

**SIXTH FRAMEWORK PROGRAMME**  
**THEMATIC PRIORITY 5**  
**FOOD QUALITY AND SAFETY**



ResistVir

Co-ordination of Research on genetic resistance to plant Pathogenic Virus, and their Vectors  
in European Crops

Project number: FOOD-CT-2005-006961

Co-ordination Action

**Deliverable 33 - Report from Expert Group 7:  
Resistance durability and management in integrated production systems**

Due date of deliverable: **M37**

Delivery data on the web site: **M46**

Actual submission date to the Commission: **M48**

Start date of the project: **February 1<sup>st</sup>, 2005**

Duration: **48 months**

Organisation name of lead contractor: **Institut National de la Recherche Agronomique (INRA)**

Project co-funded by the European Commission within the sixth Framework programme (2002-2006)	
Dissemination Level	
PU public	PU
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium ( including the Commission services)	
CO Confidential, only for members of the consortium (including the Commission services)	

# Integrated management and durability of plant resistance to viruses and their vectors: State of the art and prospects.

**Benoît Moury** (Institut National de la Recherche Agronomique -INRA), **Alberto Fereres** (Consejo Superior De Investigaciones Científicas -CSIC), **Fernando García-Arenal** (Universidad Politécnica de Madrid –UPM), **Hervé Lecoq** (Institut National de la Recherche Agronomique –INRA)

## Content

<b>Introduction</b>	<b>2</b>
<b>1. The biological scenario of plant resistance breakdown by viruses.</b>	<b>2</b>
1.1 <i>The difference steps of resistance breakdown and evolutionary forces at play</i>	2
1.2 <i>Estimation of the strength of evolutionary forces acting on plant virus populations</i>	5
<b>2. Which factors influence the durability of plant resistance to viruses?</b>	<b>8</b>
2.1 <i>Nature of plant resistances</i>	8
2.2 <i>Intrinsic virus traits</i>	9
2.3 <i>Environmental and anthropic factors</i>	10
<b>3. gg Integrated virus management to preserve the durability of plant resistance</b>	<b>11</b>
3.1 <i>Combining resistance genes to preserve their durability</i>	11
3.2 <i>Additional control methods to combine to resistance</i>	11
3.3 <i>Rules to combine different control methods</i>	13
3.4 <i>Which effects are expected from control method combinations?</i>	14
<b>4. Prospects</b>	<b>14</b>
<b>Acknowledgments</b>	<b>16</b>
<b>References</b>	<b>16</b>
<b>Table 1</b>	<b>24</b>
<b>Table 2</b>	<b>25</b>
<b>Figure 1</b>	<b>27</b>
<b>Figure 2</b>	<b>28</b>

## **Introduction**

In the absence of curative control methods against plant viruses, genetic resistance is amongst the most efficient and the most widely and easily used control methods. Resistance should therefore occupy a central place in integrated strategies for viral disease control. Although they share common objectives, *i.e.* the sustainable control of plant pathogens, resistance durability and integrated plant disease management have complex relationships (Fig. 1). When resistance genes are introduced into commercial varieties, they are often sufficiently efficient by themselves to protect crops from diseases. Consequently, as long as no resistance breakdown occurs, growers are less prone to combining the use of these resistances with additional control methods in an integrated approach. Such practices will in turn favour resistance breakdown, especially if the resistance has initially a high efficiency and exerts a strong selective pressure on the pathogen populations. This negative feedback builds a “vicious circle” which can accelerate the “boom and bust” cycle of resistance genes. In an alternative and more desirable view, considering plant resistance genes as rare resources to be preserved on the long term should be an incentive to combine resistances with other control methods to extend their durability. In turn, the integrated management strategy, often composed of control methods with quantitative effects on the pathogen population, will benefit on the long term of the additional effect of the resistance in a positive feedback (“virtuous circle”). Switching from the “vicious” to the “virtuous” circle depends as much on our knowledge of the biological and ecological processes involved in the complementary effects of plant resistance and additional control methods as on socio-economical factors.

In this report, we will review the state of the art concerning the durability of plant resistance to viruses and the relationship between integrated management of plant virus diseases and resistance durability.

### **1. The biological scenario of plant resistance breakdown by viruses.**

#### *1.1. The different steps of resistance breakdown and evolutionary forces at play.*

Assuming that the virus population is totally avirulent (*i.e.* unable to infect plants carrying the resistance) when a new resistance is released in commercial varieties, breakdown of the resistance involves three steps (Fig. 2) (only the latter two steps are relevant in case virulent variants [*i.e.* variants able to infect plants carrying the resistance] pre-exist when the new

resistance is released). The first step consists in the appearance of virulent variants in the initially avirulent viral population. As soon as these variants appear, they are in competition with the avirulent components of the viral population for their accumulation in the cells and plants where they first appeared. Only if they are competitive enough, will the virulent variants multiply enough into these plants (step 2) and have the opportunity to be transmitted to another plant and to spread in the crops (step 3) allowing epidemics to develop in resistance-carrying plants. This scenario will serve us as a canvas to identify the factors and forces involved in resistance breaking and the potential actions of additional control methods on resistance durability.

Johnson (1981) defined durable resistance as a resistance that remains effective while being extensively used in agriculture for a long period in an environment conducive to the disease. Effectiveness of a resistance is somewhat subjective. A resistance could be considered as effective if no virulent viruses have infected the plants (at least step 2 in the scenario has not been fulfilled efficiently) or if no economic damages are induced by these virulent viruses (at least step 3 in the scenario has not been fulfilled efficiently). It is generally considered that if all these steps are efficiently performed by virulent virus variants, then a breakdown of the resistance occurs. If not, the resistance will be considered as durable.

Different evolutionary forces rule the achievement of these different steps (Fig. 2). The first step, appearance of virulent variants, usually involves a small number (most often one or two) of nucleotide substitutions in the so-called avirulence gene encoded by the viral genome (Harrison, 2002). Virus avirulence genes corresponding to around 30 plant resistance genes or alleles have been identified (Table 1). Identification of avirulence genes was usually performed by reverse genetics approaches where genome regions were exchanged between DNA or cDNA clones of virulent and avirulent virus variants. Finally, directed mutagenesis of a clone of an avirulent virus isolate allows the mapping of the mutations involved in virulence properties. In almost all cases, these nucleotide substitutions correspond also to putative amino acid substitutions. Consequently, it is generally assumed that the avirulence factor is not the genome segment corresponding to the avirulence gene by itself but the protein it encodes. That hypothesis is particularly convincing when direct and specific physical interactions have been shown between the products of the resistance or susceptibility alleles of the plant and those of the avirulence or virulence alleles of the virus (Charron *et al.* 2008). However, a non-coding region of the virus genome was shown to be the avirulence factor for at least one resistance gene (Díaz *et al.* 2004). In theory, when nucleotide

substitutions at different positions in the viral genome are required for virulence, recombination or reassortment between two (or more) avirulent virus isolates could generate virulent variants. However, no such situation has been reported so far.

The second step, relating to the competition between avirulent and virulent viruses in the plants where the latter appeared, involves mainly two evolutionary forces: differential selection between the two virus variants (a component of their relative fitness) and genetic drift during the infection of the plant by the viruses. Since virulent variants are more likely to appear in plants devoid of the resistance gene (virus replication is frequently drastically reduced in resistant plants, therefore decreasing the probability that virulence mutations occur), the relative competitiveness of virulent and avirulent virus variants is usually measured in such plants. Practically, avirulent and virulent variants, differing only by the virulence mutations to avoid any side effect of additional mutations, are co-inoculated to “susceptible” plants. The relative accumulation of both virus variants is monitored during the course of the plant infection and compared to their relative concentration in the inoculum. It should be noticed that the results obtained with these mixed-inoculation experiments are frequently different from those obtained with single-inoculation experiments (*i.e.* where the avirulent and virulent variants are inoculated singly to different individual plants). Differences in virus accumulation usually increase (or appear) in mixed-inoculation experiments while these differences are smaller (or absent) in single-inoculation experiments (Desbiez *et al.* 2003; Ayme *et al.* 2006). In some cases, opposite rankings for relative accumulation of virus variants were observed between the two different protocols (Pierrugues *et al.* 2007).

The third step of resistance breaking is probably the most complex one since three different evolutionary forces can exert on the virus population: (i) spatial dissemination (migration) of the virus between plants, (ii) genetic drift during transmission events and (iii) selection imposed by vectors of the virus.

This general overview of the different forces at play during resistance breakdown suggests two comments:

- Many evolutionary parameters should be estimated to account for the whole process of resistance breakdown. Most of these are particularly difficult to estimate with precision and poorly known for plant viruses.
- The interplay between these different forces, acting on different steps of resistance breakdown and at different spatial scales, is almost impossible to comprehend by experimental approaches and requires mathematical modelling developments which,

in their turn, should be verified with experimental or observational data. Mathematical modelling outputs will also provide future hypotheses to be tested experimentally.

### *1.2. Estimation of the strength of evolutionary forces acting on plant virus populations.*

After having listed the different forces acting on virus populations and responsible for the breakdown of plant resistances, we will briefly review what is known about their quantification.

Mutation and recombination rates are difficult to estimate from natural diversity studies since they are not easy to disentangle from the action of natural selection and also because of the difficulties of estimation of generation times. The spontaneous mutation rate of plant viruses under minimum selection from the host plant was estimated only for Tobacco mosaic virus and estimated to be 0.10 to 0.13 mutations per genome per replication cycle ( $\sim 2 \times 10^{-5}$  mutations per nucleotide per replication cycle), a value similar to that of animal viruses (Malpica et al. 2002) and at least 10,000 times higher than for prokaryotes or eukaryotes. Only for Cauliflower mosaic virus, a DNA virus, do we have an estimation of the recombination rate during the infection of the host ( $2 \times 10^{-5}$  to  $4 \times 10^{-4}$  recombinations per nucleotide per replication cycle; Froissart et al. 2005). Frequent recombination was also observed for *Brome mosaic virus* and *Cucumber mosaic virus*, two RNA viruses (Bruyere et al., 2000; García-Arenal et al. 2001). The frequency of mutation or recombination in virus populations depends both on the individual mutation/recombination rate of viruses and on their population size. The total (census) population size of plant viruses can be tremendous (up to  $10^{12}$  viral particles in a single infected leaf; García-Arenal et al. 2001). Thus, given their mutation rates, population sizes and generation times, extremely high levels of diversity are expected within plant virus populations. However, the action of genetic drift and selection dramatically reduces the diversity within plant virus populations.

During the infection of individual plants by viruses, two different forces, genetic drift and selection, operate. Genetic drift occurs at any step where molecules or pathways necessary for the virus life cycle are in insufficient quantities with regard to the total virus population size. The effects of drift and selection can be difficult to distinguish, since both occur at the same steps in the virus life cycle and their effect on population diversity can be similar. Both phenomena reduce the within-population diversity but can increase the between-population diversity. However the effects of drift are stochastic and individually unpredictable, whereas

the effects of selection should be reproducible for identical environments and initial composition of the virus population. Genetic drift during plant invasion was recently shown for different plant viruses infecting different hosts (French and Stenger, 2003; Li and Roossinck, 2004; Sacristán *et al.* 2003; Monsion *et al.* in press). The effective population size, *i.e.* the number of individual viruses giving rise to the entire virus population in the plant, was found to vary from around ten for two RNA viruses (French and Stenger, 2003; Sacristán *et al.* 2003) to several hundreds for a DNA virus (Monsion *et al.* in press), which indicates the possibility of severe genetic drift when compared to the census size of virus populations which can reach  $10^{12}$  in a single plant leaf (García-Arenal *et al.* 2001). Genetic drift acting on virus populations within the infected plants might have important consequences on the further plant-to-plant spread of virus variants (Moury *et al.* 2007).

Differential selection between viral variants can be the result of constraints exerted by hosts, vectors, physical environment or by the virus itself. Because viral genes are often multifunctional, even a limited number of nucleotide changes may have strong pleiotropic effects and may be responsible for fitness costs conferred by gains of virulence (in plants devoid of the corresponding resistance). Practically, to measure the relative competitiveness of avirulent and virulent variants, viral populations differing only by the virulence mutations should be compared to avoid any side effect of additional mutations. These populations are co-inoculated to “susceptible” plants and the relative accumulation of both virus variants is monitored during the course of the plant infection and compared to their relative concentrations in the inoculum. It should be noticed that the results obtained with these mixed-inoculation experiments are frequently different from those obtained with single-inoculation experiments (*i.e.* where the avirulent and virulent variants are inoculated singly to different individual plants). Occurrence of “virulence fitness costs” was shown in several instances (Goulden *et al.* 1993; Lanfermeijer *et al.* 2003; Jenner *et al.* 2002a; Desbiez *et al.* 2003; Ayme *et al.* 2006). Other examples suggest that virulent strains can be at least as fit as avirulent ones (Chain *et al.* 2007; Sorho *et al.* 2005). However, in these latter examples, it was not determined whether mutations outside the virulence factor also contributed to fitness variations.

Given their diverse dispersal means (persistent or nonpersistent transmission by airborne insect vectors, transmission by soilborne vectors such as fungi or nematodes, contact or wound transmission, vertical seed transmission, dispersal by infected plant material

propagation or trade), diverse patterns of virus transmission from plant to plant are expected, together with different strengths of the three different evolutionary forces (migration, selection and drift) exerted on virus populations during this process.

For almost all plant viruses, the frequency versus distance distribution of migration events is unknown. Even though the dynamics of vectors can be estimated both at field and at regional scales, their viruliferous status is rarely determined and remained extremely difficult to assess in the case of transient, nonpersistent virus-vector interactions. Moreover, detecting viruses in a vector does not imply that the virus will be transmitted or even that the detected virus is in a transmissible state. However, efforts have been made to estimate the spread of nonpersistent viruses such as *Soybean mosaic virus* (Ruesink and Irwin, 1986) or *Potato virus Y* (Sigvald, 1986) as a function of aphid landing and transmission rates. Inferring migration events from observed plant infection patterns is also rendered difficult by the lack of virus variability either to identify independent migration events or to separate primary and secondary infections. As a consequence, few studies have examined the virus variability with the aim to measure their migration distances and only general tendencies are known. For example, when a spatial structure of virus diversity is observed, this suggests a lack of intense gene flow (Fargette *et al.* 2004; Fraile *et al.* 1996; Lecoq *et al.* 2005). Also, minimal distances for which spatially-structured populations are observed may indicate distance thresholds above which virus migrations are drastically reduced (Arboleda and Azzam, 2000; Azzam *et al.* 2000).

Selection acting on virus populations during plant-to-plant transmission was mostly demonstrated in the case of viruses transmitted by biological vectors. That different viral genotypes are transmitted with different efficiencies was initially shown by describing poorly transmissible or non transmissible virus variants issued from laboratory experiments (Ng and Falk 2006). Differential transmission efficiency of virus variants between different vector species was also shown experimentally (Perry *et al.* 1998). The importance of differential selection of virus variants by vectors in epidemiological conditions remains unknown although indirect evidence suggest that it could be a major evolutionary constraint on some virus populations (Moury 2004).

Genetic drift is thought to be severe during plant-to-plant transmission of viruses by vectors, which was shown experimentally for different aphid-transmitted RNA viruses (Ali *et al.* 2006; Sentandreu *et al.* 2006; Moury *et al.* 2007; Betancourt *et al.* 2008). However, an estimation of the drift intensity was obtained only for *Potato virus Y* and *Cucumber mosaic virus*, for which individual aphids were estimated to transmit less than 3.2 viral infectious units on average (Moury *et al.* 2007; Betancourt *et al.* 2008).

## 2. Which factors influence the durability of plant resistance to viruses?

According to Johnson's definition, the durability of resistances can be measured only *a posteriori* after their large-scale deployment. The ability to predict the potential durability of resistance since the beginning of the breeding programs represents a major challenge and relies on the knowledge of the factors which influence most that durability.

### 2.1. Nature of plant resistances.

Given the wide diversity of plant resistance phenotypes, mechanisms and genetic determinisms, it was logical to try to link these properties to resistance durability. No association was observed between the genetic determinism of virus resistance and its durability (García-Arenal and McDonald, 2003). On the opposite, a slightly significant overall association was noticed between the resistance phenotype and its durability, although no particular category of resistance was found more durable than others (García-Arenal and McDonald, 2003). "Extreme resistances", *i.e.* dominant resistances which reduce virus multiplication at the single cell (protoplast) level and do not exhibit hypersensitive reactions on inoculated organs, are particularly durable (Köhm *et al.* 1993; Bendahmane *et al.* 1995; Barker and Harrison, 1984; Hajimorad and Hill, 2001). However, only few members of this resistance category have been characterized so far and no general rules can be derived from these observations yet.

Based upon data obtained mainly from plant resistance to fungi, polygenic resistances are often presumed to be more durable than monogenic ones (Lindhout *et al.* 2002). This could be true also for virus resistances, but these complex resistances are less studied than mono- or oligogenic ones and there is no experimental evidence for this hypothesis. Furthermore, the reasons for higher durability of polygenic resistances are unclear. Polygenic resistances have usually quantitative effects on virus infection while many monogenic resistances have much stronger (qualitative) effects. It is unknown if the durability of these resistances is due to their polygenic or to their quantitative nature.

## 2.2. *Intrinsic virus traits.*

Considering the different evolutionary forces involved in resistance breaking (Fig. 2), it seems logical that their effects vary largely according to the different virus species. For mutation/recombination rates, genetic drift within plants and selection during plant-to-plant transmission, the scarcity of our knowledge does not allow comparisons between plant virus species (see section 1.2.). However, at least two additional properties can affect resistance durability: (i) the size of virus populations and (ii) their capacity of dissemination in the agro-ecosystem. Virus population size directly affects the probability of appearance of virulent variants. It depends on the number of host species, the frequency of these host species and the capacity of the virus to accumulate in its host plants, which are known to vary greatly between viruses. Moreover, viruses can undergo more or less severe decrease (bottlenecks) of their population size at certain steps of their infection cycles. These bottlenecks can in turn cause the extinction of many virus variants and decrease the probability of emergence of virulent variants. The differences of dissemination capacity between viruses are not known with precision. However, they can be inferred from the knowledge of the particular vectors which may be involved in virus transmission, of the potential spread of the virus in seeds or in material used for plant vegetative reproduction or from studies of the population structure of viruses. Notably, air-borne vectors can disseminate viruses on longer distances than soil-borne ones and persistent viruses can be disseminated on longer distances compared to non-persistent ones. Seed-borne viruses have the longest dispersal ranges due to human activities. The number of vector species and their prevalence are also important with regard to virus dissemination chance and distance.

These two properties, together with a third one (occurrence of “sexual reproduction”, *i.e.* recombination or reassortment) were incorporated into García-Arenal and McDonald’s “evolutionary potential” (EP) of viruses (García-Arenal and McDonald, 2003), a semi-quantitative compound index. In spite of many estimation uncertainties and oversimplifications, that index was shown to correlate with resistance durability: The higher the EP of viruses, the lower the durability of resistances targeting them. The link between the third property and resistance durability is not straightforward since sexual reproduction has not been shown to be involved directly in the generation of virulent virus variants. In addition, the frequency of recombinants or reassortants has not been shown to differ between virulent and avirulent isolates. However, when virulence is associated to a fitness cost of the virus in

plants devoid of the resistance gene, a fitness recovery could arise by secondary mutations or, maybe more rapidly, by recombination or reassortment. Then, sexual reproduction would contribute to the emergence of virulent virus variants. This was suggested to occur in the case of a resistance to *Tomato spotted wilt virus* (TSWV) in transgenic tobacco plants conferred by the nucleocapsid gene of TSWV. The ability to overcome the resistance was associated with the M RNA segment of TSWV but modified by the other two RNAs in the TSWV genome (Hoffmann *et al.* 2001). Accordingly, when a composite TSWV population was inoculated to resistant plants, the selected virulent virus variants were reassortants between the two components of the virus population (Qiu and Moyer, 1999).

Different resistance genes which target the same virus frequently show contrasted durability. By definition, the EP of viruses cannot account for these differences. In this respect, knowledge derived from the identification of virulence genes and mutations could contribute to a better prediction of resistance durability. For example, Harrison (2002) showed that the number of amino acid substitutions required for virulence was linked to the resistance durability. When two or more substitutions were necessary, durability was generally high while, when a single substitution sufficed to confer virulence, resistance durability was generally low.

### 2.3. *Environmental and anthropic factors.*

By affecting the frequency and geographic distributions of virus host plant and/or vector populations, physical environment of crops (climate, soil...) and human decisions (choice of crop species and genotypes) or behaviour (trade or introduction of infected plants or seeds) can influence strongly the prevalence of viral diseases and the durability of virus resistances. As already underlined (see section 2.2.), this can operate by increasing the population size of viruses, decreasing the impact of population bottlenecks on viruses and increasing their capacity of dissemination in the agro-ecosystem. The chance that individual plants could be infected by different virus isolates will also increase, enhancing the effects of recombination or reassortment. Insect vectors of viruses are particularly affected by climate variations and can also be transported over long distances with trade of plant material. In these last years, the worldwide expansion of thrips (*Frankliniella occidentalis*), whiteflies (*Bemisia tabaci*) or aphids (*Toxoptera citricida*) was the cause of the spread of particularly damaging plant viruses.

However, the fact that human activities can be detrimental to the durability of plant resistances to viruses suggests that rational crop management should allow preserving it.

### **3. Integrated virus management to preserve the durability of plant resistance.**

The knowledge of the potential relationships between the EP of viruses and the effect of certain environmental changes or human activities on resistance durability will be of help to define integrated strategies aiming to preserve resistance durability.

#### *3.1. Combining resistance genes to preserve their durability.*

Face to the rapid “boom and bust” cycles affecting the resistance genes exploited by breeders and growers, it was tempting to combine different resistance genes or alleles in order to increase their individual durabilities. Such strategies include the accumulation of different genes in the same plant genotype (the so-called “pyramiding” of resistance genes) and the alternation of genes at the spatial or temporal levels (rotations of crops). The efficiency of these approaches has essentially been evaluated against fungal diseases of crops (Zhu *et al.* 2000; Pink, 2002; Mundt *et al.* 2002) and remains largely to be evaluated for viruses.

#### *3.2. Additional control methods to combine to resistance.*

The different control methods that can be used and combined against plant viruses have been recently reviewed (Jones 2006 and Table 2). These include prophylactic measures, the use of agronomic practices aiming to reduce the initial sources of inoculum and/or the subsequent spread of the virus and the control of vectors (either chemical, genetic resistance, cultural or biological). Among the control methods mentioned by Jones (2006), only cross-protection seems incompatible with the use of resistances. Indeed, cross-protection requires that the plants are infected by an attenuated strain of the virus while resistance maintains plant infection at a low level.

Control methods can interfere with resistance management and promote their durability by different mechanisms (Fig. 2; Table 2). Any control method which *reduces the size of the virus populations* will decrease the risk that virulent mutant or recombinant viruses appear – resistance breakdown step 1. The use of healthy propagules (seeds, cuttings, stocks), the reduction of the number of virus reservoirs (*e.g.* weeds or volunteers) and the number of

vectors landing on the crop will mostly contribute to decrease the inoculum potential. Cultural control methods may also slow down the selection of virulent variants within the host plants by decreasing the selective pressure in favour of the virulent variants (disruptive selection). By planting mixtures of resistant and susceptible cultivars or by rotating resistance and susceptible cultivars in time or space, selection of virulent variants could be delayed – resistance breakdown. step 2.

*Reducing the rate of spread of the virus* by interfering with virus dissemination - resistance breakdown step 3 - is a major goal of additional control methods which can improve resistance durability. This is achieved by targeting the virus vectors with exclusion methods such as physical nets that prevent insects to enter the protected environment or by preventing vector landing on the crop by means of mulches or other reflective surfaces. Also, genetic resistance, agrochemicals or biopesticides are effective methods to reduce vector numbers within crops. Agrochemicals in some cases may be particularly useful to control viruses transmitted in a persistent manner; the number of virus vectors will be reduced and lower inoculum pressure will challenge resistant cultivars. For persistently-transmitted viruses, insecticides may also prevent transmission by insects landing on a crop treated by interfering with the inoculation process. For nonpersistent viruses, chemicals also may reduce vector numbers but will not avoid inoculation of the virus once the vectors land and probe on the plant because transmission will occur in seconds, before insecticides interfere with landing vectors (Raccah, 1986; Perring *et al.* 1999). Counterproductive effects of insecticides have also been noticed since their application could contribute to the spread of nonpersistent viruses by increasing vector activity and mobility (Fernández-Calvino *et al.* 2007). However, reducing the numbers of vectors will benefit the crop because fewer insects will be available to land on the resistant crop. Certain insect predators such as ladybeetles may alter the dispersal patterns of their preys acting as virus vectors, and in some cases negative effects have been reported due to an increase in the movement of the vector to neighbouring plants which increased the risk of secondary spread. Nevertheless, very limited information is available to understand how natural enemies of insect vectors may influence the spread of virus diseases. In recent years, however, the use of predators or parasitoids are becoming more and more frequent to manage insect vector populations attacking horticultural crops in protected environments. The use of non-host barriers or trap crops and intercropping of susceptible and resistant crops, the use of crop-free periods, delaying sowing/planting date and growing early maturing cultivars will also avoid vectors to visit resistant plants and

hence, increase resistance durability (Hooks and Fereres, 2006). Developing push-pull strategies (Cook et al., 2007) may allow maintaining vectors far from the target crop and reducing the risk of virus transmission. Photosensitive films and nets that interfere with insect vision are known to dramatically reduce the spread of plant viruses. More precisely, UV-absorbing films and nets have proved very effective in controlling insect-transmitted viruses by reducing immigration rate and plant-to-plant movement of insect vectors (Antignus et al., 1996; Diaz et al., 2006; Kumar and Poehling, 2006; Diaz and Fereres, 2007). Photosensitive barriers and insecticide-impregnated nets are now being tested together with biological control agents to manage pests and virus diseases in greenhouse crops.

### *3.3. Rules to combine different control methods.*

Rules to combine different resistance genes (see section 3.1.) depend on our knowledge of their mechanisms of action, their target in the virus genome and the viral mutations involved in virulence. Resistance genes acting at different steps of the virus infection cycle (inoculation process, within-cell multiplication, cell-to-cell or systemic movement, acquisition by vectors) should be the most interesting to combine. Pepper resistance to *Cucumber mosaic virus* (CMV) is a good example. No major resistance genes have been found in the pepper germplasm to control CMV. However, combining in the same genotype partial resistance genes acting on inoculation, multiplication, cell-to-cell and systemic movement provided a high level of resistance which proved durable (Palloix *et al.* 1997). Another strategy is to combine in the same genotype several resistance genes which recognize different targets in the virus genome. Evolution toward virulence will require more mutation events which would have higher chances to confer fitness penalties to the virus. When different resistance genes or alleles recognize the same avirulence gene, interactions between the mutations involved in virulence towards these different genes/alleles could however disfavour their combination (pyramiding or rotation) (Ayme *et al.* 2007).

Few general rules have been defined to combine resistances and other control methods. Jones (2006) suggested that favourable associations could be those with different selectivity or which act at different steps of the epidemics (controlling the initial sources of inoculum or the spread of the virus; Table 2). We could add that control methods acting on different evolutionary forces involved in resistance breaking would probably also be interesting (Table

2). At least as important as these considerations is the socio-economic feasibility of combining the different control methods.

#### *3.4. Which effects are expected from control method combinations?*

It is frequently assumed that positive epidemiological synergisms are obtained by combining different disease control methods (Mundt *et al.* 2002; Jones, 2006). For example, Mundt *et al.* (2002) showed synergism between resistance to potato late blight and fungicide applications or between mixture of resistant and susceptible cultivars and fungicide applications. Using exclusion nets together with insecticides has provided a very effective way to control vectors of virus diseases and other insect pests of cabbage crops (Martin *et al.*, 2006). That increased effectiveness of disease control has also important consequences on resistance durability due to the reduction of inoculum, of population size of pathogens and the increase of the effects of genetic drift during the life cycle of parasites.

Synergism is however not a general rule in integrated pest management. Lecoq and Pitrat (1983) showed additive or less than additive effects of combining resistance, weeding and the use of mulch to control CMV in muskmelon.

#### **4. Prospects.**

The quest for resistance durability predictors that could be used by breeders and growers largely relies on our knowledge of the biological mechanisms of resistance breaking and on our ability to estimate precisely the durability of the resistance genes that have already been used. As already mentioned, these mechanisms are still largely unknown and their study should be pursued. Estimating resistance durability will require long-term and large-scale ecological and epidemiological studies. Molecular ecology and epidemiology approaches were rarely applied to this purpose (Acosta-Leal *et al.* 2008; Schirmer *et al.* 2005).

Most of our knowledge of resistance breakdown and durability was obtained with resistance genes with strong, qualitative effects on plant pathogens (“major genes”). However, these major genes are certainly only the “tip of the iceberg”, considering the iceberg as the whole pool of resistance genes. The durability of quantitative resistance genes and of polygenic resistances is largely unknown while new technologies incite breeders to use them (quantitative measure of virus resistance such as real-time [RT-]PCR; mapping of quantitative

trait loci [QTLs] in plant genomes; marker-assisted selection of QTLs). The molecular nature of quantitative resistance genes is still unknown. It was suggested that these could be similar to major resistance genes (RGA or resistance gene analogs) and may represent defeated resistance genes. Another hypothesis suggests that resistance QTLs could be classical defence genes acting downstream in the resistance signalling pathway. These hypotheses were not supported by a recent meta-analysis in the case of resistances to rice blast (Ballini *et al.* 2008). It will be all the more important to analyse further the durability of quantitative resistances since some of these QTLs probably correspond to new classes of genes. The same conclusions could be reached for tolerance genes, even less extensively studied than partial resistances. Tolerance corresponds to “the ability of the host to limit the disease severity induced by a given parasite burden” (Råberg *et al.* 2007) and is usually measured as the slope of the host fitness against infection level. Given that tolerance does not affect the capacity of reproduction of the parasite within its host, it is often assumed that it does not exert any selective pressure on the parasite population (Råberg *et al.* 2007). At least one example shows that a tolerance gene can indeed exert a very high selective pressure on a plant virus population and that its durability can be rapidly impaired (Desbiez *et al.* 2003). Clearly, additional data are required about the durability of tolerance genes and about the possibilities to combine resistance and tolerance in an integrated approach.

Given the complexity of the mechanisms involved (Fig. 2), assessing the risks of resistance breaking and defining strategies to combine resistances to additional control methods will be difficult by purely experimental approaches. Mathematical modelling approaches are therefore required. Until now, the use of models in plant virology was essentially restricted to the description of virus epidemics (but see for example Escriu *et al.* 2003; Gilligan *et al.* 2007; Moury *et al.* 2007). This field of research should benefit from models aiming to predict the outcome of different control strategies as recently developed for animal viruses (Ferguson *et al.* 2001, 2005; Morris *et al.* 2001). Indeed, analogies can be made between the impacts of control methods on plant and animal virus populations. Plant qualitative resistance is comparable to vaccination, partial resistance comparable to the use of antiviral drugs and roguing and weeding to the culling practice in livestock. This will require more detailed estimations of the various evolutionary and epidemiological parameters involved in resistance breaking and of their variance. Notably, analyses of the variations of plant virus fitness and aggressiveness under various environmental conditions and control strategies are still underdeveloped. Increasing knowledge of the virulence factors and mutations of plant virus

should also allow for the integration of evolution processes into epidemiological models (Galvani *et al.* 2003; Débarre *et al.* 2007). Various outputs can be expected from these models: Estimation of the relative importance of, and interplay between the various mechanisms that operate during resistance breaking, comparison of the potential durability of different resistance genes or mechanisms, definition of predictors of resistance durability, definition of integrated strategies to avoid resistance breakdown, estimation of the economical and ecological costs and benefit of integrated strategies.

## Acknowledgments

Support was provided by the EU-FP6 Co-ordination action ResistVir “Co-ordination of Research on Genetic resistance to Plant Pathogenic Viruses and their Vectors in European Crops”, Contract No. FOOD-CT-2005-514048.

## References

- Acosta-Leal, R., Fawley, M.W., Rush, C.M. (2008). Changes in the intrasolate genetic structure of Beet necrotic yellow vein virus populations associated with plant resistance breakdown. *Virology* 376:60–68.
- Ali A, Li H, Schneider WL, Sherman DJ, Gray S, Smith D, Roossinck MJ (2006) Analysis of genetic bottlenecks during horizontal transmission of *Cucumber mosaic virus*. *J. Virol.* 80:8345-8350.
- Antignus, Y., Mor, N., Joseph, R.B., Lapidot, M., Cohen, S. (1996). Ultraviolet-absorbing plastic sheets protect crops from insects pests and from virus diseases vectored by insects. *Environ. Entomol.* 25: 919-924.
- Arboleda, M., and Azzam, O. (2000). Inter- and intra-site diversity of natural field populations of rice tungro bacilliform virus in the Philippines. *Arch. Virol.* 145:275–289.
- Ayme V, Souche S, Caranta C, Jacquemond M, Chadœuf J, Palloix A, & Moury B. (2006). Different mutations in the VPg of Potato virus Y confer virulence on the *pvr2<sup>3</sup>* resistance in pepper. *Mol. Plant Microbe Interact.* 19:557-563.
- Ayme V, Petit-Pierre J, Souche S, Palloix A & Moury B (2007). Molecular dissection of the potato virus Y VPg virulence factor reveals complex adaptations to the *pvr2* resistance allelic series in pepper. *Journal of General Virology* 88:1594-1601.
- Azzam, O., Yambao, M. L. M., Muhsin, M., McNally, K. L., and Umadhay, K. M. L. (2000). Genetic diversity of rice tungro spherical virus in tungro-endemic provinces of the Philippines and Indonesia. *Arch. Virol.* 145:1183-1197.
- Ballini, E., Morel, J.-B., Droc, G., Price, A., Courtois, B., Notteghem, J.-L., Tharreau D. (2008). A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance *Mol. Plant Microbe Interact.* 21:859–868.
- Barker, H., Harrison, B.D. (1984). Expression of genes for resistance to potato virus Y in potato plants and protoplasts. *Ann. Appl. Biol.* 105:539-545.
- Bendahmane, A., Kohm, B. A., Dedi, C. & Baulcombe, D. C. 1995 The coat protein of potato virus X is a strain-specific elicitor of Rx1-mediated virus resistance in potato. *Plant J.* 8:933-941.
- Bendahmane, A., Querci, M., Kanyuka, K. & Baulcombe, D. C. 2000 Agrobacterium transient expression system as a tool for the isolation of disease resistance genes: application to the Rx2 locus in potato. *Plant J.* 21:73-81.

- Berzal-Herranz, A., De la Cruz, A., Tenllado, F., Díaz-Ruiz, J. R., López, L., Sanz, A. I., Vaquero, C., Serra, M. T. & García-Luque, I. 1995 The *Capsicum* L3 gene-mediated resistance against the tobamoviruses is elicited by the coat protein. *Virology* 209:498-505.
- Betancourt, M., Fereres, A., Fraile, A., García-Arenal, F. (2008). Estimation of the effective number of founders that initiate an infection after aphid transmission of a multipartite plant virus. *J. Virol.* in press.
- Bruun-Rasmussen, M., Møller, I. S., Hensen, J. K. R., Lund, O. S. & Johansen, I. E. 2007 The same allele of translation initiation factor 4E mediates resistance against two *Potyvirus* spp. in *Pisum sativum*. *Mol. Plant Microbe Interact.* 20:1075-1082.
- Bruyere A., Wantroba M., Flasiński S., Dzianott A., Bujarski J.J. (2000). Frequent homologous recombination events between molecules of one RNA component in a multipartite RNA virus. *J. Virol.* 74:4214–4219.
- Chain F, Riault G, Trottet M, Jacquot E. (2007). Evaluation of the durability of the Barley yellow dwarf virus-resistant Zhong ZH and TC14 wheat lines. *Eur. J. Plant Pathol.* 117:35-43.
- Charron C., Nicolai M., Gallois J.-L., Robaglia C., Moury B., Palloix A., Caranta C. (2008). Natural variation and functional analyses provide evidence for co-evolution between plant eIF4E and potyviral VPg. *Plant J.* 54:56-68.
- Chiba, S., Miyanishi, M., Andika, I. B., Kondo, H. & Tamada, T. 2008 Identification of amino acids of the beet necrotic yellow vein virus p25 protein required for induction of the resistance response in leaves of *Beta vulgaris* plants. *J. Gen. Virol.* 89:1314-1323.
- Cook, S.M., Khan Z.R., Pickett, J.A. (2007). The use of push-pull strategies in integrated pest management. *Ann. Rev. Entom.* 52:375-400.
- Diaz, B.M., Biurrun, R., Moreno, A., Nebreda, M., Fereres, A. (2006). Impact of ultraviolet-blocking plastic films on insect vectors of virus diseases infesting crisp lettuce. *Hortscience* 41: 711-716.
- Diaz, B.M., Fereres, A. (2007). Ultraviolet-blocking materials as a physical barrier to control insect pests and plant pathogens in protected crops. *Pest Technology* 1:85-95.
- Débarre F., Bonhoeffer S. & R. R. Regoes. 2007. The effect of population structure on the emergence of drug resistance during influenza pandemics. *J. R. Soc. Interface* 4:893-906.
- Desbiez, C., Gal-On, A., Girard, M., Wipf-Scheibel, C., and Lecoq, H. (2003). Increase in *Zucchini yellow mosaic virus* symptom severity in tolerant zucchini cultivars is related to a point mutation in P3 protein and is associated with a loss of relative fitness on susceptible plants. *Phytopathology* 93:1478-1484.
- Diaz, J., Nieto, C., Moriones, E., Truniger, V., Aranda, M. (2004). Molecular characterization of a Melon necrotic spot virus strain that overcomes the resistance in melon and nonhost plants. *Molecular Plant-Microbe Interactions* 17:668-75.
- Eggenberger, A.L., Hajimorad, M.R., Hill J.H. (2008). Gain of virulence on Rsv1-genotype soybean by an avirulent soybean mosaic virus requires concurrent mutations in both P3 and HC-Pro. *Mol. Plant-Microbe Interact.* 21:931-936.
- Escriu, F., Fraile, A., García-Arenal, F. (2003). The evolution of virulence in a plant virus. *Evolution* 57:755-765.
- Fargette, D., Pinel, A., Abubakar, Z., Traoré, O., Brugidou, C., Fatogoma, S., Hébrard, E., Choisy, M., Séré, Y., Fauquet, C., and Konaté, G. (2004). Inferring the evolutionary history of *Rice yellow mottle virus* from genomic, phylogenetic, and phylogeographic studies. *J. Virol.* 78:3252-3261.
- Ferguson N. M., Donnelly C. A. & R.M. Anderson. (2001). The foot-and-mouth epidemic in Great Britain: pattern of spread and impact of interventions. *Science* 292:1155–1160.
- Ferguson N.M., Cummings D.A.T., Cauchemez S., Fraser C., Riley S., Meeyai A., Iamsrithaworn S., D. Burke. (2005). Strategies for containing an emerging influenza pandemic in Southeast Asia. *Nature* 437:209-214.
- Fernández-Calvino, L., López-Abella, D., López-Moya, J.J. (2007). Integrated management of insect-borne viruses by means of transmission interference as an alternative to pesticides. In: General concepts in integrated pest and disease management. Eds: Ciancio, A., Mukerji, K.G., Springer, pp. 269-293.
- Fraile, A., Malpica, J. M., Aranda, M. A., Rodríguez-Cerezo, E., and García-Arenal, F. (1996). Genetic diversity in tobacco mild green mosaic tobamovirus infecting the wild plant *Nicotiana glauca*. *Virology* 223:48-155.

- French, R., and Stenger, D. C. (2003). Evolution of *Wheat streak mosaic virus*: dynamics of population growth within plants may explain limited variation. *Ann. Rev. Phytopathol.* 41:199-214.
- Froissart, R., Roze, D., Uzest, M., Galibert, L., Blanc, S., and Michalakis, Y. (2005). Recombination every day: abundant recombination in a virus during a single multi-cellular host infection. *PLoS Biol* 3:e89.
- Galvani, A. P. (2003). Epidemiology meets evolutionary ecology. *Tr. Ecol. Evol.* 18:132-139.
- García-Arenal, F. & McDonald, B. A. 2003 An analysis of the durability of resistance to plant viruses. *Phytopathology* 93:941-952.
- García-Arenal, F., Fraile, A., and Malpica, J.M. (2001). Variability and genetic structure of plant virus populations. *Ann. Rev. Phytopathol.* 39:157-186.
- Genda, Y., Kanda, A., Hamada, H., Sato, K., Ohnishi, J., Tsuda, S. (2007). Two amino acid substitutions in the coat protein of *Pepper mild mottle virus* are responsible for overcoming the L4 gene-mediated resistance in *Capsicum* spp. *Phytopathology* 97:787-793.
- Gilardi, P., Garcia-Luque, I., Serra, M. T. (2004). The coat protein of tobamovirus acts as elicitor of both L2 and L4 gene-mediated resistance in *Capsicum*. *Journal of General Virology* 85:2077-2085.
- Gilligan, C. A., Truscott, J. E. & Stacey, A. J. 2007. Impact of scale on the effectiveness of disease control strategies for epidemics with cryptic infection in a dynamical landscape: an example for crop disease. *J. R. Soc. Interface*, 4:925-934.
- Goulden, M.G., Köhm, B.A., Santa Cruz S., Kavanagh, T.A., Baulcombe, D.C. (1993). A feature of the coat protein of potato virus X affects both induced virus resistance in potato and viral fitness. *Virology* 197:293-302.
- Hajimorad, M.R., Hill, J.H. (2001). Rsv1-mediated resistance against *Soybean mosaic virus-N* is hypersensitive response-independent at inoculation site, but has the potential to initiate a hypersensitive response-like mechanism. *Mol. Plant-Microbe Interact.* 14: 587-598.
- Hajimorad, M.R., Eggenberger, A.L., Hill J.H. (2008). Adaptation of *Soybean mosaic virus* avirulent chimeras containing P3 sequences from virulent strains to Rsv1-genotype soybeans is mediated by mutations in HC-Pro. *Mol. Plant-Microbe Interact.* 21:937-946.
- Harrison, B. D. 2002 Virus variation in relation to resistance breaking in plants. *Euphytica* 124:181–192.
- Hébrard, E., Pinel-Galzi, A., Bersoult, A., Sire, C. & Fargette, D. 2006 Emergence of a resistance-breaking isolate of *Rice yellow mottle virus* during serial inoculations is due to a single substitution in the genome-linked viral protein VPg. *J. Gen. Virol.* 87:1369-1373.
- Hoffmann, K., Qiu, W.P., Moyer, J.W. (2001). Overcoming host- and pathogen-mediated resistance in tomato and tobacco maps to the M RNA of *Tomato spotted wilt virus*. *Mol. Plant-Microbe Interact.* 14:242-249.
- Hooks, C.R.R., Fereres, A. (2006). Protecting crops from non-persistently aphid-transmitted viruses: A review on the use of barrier plants as a management tool. *Virus Research* 120:1-16.
- Jefferies S.P., King B.J., Barr A.R., Warner P., Logue S.J., Langridge P. 2003. Marker assisted backcross introgression of the *Yd2* gene conferring resistance to Barley yellow dwarf virus in barley. *Plant Breeding* 122:52–56.
- Jenner, C. E., Sanchez, F., Nettleship, S. B., Foster, G. D., Ponz, F. & Walsh, J. A. (2000). The cylindrical inclusion gene of turnip mosaic virus encodes a pathogenic determinant to the Brassica resistance gene TuRB01. *Mol. Plant Microbe Interact.* 13:1102-1108.
- Jenner, C. E., Wang, X., Ponz, F. & Walsh, J. A. (2002a). A fitness cost for *Turnip mosaic virus* to overcome host resistance. *Virus Res.* 86:1-6.
- Jenner C.E., [Tomimura K.](#), [Ohshima K.](#), [Hughes S.L.](#), [Walsh J.A.](#) (2002b). Mutations in *Turnip mosaic virus* P3 and cylindrical inclusion proteins are separately required to overcome two *Brassica napus* resistance genes. *Virology* 300:50-59.
- Johansen I.E., [Lund O.S.](#), [Hjulsager C.K.](#), [Laursen J.](#) (2001). Recessive resistance in *Pisum sativum* and potyvirus pathotype resolved in a gene-for-cistron correspondence between host and virus. *J. Virol.* 75:6609-6614.
- Johnson, R. (1981). Durable resistance: Definition of, genetic control and attainment in plant breeding. *Phytopathology* 71:567-568.

- Jones, R.A.C. (2006). Control of plant virus diseases. *Adv. Virus Res.* 67:205-244.
- Jridi C., Martin J.F., Marie-Jeanne V., [Labonne G.](#), [Blanc S.](#) (2006). [Distinct viral populations differentiate and evolve independently in a single perennial host plant.](#) *J. Virol.* 80:2349-2357.
- Kavanagh T, Goulden M, Santa Cruz S, Chapman S, Barker I, Baulcombe D (1992). Molecular analysis of a resistance-breaking strain of Potato virus X. *Virology* 189:609-617.
- Keller K E, Johansen I E, Martin R R, and Hampton R O (1998). Potyvirus genome-linked protein (VPg) determines Pea seed-borne mosaic virus pathotype-specific virulence in *Pisum sativum*. *Molecular Plant-Microbe Interactions* 11: 124-130.
- Kim C.H., Palukaitis P. (1997). The plant defense response to cucumber mosaic virus in cowpea is elicited by the viral polymerase gene and affects virus accumulation in single cells. *EMBO J.* 16:4060-4068.
- Köhm, B.A., Goulden, M.G., Gilbert, J.E., Kavanagh, T.A., Baulcombe, D.C. (1993). A potato virus X resistance gene mediates an induced, nonspecific resistance in protoplasts. *Plant Cell* 5:913-620.
- Kühne, T., Shi, N., Proeseler, G., Adams, M. J. & Kanyuka, K. 2003 The ability of a bymovirus to overcome the rym4-mediated resistance in barley correlates with a codon change in the VPg coding region on RNA1. *J. Gen. Virol.* 84:2853-2859.
- Kumar, P., Poehling, H.M. (2006). UV-blocking plastic films and nets influence vectors and virus transmission on greenhouse tomatoes in the humid tropics. *Environ. Entomol.* 35: 1069-1082.
- Lanfermeijer F.C., Dijkhuis J., Sturre M.J.G., Haan P., Hille J. (2003). Cloning and characterization of the durable *Tomato mosaic virus* resistance gene *Tm-2<sup>2</sup>* from *Lycopersicon esculentum*. *Plant Mol. Biol.* 52:1039–1051.
- Lecoq, H. and Pitrat, M. (1983). Field experiments on the integrated control of aphid-borne viruses in muskmelon. In: *Plant Virus Epidemiology*, Eds: Plumb, R.T., and Thresh, J.M., Blackwell Scientific Publications, Oxford, pp. 169-176.
- Lecoq, H., Desbiez, C., Wipf-Scheibel, C., Costa, C. and Girard, M. (2005). Molecular epidemiology of *Watermelon mosaic virus* (WMV, *Potyvirus*) in cucurbits: from simple to complex patterns. *IX International Plant Virus Epidemiology Symposium*, Lima, Peru, April 4-7, 2005, Abstract p. 53.
- Li, H., and Roossinck, M. J. (2004). Genetic bottlenecks reduce population variation in an experimental RNA virus population. *J. Virol.* 78:10582-10587.
- Lindhout, P. (2002). The perspectives of polygenic resistance in breeding for durable disease resistance. *Euphytica* 124:217-226.
- Malcuit, I., Marano, M. R., Kavanagh, T. A., De Jong, W., Forsyth, A. & Baulcombe, D. C. (1999). The 25-kDa movement protein of PVX elicits Nb-mediated hypersensitive cell death in potato. *Mol. Plant Microbe Interact.* 12:536–543.
- Malpica, J. M., Fraile, A., Moreno, I., Obies, C. I., Drake, J. W., and García-Arenal, F. (2002). The rate and character of spontaneous mutation in an RNA virus. *Genetics* 162:1505–1511.
- Margaria, P., Ciuffo, M., Pacifico, D. & Turina, M. (2007). Evidence that the nonstructural protein of *Tomato spotted wilt virus* is the avirulence determinant in the interaction with resistant pepper carrying the *Tsw* gene. *Mol. Plant Microbe Interact.* 20:547-558.
- Martin, T., Assogba-Komlan, F., Houndete, T., Hougard, J.M., Chandre, F. (2006). Efficacy of mosquito netting for sustainable small holders' cabbage production in Africa. *J. Econ. Entom.* 99:450-454.
- Meshi, T., Motoyoshi, F., Adachi, A., Watanabe, Y., Takamatsu, N. & Okada, Y. (1988). Two concomitant base substitutions in the putative replicase genes of *Tobacco mosaic virus* confer the ability to overcome the effects of a tomato resistance gene, Tm-1. *EMBO J.* 7:1575-1581.
- Meshi, T., Motoyoshi, F., Maeda, T., Yoshiwoka, S., Watanabe, H. & Okada, Y. (1989). Mutations in the tobacco mosaic virus 30-kD protein gene overcome Tm-2 resistance in tomato. *Plant Cell* 1, 515–522.
- Mestre, P., Brigneti, G. & Baulcombe, D. C. 2000 An Ry-mediated resistance response in potato requires the intact active site of the NIa proteinase from *Potato virus Y*. *Plant J.* 23:653-661.
- Monsion, B., Froissart, R., Michalakakis, Y., Blanc, S. (2008). Large bottleneck size in *Cauliflower mosaic virus* populations during host plant colonization. *PLoS Pathogens*, in press.

- Morris R. S., Wilesmith J.W., Stern M.W., Sanson R.L. & M.A. Stevenson. (2001). Predictive spatial modelling of alternative control strategies for the foot-and-mouth disease epidemic in Great Britain, *Veterinary Record*, 149:137–144.
- Moury B (2004). Differential selection of genes of *Cucumber mosaic virus* subgroups. *Molecular Biology and Evolution* 21:1602-1611.
- Moury, B., Morel, C., Johansen, E., Guilbaud, L., Souche, S., Ayme, V., Caranta, C., Palloix, A. & Jacquemond, M. (2004). Mutations in *Potato virus Y* genome-linked protein determine virulence toward recessive resistances in *Capsicum annuum* and *Lycopersicon hirsutum*. *Mol. Plant Microbe Interact.* 17:322-329.
- Moury B, Fabre F & Senoussi R. (2007). Estimation of the number of virus particles transmitted by an insect vector. *Proc. Natl. Acad. Sci. USA* 104:17891-17896.
- Mundt, C.C., Cowger, C., Garrett K.A. (2002). Relevance of integrated disease management to resistance durability. *Euphytica* 124:245-252.
- Ng J.C.K., Falk B.W. (2006). Virus-vector interactions mediating nonpersistent and semipersistent transmission of plant viruses. *Ann. Rev. Phytopath.* 44:183-212.
- Nicolas O., [Dunnington S.W.](#), [Gotow L.F.](#), [Pirone T.P.](#), [Hellmann G.M.](#) (1997). Variations in the VPg protein allow a potyvirus to overcome *va* gene resistance in tobacco. *Virology* 237:452-459.
- Padgett, H. S., Watanabe, Y., Beachy, R. N. 1997 Identification of the TMV replicase sequence that activates the N gene-mediated hypersensitive response. *Mol. Plant Microbe Interact.* 10:709–715.
- [Palloix](#), A., Daubèze, A.M., Lefebvre, V., Caranta, C., Moury, B., Pflieger, S., Gebre-Selassie, K., Marchoux, G. (1997). Construction de systèmes de résistance aux maladies adaptés aux conditions de culture chez le piment. *C.R. Acad. Agric. Fr.* 83:87–98.
- Perring, T.M., Gruenhagen, N.M., Farrar, C.A. (1999). Management of plant virus diseases through chemical control of insect vectors. *Ann. Rev. Entomol.* 44:457-481.
- Perry, K. L., Zhang, L., and Palukaitis, P. (1998). Amino acid changes in the coat protein of cucumber mosaic virus differentially affect transmission by the aphids *Myzus persicae* and *Aphis gossypii*. *Virology* 242:204–210.
- Pierrugues O., [Guilbaud L.](#), [Fernandez-Delmond I.](#), [Fabre F.](#), [Tepfer M.](#), [Jacquemond M.](#) (2007). Biological properties and relative fitness of inter-subgroup cucumber mosaic virus RNA 3 recombinants produced *in vitro*. *J. Gen. Virol.* 88:2852-2861.
- Pink, D.A.C. (2002). Strategies using genes for non durable disease resistance. *Euphytica* 124:227-236.
- Qiu, W., Moyer, J.W. (1999). Tomato spotted wilt tospovirus adapts to the TSWV N gene-derived resistance by genome reassortment. *Phytopathology* 89:575-582.
- Raberg L., [Sim D.](#), [Read A.F.](#) (2007). Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science* 318:812-814.
- Raccah, B. (1986). Nonpersistent viruses: epidemiology and control. *Adv. Vir. Res.* 31:387-429.
- Ren, T., Qu, F., Morris, T.J. (2000). HRT gene function requires interaction between a NAC protein and viral capsid protein to confer resistance to *Turnip crinkle virus*. *Plant Cell* 12:1917–1926.
- Ruesink W.G., Irwin M.E. (1986). Soybean mosaic virus epidemiology: A model and some implications, pp. 295-313. In G. D. McLean, R. G. Garrett and W. G. Ruesink [eds.], *Plant Virus Epidemics: Monitoring, Modelling and Predicting Outbreaks*, Academic Press ed.
- Sacristán, S., Malpica, J. M., Fraile, A., García-Arenal, F. (2003). Estimation of population bottlenecks during systemic movement of *Tobacco mosaic virus* in tobacco plants. *J. Virol.* 77:9906–9911.
- Saito T, Meshi T, Takamatsu N, Okada Y (1987). Coat protein gene sequence of tobacco mosaic virus encodes a host response determinant. *Proc. Natl. Acad. Sci. USA* 84:6074-6077.
- Schirmer A, Link D, Cognat V, Moury B, Beuve M, Meunier A, Bragard C, Gilmer D & Lemaire O. (2005). Phylogenetic analysis of isolates of beet necrotic yellow vein virus collected worldwide. *Journal of General Virology*, 86:2897-2911.
- [Sentandreu V.](#), [Castro J.A.](#), [Ayllon M.A.](#), [Rubio L.](#), [Guerra J.](#), [Gonzalez-Candelas F.](#), Moreno P., [Moya A.](#) (2006). [Evolutionary analysis of genetic variation observed in \*Citrus tristeza virus\* \(CTV\) after host passage.](#) *Arch. Virol.* 151:875-894.

- Seo, Y.-S., Rojas, M.R., Lee, J.-Y., Lee, S.-W., Jeon, J.-S., Ronald, P., Lucas, W. J., Gilbertson R. L. (2006). A viral resistance gene from common bean functions across plant families and is up-regulated in a non-virus-specific manner. *Proc. Natl. Acad. Sci. USA* 103:11856-1186.
- Sigvald, R. (1986). Forecasting the incidence of Potato virus Y, pp. 419-441. In Eds., G. D. McLean, R. G. Garret and W. G. Ruesink [eds.], *Plant Virus Epidemics - Monitoring, Modelling and Predicting Outbreaks*, Academic Press.
- Sorho F., [Pinel A.](#), [Traore O.](#), [Bersoult A.](#), [Ghesquiere A.](#), [Hébrard E.](#), [Konate G.](#), [Sere Y.](#), [Fargette D.](#) (2005). Durability of natural and transgenic resistances in rice to Rice yellow mottle virus. *Eur. J. Plant Pathol.* 112:349-359.
- Takahashi H., [Suzuki M.](#), [Natsuaki K.](#), [Shigyo T.](#), [Hino K.](#), [Teraoka T.](#), [Hosokawa D.](#), [Ehara Y.](#) (2001). Mapping the virus and host genes involved in the resistance response in cucumber mosaic virus-infected *Arabidopsis thaliana*. *Plant and Cell Physiology* 42:340-347.
- Weber, H., Schultze, S. & Pfitzner, A. J. 1993 Two amino acid substitutions in the tomato mosaic virus 30-kilodalton movement protein confer the ability to overcome the *Tm-2<sup>2</sup>* resistance gene in the tomato. *J. Virol.* 67:6432–6438.
- Zhu Y.Y., Chen H.R., Fan J.H., Yang S.S., Hu L.P., Leung H., Mew T.W., Teng P.S., Wang Z.H., Mundt C.C. (2000). Genetic diversity and disease control in rice. *Nature* 406:718-722.

**Table 1:** Viral genes determining virulence or avirulence towards resistance genes or alleles in plants.

Plant and resistance allele	Virus	Avirulence gene	Reference
<b>Dominant genes</b>			
	<b><i>Benyvirus</i></b>		
Sugarbeet <i>Rz-1</i>	<i>Beet necrotic yellow vein virus</i>	P25 protein	Chiba <i>et al.</i> (2008)
	<b><i>Carmovirus</i></b>		
<i>Arabidopsis thaliana</i> HRT	<i>Turnip crinckle virus</i>	Capsid	Ren <i>et al.</i> (2000)
	<b><i>Cucumovirus</i></b>		
<i>Arabidopsis thaliana</i> RCY1	<i>Cucumber mosaic virus</i> (CMV)	Capsid	Takahashi <i>et al.</i> (2001)
Cowpea <i>Cry</i>	CMV	RNA-dependent RNA polymerase (2a protein)	Kim and Palukaitis (1997)
Common bean <i>RT4-4</i>	CMV	RNA-dependent RNA polymerase (2a protein)	Seo <i>et al.</i> (2006)
	<b><i>Potexvirus</i></b>		
Potato <i>Nb</i>	<i>Potato virus X</i> (PVX)	Movement protein (25 kDa)	Malcuit <i>et al.</i> (1999)
Potato <i>Nx</i>	PVX	Capsid	Kavanagh <i>et al.</i> (1992)
Potato <i>Rx1</i>	PVX	Capsid	Goulden <i>et al.</i> (1993)
Potato <i>Rx2</i>	PVX	Capsid	Bendahmane <i>et al.</i> (2000)
	<b><i>Potyvirus</i></b>		
Oilseed rape <i>TurB01</i>	<i>Turnip mosaic virus</i> (TuMV)	Helicase (protéine CI)	Jenner <i>et al.</i> (2000)
Oilseed rape <i>TurB03</i>	TuMV	P3 protein	Jefferies <i>et al.</i> (2003)
Oilseed rape <i>TurB04</i>	TuMV	P3 protein	Jenner <i>et al.</i> (2002b)
Oilseed rape <i>TurB05</i>	TuMV	Helicase (CI protein)	Jenner <i>et al.</i> (2002b)
Zucchini <i>Zym</i>	<i>Zucchini yellow mosaic virus</i>	P3 protein	Desbiez <i>et al.</i> (2003)
Potato <i>Ry</i>	PVY	NIa protease	Mestre <i>et al.</i> (2000)
Soybean <i>Rsv-1</i>	<i>Soybean mosaic virus</i>	HcPro and P3 proteins	Eggenberger <i>et al.</i> (2008); Hajimorad <i>et</i>

			<i>al.</i> (2008).
	<b><i>Tobamovirus</i></b>		
Pepper <i>L</i> <sup>2</sup>	<i>Paprika mild mottle virus</i> (PaMMV) / <i>Pepper mild mottle virus</i> (PMMoV)	Capsid	Gilardi <i>et al.</i> (2004)
Pepper <i>L</i> <sup>3</sup>	PMMoV	Capsid	Berzal-Herranz <i>et al.</i> (1995)
Pepper <i>L</i> <sup>4</sup>	PMMoV	Capsid	Genda <i>et al.</i> (2007)
Tobacco <i>N</i>	<i>Tobacco mosaic virus</i> (TMV) / <i>Obuda pepper virus</i>	Helicase	Padgett <i>et al.</i> (1997)
Tobacco <i>N'</i>	TMV	Capsid	Saito <i>et al.</i> (1987)
Tomato <i>Tm-1</i>	TMV	RNA-dependent RNA polymerase	Meshi <i>et al.</i> (1988)
Tomato <i>Tm-2</i>	TMV	Movement protein	Meshi <i>et al.</i> (1989)
Tomato <i>Tm-2</i> <sup>2</sup>	<i>Tomato mosaic virus</i>	Movement protein	Weber <i>et al.</i> (1993)
	<b><i>Tospovirus</i></b>		
Pepper <i>Tsw</i>	<i>Tomato spotted wilt virus</i>	NSs protein	Margaria <i>et al.</i> (2007)
<b>Recessive genes</b>			
	<b><i>Bymovirus</i></b>		
Barley <i>rym-4</i>	<i>Barley yellow mosaic virus</i>	Viral protein genome-linked (VPg)	Kühne <i>et al.</i> (2003)
	<b><i>Potyvirus</i></b>		
Pepper <i>pvr2</i> <sup>1</sup>	<i>Potato virus Y</i> (PVY)	NIa viral protein genome-linked (VPg)	Moury <i>et al.</i> (2004)
Pepper <i>pvr2</i> <sup>2</sup>	PVY	NIa viral protein genome-linked (VPg)	Moury <i>et al.</i> (2004)
Pea <i>sbm-1</i>	<i>Pea seed-borne mosaic virus</i> (PSbMV)	NIa viral protein genome-linked (VPg)	Keller <i>et al.</i> (1998)
Pea <i>sbm-2</i>	PSbMV	P3 protein	Johansen <i>et al.</i> (2001)

Tobacco <i>va</i>	<i>Tobacco vein mottling virus</i>	N1a viral protein genome-linked (VPg)	Nicolas <i>et al.</i> (1997)
Tomato <i>pot-1</i>	PVY	N1a viral protein genome-linked (VPg)	Moury <i>et al.</i> (2004)
Bean <i>wlv</i>	<i>Bean yellow mosaic virus</i>	N1a viral protein genome-linked (VPg)	Bruun-Rasmussen <i>et al.</i> (2007)
	<b><i>Sobemovirus</i></b>		
Rice <i>rymv-1</i>	<i>Rice yellow mottle virus</i>	Viral protein genome-linked (VPg)	Hébrard <i>et al.</i> (2006)
	<b><i>Tombusvirus</i></b>		
Melon <i>nsv</i>	<i>Melon necrotic spot virus</i>	3' untranslated region	Díaz <i>et al.</i> (2004)

**Table 2:** Efficiency and selectivity of different virus control methods and their potential actions on evolutionary forces involved in emergence of resistance-breaking isolates (modified after Jones 2006).

Control method	Measure	Selectivity		Initial amount of inoculum		Rate of spread		Action on evolutionary forces involved in R breaking <sup>b</sup>
		Low	High	External	Internal	Early	Late	
Host resistance	to the virus, partial	- <sup>a</sup>	+	-	-	+	-	↓ 1,2,5,6 ↑ 3
	to the virus, complete	-	+	-	-	+	+	
	to the vector, antibiosis	-	+	-	-	+	+	↓ 5,6
	to the vector, antixenosis	-	+	-	-	+	+	↓ 5,6
Host tolerance		+	+	-	-	-	-	↑ 1,2,3,5,6
Chemical	Regular foliar application	+	+	-	-	+	+	↓ 5,6
	At, before or directly after planting	+	+	-	-	+	-	↓ 5,6
	Oils and repellents, regular foliar application	+	-	-	-	+	+	↓ 5,6
Cultural, phytosanitary	Hygiene	+	-	+	+	+	-	↓ 5,6
	Roguing	+	+	-	+	+	-	↓ 1,2,5,6
	Healthy propagules	+	+	-	+	+	-	↓ 1,2,5,6
Cultural, agronomic	Isolation, safe planting distances	+	-	+	-	+	-	↓ 5,6
	Plant unwind, non-host barrier, large field size	+	-	+	-	+	-	↓ 5,6

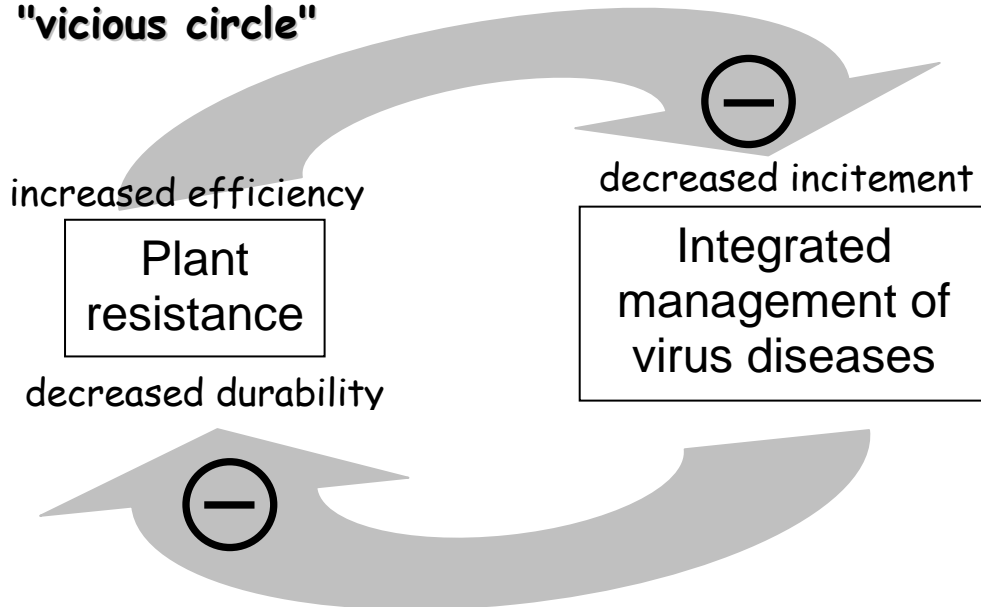
	Mixture with non-host/resistant host	+	-	+	+	+	+	↓ 5,6
	Manipulate sowing date	+	-	-	-	+	-	↓ 6
	Groundcover, reflective surfaces	+	-	-	-	+	-	↓ 5,6
	Early canopy cover, high plant density, narrow row spacing	+	-	-	+	-	+	↓ 5,6
	Early harvest, early maturing cultivar	+	-	-	-	-	+	↓ 1,2,5,6
	Crop and weed free period, crop rotation	+	-	+	+	+	-	↓ 6
Biological	Cross protection	-	+	-	-	+	+	Incompatible
	Biopesticide, predator, parasite or pathogen	+	+	-	-	-	+	↓ 5,6
Legislation	Quarantine	+	-	+	-	+	-	↓ 5,6

<sup>a</sup> “+” indicates existence and “-“ absence of control measures corresponding to each action.

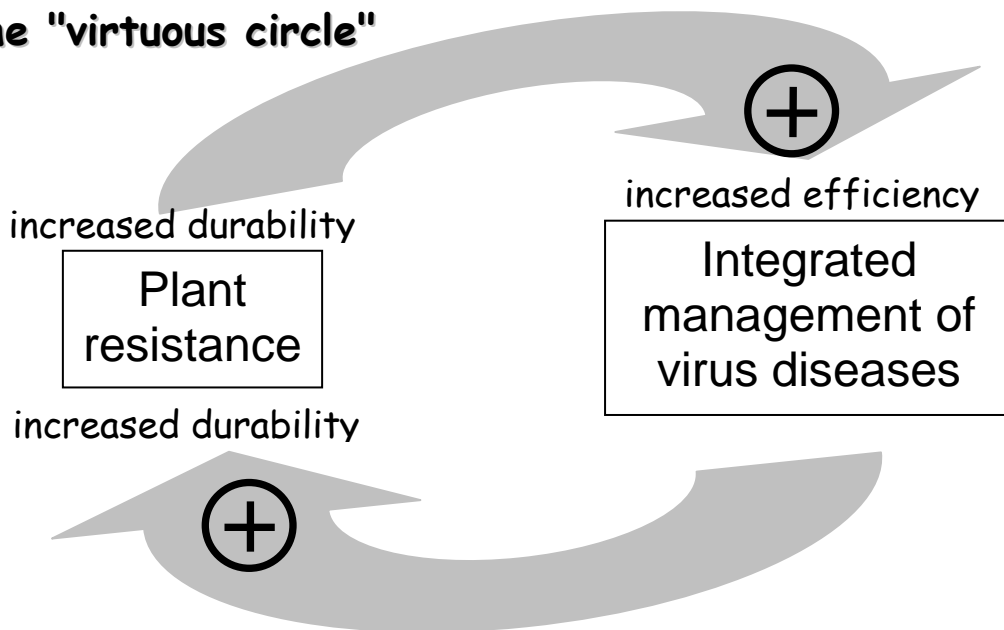
<sup>b</sup> Only direct effects are mentioned. Additional secondary effects probably also exist. For example, slowing down the rate of virus spread will also decrease the census size of virus populations. “↓” indicates a reduction of the risk for resistance durability associated to the indicated evolutionary forces (Fig. 2). “↑” indicates an increase of the risk for resistance durability associated to the indicated evolutionary forces.

**Figure 1:** Two possible relationships between the durability of plant resistance to pathogens and integrated management of diseases.

### The "vicious circle"



### The "virtuous circle"



**Figure 2:** The biological scenario of breakdown of plant resistances to viruses, evolutionary forces involved and potential effects of additional control methods.

