Risk Assessment: Do We Let History Repeat Itself?

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Sustainable agriculture is a form of agriculture with minimal ecological impact that maintains a productive agroecosystem while realizing maximum economic returns. Plant disease control plays an important role in the economics of sustainable agriculture.

Since no curative measures can be taken once viruses infect plants, control has concentrated on several different measures aimed at reducing virus spread, removing virus sources, breeding for virus resistance, providing disease-free starting material (e.g., seed or budwood indexing), providing disease escape by varying cultural methods, etc. This variety of unrelated measures makes virus control labor-intensive and therefore expensive.

When genetic engineering evolved as a tool for introducing genes into agronomically important plants, engineering virus resistance (cross-protection) (10) became both a high priority and one of the first successful applications of biotechnology in plant agriculture. Although the actual mechanism of cross-protection or genetically engineered cross-protection is unknown, many laboratories around the world are engineering transgenic plants to express the coat protein of one or many different viruses in the hope that the plants will exhibit cross-protection against subsequent virus infection in the field. In all likelihood, this will lead to a large number of requests for environmental introductions of engineered plants without a risk assessment of the sort advocated here, as necessary to ensure a sustainable and productive agroecosystem. In this communication I want to specifically examine issues related to genetically engineered cross-protection and to insect transmission of plant viruses.

There are several characteristics of a virus or its replication that impinge on the assessment of the possible dangers of the release of virus coat-protein-transgenic (Cp-transgenic) plants in the environment.

When we take into consideration that the coat protein of several important plant viruses plays a role in determining virus transmissibility by insect vectors, two replicative phenomena need to be weighed in the risk assessment of Cp-transgenic plants:

1. Transcapsidation (Fig. 1) of virus RNAs in mixed infections occurs in nature and has led to altered virus-vector specificities (8,9,17,18).
2. Template switching in vivo has been described as common for picornaviruses and, recently, for plant viruses (1,3).

If these two phenomena are active alone or in combination, virus infections of Cp-transgenic plants in the agroecosystem could lead to altered vector specificities in the progenies of viruses infecting these plants, or novel virus genomes could arise by recombination between transgenic mRNAs and infecting viral RNAs. In monocultures of Cp-transgenic plants every virus infection is in essence a double infection (in regard to the Cp gene). The probabilities for recombination are increased considerably when compared to monocultures of nontransgenic plants (15).

Although critical questions concerning the mechanism of cross-protection and engineered cross-protection are still unanswered (2), it is quite obvious that plants expressing viral coat protein are exhibiting potentially agronomically useful levels of disease resistance. It is therefore only a matter of time before we will see engineered cross-protection used in sustainable agriculture. However, the advances made may be negated by the problems that we might have created.

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Dependent transmission mechanisms. Heterologous encapsidation, phenotypic mixing (Fig. 1 [3]) and transcapsidation (Fig. 1 [2]) are used here sensu Rochow (19). For an in-depth treatment of dependent transmission as well as the phenomena of heterologous encapsidation in relation to vector transmission of plant viruses, the reader is referred to Rochow (18), Falk and Duffus (9), and Creamer and Falk (8).

Heterologous encapsidation leading to changes in transmissibility is a unique and significant feature of aphid plant-virus relationships for at least two reasons. First, all three types of aphid-virus relationships—nonpersistent, semipersistent, and persistent—include examples of the dependent-transmission phenomenon. Second, the examples include many crops, virus groups, and species of aphid.

Since transcapsidation or phenotypic mixing may change the vector range of a virus, heterologous encapsidation could lead to the creation of an apparently "new" disease. Although this is well described under conditions of mixed infections, one must also consider the possibility of a similar phenomenon in Cp-transgenic plants. The situation in a Cp-transgenic plant is depicted in Fig. 1, where the virus (V) replication is neither temporally nor spatially separate from the transgenetically produced coat protein (Cp). Transgenic coat protein (Cp in Fig. 1) in the viral capsid may therefore make the nucleic acid from particle V transmissible by aphid species different from those that normally transmit particle V by either transcapsidation (particle 2) or phenotypic mixing (particle 3). If this different aphid species has a plant-host range distinct from those of the usual vector, nucleic acid of particle V could thus be introduced into a "new" host range, where it can initiate replication. If particle 2 or 3 alone was transmitted to the new host, the resulting infection would produce only particles similar to V. But such particles, now in a host distinct from the original, could be acquired and transmitted by still other aphid species that feed on the new host and not on the original host range of V. Thus, particle V could become transmissible by an aphid species to a plant-host range, both quite different from the plant and aphid involved in the original

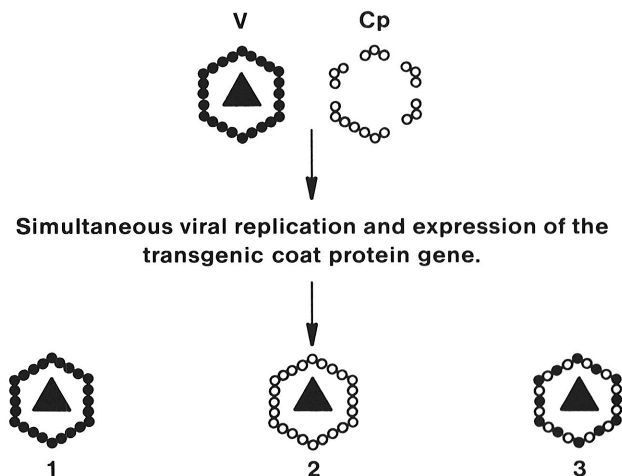

Fig. 1. Possible combinations of viral RNA and capsid proteins in the progeny virus in a virus-infected coat-protein-transgenic plant. Shown are the infecting virus (**V**) and its identical offspring (**1**), the coat protein (**Cp**) for which the plant is transgenic, and the possible capsid combinations when transcapsidation (**2**) or phenotypic mixing (**3**) occurs (19).

infection. Because of the change of both host and vectoring aphid, the identity of virus V before and after these events might not be easily recognized. Thus, the net result would be an expansion of both the host and vector range of the virus.

Heterologous encapsidation is common within the luteovirus group when mixed infections occur (8,9) and has now been shown to occur in CP-transgenic plants (16). The possibilities of heterologous encapsidation become even more menacing when dependent transmission occurs among viruses of different, unrelated groups, such as pea enation mosaic virus, the helper, and bean yellow veinbanding virus (4). Pea leaf roll virus, a typical luteovirus, can also serve as a helper in this complex. The evidence regarding the mechanism of persistent virus transmission suggests that specific coat proteins are involved in the specificity of aphid transmissibility (6,11,12,13,18).

Heterologous encapsidation has been emphasized here in relation to aphid-transmissible viruses mainly because it directly affects transmissibility and vector specificity. However, for most plant viruses we have little knowledge of the functions of coat protein other than that of protecting the RNA. Thus it is important to study the role of coat protein in the transmission of heterologously encapsidated viruses.

Template switching. Research results from animal RNA viruses (7,14) as well as from plant RNA viruses (1,3) indicate that recombination between viral RNAs occurs at relatively high frequencies during replication. Two different mechanisms for this recombination have been suggested: 1) enzymatic cutting and religation and 2) a "copy choice" during replication between advantageously proximal templates. The latter mechanism seems to be generally favored for recombination in picornaviruses.

When CP-transgenic plants become infected with viruses related to the one providing the Cp gene in the transgenic plant, template switching might occur between the transgenic mRNA and the replicating viral RNA. Template switching might be more likely if the transgenic sequences included the 3′ nontranslated part of the viral genomic RNA (the replicase initiation site), facilitating promiscuous initiation on the transgenic message by the replicase of the infecting virus. It is clearly possible that a combination of heterologous encapsidation and template switching in released Cp-transgenic plants could lead to the formation of "new" viruses with altered vectors and host ranges and new combinations of genes. I will not dwell here on the potential problems with viroid or satellite cDNA-transgenic plants, in which the RNAs that are produced can replicate and ameliorate the symptoms of either the viroids or the satellite-associated viruses. Specifically in the case of satellite RNAs, where their amplification is virus-dependent and recombination during replication has been amply documented (5), problems may arise that are somewhat different from those described here, but they are at least as real and worthy of assessment.

Risk assessment. Funding for risk assessment of these or other scenarios in regard to Cp-transgenic plants depends to a great extent on the acceptance of these ideas by our peers. Since there appears to be no research that specifically addresses these concerns or documents their irrelevance, we depend on rather uninformed peer review and therefore on uninformed risk assessment.

I advocate that it is better to base our risk assessment on experimentation than to base it on popular opinions. The once generally held belief that coat protein had nothing to do with cross-protection (a result of the lack of experimentation) was the reason that advances in the area of engineered cross-protection, until recently, came notwithstanding the absence of federal (peer-reviewed) funding.

Other questions that should be raised address the issue of the efficacy of monogenic Cp gene-engineered resistances against the background of the experience over the past 70 years in breeding for such resistance traits. Furthermore, the determination of liabilities as to environmental contamination with genes desirable in one crop but not in related crops or its weed relatives is going to be an important currently unanswered issue. Answers to these questions should be formulated for successful application of the technologies now available.

We, as scientists working in the field, should be in the forefront of risk assessment. Rather than dismissing questions from USDA, APHIS, or EPA on the danger of coat protein in transgenic plants entering the food chain, we should be asking the relevant questions. We are developing biotechnology for and with industry; its success is our success, and that success determines our continued support. If applications of our technologies require the formulation of pertinent questions on risk assessment, let us do it now rather than thirty years from now. History indicates that the euphoria of the moment tends to silence the questioning voices. It has happened with chlorinated hydrocarbons and countless other products that were not assessed adequately or in a technically pertinent manner when they became available. Let us assess the risk *now* and do it right to provide the basis for the successful applications of these technologies without repeating the mistakes of the past.

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