COMPARATIVE PLANT VIROLOGY

SECOND EDITION

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CHAPTER

14

Conventional Control

A range of control measures are used to mitigate the considerable losses that plant viruses cause to crops.

OUTLINE

I. INTRODUCTION

The use of fungicidal chemicals to protect crop plants from infection or minimise invasion is an important method for the control of many fungal diseases. No such direct method for the control of virus diseases is yet available. Most of the procedures that can be used effectively involve measures designed to reduce sources of infection inside and outside the crop, to limit spread by vectors, and to minimise the effect of infection on yield. Generally speaking, such measures offer no permanent solution to a virus disease problem in a particular area. Control of virus disease is usually a running battle in which organisation of control procedures, care by individual growers, and cooperation among them is necessary year after year. The few exceptions are where a source of resistance to a particular virus has been found in, or successfully incorporated into, an agriculturally useful cultivar. This is becoming of increasing importance with the development of transgenic protection of plants against viruses that are discussed in Chapter 15. Even with conventional and transgenic resistance, protection may not be permanent when new strains of the virus arise that can cause disease in a previously resistant cultivar.

Correct identification of the virus or viruses infecting a particular crop is essential for effective control measures to be applied. Of major importance in designing a strategy for controlling a virus in a specific crop is an understanding of the epidemiology of that virus that was discussed in Chapter 13. This enables disease outbreaks to be forecasted (Box 14.1).

The three major approaches to conventional control of plant viruses are the removal or avoidance of sources of infection, protecting plants from systemic infection, and deployment of resistance.

BOX 14.1

DISEASE FORECASTING

An understanding of the epidemiology and ecology of some major crop virus diseases has led to procedures for forecasting potential epidemics. This is very useful in implementing control measures. There are two main approaches to forecasting: monitoring the progress of a disease and developing mathematical models.

Monitoring Virus Disease Progress

Many large-scale farmers routinely monitor their crops and apply control measures at an appropriate time. However, as virus diseases take several days or even weeks to show symptoms after infection, the application of control measures based on symptom appearance can be too late. It also depends on the correct diagnosis of the disease and knowledge of how it is spread.

Mathematical Modelling

There are an increasing number of mathematical models directed at forecasting the outcome of the spread of a disease into an agronomic situation. Basically, the two types of models are prediction models, to predict a possible epidemic, and simulation models, to understand the factors that give rise to and control a given situation. A model is developed to answer specific questions, and there is no general model to predict the potential and outcome of all potential viral epidemics. In developing a model, as many factors as can be predicted are taken into account. These include, knowledge on the virus, its vector, virus-vector interaction, type of crop, the cropping system, and various environmental factors that can impact on these biological factors. A good model enables one to make strategic management decisions on whether the problem is going to be significant and, if so, when and how to deal with it.

The efficiency of prediction from even a good model is only limited by the amount and reliability of the data fed into it. The data must be obtained from various sources and collated. An example is a model for predicting virus yellows disease of sugar beet in the United Kingdom that is based on the preceding winter weather (especially the number of frost days) and the dates when the aphid vectors begin their spring migration and region in which the beet is being grown (eastern, western, and northern regions). This model has been refined to allow for the numbers of migrating *Myzus persicae*, the major vector.

II. AVOIDING INFECTION

A. Removal of Sources of Infection

It is obvious that there will not be a virus problem if the crop is free of virus when planted and when there is no source of infection near enough to allow it to spread into the crop. Sources of infection are discussed in Chapter 13.

To eliminate these sources, it may be worthwhile to remove infected plants (rogue) from a crop. If the spread is occurring rapidly from sources outside the crop, roguing the crop will have no beneficial effect. In certain situations, roguing may increase disease incidence by disturbing vectors on infected plants. In many crops, newly infected plants may be acting as sources of virus for further vector infection before they show visible signs of disease.

Most of the successful eradication schemes have been on tree crops. The following are among the factors that dictate success:

- Relatively small numbers of infected trees and infection foci
- Low rate of natural spread
- Good data on extent and distribution of infection
- Rapid, reliable, and inexpensive diagnostic procedure for the virus and resources for rapid and extensive surveys and tree removal.

Two examples of roguing and eradication schemes are given in Box 14.2.

B. Virus-Free Seed

Where a virus is transmitted through the seed, such transmission may be an important source of infection, since it introduces the virus into the crop at a very early stage, allowing infection to be spread to other plants while they are still young. In addition, seed transmission introduces scattered foci of infection throughout the crop. Where seed infection is the main or only source of virus, and where the crop can be grown in reasonable isolation from outside sources of infection, virus-free seed may provide a very effective means for control of a disease.

Lettuce mosaic virus is a good example of controlling a virus problem through clean seed. Crops grown from virus-free seed in California had a much lower percentage of mosaic at harvest than adjacent plots grown from standard commercial seed. To obtain effective control by the use of virus-free or low-virus seed, a certification scheme is necessary, with seed plants being grown in appropriate isolation.

C. Virus-Free Vegetative Stocks

For many vegetatively propagated plants, the main source of virus is chronic infection in the plant itself. With such crops, one of the most successful forms of control has involved the development of virus-free clones—that is, clones free of the particular virus under consideration. Two problems are involved. First, a virus-free line of the desired variety with good horticultural characteristics must be found. When the variety is 100 percent infected, attempts must be made to free a plant or part of a plant from the virus. Second, having obtained a virus-free clone, a foundation stock or "mother" line must be maintained virus free, while other material is grown up on a sufficiently large scale under conditions where reinfection with the virus is minimal or does not take place. These stocks are checked that they are "virus free" (e.g., below a set level of detected virus) and are then used for commercial planting.

As a plant is usually infected with a virus for life, various techniques have to be used to free them of virus, including heat therapy in which the plant is kept at a temperature usually in the range of 35 to 40°C for periods of weeks, meristem tip culture, taking advantage of the fact

BOX 14.2

ROGUING AND ERADICATION CONTROL OF PLANT VIRUSES

Banana Bunchy Top Virus (BBTV)

One of the most successful examples of disease control by roguing of infected crop plants has been the reduction in incidence of BBTV in bananas in eastern Australia. Legislation to enforce destruction of diseased plants and abandoned plantations was enacted in the late 1920s. Within about 10 years, the campaign was effective to the point where bunchy top disease was no longer a limiting factor in production. The success of the scheme was attributed to the absence of virus reservoirs other than bananas, together with a small number of wild bananas; knowledge that the primary source of the virus was planting material and that spread was by aphids; cultivation of the crop in small, discrete plantations rather than as a scattered subsistence crop; strict enforcement of strong government legislation; and the cooperation of most farmers.

Cocoa Swollen Shoot Virus (CSSV)

Cocoa swollen shoot disease (CSSD) is caused by CSSV. The disease was discovered in Ghana in 1936 and is one of the most devastating scourges of cocoa, in the 1940s threatening to wipe out the cocoa industry in what is now Ghana. A massive nationwide eradication campaign began in 1946 after it had been shown that the swollen shoot and dieback disease was caused by a virus that is spread from tree to tree by several species of mealybugs (*Pseudococcidae*). The eradication campaign has continued to the present time, but there have been serious interruptions and discontinuities. Thus, financial resources and personnel who could have otherwise been used to improve the standard of husbandry, raise cocoa production, and make improvements of other crops in the agricultural sector have been diverted into eradicating the disease by cutting out diseased and at times neighbouring "contact" trees. By the 1980s, more than 190 million trees had been removed, but despite massive expenditure, swollen shoot was more prevalent in Ghana than ever before. Among the main problems were the financial and logistic problems in mounting and sustaining such a large and complex eradication programme, lack of cooperation from farmers who were reluctant to lose several years' production, and lack of detailed epidemiological information. Since then the main approach to control is by trying to find and deploy resistance to the virus.

that most viruses do not invade the plant meristem (see Chapter 9), and chemotherapy, by treating select plants with antiviral compounds such as an analogue of guanosine (ribavirin, also called virazole) in combination with *in vitro* tissue culture. Such techniques are only used on elite material of high-cost crops such as soft fruit and flowers. It is very important to include long-term virus testing into the programme for producing virus-free mother plants and also to maintain the nuclear stock in a virus-free environment.

D. Modified Agronomic Practices

Virus infection can be reduced by modifying agronomic practices such as breaking the infection where one major susceptible annual crop or group of related crops is grown in an area and where these are the main hosts for a virus in that area by ensuring that there is a period when none of the crop is grown. A good example of this is the control of planting date of the winter wheat crops in Alberta to avoid overlap with the previous spring- or winter-sown crop (Figure 14.1). This procedure, together with elimination of volunteer wheat and barley plants and grass hosts of *Wheat streak mosaic virus* before the new winter crop emerges, can give good control in most seasons.

Other approaches include changing planting dates to avoid young plants being exposed to major migrations of the insect vector and using close-spaced planting to reduce the attractiveness to flying aphids.

E. Quarantine Regulations

Most agriculturally advanced countries have regulations controlling the entry of plant material to prevent the introduction of diseases and pests not already present. Many countries now have regulations aimed at excluding specific viruses and their vectors, sometimes from specific countries or areas. The setting up of quarantine regulations and providing effective means for administering them is a complex problem. Economic and political factors frequently have to be considered. Quarantine measures may be well worthwhile with certain viruses, such as those transmitted through seed, or in dormant vegetative parts such as fruit trees and bud wood.

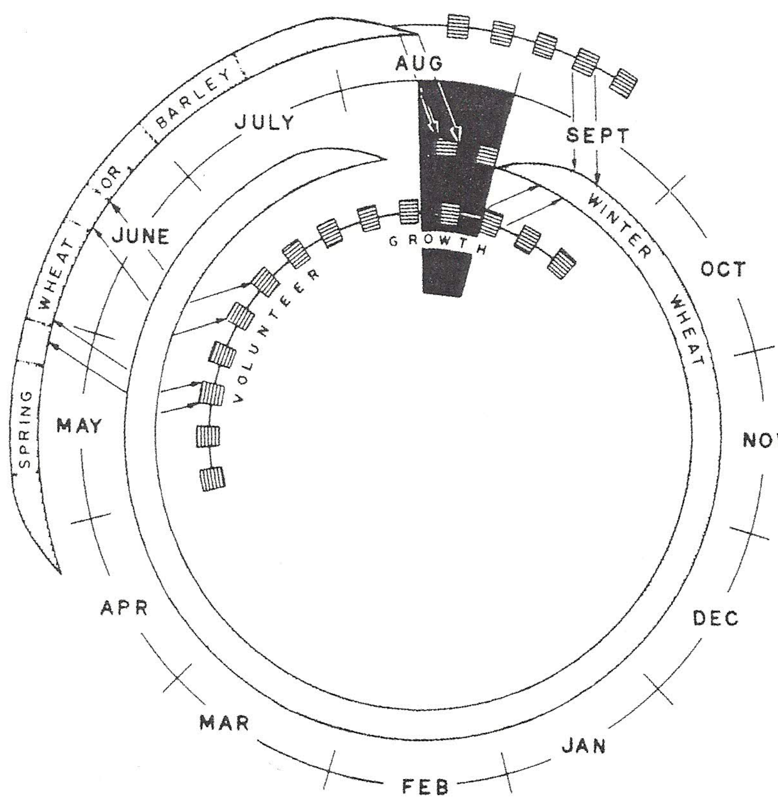

FIGURE 14.1 Wheat streak mosaic disease cycle. Preventing the infection of winter wheat in the autumn is the key to controlling this disease in southern Alberta. Dark area period during which effective control can normally be achieved; broken hatched bands, problems presented by volunteer seedlings, early-seeded winter wheat, and/or late-maturing spring wheat or barley; arrows, transfer of virus by windblown mites. (Diagram courtesy of T.G. Atkinson.)

The value of quarantine regulations will depend to a significant degree on the previous history of plant movements in a region. For example, active exchanges of ornamental plants between the countries of Europe have been going on for a long period, leading to an already fairly uniform geographical distribution of viruses infecting this type of plant. On the other hand, the European Plant Protection Organisation found it worthwhile to set up quarantine regulations against fruit tree viruses not already recorded in Europe.

In spite of many countries having regulations designed to prevent the entry of damaging viruses, they can spread internationally very rapidly. A good example is the rhizomania disease of sugar beet, shown in Figure 13.7.

III. STOPPING THE VECTOR

As described in Chapter 12, plant viruses are usually transmitted by arthropod vectors, but some are transmitted by fungal vectors, and others, particularly *Tobacco mosaic virus* (TMV) and *Tomato mosaic virus* (ToMV), may be transmitted mechanically (by "human vectors"). Once TMV or ToMV enters a crop like tobacco or tomato, it is very difficult to prevent its spread during cultivation and particularly during such processes as tying-up of plants. Control measures consist of treatment of implements and washing of the hands. Workers' clothing may become heavily contaminated with TMV and thus spread the virus by contact.

A. Air-Borne Vectors

Before control of virus spread by air-borne vectors can be attempted, it is necessary to identify the vector. This information has sometimes been difficult to obtain. Not uncommonly, it is an occasional visitor rather than a regular coloniser that is the main or even the only vector of a virus. Furthermore, some aphid species are more efficient vectors than others. For instance, the brown citrus aphid (*Toxoptera citricida*) is a much more efficient vector of *Citrus tristeza virus* (CTV) than is the melon aphid (*Aphis gossypii*; see Figure 14.2).

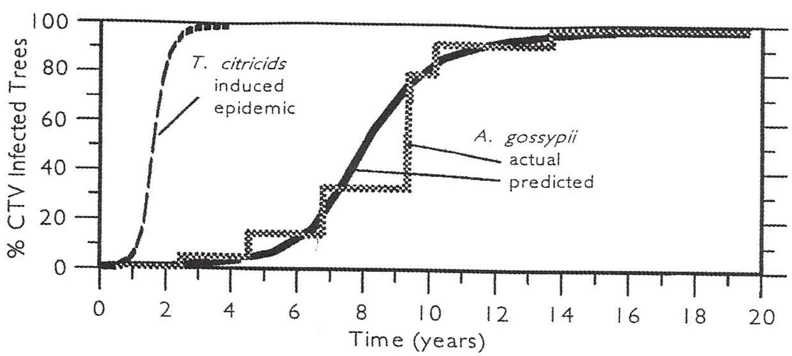

FIGURE 14.2 Comparative increase of *Citrus tristeza virus* infection in field situations when vectored by the brown citrus aphid (*Toxoptera citricida*), an efficient vector, and the melon aphid (*Aphis gossypii*), a less-efficient vector. Data for the brown citrus aphid were taken from test plots in Costa Rica and the Dominican Republic. Data for the melon aphid were taken from surveys and experimental plots in Spain, Florida, and California. Initial infection levels were less than 1%. Note "stairstep" progression in infection with the melon aphid, which is believed to correspond to periodic heavy aphid migrations. [From Garnsey *et al.* (1998; in *Plant virus disease control*, A. Hadidi, R.K. Khetarpal, and H. Koganezawa, Eds., pp. 639–658, APS Press, St. Paul, MN).]

1. *Insecticides*

The application of insecticides is currently one of the main ways of controlling insect pests of plants. To prevent an insect from causing direct damage to a crop, it is necessary only to reduce the population below a damaging level. Control of insect vectors to prevent infection by viruses is a much more difficult problem, as relatively few winged individuals may cause substantial spread of virus. Contact insecticides would be expected to be of little use unless they were applied very frequently. Persistent insecticides, especially those that move systemically through the plant, offer more hope for virus control. Viruses are often brought into crops by winged aphids, and these may infect a plant during their first feeding, before any insecticide can kill them. When the virus is nonpersistent, the incoming aphid, when feeding rapidly, loses infectivity anyway, so killing it with insecticide will not make much difference to infection of the crop from the outside. On the other hand, an aphid bringing in a persistent virus is normally able to infect many plants, so killing it on the first plant will reduce spread.

As far as subsequent spread within the crop is concerned, similar factors should operate. Spread of a virus that is nonpersistent should not be reduced as much by insecticide treatment as a persistent virus where the insect requires a fairly long feed on an infected plant. Thus, spread of the persistent *Potato leaf roll virus* (PLRV) in potato crops was substantially reduced by appropriate application of insecticides, but spread of the nonpersistent *Potato virus Y* (PVY) was not.

As discussed in Box 14.1, disease forecasting data can be an important factor in the economic use of insecticides. Sometimes a long-term programme of insecticide use aimed primarily at one group of viruses will help in the control of another virus. Thus, the well-timed use of insecticides in beet crops in England, aimed mainly at reducing or delaying the incidence of yellows diseases (*Beet yellows virus* and *Beet mild yellows virus*), has also been a major factor in the decline in the importance of *Beet mosaic virus* in this crop. A warning scheme to spray against the vectors of beet yellows viruses was initiated in the United Kingdom in 1959 and is based on monitoring populations of aphids in crops from May until early July.

As well as the problems just described, there may be other adverse biological and economic consequences related to the use of insecticides, including development of resistance by the target insect to the insecticide, resurgence of the pest once the insecticide activity has worn off, and possible effects on humans and other animals in the food chain.

2. *Insect Deterrents*

The application of various chemicals or materials can deter aphids from landing on or feeding on crop plants. Spraying mineral oils on plants affects the feeding behaviour of aphids and leafhoppers and can give some protection against nonpersistent viruses. Derivatives prepared from the pheromone (E)-ß-farnesene and related compounds have been shown to interfere with the transmission of PVY by *Myzus persicae* in glasshouse experiments. Laying aluminium strips on the ground between crop rows repels aphids coming into the crop through reflecting UV light.

3. *Agronomic Techniques*

A tall cover crop will sometimes protect an undersown crop from insect-borne viruses. For example, cucurbits are sometimes grown intermixed with maize. It is thought that incoming aphids land on the barrier crops, feed briefly, and either stay there or fly away.

A major approach is the use of crops bred for resistance to insect pests. Sources of resistance have been found against most of the air-borne vector groups. The basis for resistance to the vectors is not always clearly understood, but some factors have been defined. In general terms, there are two kinds of resistance relevant to the control of vectors. First, *nonpreference* involves an adverse effect on vector behaviour, resulting in decreased colonisation, and second, *antibiosis* involves an adverse effect on vector growth, reproduction, and survival after colonisation has occurred. These two factors may not always be readily distinguished. Some specific mechanisms for resistance are sticky material exuded by glandular trichomes, such as those in tomato; heavy leaf pubescence in soybean; A-type hairs on *Solanum betrhaultii* that when ruptured, entrap aphids with their contents and B-type hairs on the same host, which entangle aphids, making them struggle more and so rupture more A-type hairs; inability of the vector to find the phloem in *Agropyron* species; and interference with the ability of the vector to locate the host plant. For example, in cucurbits with silvery leaves, there was a delay of several weeks in the development of 100 percent infection in the field with *Cucumber mosaic virus* (CMV) and *Clover yellow vein virus*. This effect may be due to aphids visiting plants with silvery leaves less frequently because of their different light-reflecting properties.

There may be various limitations on the use of vector-resistant cultivars: Sometimes such resistance provides no protection against viruses. For example, resistance to aphid infestation in cowpea did not provide any protection against *Cowpea aphid-borne mosaic virus*. If a particular virus has several vector species, or if the crop is subject to infection with several viruses, breeding effective resistance against all the possibilities may not be practicable, unless a nonspecific mechanism is used (e.g., tomentose leaves). Perhaps the most serious problem is the potential for new vector biotypes to emerge following widespread cultivation of a resistant cultivar, as may happen following the use of insecticides (Box 14.3).

BOX 14.3

INSECT BIOTYPES OVERCOMING PLANT RESISTANCE

This breakdown of plant resistance to insects is well illustrated by the history of the rice brown planthopper (*Nilaparvata lugens*) (BPH). With the advent of high-yielding rice varieties in Southeast Asia in the 1960s and 1970s, the rice BPH and *Rice grassy stunt virus*, which it transmits, became serious problems. Cultivars containing a dominant gene (*Bph1*) for resistance to the hopper were released about 1974. Within about three years, resistance-breaking populations of the hopper emerged. A new, recessive resistance gene (*bph2*) was exploited in cultivars released between 1975 and 1983. They were grown successfully for a few years until a new hopper biotype emerged that overcame the resistance. A study of the adaptation of three colonies of *N. lugens* to rice cultivars containing different resistance genes showed that the *bhp1* and *bhp3* resistance genes were overcome more readily by colonies that had been exposed for about 10 years to those genes. However, rice cultivar IR64 which contained *bph1* and some minor resistance genes showed a greater durability of resistance than other cultivars. DNA markers to BPH resistance genes are being used in breeding programmes.

B. Soil-Borne Vectors

Most work on the control of viruses transmitted by nematodes and fungi has centred on the use of soil sterilization with chemicals. However, several factors make general and long-term success unlikely:

- Huge volumes of soil may have to be treated.
- A mortality of 99.99 percent still leaves many viable vectors.
- The use of some of the chemicals involved has been banned in certain countries, and such bans are likely to be extended.

In any event, chemical control can be justified economically only for high-return crops or crops that can remain in the ground for many years. However, some recent advances in nematode control procedures may be applicable to the control of viruses that they transmit and may be adaptable to the control of fungus-transmitted viruses.

1. *Nematodes*

There are four basic strategies for nematode control:

1. Exclusion or avoidance usually by quarantine.
2. Reduction of the initial population density by cultural approaches such as use of clean planting stock or crop rotation with a break crop of a species that is not a host for the target nematode, by chemical nematicides, by biological tactics such as introducing biological agents antagonistic to nematodes and organic amendments, or by the use of nematode-resistant crop varieties that will reduce nematode populations.
3. Suppression of nematode reproduction by chemicals, organic amendments, and certain natural and transgenic resistance traits.
4. Restriction of the current or future crop damage by nematode resistance. However, tolerant cultivars will reduce crop damage due to nematode feeding but will not reduce the chances of virus infection.

2. *Fungi*

Fungus-transmitted viruses are important in two agronomic situations: nutrient or aquatic systems, and fields. The major control measures are the use of three types of chemicals: surfactants, heavy metals, and sometimes fungicides for use in nutrient or aquatic systems; soil amendments and fungicides to control the fungal vector in the soil; and soil partial sterilants or disinfectants to reduce the active and resting spore stages of fungal vectors in the field. In general, attempts to control infection with viruses having fungal vectors by application of chemicals to the soil have usually not been successful.

IV. PROTECTING THE PLANT

Even if sources of infection are available and the vectors are active, a third kind of control measure is available: protecting inoculated plants from developing systemic disease. There are essentially three approaches that have been used to protect plants, using a mild strain of the virus (termed cross-protection or mild strain protection), the use of chemicals, and genetic protection (conventional resistance and transgenic resistance).

A. Protection by a Plant Pathogen

Inoculation of plants with either a mild virus strain or with satellite RNA (termed cross-protection) has been used to protect against severe virus strains. The phenomenon of cross-protection is described in Chapter 10. Infection of a plant with a strain of virus causing only mild disease symptoms (the protecting strain; also known as the mild, attenuated, hypovirulent, or avirulent strain) may protect it from infection with severe strains (the challenging strain). Thus, plants might be purposely infected with a mild strain as a protective measure against severe disease.

Although such a procedure could be worthwhile as an expedient in very difficult situations, it is not to be recommended as a general practice for the following reasons:

- So-called mild strains often reduce yield by about 5 to 10 percent.
- The infected crop may act as a reservoir of virus from which other more sensitive species or varieties can become infected.
- The dominant mild strain of virus may change to a more severe type in some plants.
- Serious disease may result from mixed infection when an unrelated virus is introduced into the crop.
- For annual crops, introduction of a mild strain is a labour-intensive procedure.
- The genome of the mild strain may recombine with that of another virus, leading to the production of a new virus.

In spite of these difficulties, the procedure has been used successfully, at least for a time, with some crops. A suitable mild isolate should have the following properties:

- It should induce milder symptoms in all the cultivated hosts than isolates commonly encountered and should not alter the marketable properties of the crop products.
- It should give fully systemic infections and invade most, if not all, tissues.
- It should be genetically stable and not give rise to severe forms.
- It should not be easily disseminated by vectors to limit unintentional spread to other crops.
- It should provide protection against the widest possible range of strains of the challenging virus.
- The protective inoculum should be easy and inexpensive to produce, check for purity, provide to farmers, and apply to the target crops.

Mild protecting strains are produced from naturally occurring variants, from random mutagenesis, or from directed mutagenesis of severe strains. The control of CTV provides the most successful example for the use of cross-protection (Box 14.4).

Satellite viruses and RNAs are described in Chapter 3, and, as far as potential biocontrol agents, fall into three categories: those that enhance the helper virus symptoms, those that have no effect, and those that reduce the helper virus symptoms. The last one has potential as a control agent. Most of the work has focussed on the satellites of CMV with some successes in field application, especially against the necrogenic satCMV (CARNA5) described in Chapter 3.

However, there has been concern over the durability of using satellites as biocontrol agents. There is a wide range of necrogenic and other virulent strains of satCMV. Passage of a benign satellite of CMV through *Nicotiana tabacum* led to the satellite rapidly mutating to a pathogenic form and mutations of a single or a few bases can change a nonnecrogenic variant to a necrogenic one (Figure 14.3). Necrogenic variants of the CMV satellite have a greater virulence than nonnecrogenic variants, but, as they depress the accumulation of the helper virus more than do nonnecrogenic variants, the necrogenic variants are not so efficiently aphid transmitted.

B. Antiviral Chemicals

Considerable effort has gone into a search for inhibitors of virus infection and multiplication that could be used to give direct protection to a crop against virus infection in the way that

BOX 14.4

CONTROL OF *CITRUS TRISTEZA VIRUS* BY CROSS-PROTECTION

Worldwide, *Citrus tristeza virus* (CTV) is the most important virus in citrus orchards. In the 1920s, after its introduction to South America from South Africa, the virus virtually destroyed the citrus industry in many parts of Argentina, Brazil, and Uruguay. The application of cross-protection by inoculation with mild CTV isolates in Brazil proved to be successful particularly with Pera oranges, with more than 8 million trees being planted in Brazil by 1980. Protection continues in most individual plants through successive clonal generations. However, in an eight-year assessment of the ability of four mild isolates to suppress severe CTV isolates in Valencia sweet orange on sour orange rootstock in Florida, about 75 percent of the mild-strain protected trees had severe symptoms compared with about 85 percent of the unprotected trees. The use of the same isolates gave better protection of Ruby Red grapefruit on sour orange rootstock. Thus, there are differences in the responses of the scion:rootstock combination, but it is also important to have a compatible mild strain. The search for improved attenuated strains of the virus continues, and the technique is being adopted in other countries.

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Y Sat-RNA(a)        CUAAGGCUUAUGCUAUGCUGAUCUCCGUGAAUGUCUAUACAUUCCUCUACAGGACCC   necrogenic
                                ⇓                                                   ⇓
                                U                                              ameliorative

Y Sat-RNA(b)        CUAAGGCUUAUGCUAUGCUGAUCUCCGUGAAUGUCUAUACAUUCCUCUACAGGACCC   necrogenic
                         ⇓    ⇓ ⇓                                                   ⇓
                         A    G U                                              ameliorative

Wl1 Sat-RNA(c)      CUUAGACUUAGGUUAUGCUGAUCUCCGUGAAUGUCUACACAUUCCUCUACAGGACCC   ameliorative
                         ⇓    ⇓ ⇓                                                   ⇓
                         G    U C                                               necrogenic

R Sat-RNA(d)        CUAAGGCUUAUGCUACGCUGAUCUCCGUGAAUGUCUA.UCAUUCCUC.ACAGGACCC   ameliorative
                                   ⇓                                                ⇓
                                   U                                            necrogenic
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FIGURE 14.3 Alignment of the 55 3′-terminal residues of the CMV satellite RNA variants mutated from a necrogenic form towards a nonnecrogenic one or vice versa. The arrows indicate the positions found to be determinant for necrogenicity. [From Jacquemond and Tepfer (1998; in *Plant virus disease control,* A. Hadidi, R.K. Khetarpal, and H. Koganezawa, Eds., pp. 94–120, APS Press, St. Paul, MN).]

fungicides protect against fungi. There has been no successful control on a commercial scale by the application of antiviral chemicals due to these major difficulties:

- An effective compound must inhibit virus infection and multiplication without damaging the plant. This is a major problem, as virus replication is so intimately bound up with cell processes and any compound blocking virus replication is likely to have damaging effects on the host.
- An effective antiviral compound would need to move systemically through the plant if it is to prevent virus infection by invertebrate vectors.
- A compound acting systemically would need to retain its activity for a reasonable period. Frequent protective treatments would be impracticable. Many compounds that have some antiviral activity are inactivated in the plant after a time.
- For most crops and viruses, the compound would need to be able to be produced on a large scale at an economic price. This might not apply to certain relatively small-scale, high-value crops, such as greenhouse orchids.
- For use with many crops, the compound would have to pass food and drug regulations. Many of the compounds that have been used experimentally would not be approved under such regulations.

Because of these difficulties, there are only a few cases of the use of chemicals to produce virus-free stock plants.

V. CONVENTIONAL RESISTANCE TO PLANT VIRUSES

A. Introduction

When one considers the advantages and disadvantages of the control measures just described, it is obvious that the use of crop plants that are resistant to viruses is likely to be the most promising approach. Thus, for many years plant breeders have been attempting to produce virus-resistant varieties. There are two sources of resistance gene: natural ones from sexually compatible species and nonconventional ones introduced by genetic modification; the latter is discussed in Chapter 15.

There are three types of genes that plant breeders consider for control of plant viruses: those conferring immunity, those conferring field resistance, and those conferring tolerance (Box 14.5). Some molecular aspects of these virus:plant interactions are discussed in Chapter 10.

The following points concerning the effects of host genes on the plant's response to infection emerge from many different studies:

- Both dominant and recessive Mendelian genes may have effects. However, while most genes known to affect host responses are inherited in a Mendelian manner, cytoplasmically transferred factors may sometimes be involved.
- There may or may not be a gene dose effect.
- Genes at different loci may have similar effects.
- The genetic background of the host may affect the activity of a resistance gene.
- Genes may have their effect with all strains of a virus or with only some.
- Some genes influence the response to more than one virus.
- Plant age and environmental conditions may interact strongly with host genotype to produce the final response.
- Route of infection may affect the host response. Systemic necrosis may develop following introduction of a virus by grafting into a high-resistant host that does not allow systemic spread of the same virus following mechanical inoculation.
- Resistance originally thought to be to the virus may be really to the vector.

BOX 14.5

TYPES OF RESISTANCE TO A PLANT VIRUS

There are three main types of resistance and immunity to a particular virus:

1. *Immunity* involves every individual of the species; little is known about the basis for immunity, but it is related to the question of the host range of viruses discussed in Chapter 2.
2. *Cultivar resistance* describes the situation where one or more cultivars or breeding lines within a species show resistance, whereas others do not.
3. *Acquired or induced resistance* is present where resistance is conferred on otherwise susceptible individual plants following inoculation with a virus.

Some authors have considered that immunity and cultivar resistance are based on quite different underlying mechanisms. However, studies with a bacterial pathogen in which only one pathogen gene was used show that for this class of pathogen at least the two phenomena have the same basis.

B. Genetics of Resistance to Viruses

Resistance to viruses in many crop virus combinations is controlled by a single dominant gene (Table 14.1). However, this may merely reflect the fact that most resistant cultivars were developed in breeding programmes aimed at the introduction of a single resistance gene. Furthermore, incomplete dominance may be a reflection of gene dosage or be due to environmental factors. Some specific examples of dominant and recessive genes for resistance were shown in Table 10.2. The current reports about the mechanisms of resistance by either total immunity or hypersensitive response were also discussed in Chapter 10.

In several plant species, the resistance virus resistance genes are clustered to specific loci on the chromosomes; in this, virus resistance resembles that for fungi. For instance, in *Pisum sativum*, the resistances to the lentil strain of *Pea seed-borne mosaic virus, Bean yellow mosaic virus, Watermelon mosaic virus-2, Clitoria yellow vein virus*, and *Bean common mosaic virus* NL-8 strain (all potyviruses) are controlled by tightly linked recessive genes on chromosome 2.

TABLE 14.1 Summary of Number of Virus Resistance Genes Reported.

Resistance Gene	Monogenic	Oligo- or Polygenic
Dominant	81	10
Recessive	43	20
Incompletely dominant	15	6
(Nature unknown)	—	4
Total number of resistance genes	139	40

From Khetarpal *et al.* (1998; in *Plant virus disease control*, A. Hadidi, R.K. Khetarpal, and H. Koganezawa, Eds., pp. 14–32, APS Press, St. Paul, MN).

C. Tolerance

The classic example of genetically controlled tolerance is the Ambalema tobacco variety. TMV infects and multiplies through the plant, but in the field, infected plants remain almost normal in appearance. This tolerance is due to a pair of independently segregating recessive genes, r_{m1} and r_{m2}, and perhaps to others, as well with minor effects. On the other hand, tolerance to *Barley yellow dwarf virus* is controlled by a single dominant gene in barley, with different alleles giving different degrees of tolerance.

D. Use of Conventional Resistance for Control

A review of the consideration in a breeding programme for resistance to an important virus—that causing rhizomania of sugar beet—is given in Scholten and Lange (2000). Many of the aspects that they discuss are applicable to breeding programmes for resistance to other viruses. In this section, we examine the application of conventional resistance to the control of viruses.

1. *Immunity*

Although many searches have been made, true immunity against viruses and viroids, which can be incorporated into useful crop cultivars, is a rather uncommon phenomenon.

2. *Field Resistance*

Where suitable genes can be introduced into agriculturally satisfactory cultivars, breeding for resistance to a virus provides one of the best solutions to the problem of virus disease. However, there are two major problems. It has proved difficult to find resistance genes in species that are sexually compatible with the crop species. There have been widespread searches in wild species for such genes, and techniques for wide crosses, such as embryo rescue, have been used. Chemical and radiation mutagenesis of the crop plant has also been used to provide useful resistance

The second problem has been the durability of resistance. How long can the gene be deployed successfully before a resistance breaking (virulent) strain of the virus emerges? Of 87 host-virus combinations for which resistance genes have been found, more than 75 percent of those tested were overcome by virulent virus isolates. Fewer than 10 percent of the resistance genes have remained effective when tested against a wide range of virus isolates over a long period. However, some of the virulent isolates were found only in laboratory tests rather than field outbreaks.

The costs of a breeding programme must be weighed against the possible gains in crop yield. Many factors are involved, such as the seriousness of the virus disease in relation to other yield-limiting factors; the "quality" of the available resistance genes—for example, resistance genes against CMV are usually "weak" and short-lived, which may be due, at least in part, to the many strains of CMV that exist in the field; the importance of the crop (compare, for instance, a minor ornamental species with a staple food crop such as rice); and crop quality. Good virus resistance that gives increased yields may be accompanied by poorer quality in the product, as happened with some TMV-resistant tobacco cultivars.

The difficulties in finding suitable breeding material are compounded when there are strains of not just one but several viruses to consider. Cowpeas in tropical Africa are infected to a significant extent by at least seven different viruses. In such circumstances, a breeding programme may utilise any form of genetic protection that can be found. Sources of resistance, hypersensitivity, or tolerance have been found for five of the viruses. However, several of these viruses have different strains or isolates that may break resistance to other isolates. There is, of course, the further problem of combining these factors with multiple resistance to fungal and bacterial diseases. For example, genetic resistance to TMV, cyst nematodes, root-knot nematodes, and wildfire from *Nicotiana repanda* has been incorporated into *N. tabacum*.

3. *Tolerance*

Where no source of genetic resistance can be found in the host plant, a search for tolerant varieties or races is sometimes made. However, tolerance is not nearly as satisfactory a solution as genetic resistance for several reasons:

- The infected tolerant plants may act as a reservoir of infection for other hosts. Thus, it is bad practice to grow tolerant and sensitive varieties together under conditions where spread of virus may be rapid.
- Large numbers of virus-infected plants may come into cultivation. The genetic constitution of host or virus may change to give a breakdown in the tolerant reaction.
- The deployment of tolerant varieties removes the incentive to find immunity to the virus until the tolerance breaks down in an "out of sight, out of mind" attitude.
- Virus infection may increase susceptibility to a fungal disease (see Chapter 10).

However, tolerant varieties may yield very much better than standard varieties where virus infection causes severe crop losses and where large reservoirs of virus exist under conditions where they cannot be eradicated. Thus, tolerance has, in fact, been widely used. Cultivars of wheat and oats commonly grown in the midwestern United States have probably been selected for tolerance to BYDV in an incidental manner because of the prevalence of the virus.

VI. STRATEGIES FOR CONTROL

Three kinds of situations are of particular importance: annual crops of staple foods such as grains and sugar beet that are either grown on a large scale or are subsistence crops and that under certain seasonal conditions may be subject to epidemics of viral disease; perennial crops, mainly fruit trees with a big investment in time and land, where spread of a virus disease, such as citrus tristeza or plum pox, may be particularly damaging; and high-value cash crops such as tobacco, tomato, cucurbits, peppers, and a number of ornamental plants that are subject to widespread virus infections.

With almost all crops affected by viruses, an integrated and continuing programme of control measures is necessary to reduce crop losses to acceptable levels. Such programmes will usually need to include elements of all three kinds of control measure just discussed. In developing strategies for the integrated approach, it is essential to have a full understanding of the disease, its epidemiology and ecology, and the pathogen, its genetic makeup and functioning and its potential for variation.

VII. VIRUSES OF OTHER KINGDOMS

Some of the ways of controlling animal viruses, such as avoidance of infected individuals, are the same as those described previously for plant viruses. However, plants do not have an innate immune system, so control by immunisation is not a viable approach. As we will see in Chapter 15, there is an analogous approach in plants to immunisation in that they can be transformed to activate the RNA silencing defence system. Also, they can be transformed to produce antibodies that have been shown to mitigate some viruses.

Although there are examples of genetic resistance to viruses infecting vertebrates, more effort is put into control by immunisation and chemoprophylaxis. There are some examples of breeding virus resistance genes into invertebrates (e.g., shrimps).

VIII. SUMMARY

- There are four basic approaches to controlling plant virus diseases: avoiding infection, stopping the vector, protecting the plant, and breeding for resistance.

- The first two approaches involve agronomic practices such as using clean planting material, changing the planting time, and using insecticides against vectors.
- Insecticides are better at preventing the spread of viruses with a persistent interaction with their vector than those with a nonpersistent interaction.
- Plants can be protected by inoculating them with a mild strain of the virus (cross-protection). This is only viable with high-cost perennial crops.
- Breeding for resistance is considered to be the best approach but has the difficulties of sources of resistance genes in sexually compatible species and the durability of resistance.

Further Reading

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