

## Virus Diseases of *Rubus* and Strategies for Their Control

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### Abstract

Over 30 virus and virus-like diseases infect *Rubus* species. In all instances, the number one priority for virus control is to establish plantings with virus-free material. Many aphid-borne viruses remain uncharacterized, due in part to their limited host range and in part to the limited number of people working on them. Breeding for aphid resistance has been successful in controlling the incidence and spread of aphid-borne viruses, primarily because within a geographic region, a single vector is responsible for transmitting these viruses. There are four nematode-borne viruses in Europe and three in North America that can cause serious diseases in *Rubus* species. Control of nematode-borne viruses was enhanced greatly with the introduction of soil fumigants to reduce nematode populations. However, as some of these chemicals are being removed from the market for environmental reasons, alternative control measures are needed. There are sources of genetic resistance to some of the nematode vectors but the use of these resistant genes has been a low priority in breeding programs. There are two pollen-borne viruses in *Rubus*, *Raspberry bushy dwarf virus* (RBDV) and *Tobacco streak virus*, the latter being of minor importance. RBDV has been controlled through the use of gene *Bu* that confers resistance to D-type isolates of RBDV. RBDV has become a serious problem in all raspberry production areas over the past 10 years due to the planting of newer cultivars that lack RBDV resistance and the presence of a new strain of RBDV that overcomes the resistance conferred by the *Bu* gene. With developments in molecular biology, new sources of virus resistance derived from the pathogens themselves have been developed for a large number of viruses. In *Rubus*, this type of resistance is being developed for RBDV in Scotland and North America and for *Tomato ringspot virus* in North America. With RBDV, we know that approximately 1/3 of the transgenic plants remain virus-free after grafting twice with RBDV-infected scions. These still need to be evaluated for fruit quality, yield and field resistance. At this time, it appears that genetically engineered virus resistance to the important viruses of *Rubus* will be developed, however, whether fruit produced from such plants will be accepted socially remains to be determined.

### INTRODUCTION

Rather than discuss the 30 or more viruses that infect *Rubus* individually, the most logical and efficient way to present the information is to group the viruses based on their vectors. Control methods for viruses transmitted by the same type of vector will be quite similar despite the virus. There are three main means of transmission for viruses that infect *Rubus*, by nematodes, aphids and pollen. Much of the work on the viruses of *Rubus* has been done in Europe and North America. As a result there is little information on the viruses that occur in *Rubus* in other parts of the world. Several of the aphid-borne viruses of *Rubus* have been reported in the southern hemisphere, but not of their vector. It is important that researchers and growers in these areas not become complacent about these viruses since it is quite possible that an aphid endemic to these regions may vector one or more of the viruses. Rubus stunt and black raspberry witches broom are phytoplasmas that are transmitted by leafhoppers (Ellis et al., 1991) and will not be discussed here. The phytoplasma of greatest importance is Rubus stunt which occurs in Europe but has not

been reported from the western hemisphere or Australasia. The vector can be controlled with insecticides and because the pathogen has a long latent period in the vector, frequent sprays are not required. For excellent photographs of the symptoms caused by viruses in *Rubus* readers are referred to previous publications (Converse, 1987; Ellis et al., 1991).

### **APHID-BORNE VIRUSES**

Raspberry mosaic disease is caused by a complex of 2-4 viruses, each of which is transmitted by *Amphorophora* species. In North America, *Black raspberry necrosis virus* (BRNV) and *Rubus yellow net virus* (RYNV), vectored by *A. agathonica*, have been shown to cause the mosaic disease (Stace-Smith, 1956). These viruses cause the most damage in black raspberry (*R. occidentalis*) and only mild symptoms in red raspberry in North America, with few if any symptoms in blackberry. The *Ag1* gene for resistance to *Amphorophora* has been very effective in controlling the aphid-borne viruses in the Pacific Northwest of North America. A new biotype able to reproduce on cultivars containing the *Ag1* gene was first observed in the early 1990s but it has not emerged as a major component of the raspberry aphid populations in the area. In Europe, the disease (also referred to as raspberry veinbanding mosaic disease) is caused by a combination of RYNV and *Raspberry leaf mottle virus* (RLMV) (Jones, 1991). Symptoms of mosaic were intensified when BRNV or BRNV plus *Raspberry leaf spot virus* (RLSV) were combined with the RYNV plus RLMV combination. RLMV and RLSV, as single infections, can cause a severe chlorotic spotting, leaf distortion and stunting of plants of susceptible cultivars. The discrepancy between which viruses cause raspberry mosaic between North America and Europe may be due to environmental factors, severity of isolates used in the studies, or the presence of mixed infections in some virus cultures used in the studies. Also, in Europe there are four biotypes of the vector *A. idaei* that overcome different resistance genes. Until recently, plants with the A10 gene were resistant to *A. idaei*, but there is a new biotype that now survives on plants with this gene.

To differentiate the four viruses that can be involved in the Raspberry Mosaic Complex it is necessary to transmit the viruses to indicator plants by grafting or using aphids. In black raspberry, *Rubus occidentalis*, RYNV shows an uneven growth of the two basal leaflets of the trifoliates, and can show a yellowing of the veins when young plants are inoculated using aphids; the symptoms are less distinctive if transmission is by grafting. Since *A. idaei* does not feed on *R. occidentalis*, the indicator *R. macraei*, which also develops a vein netting with RYNV infection, is used as an indicator for RYNV in Europe. BRNV, RLMV and RLSV all cause a tip necrosis in black raspberry and need to be differentiated on red raspberry cultivars (Converse, 1987; Ellis et al., 1991). RLMV causes symptoms in 'Malling Landmark' but not in 'Norfolk Giant', whereas, RLSV causes symptoms in 'Norfolk Giant' but not in 'Malling Landmark'. BRNV does not cause symptoms in either of these two cultivars. Detection of these four viruses is by leaf grafting or aphid transmission. Recently, primer pairs have been developed to detect RYNV by PCR (Jones et al., 2001).

*Raspberry leaf curl virus* (RLCV), transmitted by *Aphis rubicola*, is found only in North America, primarily east of the Rocky Mountains. Infected plants are severely stunted, produce very little fruit of poor quality and are susceptible to winter damage. Symptoms include distortion, some chlorosis and curling of leaves and very poor fruit set. Raspberries show symptoms of severe dwarfing and curled foliage after the initial year of infection. Detection is by leaf grafting onto wineberry (*R. phoenicolasius*) (Stace-Smith and Converse, 1987).

*Raspberry vein chlorosis virus* (RVCV), transmitted by *A. idaei*, is apparently widespread in Europe. Symptoms include chlorosis of minor veins. The virus appears to be a rhabdovirus based on its morphology and is transmitted in a persistent and propagative manner (Jones et al., 1987). *Cucumber mosaic virus* has also been reported to infect *Rubus* but it is rare and appears to do little damage (Jones, 1987).

### **Control of Aphid-Borne Viruses**

There is no immunity to the raspberry mosaic complex though there are a number of tolerant cultivars. A single dominant gene for resistance to *A. agathonica* has been quite effective in North America, however, recently a new biotype of the aphid arose that feeds on *R. idaeus* cultivars containing the *Ag<sub>1</sub>* resistance gene. Whether this new biotype of *A. agathonica* will become a major component of the raspberry aphid complex remains to be determined. It could drastically change the priority for breeding aphid resistance in the Pacific Northwest. In Europe, four biotypes of *A. idaei* have been characterized and several genes provide resistance to all four biotypes. There appears to be a fifth biotype of *A. idaei* as cultivars with the A10 gene are being colonized by the aphid. The presence of multiple biotypes of the vector aphid will make breeding for resistance more challenging. Cultivars with resistance to aphid colonization do not become infected in the field except under very high levels of disease pressure and then the infection rate is very slow (Stace-Smith, 1984). Aphid resistance is the method of choice for controlling the raspberry mosaic complex. There is resistance to the vector of RLCV but very little work has been done with this resistance and little is known about its inheritance. RVCV immunity is reported but not used so far to develop resistant commercial cultivars.

Breeders, other researchers and growers all need to follow quarantine protocols to prevent the spread of these viruses across natural barriers such as oceans. For growers, use of certified plants of immune or tolerant cultivars, use of aphid resistant cultivars and isolation from infected sources are the best means of reducing the impact of these viruses. Insecticides may reduce the spread within a field but likely will not prevent infection from becoming established in a field since the viruses are transmitted in a semi-persistent manner. Thus, the virus would likely be transmitted before an aphid is killed by the insecticide. As chemicals are removed from the market, control will depend more on breeding for virus or aphid resistance and on cultural practices.

### **NEMATODE-BORNE VIRUSES**

There are four nematode-borne viruses of *Rubus* in Europe, *Arabis mosaic virus* and *Strawberry latent ringspot virus* (SLRSV), vectored by *Xiphinema* species and *Raspberry ringspot virus* (RpRSV) and *Tomato black ring virus* (TBRV), vectored by *Longidorus* species. These viruses do not occur naturally in North America but have been introduced in other crops such as ornamentals and grapevines. In North America, *Tomato ringspot virus* (ToRSV), *Tobacco ringspot virus* (TRSV), and *Cherry rasp leaf virus* (CRLV), all transmitted by *Xiphinema americanum*, are known to infect *Rubus spp.* TRSV has been reported from blackberry and ToRSV from red and black raspberry, CRLV from red raspberry. *Cherry leaf roll virus* (CLRV) has been reported in *Rubus* from Europe and New Zealand. These viruses tend to cause oval patches of very weak or dead plants in fields. Since they are transmitted by nematodes, movement tends to be more rapid along rows than between rows as soil is moved by equipment along rows. Symptoms of infection by these viruses often include a leaf mottling, sometimes a leaf distortion, weakened plants and few small crumbly fruit are produced.

### **Control of Nematode-Borne Viruses**

Preplant site preparation combined with the use of certified planting material is the key to control of nematode-borne viruses. Over the past several decades, soil fumigation prior to planting has been used to control nematodes. This is very effective at eliminating the nematodes from the upper layer of soil but will not kill nematodes that are deep in the soil profile. After replanting the surviving nematodes will move up and down the profile with the water table and encounter roots of the new plants and be able to transmit virus. Thus, soil fumigation is often a short term (3-5 years) control of these viruses. These viruses infect a wide range of broadleaf weeds and can be pollen and seed-borne. Thus, weed control can reduce the spread of these viruses. The nematodes can be moved on equipment within and between fields. Starting field operations in the cleanest fields as far as disease is concerned is always a good idea. Roguing of infected plants, plus 5 or 6

plants on either side of those that show symptoms, followed by spot treatment with nematicide can be useful to treat small areas of infection. Crop rotation with crops that are nonhosts for the virus can be used to eliminate virus from the nematodes. The nematodes that vector viruses rarely reach populations where they do significant damage on their own in raspberry plantings, so getting rid of the virus is all that is really necessary in most cases. For the breeders, growers and researchers, remember to follow those quarantine regulations.

### **POLLEN-BORNE VIRUSES**

*Raspberry bushy dwarf virus* (RBDV) and *Tobacco streak virus* (TSV) are pollen-borne viruses that infect *Rubus*. TSV is of minor importance and will not be discussed further as methods of controlling RBDV will also be effective in controlling TSV. RBDV is the most common virus of *Rubus* worldwide, it infects red raspberry, black raspberries, hybrid berries and blackberries. On its own in plants, it rarely causes a “bushy dwarf” symptom. The initial report of the virus was likely from plants doubly infected with RBDV and BRNV, though at the time, BRNV was not known. RBDV may cause a crumbly fruit symptom with foliar symptoms ranging from none to a bright yellow chlorosis and in a few cultivars there is a stunting of the plants. Not all infected plants show crumbly fruit. In blackberries, such as ‘Marion’, it can cause a severe drupelet abortion with very few drupelets produced in some plants. As in red raspberry there may or may not be chlorosis and the degree of drupelet abortion varies by cultivar. Yields can be reduced by 40% even though fruit number is not affected (Strik and Martin, 2002). In black raspberry, RBDV does not cause significant fruit losses or symptoms but can reduce cane number and vigor. The poor fruit set is not due to pollen abortion but rather to drupelet abortion that leads to the crumbly fruit symptom.

RBDV can spread through raspberries quite rapidly, for example, fields of ‘Meeker’ red raspberry can become 100% infected in 5-6 years (Martin, 1998). There appears to be an environmental component that contributes to the rate of spread of RBDV since in some areas the spread of RBDV in ‘Meeker’ can be quite slow. RBDV is also seed-borne in red raspberry with seed transmission occurring when either the male or female parent is infected. The rate of seed transmission increases if both parents are infected and is also influenced greatly by genotype. In addition to transmission of RBDV to the seed from infected pollen, the virus is also readily transmitted from pollen to the pollinated plant, thus the virus spreads from plant to plant in the field via pollen. The exact mechanism of this spread is unknown.

There are three strains of RBDV, the common or D strain, which are usually found in red raspberry, the B strain which is serologically distinct, and from black raspberry and the RB (resistance breaking) strain which is serologically indistinguishable from the D strain but overcomes the resistance gene *Bu*, in red raspberry (Barbara et al., 1984). Most cultivars grown in the UK from 1970 to 1990 carried the *Bu* gene for resistance and the virus had become of minor significance there by 1990 and was of minor importance in North America prior to the early 1990s. In the Pacific Northwest of North America, ‘Meeker’ became the dominant cultivar in the early 1990s due to improved root rot resistance and fruit quality. However, it is susceptible to RBDV and by 1995 growers were observing crumbly fruit that was due to RBDV infection. Though most programs use RBDV resistant material as parents in their breeding programs, there are very few new cultivars being released with the *Bu* gene for RBDV resistance. There appear to be horticulturally negative traits closely linked to the *Bu* gene.

### **Control of Pollen-Borne Viruses**

The best means of controlling RBDV is the use of immune cultivars, however, with the occurrence of the RB strains of RBDV this may not be a suitable strategy in all locations. Planting large blocks of certified material should also reduce the rate of spread combined with placement of beehives in the center of the fields. In this way most bee visits will be to flowers of RBDV-free plants and the movement of virus will be slowed.

This will probably only be effective with quite large fields. A second strategy to increase the time between planting and infection by RBDV is to isolate new fields from established fields of *Rubus*. In many cases, the native *Rubus* may not be a critical component if the flowering times of native and commercial *Rubus* do not overlap.

RBDV infection is a different problem for breeders than for growers. Growers are not concerned about seed transmission but this is an important consideration for breeders. A strategy that has been used in the breeding programs in the Pacific Northwest of North America is for the breeders to have the parent material tested for RBDV, TSV and TomRSV (seed-borne viruses of *Rubus* in this area) prior to making crosses. The breeders then select plants that are free of the seed-borne viruses as parents and in this way the progeny all start out free of these viruses. This involves much less testing than assaying the progeny plants for these viruses. This type of screening was started in the British Columbia program more than 20 years ago and has since been adopted in the programs in Oregon and Washington as well.

The use of biotechnology to engineer resistance to RBDV is being studied in two laboratories (Jones et al., 1998; Martin and Mathews, 2001). 'Meeker' raspberry transformed with sequences of the RBDV coat protein, mutated movement protein and nontranslatable RNA have been produced. In graft transmission trials about 40% (53/141) of the developed lines were resistant to RBDV. In field trials many of the transgenic lines were off-type but lines that appear true-to-type are being evaluated further. It will be interesting to determine if this engineered resistance is effective against the RB strains of RBDV. Since the sequence similarity between the RB strain and the type strain is quite high (>95%) this resistance should be effective against both strains.

### **Minor Viruses of *Rubus***

*Blackberry calico virus* is quite common in *Rubus* in the Pacific Northwest of North America but does not have an economic affect. *Blackberry calico virus* is widespread in blackberry plantings, producing a very striking yellow line pattern on the leaves. The impact of this virus in mixed infections with other viruses is unknown. There is very little effort at controlling *Blackberry calico virus* other than the use of certified stock. *Apple mosaic virus* can cause a brilliant yellow leaf symptom but is relatively rare and does not cause a fruit symptom. *Wineberry latent virus* has been reported from wineberry (*R. phoenicolasius*) in Scotland but is very rare, it may be related to blackberry calico.

### **DISCUSSION**

As described above, there are many viruses that can infect *Rubus*, however, from a practical point of view there are relatively few things that can be done to minimize the impact of the diseases caused by these viruses. There are no chemical means to control plant viruses directly, so once field plants become infected the only way to get rid of the virus is to remove the infected plants. First, the virus involved must be identified so the appropriate control measures can be taken. With nematode-borne and aphid-borne viruses there are some chemicals that can be used to control the virus vectors and reduce virus spread. For the pollen-borne viruses there is very little that can be done with a chemical as part of a control strategy.

As fumigants and other pesticides are being removed from the market, alternative environmentally friendly means of control will be needed. Future challenges will include more breeding efforts to develop cultivars resistant to some of the viruses that have been controlled by soil fumigation or with insecticides. Recent developments with many crops have shown that biotechnology can be used to develop resistance to a wide range of viruses and multiple virus resistances can be developed in the same plant. As these are vegetatively propagated crops, the genetic engineering approach is more straightforward than with seed crops. Perhaps the biggest question with this technology is not whether it will be effective, but rather will the public accept foods produced using the technology. If acceptable, biotechnological approaches to virus control in *Rubus* would mean that

breeders could concentrate on selecting complex traits such as yield, flavor, color etc. that, at this time, are not readily amenable to manipulation through genetic engineering.

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