum* species may be present in the same field. Among the more important barriers are ploidy differences that may prevent or rather restrict gene flow. Ploidy effects on gene spread are rather complex. One has to distinguish between real ploidy, based on numbers of genomes (numbers of sets of 12 chromosomes) and effective ploidy (EBN = endosperm balance number) based on cytological and crossing behavior. In cultivated autotetraploids (*S. tuberosum* subspecies), real and effective ploidy are equal, i.e. the number of genomes and the EBN numbers are equal (each = 4). Likewise, many diploid species have two genomes and an EBN number of 2. On the other hand, allotetraploids have four genomes but and EBN number of 2, hence they function as diploids. Also, some diploid species have an EBN number of one. As a general rule, unless functional 2n gametes are produced by the parent with the lower EBN number, species with equal EBN numbers can be crossed readily and produce viable hybrids due to a balanced endosperm ratio of 2 female : 1 male EBN. Examples of unsuccessful combinations (EBN numbers in brackets) are autotetraploid (4) x allotetraploid (2). autotetraploid (4) x diploid (2). and 2 EBN diploids x 1 EBN diploids. In these cases, the hybrid endosperm is unbalanced and degenerates. At times it is possible to "rescue" the embryos by *in vitro* culture. These observations lead to the conclusion that in nature gene flow generally can occur only between species with equal, effective ploidy, such as autotetraploids x autotetraploids, 2 EBN diploids x 2 EBN diploids, and 1 EBN diploids x 1 EBN diploids.

3. Crosses between remotely related species may be difficult or unsuccessful, even if species have the same effective ploidy. Various pre- and post-fertilization barriers exist which prevent or hamper the production of viable hybrids. In such cases, barriers may be overcome or circumvented by several experimental manipulations such as mechanical, chemical or physical treatments, extensive reciprocal matings in diverse environments, embryo rescue, the use of bridging species and somatic hybridization. All of these treatments have been successful in breeding programs but generally do not apply to spontaneous hybridization between remote species in nature.

The above information indicates that interspecific hybridization and gene flow occur in nature regularly between compatible species, but very rarely between distantly related Species. With distantly related species, a breeder/researcher usually must go to considerable trouble to carry out artificial hybridizations and to force gene flow. Given this information, it is possible to outline ways in which such gene flow can be promoted, restricted, or prevented. For example:

1. When interfertile species are present and environmental condition are appropriate, gene flow from one plant or *species to* another will commonly occur. Thus in the gene centers (Andes region. Mexico) gene flow between interfertile planu is quite common, whereas in the northern U. S. (*e.g.* Wisconsin, Idaho, or Maine), where wild relatives are not only distantly related but also sexually incompatible, gene flow from experimental fields into natural ecosystems is virtually excluded.

2. Effective gene flow from a donor to a recipient plant requires female fertility in the recipient and male fertility in both donor and recipient. Most pollinations of potato flowers are by bumble bees. The bees are not attracted to male-sterile flowers. Thus the use of male-sterile lines such as Russet Burbank can reduce *gene* flow substantially.

3. Conversely, high female and male fertility, self-incompatibility, an abundance of active bas, relatively cool summer temperatures, and synchronous flowering of donor and recipient plants will promote interplant gene exchange.

4. Hot summer temperatures can limit pollen survival. Very cold winters *can* prevent survival of tubers *or seedlings.* Conversely, in mild climates the survivors may grow as volunteers the next season. In that case, rotation of the crops and elimination of volunteers will limit potential for spread.

B. Experimental measurement of outcrossing in transgenics

After the presentation by Prof. Hermsen, the discussion turned to evidence from planned experiments that *can shed* light on the question of frequency and extent of gene transfer under typical agricultural conditions. Experiments to examine this point are being planned *or* conducted in a number of U. S. and foreign laboratori'es. Drs. Wm. Belknap (USA), A. Connor (New Zealand), and J. Sheffler (U.K.) provided information to members of the workshop.

In New Zealand, Conner and his co-workers are examining pollen dispersal from transgenic potatoes (See Tynan et al. 1990. J. Genet. and Breed. 44: 303-306.) In experiments with fertile, untransformed potato lines of the same cultivars as *the* transformed lines ,they measured the distances of pollen travel by recovery of plants *that* acquired the recombinant constructs in the field. Within a field of interplanted non-transgenic and transgenic potatoes, the pollen dispersal was approximately 1% (51 of 4.476 tested plants). Moving from the plot to edge rows, dispersal was much lower. Within 45 meters of transgenics, 19 of 40,771 tested *seedlings* (0.0596) showed presence of the recombinant construct. Beyond 4.5 meters, no transfers occurred in their tests (0 transfers in 16.034 plants tested). In other tests, attempts to cross tk transgenic plants with nightshade (*S. nigrum*) with conventional techniques were not successful. In that regard Dr. Hoogendoorn of the CPRO in Wageningen reported that her colleagues Eijlander and Stiekema obtained progeny of a cross with cv. Desiree and *S. nigrum* only by resorting to very early *embryo* rescue and *in vitro* culture of the embryos.

Similar data indicating a very low probability of spreading of genes from transgenic plants even under ideal conditions were obtained by the PROSAMO (Planned Release of Selected and Modified Organisms) project being performed by the Cambridge Laboratory at the John Innes Inst., Norwich, UK. Dr. Scheffler reported that *two* seedlings (of 2,400 tested) containing the recombinant DNA were found downwind within 10 meters of the transgenics. These two plants may have *come* from a single pollination. No other recombinant DNA containing seedlings were found in buffer rows 10 meters from the transgenics *on* the other three sides of the plot. Furthermore, no transfers of recombinant genes to potatoes 20 meters from the plot in any direction wen detected, even in tk downwind rows. A *large* experiment was in progress in the summer of 1991 to test the possibilities of escape on a much larger scale. Also, tests are underway to test possible gene transfer to other related Solanaceous species (tobacco, tomato, *Datura*, nightshade, petunia, and eggplant)

In the U. S., a number of tests are planned by Dr. Belknap and his collaborators in major potato growing areas. The experimental sites differ widely in temperature, rainfall, and winter temperatures. These experiments will also involve selectable markers (GUS and herbicides) and trapping with female-fertile cultivars. The key questions again will be how far pollen is dispersed.

In conclusion, it appears that even when fertile cultivars are used, the probability of spread beyond 10 meters is extremely low. The use of trap rows and male-sterile lines will further reduce the possibilities of gene spread even further. Finally, growing potatoes in the northern united states, where compatible wild species are not present, will further reduce chances of spread Therefore. effective means of controlling gene spread in field tests are available.

C. Possible transfer of **genes from plants to bacteria**

The above considerations all dealt with the transfer of genes from plant to plant by crossings. Theoretically, microbiological or insect vectors could also transfer genes from a transgenic plant to another plant. In that regard, the prospects of plant genes being transferred to a bacterium which would then transfer the gene to another plant were discussed by Dr. Fratenelli, with specific reference to experiments with soft rotting bacteria. Slices were obtained from tubers of a potato transformed with a gene for ampicillin resistance fused to a procaryotic promoter. The slices were inoculated with *Erwinia chrysanthemi*. The bacteria were isolated and plated on ampicillin-containing media to test for transfer of resistance from the plant tissue to the bacteria. No transfer of resistance was found in 4 separate experiments, each utilizing 10^{12} bacteria.

II Consequences of transfer of genes from transgenic plants to other plants.

The consequences of an "escape" of recombinant DNA from a transgenic plant are directly related to the type of genetic material inserted in the crop plant. Because of this, the workshop participants considered the types of experiments currently being planned with potatoes. Discussions were also held on potential problems other than escape by intercrossing or winter survival. In this area, particular attention was paid to the question of whether there could be adverse consequences of viral coat protein experiments.

A. Kinds of experiments being done.

To initiate discussion, Dr. Fraley presented the rationale for applying biotechnology to agriculture and, specifically, potatoes. He stated that there is a great need for increasing the world food supply. If the world population doubles by 2030, production of food may be severely limiting and crop improvement will be critically important to meet the demand. He also indicated that a number of technologies, including breeding, discovery of new chemicals, improved management as well as biotechnology, can contribute to the maintenance of adequate food production. In particular, ways that potato can be improved include obtaining new pest and disease resistances as well as improving food processing characteristics. Additional modifications may open new avenues for products. For example. it may be possible to produce exotic chemicals by engineering in novel biosynthetic genes.

One very active area for experimentation at present is the use of Bt toxin for Colorado potato beetle control. Plants transformed with the bacterial genes are being tested for resistance to the beetles, and dramatic examples of control have been obtained Another area of active research is the transformation of plants with genes for viral coat proteins to control viruses. A recent outbreak of PVY net necrosis in Canada and the U. S. is a possible target for this gene technology. The economic threat of this outbreak is very large and impressive instances of control of Viruses by transformed plants have been obtained. Other viruses such as potato leaf roll virus (PLRV) may also be controlled by this method. In addition to the control of serious diseases, another advantage of this technology could be the reduced need for multiple insecticide sprays to kill aphids and beetles on transgenic plants.

Discussions then focused on the question of whether these genes being inserted into crops be a threat in some way? For example, is there evidence that Bt toxin genes in plants will be a major problem? Current toxicity data indicate that the materials are safe to humans. Thus, the key question for the future may be whether insect resistance to the toxin can be avoided.

B. Transgenic plants expressing viral coat proteins.

Dr. Fraley also indicated that another advana could be the genetic engineering of potato for virus resistance, a possibility which stems from the phenomenon of cross protection. Infection by one virus has long been known to interfere with infection by another. It appears that the coat protein of viruses in plants effectively blocks infection and when coat protein genes are inserted into plants such as potato or tobacco, infection can be prevented. The exact mechanisms that operate are currently unknown, although since naked virus particles can infect whereas coated particles do not. indicating that some aspect of uncoating seems to be involved. Whatever the mechanism, the procedure does seem to work to prevent virus damage and in the future millions of transgenic plants may be growing on thousands of acres.

The background of cross protection and his concerns regarding some aspects of the experiments with virus coat protein containing plants were presented by Dr. de Zoeten, the keynote speaker for the third session. Can it be that the very high density of transgenic plants could lead to viruses with different host ranges? Will the ubiquitous presence of the coat protein lead to a finite number of trancapsidations and then new host ranges because of altered insect vectors. Will template switching also occur? Dr. de Zoeten indicated that in his opinion it is necessary to carry out more research to test for potential adverse effects of these changes.

In discussion that followed de Zoeten's talk it became apparent that there was substantial disagreement Many agreed that it was quite likely that template switching and transcapsidation could and probably would occur. However, there was little agreement on whether this would be a threat. Many felt that such changer have always gone on as a mechanism by which viruses evolve and would continue to do so without a major increase in frequency due to deployment of transgenics. Others felt that this point should be tested.

After the conference, a number of virologists were consulted to obtain their opinions. A list of those consulted and a synopsis of their comments is attached as an appendix. Since all members of the group did not take part in these discussions, it is appropriate to indicate the following are only the impressions of the Chair and not that of the workshop.

In general, the responses of the virologists were as varied as the opinions expressed at the meeting. Many felt that Dr. de Zoeten had raised several valid points. Of these, some felt that any potential dangers were overstated others felt that his points were well made and deserved experimental examiniations. Several reported results of experiments to test the safety of the constructs, although they had not published these results as yet. There appeared to be a general tendency to have considered Dr. de Zoeten's discussion in Phytopathology and to have looked at the particular system that they were working with. Finally, there was also some support for additional experiments and additional funding for risk assessment experiments. A workshop on the topic of antiviral constructs would be an appropriate way of considering this matter further.

III. How can any adverse consequences be eliminated or minimized?

A. The role of experimentation

The experiments to measure gene spread outlined above will provide data on actual frequencies of gene transfers from transgenic potatoes to other plants by sexual crossing. These data can serve as a basis for recommendations for isolation distances, locales for growing transgenics, and even k y potato varieties to use for experiments The objectives of these recommendations will be to decrease significantly the likelihood of escape of recombinant constructs from transgenics. Also, as Dr. Shewmaker emphasized in her consideration of plans currently being made by companies, there is a need for insuring the physical security of an experiment, the oversight of the experiment by responsible parties, and the procedures for preventing propagation in future years.

Potato appears to be an ideal plant **for** field release experiments. it can be grown in areas where virtually no wild relatives that **will** cross with the plant are present It can be maintained and grown clonally. thus obviating the necessity **for seed** production or crossing. Tubers will winter-kill or **can** be easily spotted and killed in future years. Because of this there are a number of procedures that could be recommended for **insuring the** safe release of transgenic lines. Some **recommendati'ons** resulting from these discussions **are** given below in the section on conclusions.

IV Public perception of dangers of **transgenic** plants.

Although the main **focus of the** mating was to discuss the potential **for spread of recombinant** DNA from a **transformed** potato plant to another plant. **and** the consequences **of** such **spread.** there was some consideration of the public perception of these experiments. Public input into discussions appears to be a common process in several nations' schemes **for** consideration of release experiments. In regard *to* the public, **Dr.** Rissler **stated** her opinion that there is considerable **skepticism** as to the potential advantages **and** *safety* of biotechnology. She also indicated that *some* wonder if biotechnology is really **to sustain food supplies or will** some **form of** sustainable agriculture suffice. Later, in correspondence with the chairperson, **she** provided her opinion **that the** public's mistrust **of** this technology **is** complex, related to its different views of nature and comes partially from its lack of trust in the institutions promoting and developing this technology. **She does** not **feel** that the public mistrust is based solely *on* the lack **information** or *on* erroneous information but on different interpretations of this **information. The** discussions *at* the meeting illustrated **the** difficulties of dialogue between people with disparate views, while *at* the same time, also indicated the **need** to maintain reasonable dialogues.

Dr. Gebhart **spoke of** the public **concerns** in **Germany. Demonstrations at** the **gates of the Max-Planck-Insitut accompanied two** field releases **of** petunia plants containing the Maize-AC transposable element **and** the **NPTII** gene **for** kanamycin **resistance.** These demonstrations have resulted in **increased** physical **security for the** site. **These** kinds **of demonstrations** also may **make** future release experiments very difficult in Germany. **In the** past few **years there have also** been reports from the **Netherlands** on the **destruction** of field experiments that **were designed to** test **for somaclonal** variation **in potato plants transformed** with the **NPTII gene.** It was *clear* to **all** that there **are** public concerns in Europe **regarding** the *safety* of biotechnology. In general, it appears that public **reactions** in **the** U. S. **are** not as violent as in some **areas of Europe. This may reflect a longer history of** field releases. Certainly, the early releases of the ice-minus ***Pseudomonas* strains** in California **were also accompanied** by violent **expressions of** public **concerns** ad **even destruction of** experiments.

Several workshop members indicated that public concerns with consuming transgenic products or utilizing the technology *can* be quite positive. For **example, Dr. Conner** *stated* that **a majority (85%) of** those polled in New Zealand had no qualms **about** eating transgenic potatoes. Dr. Vayda **also** carried out an **informal** survey **of consumers** in **Maine.** There the **key question in people's** minds **seemed** to be what **had been added by** the **transformation** rather **than** the potatoes being transgenic per *se*.

In addition to **providing** new means of control, there is also an obvious **potential for** profit from sales **of these** transformed **materials, both in the United States** ad **abroad.** This is recognized not only by **commercial** companies but **also** by developing **countries which,** *according* **to Dr.** Dodds, would very much like to buy the technology. He stated that although the **materials** may **save** money **for** growers **in industrialized** countries, it could literally permit **survival** of the *crop* in developing counmes where **severe disease pressures exist.**

Conclusions and Recommendations.

The majority of the workshop members would **like to see molecular** biological improvements **of** potato proceed. **They** would like, however, ***to* see that the experiments are designed** *to* be as safe as possible. There is **a need** for careful, **rational assessment** of the safety **of experiments, and** adequate **funding to provide for** such experiments. Many also feel that the only chance we have to limit the spread of a gene is in the small scale experiments. Once large scale releases have been carried out it will be very difficult if not impossible to limit the spread should it be necessary to do so. Finally, it will be necessary to demonstrate to the public that the experiments planned by scientists in universities and companies are going to be safe. This can only be done by assuring that the experiments are indeed safe. some additional conclusions and recommendations stemming from these conclusions by the workshop members are as follows:

1. outside of very limited areas of the Southwest, there are no wild *Solanum* species in the United States that will hybridize with fertile potato lines. In areas where hybridization does occur (Mexico, Peru, Southwestern U. S. etc.), potential outcrossing into "Weedy" species could be a concern. However, this should not be the case in the main potato growing areas of the United States and Canada. It follows, therefore, that it is not necessary to limit plantings of transgenics to only male-sterile lines except in those areas where risk of outcrossing does occur.

2. Currently available experimental information indicates that spread of pollen is very limited. Therefore. with fertile transgenic plants, border rows extending 5-10 meters from the plot will be sufficient for trapping pollen from the transgenics. These should be used in field experiments.

3. Some survival of vegetative propagules and true seed of transgenic lines can be expected in major potato growing areas, although severe winters will decrease the likelihood of tuber survival in some areas. Therefore. rotation of crops and killing of volunteers should be practiced routinely.

4. Risk assessment experiments should be carried out in the major potato growing areas of the U. S. and Canada on realistic field scales and with adequate funding. These should be carried out promptly so that the risk can be adequately assessed prior to large scale or commercial release of transgenics. In particular, the assessment of transgenics containing portions of viral genomes should be *encouraged* and those virologists with information on their constructs should be encouraged to publish this information.

Addendum

After the workshop, a number of plant virologists in addition to those present at the workshop were contacted. A copy of the letter that was sent *to* these persons is attached. Information on this topic was obtained from the following:

J. Bujarski, N. Illinois Univ.
Paul Ahlquist, Univ. of Wisconsin-Madison
Tom German, Univ. of Wisconsin-Madison
O. W. Barnett, Clemson Univ.
Wm. Dougherty, On. State
Roger Beachy, Scripps
S. Gal, Michigan State
Phil Berger, Univ. of Idaho
John Hammond, ARS, Beltville
Pete Thomas, ARS, Prosser, Wash.
David Robinson, SCRI, Dundee
Andy Jackson, Univ. of California-Berkeley
Milt Zaitlain, Cornell Univ.
Peter Palukaitis, Cornell Univ.
Christine Shewmaker, Calgene
Geoff Keyes, Monsanto
G. A. de Zoeten, Michigan State

This addendum is a summary of some of the information provided and comments made by the above as interpreted by J. P. Helgeson.

The basic questions are whether transcapsidation or template switching might occur and, if so, would these events lead *to* novel viruses or altered host ranges that eventually could lead to problems in the field The focus of the comments was on a letter to the Editor of Phytopathology sent by Prof. de Zoeten (Phytopathology. 1991.81:585-586) The "letter" format has been used io the past to publish views and make suggestions. The intent in this case was clearly to call for an evaluation of risk assessment for plants containing pieces of viral genes.

There appears to be general agreement that transencapsidation does occur in some instances, although the evidence in potato appears to be weak at present. For example, a recent report by Bourdin and Lecoq (Phytopathology. 1991.81: 1459-1464) provides evidence that this mechanisms my allow a previously non-aphid transmissible zucchini yellow mosaic virus to become aphid transmissible. Most of those consulted would not *agree* that this would be a major problem. The level of coat protein in a transgenic plant appears to be extremely low in many cases. Thus, although the numbers of plants in an all-transgenic field would be high, the chances of effective transencapsidation may be rather low. Furthermore, even if transported to a new plant, the viral specificity will be imparted by the nucleic acid, not the protein.

Many of those consulted also agreed that recombination also occurs, and perhaps is a major factor in virus evolution. But most would discount this because of a feeling that most if not all of the recombinations have already occurred in nature. Also, some efforts are currently being made to test these possibilities and, because of negative results, they have not been published in full. In particular, Beachy's lab has been active in this area.

A related possibility is reported in a paper in press in Virology by S. Gal, B. Pisan, T. Hohn. N. Grimsley and B. Hohn of the Freidrich Meischer-Institut. They were able to reconstitute Cauliflower mosaic virus by inoculating *Brassica napus* plants containing one part of the CaMV genome with *Agrobacterium tumefaciens* containing the other part of the CaMV genome. This is not recombination of RNA. of course, but it does indicate that recombinations of viral nucleic acids do occur *in vivo* if the experiments are designed to permit this to happen.

Most of those consulted felt that some careful attention to risk assessment is necessary and should be supported. The problems of trying to "prove" negatives and to look for uncommon events appeared to be daunting. Some felt that instead it would be appropriate to look for ways for constructing transformed plants with the objective of making recombinations or transencapidations impossible.

In conclusion, it appears to this observer that de Zoeten's letter has already succeeded in stimulating some of the studies thought essential. Several of those consulted said that they were either proposing to do experiments in this area or are doing them.

ATTENDEES OF POTATO MEETING IN SCOTLAND, AUGUST, 1991

Dr. Mike Vayda
Dept. BMMB, Hitchner Hall
Univ. of Maine
Orono, ME 04469 USA

Dr. Jodi schemer
JI Centre, Cambridge Laboratory
Colney Lane
Norwich NR4 7UJ, UK

Dr. Howard Davies
Scottish Crop Research Institute
Invesgowrie
Dundee, U.K. DD25DA

Dr. Herman Wenzler
Frito-Lay, Inc.
4295 Tenderfoot Road
Rhinelander, WI 54501

Dr. Mike Kirkman
Walkers Crisps LTD
Feature Road
Thurmaston
Leicester, LE4 8BS

Dr. Peter Harkett
McCain Foods (GB) LTD
(Agronomy Department)
Burton Road, Easton
Grantham Lincs NG33 5AY

Dr. Eric Bonnel
Research Manager
GERMICOPA S.A.
8. rue Olivier de Serres
29103 QUIMPER FRANCE

Dr. J. Hoogendoorn
Centre for Plant Breeding and Reproduction Research (CPRO-DLO)
PO Box 16,6700 AA Wageningen
Netherlands

Dr. Liz Paul
ICI seeds
Jealott's Hill Research Station
Bracknell, Berks. UK8

Dr. Robb Fraley (BB3B)
Monsanto Company
700 Chesterfield Village Parkway
St Louis, MO 63198 USA

Dr. Milan Hrovda
Res. Inst. Crop Production
Omovsh '507
Pragueg, Ruzyne
CS-16106

Dr. H. Dickinson
Agricultural Scientific Services
Scottish Office Agriculture
and Fisheries Department
East Craigs, Edinburgh EH12 4BN

Dr. Jane Rissler
National Wildlife Federation
1400 16th Street, N.W.
Washington, DC 20036

Dr. John Dodds
Dept. of Crop and Soil Sci.
Michigan State Univ.
East Lansing, MI 48824

Dr. Laurent Farinelli
Station Federale De Recherches
Agronomiques de Changins
CH-1260 NYON
Switzerland

Dr. Joe Sowokinos
Univ. of Minnesota
RRV Potato Res. Lab
E. Grand Forks, MN 56721

Dr. David Hannapel
Iowa State University
Dept. of Horticulture
Ames, IA 50011 USA

Dr. Quentin B. Kubicek
USDA APHIS BBEP
6505 Belcrest Road
Hyattsville, MD 20762

Dr. David Robinson
Scottish Crop Research Institute
Invergowrie, Dundee DD2 5D

Dr. Michel Perombelon
Scottish Crop Research Institute
Invergowrie
Dundee U.K. DD25DA

Dr. Wayne Powell
S.C.R.I.
Invergowrie
Dundee UK DD25DA

Dr. Christiane Gebhardt
MPI fur Zuchtingsforschung
Carl von Linne Weg
D-5000 Koln 30. Germany

Dr. Mike Burrell
Advanced Technologies (Cambridge) LTD
Science Park, Milton Road
Cambridge UK

Dr. Barbara Johnson
Potato Marketing Board
Broad Field House
4 Between Towns Road
Cowley,Oxford UK

Dr. P. van der Linde
RZ research.
PO Box 2, 9123 ZR
Metslawier, The Netherlands

Dr. G.A. deZoeten
Dept. of Botany & Plant Pathology
Michigan State University
East Lansing MI 48824-1312

Dr. Karin M. Blok
Dept of Genetics
Free University Amsterdam
1021 HV Amsterdam
The Netherlands

Dr. William D. Park
Biochemistry & Biophysics
Texas A&M University
College Station, TX 77843

Dr. Nicola M. Ayres
Biochemistry & Biophysics
Texas A&M University
College Station, TX 77843

Dr. Fiona Fraser
British Sugar Technical Centre,
Nowich Research Park
Colney Lane
Nowich. England NR4 7UB

Dr. Christine K. Shewmaker
Calgene Inc.
1920 Fifth Street
Davis, CA 95616

Dr. Bill Belknap
USDNARS
Western Regional Research Center
800 Buchanan Avenue
Albany, CA 94710

Dr. Tony Conw
DSIR Crop Research
Private Bag
Christchurch
New Zealand

Dr. John Helgeson
USDNARS, Dept of Plant Pathology
Univ. of Wisconsin
Madison, WI 53706