Viruses and Virus Diseases of *Rubus*

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Blackberry and raspberry are members of the family Rosaceae. They are classified in the genus *Rubus,* which comprises hundreds of species and has a center of origin in the Far East. *Rubus* is divided into 15 subgenera with blackberries classified in the *Rubus* (formerly *Eubatus*) and raspberries in the *Idaeobatus* subgenera (41). Blackberry plants are generally larger and more vigorous than raspberries and are classified in three broad categories based on growth habit: trailing, semi-erect, and erect. Blackberry and raspberry canes typically have spines, commonly called thorns for blackberry and black raspberry.

Rubus species are propagated vegetatively and are subject to infection by viruses during development, propagation, and fruit production stages. Reports of initial detection and symptoms of more than 30 viruses, virus-like diseases, and phytoplasmas affecting *Rubus* spp. were reviewed more than 20 years ago (17,18). Initially, most of the virus diseases of *Rubus* were described based upon symptoms on the virus indicators *Rubus occidentalis* (black raspberry), *R. henryii*, and *R. idaeus* 'Lloyd George' and 'Malling Landmark' following graft inoculation (114). Since the last review on *Rubus* viruses (18), significant progress has been made in the molecular characterization of many of the viruses that infect *Rubus* spp. Vectors of the newly described virus can be inferred based on its phylogenetic relationships with better-characterized viruses that are closely related, but in most cases they still need to be confirmed in transmission studies. Prior to 2000, complete or partial molecular data existed only for some of the nematode-transmitted viruses that infect *Rubus* (*Arabis mosaic virus* [ArMV; 63], *Raspberry ringspot virus* [RpRSV; 104], *Strawberry latent ringspot virus* [SLRSV; 29,59], *Tobacco ringspot virus* [TRSV; 14,94,139], and *Tomato ringspot virus* [ToRSV; 95,96,138]). Currently, reverse transcription–polymerase chain reaction (RT-PCR) detection methods are available for most of the viruses known to infect *Rubus*. There are two caveats to this statement: (i) for many of the recently identified viruses, only a few isolates have been studied, and thus, assay reliability is unknown given the limited knowledge of virus diversity; and (ii) many new viruses of *Rubus* have been characterized since 2005, and it is likely that more, previously unknown viruses, are yet to be identified. The goals of this article are to update the knowledge on previously characterized viruses of *Rubus*, highlight recently described viruses, review the virus-induced symptoms, describe the advances made in their detection, and discuss our knowledge about several virus complexes that cause serious diseases in *Rubus*. Virus complexes have been identified recently as the major cause of diseases in blackberries and raspberries. The names, acronyms, vectors, classification, currently recommended laboratory-based detection methods, and distribution of these viruses discussed below are summarized in Table 1.

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Blackberry Yellow Vein Disease (BYVD) Complex and Associated Viruses

BYVD has emerged in the last decade in the southern part of the United States. Symptoms seen on affected blackberry include: vein yellowing/feathering, leaf mottling, oak-leaf pattern, and ringspots (Figs. 1 and 2). In most cases, symptoms are observed in a few leaves rather than throughout the canopy, and become more noticeable as the season progresses. Yellowing develops along the main veins of the leaflets, being barely noticeable early in the season, and progresses to cover the majority of the leaf blade. Depending on the growing season, affected areas may turn necrotic. In severe cases, BYVD may lead to plant death, although in general, the most severe aspect of the disease is a decline in productivity that has led to a 5- to 7-year rotation in areas (Southeast and Mid-south United States) where previous plantings remained productive for more than two decades.

BYVD symptoms were anecdotally attributed to TRSV, a widespread virus in areas where the disease is prevalent. Upon closer observation, testing, and nematode transmission of TRSV to ensure single infection, followed by grafting to multiple cultivars, showed that TRSV is typically asymptomatic in blackberry (R. Gergerich, *unpublished*). This led to further study of the agents/viruses associated with the disease and revealed its complexity. Double-stranded RNA was extracted from a number of symptomatic plants, and a

new virus was found in all of them (67). After the development of detection protocols and additional testing, the new virus was still found present in all plants, alone or in association with other blackberry viruses, typically TRSV and/or *Raspberry bushy dwarf virus* (RBDV). The new virus belonged to the genus *Crinivirus*, family *Closteroviridae*. Members of this genus are transmitted by whiteflies, are not mechanically transmissible, and are recalcitrant to purification. While working on the characterization and epidemiology of the virus, asymptomatic nursery material was found infected with the virus (118), indicating that it causes an asymptomatic infection in certain cultivars, under certain environmental conditions, or there are additional viruses that can synergistically cause disease with the crinivirus. Those parameters were evaluated and several additional viruses were identified in affected plants. None of the new viruses appear to cause symptoms in single infections but cause disease when found in mixed infections with one or more additional viruses, the identity of which is not as important as the sheer number of viruses infecting plants (R. R. Martin, S. Sabanadzovic, and I. E. Tzanetakis, *personal observations*). It is important to note that identical symptoms are observed in plants infected with different sets of viruses and grown under different environmental conditions. In general terms, symptom severity is closely associated with the number of viruses infecting plants. Below we present the most important viruses as a function of their prevalence in BYVD-affected plants over wide geographic areas.

***Blackberry yellow vein-associated virus* (BYVaV).** The first virus found associated with BYVD was BYVaV, a bipartite crinivirus with the smallest genome among the sequenced members of the genus with the replication-associated proteins encoded by RNA1 and those involved in encapsidation, movement, and other functions found in RNA2 (130). BYVaV is the most widespread among all viruses associated with the disease and has been detected in plants in Arkansas, Florida, Georgia, Kentucky, North and South Carolina, Tennessee, Mississippi, West Virginia, and California in both cultivated and wild blackberries. Although BYVaV is closely associated with the disease, it is symptomless in single infections. Also, it does not cause any symptoms when grafted onto black raspberry cv. Munger. This may explain why BYVaV was detected in several nurseries across the United States the first few years after its discovery and may have accounted for the prevalence of the virus in production fields, including some that tested positive during their establishment year (118). BYVaV belongs to a group of criniviruses that infects berry crops and is transmitted by whiteflies in the genus *Trialeurodes* (87). A survey of 27 plant species found in blackberry seedling fields with a high incidence of BYVaV failed to identify an alternative host for the virus (87). These results suggest that wild blackberries are the major reservoir for the virus and play an important role in the dissemination of the virus in the field. A diversity study was performed using isolates from five states, including wild blackberries. Four regions of the genome were studied, comprising about 30% of the virus genome. This study revealed moderate levels of diversity in virus populations indicative of distinct virus isolates and also revealed evidence of recombination between isolates (88).

Table 1. *Rubus* viruses, names, acronyms, natural mode of transmission, genera, means of laboratory testing, and regional occurrence

Virus name	Acronym	Mode of transmission	Genus	Laboratory detection[b]	Regional occurrence[a]					References
					NA	SA	Europe	Asia	Aust/NZ	
Apple mosaic	ApMV	Pollen, seed	Ilarvirus	ELISA, RT-PCR	+	+	+	+	+	6, 35
Arabis mosaic	ArMV	Nematode, seed	Nepovirus	ELISA, RT-PCR	+	+	+	+	+	68, 76, 77
Beet pseudo yellows	BPYV	Whitefly	Crinivirus	RT-PCR	+	+	+	+	+	127, 137
Blackberry chlorotic ringspot	BCRV	Pollen, seed	Ilarvirus	RT-PCR	+		+			50, 87
Blackberry virus E	BVE		Unassigned	RT-PCR	+					101
Blackberry virus S	BlVS		Marafivirus	RT-PCR	+					99
Blackberry virus Y	BVY		Brambyvirus	RT-PCR	+					119, 120
Blackberry yellow vein-associated	BYVaV	Whitefly	Crinivirus	RT-PCR	+					118, 130
Black raspberry necrosis	BRNV	Aphid	Unassigned	RT-PCR	+		+		+	33, 39, 53, 110
Cherry leaf roll	CLRV	Nematode, pollen, seed	Nepovirus	ELISA, RT-PCR	+	+	+	+	+	3, 54
Cherry rasp leaf	CRLV	Nematode	Cheravirus	RT-PCR	+					114
Grapevine Syrah virus 1	GSyV-1		Marafivirus	RT-PCR	+	+				2, 27, 100
Impatiens necrotic spot	INSV	Thrips	Tospovirus	ELISA, RT-PCR	+	+	+	+	+	83, 124
Raspberry bushy dwarf	RBDV	Pollen, seed	Idaeovirus	ELISA, RT-PCR	+	+	+	+	+	16, 114
Raspberry latent	RpLV	Aphid	Unassigned	RT-PCR	+					90
Raspberry leaf blotch	RLBV	Mites	Emaravirus	RT-PCR			+			73
Raspberry leaf curl	RpLCV	Aphid	No Info.		+					114
Raspberry leaf mottle	RLMV	Aphid	Closterovirus	RT-PCR	+		+			8, 71, 125
Raspberry ringspot	RpRSV	Nematode, pollen, seed	Nepovirus	ELISA, RT-PCR			+	+		80, 114
Raspberry vein chlorosis	RVCV	Aphid	Rhabdovirus	RT-PCR			+		+	55, 69, 114
Rubus canadensis virus 1	RuCV-1		Foveavirus	RT-PCR	+					102
Rubus yellow net	RYNV	Aphid	Badnavirus	RT-PCR	+		+			49, 114
Sowbane mosaic virus	SoMV	Pollen, seed	Sobemovirus	ELISA, RT-PCR	+	+	+	+	+	56, 69
Strawberry latent ringspot	SLRSV	Nematode	Unassigned	ELISA, RT-PCR	+	+	+	+	+	1, 30, 34, 129
Strawberry necrotic shock	SNSV	Thrips, pollen, seed	Ilarvirus	ELISA, RT-PCR	+		+	+	+	62, 106, 126
Tobacco ringspot	TRSV	Nematode, pollen, seed	Nepovirus	ELISA, RT-PCR	+	+	+	+	+	114
Tomato black ring	TBRV	Nematode, pollen, seed	Nepovirus	ELISA, RT-PCR			+	+		65, 78, 114
Tomato ringspot	ToRSV	Nematode, pollen, seed	Nepovirus	ELISA, RT-PCR	+	+	+	+	+	4, 114
Wineberry latent/ Blackberry calico	WLV/ BCV	Unassigned			+		+			114

[a] Regional occurrence included all hosts of the viruses and not only Rubus; NA = North America, SA = South America, Aust = Australia, NZ = New Zealand.

[b] Only laboratory tests are listed, for a list of biological indicators for grafting or mechanical inoculation see Stace-Smith (114).

***Blackberry virus Y* (BVY).** At the time it was realized that BYVaV was not the sole cause of BYVD, plants with typical disease symptoms were examined under an electron microscope for cytopathology indicative of virus infection, and typical potyviral inclusion bodies and elongated particles were observed (120). The virus identified was BVY, the largest member of the family *Potyviridae* sequenced to date that encodes a polyprotein similar to other potyviruses except that it also includes an AlkB domain embedded in the P1 coding area. Sequence comparisons and phylogenetic analysis showed that BVY belongs to a distinct clade of the family and is only distantly related to other potyviral genera, sharing less than 35% amino acid identity over the length of the polyprotein and represents the type species of a new genus (*Brambyvirus*; 120). As with several of the newly identified viruses associated with BYVD, BVY is symptomless in black raspberry cv. Munger and in red raspberry cv. Meeker (117). The importance of BVY in BYVD depends on the locality. BVY is a prevalent virus in Arkansas but has only been found in a small number of samples in Tennessee, North and South Carolina, and Mississippi (I. E. Tzanetakis, B. Poudel, and R. R. Martin, *unpublished*). Notwithstanding, BVY has major effects in plants co-infected with BYVaV, as in several cases plants die soon after infection with the two viruses (119).

***Beet pseudo-yellows virus* (BPYV).** Like BYVaV, BPYV belongs to a subgroup of criniviruses that infect berry crops. It has a very similar genome organization to BYVaV, but unlike its close relative which is known to only infect blackberry, the host range of BPYV includes strawberry and blackberry (127,132) as well as non-rosaceous hosts such as beet and spinach (137). BPYV is known to be transmitted by the greenhouse whitefly (*Trialeurodes vaporiorum*) very efficiently when compared to transmission of other criniviruses (131). The virus has been found in blackberry in Arkansas and the Carolinas, but at much lower incidence when compared to BYVaV (I. E. Tzanetakis, B. Poudel, and R. R. Martin, *unpublished*). Given the cosmopolitan presence of the virus and its wide host range (131), which includes several weeds that are often found in blackberry fields, it is possible that the virus may be transmitted with additional vector species—also potential vectors of BYVaV—that are not as efficient in BPYV transmission as *T. vaporiorum*. BPYV may become of greater concern in the future if the greenhouse whitefly becomes naturalized in the southeastern United States as it has done in the Southwest.

***Blackberry chlorotic ringspot virus* (BCRV).** BCRV was discovered in blackberry in Scotland and in rose in the United States (50,123). The virus belongs to subgroup 1 of the genus *Ilarvirus* and is most closely related to other *Rubus*-infecting ilarviruses such as *Strawberry necrotic shock virus* and *Tobacco streak virus* (128). The BCRV genome is typical for an ilarvirus with three genomic RNA segments that encode proteins with methyltransferase and helicase motifs and an RNA dependent RNA polymerase, a movement, and a coat protein. As with other members of *Ilarvirus* subgroup 1, BCRV encodes a silencing suppressor pro-

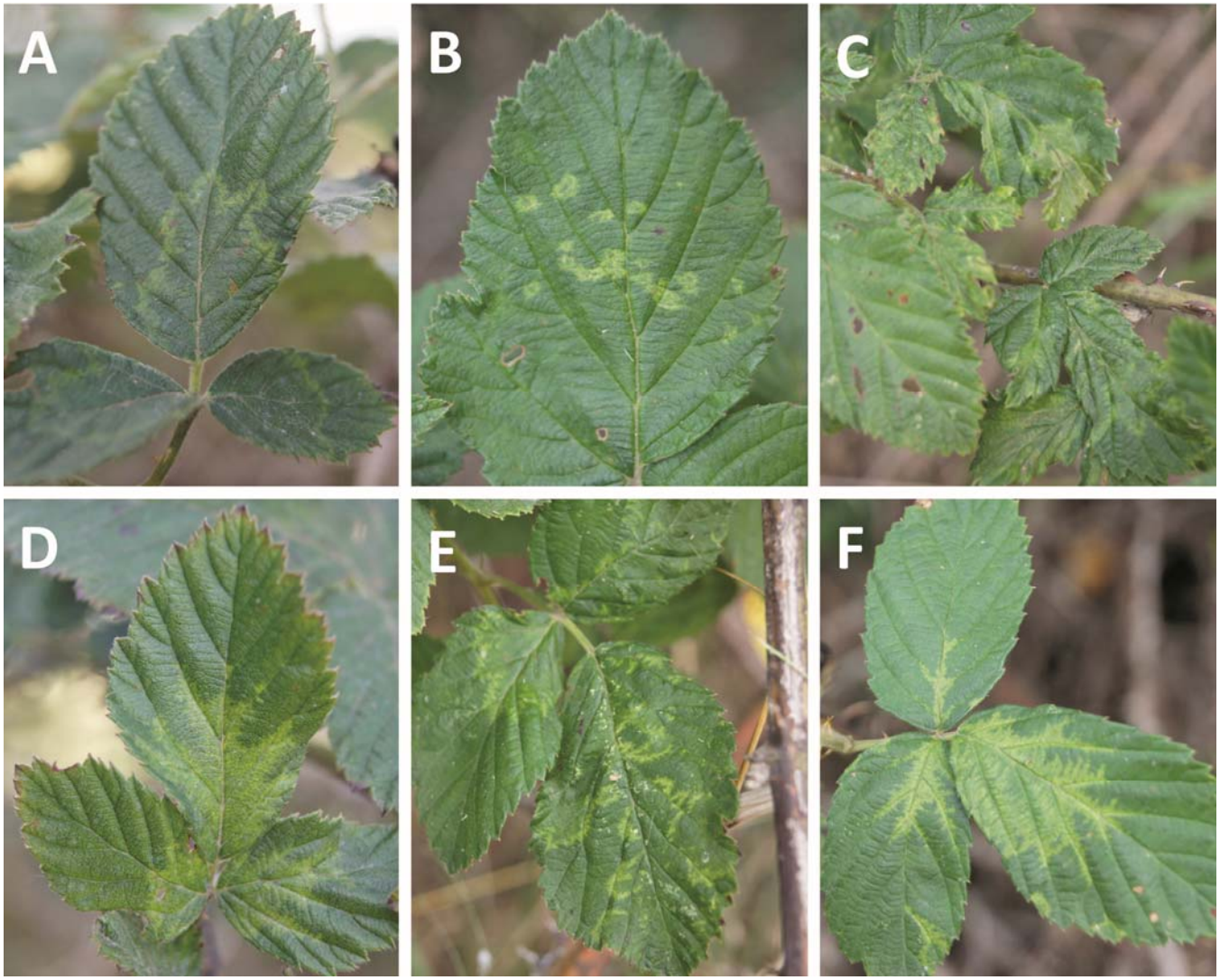

Fig. 1. Wild blackberry plant with symptoms typical of blackberry yellow vein disease. **A,** Oak leaf pattern; **B and E,** ring spot symptoms; **C,** leaf mottling; and **D and F,** vein yellowing and chlorotic feathering patterns.

tein (128). Although BCRV and SNSV show about 63% amino acid identity in the coat protein, no serological cross-reactivity between the two viruses has been observed using available antisera (50). Symptomatic blackberries from Scotland showed line pattern and ringspot symptoms. When American isolates were transmitted by grafting onto American germplasm, no symptoms were observed (87). This may be because of the pathogenicity differences of individual isolates, or different germplasm reaction to the virus or the possibility that the symptomatic plants in Scotland were also infected with another virus(es). A survey of several isolates collected from blackberry and rose across the United States revealed minimal diversity between isolates, although the New World population of the virus is very different from the only European isolate sequenced (87). The virus is widespread in the eastern part of the United States, especially among wild roses where the vast majority of the samples tested were infected with the virus. Given that there are several reports of thrips and bees transmitting ilarviruses as a function of moving pollen from infected to healthy plants (11,105), the wild rose population may be a significant reservoir of the virus. As seed is another means of transmission of ilarviruses, several hundred seeds from rose, blackberry, and herbaceous plants were tested, and the virus was found to be transmitted at high rates (87). The virus was found to be the second most widespread virus in BYVD-affected plants and has been identified in Arkansas, Florida, North and South Carolina, Missouri, Oklahoma, and Oregon (I. E. Tzanetakis, S.W. Scott, B. Poudel, *unpublished*).

***Blackberry virus E* (BVE).** BVE was recently found in blackberry plants exhibiting severe disease symptoms in Mississippi. It is phylogenetically close to members of the genus *Allexivirus* and several partially characterized flexiviruses, but its final taxonomic placement within the family *Alphaflexiviridae* is yet to be determined (101). BVE contains all allexivirus protein orthologs but lacks the most 3′-proximal open reading frame (ORF) present in all extant allexiviruses and is characterized by nucleic binding properties and a role in gene silencing. In addition to Mississippi, the virus has been found in wild and cultivated blackberry in Arkansas, North and South Carolina, and Virginia (I. E. Tzanetakis, S. Sabanadzovic, N. Abou Ghanen-Sabanadzovic, *unpublished results*). In several cases, BVE was found to co-infect plants with BVY and/or BYVaV.

An Arkansas isolate showed high diversity to the Mississippi isolate with <85% nucleotide identity (I. E. Tzanetakis and S. Sabanadzovic, *unpublished*). This may indicate that the presence of the virus in production fields has been underestimated since the genome diversity may have accounted for some false negative results during the survey (I. E. Tzanetakis, S. Sabanadzovic, N. Abou Ghanen-Sabanadzovic, *unpublished results*).

***Blackberry virus S* (BlVS).** BlVS is a member of the genus *Marafivirus* in the family *Tymoviridae*. The 6.5-kb-long genomic RNA of BlVS is polyadenylated, cytosine-rich, and contains a single ORF with all the motifs identified in other marafiviruses. BlVS is phylogenetically related to *Oat blue dwarf virus* and *Citrus sudden death-associated virus*. The virus was originally identified in several symptomatic samples of wild blackberries collected in the Great Smoky Mountains National Park in Tennessee (99). A survey carried out in Mississippi showed the presence of this virus in a few commercial blackberry samples. All BlVS plants identified in the original study showed BYVD-like symptoms. However, in agreement with previous observations on the complex etiology of this disease, all of these BYVD-affected plants containing BlVS were also infected with multiple viruses (BYVaV, BVY, BE, and other viruses yet to be characterized; S. Sabanadzovic, *unpublished*). The vector of BlVS is yet to be determined. Nevertheless, the involvement of leafhoppers, known to transmit most marafiviruses, is suspected.

***Impatiens necrotic spot virus* (INSV).** INSV belongs to the genus *Tospovirus*, viruses with an ambisense RNA genome divided into three genomic molecules encoding five ORFs and six proteins. INSV was first identified as a strain of *Tomato spotted wilt virus,* and it was not until the 1990s that it was shown to be a distinct species (61), and it is now known that the virus is established around the world (83). The two viruses share significant genomic similarities, and antisera raised against one can react to the other, leading to the original confusion around the identity of INSV. The host range of INSV includes several hundred species, both monocots and dicots. As a tospovirus, INSV can replicate in its thrips vectors, but can be acquired only in the first or second instars. Members of the genus *Frankliniella* (*F. occidentalis*, western flower thrips, *F. fusca*, tobacco thrips, and *F. intonsa*, flower thrips) have proven efficient vectors of the virus with efficiencies reaching 60% in experiments with *F. occidentalis* adults (23,81). INSV was recently reported in blackberry (124). Whether transmission to blackberry in the field is because of transmission during pruning (mechanical) or direct thrips feeding is yet to be determined. More than 30% of BYVD-affected plants in the southeastern United States were found to be infected with INSV in an enzyme-linked immunosorbent assay (ELISA)-based study (37), but results were not verified by an alternative detection method. On the contrary, in an ongoing study using both ELISA and RT-PCR, INSV was detected in a significantly smaller fraction of the samples tested (I. E. Tzanetakis and H. Barruck, *unpublished*).

***Tobacco ringspot virus* (TRSV).** One of the most important viruses of blackberry in the United States, TRSV infects hundreds of monocot and dicot plant species, ranging from blackberry and blueberry to soybean, pepper, and iris (113). TRSV is considered a New World virus but now has a worldwide distribution, probably because of the movement of infected plants and soil that harbor its vectors. TRSV was first discovered in 1917 (33). It is a member of subgroup A of the genus *Nepovirus*, family *Secoviridae*, with a genome comprising two polyadenylated positive-strand RNA molecules encapsidated in spherical virions of ~28 nm (95,96). TRSV is efficiently transmitted by pollen and seed (in some crops with 100% efficiency) and by nematodes in the genus *Xiphinema* (dagger nematodes), primarily members of the *X. americanum* complex. The first report in *Rubus* came from North Carolina, where TRSV was found infecting wild blackberry (97). Until a few years ago, TRSV was thought to be the causal agent of BYVD. Although the virus is readily found in several plants exhibiting disease symptoms in the Carolinas, it is not as prevalent in other areas. TRSV is symptomless or produces only mild symptoms in single infections in recently released blackberry cultivars that have been tested but can have a great effect and cause severe symptoms when found in plants co-infected with the other viruses associated with the disease. Other than the obvious implication on the onset of the disease, TRSV is readily seed and pollen transmissible; thus, it

Fig. 2. Blackberry cv. Chocktaw showing symptoms on young leaves caused by co-infection of *Blackberry yellow vein-associated virus*, *Tobacco ringspot virus,* and other, as yet uncharacterized, viruses.

can move horizontally and vertically vast distances with the renewed interest in germplasm sharing among plant breeders.

Raspberry Mosaic Disease (RMD) Complex and Associated Viruses

RMD is used to describe a range of diseases caused by various combinations of viruses transmitted by aphids. In the field, symptoms caused by aphid-borne virus complexes vary by the virus(es) present and cultivar, and as a result multiple names have been used to describe these viruses. Generally, symptoms are most severe on black raspberry (Fig. 3), least severe on blackberry, and intermediate on red raspberry cultivars. In black raspberry, these viruses lead to a severe decline, which often results in plantings that are only productive for 2 to 4 years. Wild and cultivated blackberries can be infected with any or all of the RMD component viruses but generally are considered tolerant and do not show visual symptoms, although lower yield and poor fruit quality may occur (114). However, when infected with one or more of the RMD viruses plus RBDV, some blackberry cultivars develop severe druplet abortion and/or chlorosis (Fig. 3). Other native *Rubus* spp. can serve as reservoirs of these viruses, but their impact on these hosts is not known. As more molecular tools become available to identify and differentiate the viruses that occur in *Rubus* spp., it is likely that the accepted definitions of what viruses cause which diseases will be modified. The current picture of RMD in North America and Europe is that at least three viruses are involved in the disease including: *Black raspberry necrosis virus* (BRNV, unassigned genus, family *Secoviridae*), *Raspberry leaf mottle virus* (RLMV, genus *Closterovirus,* family *Closteroviridae*), and *Rubus yellow net virus* (RYNV, genus *Badnavirus,* family *Caulimoviridae*), with distinct strains of each of these viruses occurring on both continents. The viruses of the RMD complex are transmitted readily by certain species of the genus *Amphorophora.* In North America, *A. agathonica* is an efficient vector for all three viruses (Fig. 4; 114). In Europe, the major vector is *A. idaei*, and five biotypes of this aphid have developed in response to the deployment of a number of resistance genes in breeding programs. The RMD complex in other growing regions has not been examined in detail, and the occurrence of these viruses is unknown.

***Black raspberry necrosis virus* (BRNV).** The nucleotide sequence of a North American and a European isolate of BRNV has been determined (39,72). BRNV belongs to the family *Secoviridae*. It has two viral RNA molecules encapsidated in 30-nm spherical particles. Detection of this virus by RT-PCR is possible; however, analysis of isolates from Tennessee (98) and the UK has shown significant sequence variation, which could affect the utility of this test in different geographical locations and highlights the necessity to develop tests that will detect all known isolates. Interestingly, the sequenced isolate of BRNV does not induce tip necrosis, raising the possibility that previous studies on the early characterization and naming of this virus may have inadvertently used mixed virus cultures where not all agents were identified.

BRNV is an aphid-transmitted virus originally described in 1955 (110) as the causal agent of tip necrosis in infected black raspberry plants and is widespread in areas with raspberry-growing history (55). In the UK, BRNV has been found to be one of the first viruses to infect new raspberry plantations, sometimes reaching 100% infection in the first year of growth (53). In Oregon, BRNV is the major component of decline in black raspberry plantings. BRNV is symptomless in several commercial *Rubus* species commonly grown alongside black raspberry, including red raspberry (*R. idaeus*), Evergreen blackberry (*R. laciniatus*), and 'Marion' blackberry (a complex hybrid with *R. idaeus, R. armeniacus,* and *R. ursinus* in its background), Himalaya blackberry (*R. armeniacus*), a weedy blackberry, and Pacific blackberry (*R. ursinus*), found growing in field perimeters and hedgerows but also other diverse hosts like western bracken fern (*Pteridium aquilinum* var. *pubescens*) and perennial sowthistle (*Sonchus arvensis*) (38).

Aphid resistance has been very effective at controlling the aphid-transmitted viruses in red raspberry (22,41), but had not been utilized in black raspberry. Recent investigations into aphid resistance in *R. occidentalis* germplasm collected from throughout its natural range, the eastern United States and Canada, has resulted in the identification of three populations that exhibit resistance to the large raspberry aphid, *Amphorophora agathonica* (25). Based on genetic analysis, it appears there are two distinct sources of resistance to this aphid in *R. occidentalis*. At this time, it is not clear if the third source is a distinct gene for resistance or the same as one of the other two. These sources of resistance are currently being employed in raspberry breeding programs (C. E. Finn, *personal communication*).

***Raspberry leaf mottle virus* (RLMV).** RLMV and Raspberry leaf spot virus (RLSV) have been reported to be widespread in the UK and to cause latent (symptomless) infection in many red raspberry cultivars but to produce diagnostic symptoms in a few cultivars. For example, RLSV produced pronounced angular chlorotic spots in cvs. Glen Clova and Norfolk Giant but was symptomless in cv. Malling Landmark. Conversely, RLMV induced angular chlorotic spots in Malling Landmark but was symptomless in Glen Clova and Norfolk Giant. However, they share other properties, i.e., they cause tip necrosis in black raspberry but can be differentiated from BRNV because they are symptomless in most red raspberry cultivars, are aphid-transmissible but not mechanically transmissible, and are fairly susceptible to heat treatment.

Recently, virus sequences were obtained from a symptomless Glen Clova plant in a breeding collection in North America and given the name Raspberry mottle virus (RMoV) based on the symptoms induced in black raspberry cv. Munger (125). RMoV is a member of the genus *Closterovirus*, family *Closteroviridae,* which have a very large RNA genome packaged into a long,

Fig. 3. Left: Black raspberry cv. Munger showing leaf symptoms caused by co-infection of *Black raspberry necrosis virus* and *Raspberry leaf mottle virus*. Right: Healthy plant.

Fig. 4. *Amphorophora agathonica,* an efficient vector of the raspberry mosaic complex viruses in the United States.

flexuous particle. Further analysis of plants in the UK infected with RLMV or RLSV, or showing symptoms of RMD, demonstrated that RLSV, RLMV, and RMoV are strains of the same virus and should be grouped together as strains of RLMV (71). In addition, in the UK, two main sequence variants of the virus were identified, sometimes occurring as a mixture in an individual plant. Recombinants between the two variants were also identified. It is likely that the small differences in host reaction originally used to differentiate RLSV and RLMV correspond to sequence variation between virus strains. Additional RLMV variants were identified in studies carried out on North American field-grown red and yellow raspberry plants showing mottling symptoms (125) and *Ribes* in Europe (8). These results have made it possible to produce a broad-spectrum RT-PCR that can detect diverse virus isolates.

***Rubus yellow net virus* (RYNV).** RYNV is a member of the genus *Badnavirus* in the family *Caulimoviridae* and is restricted to *Rubus* spp. It can only be transmitted by aphids or grafting. Partially purified virus preparations from red raspberry revealed bacilliform-shaped virus particles with dimensions of 80-150 × 25-30 nm. Partial virus sequence was obtained by amplification of DNA extracted from these particles with degenerate badnavirus-specific PCR primers, enabling a diagnostic test to be designed (49). RYNV appears to infect all red raspberry cultivars and probably all blackberry and hybrid berry cultivars, either causing no symptoms or a faint vein netting of leaves. In *R. occidentalis* cv. Munger, RYNV causes uneven growth of the basal leaflets of the trifoliates and vein chlorosis that give a yellow netted appearance to the leaves. The virus is a major component of the complex that causes raspberry vein-banding mosaic disease, which severely affects plant vigor and yield. In North America, RYNV spreads rapidly in areas with high populations of the large raspberry aphid and, combined with BRNV, has been reported to cause RMD (114). However, recently RLMV has been found to be widespread in the Pacific Northwest and likely contributes to the RMD in this area (89). Recent studies indicate that RYNV could be integrated into the host genome (R. R. Martin, D.F Quito-Avila, and N. Mosier, *unpublished*) in a manner similar to that reported for *Banana streak virus* and other badnaviruses (36,82).

Raspberry Crumbly Fruit Complex and Associated Viruses

Since the mid-1990s, crumbly fruit has become an increasingly serious problem in raspberry production in northern Washington and British Columbia. Initially thought to be caused by RBDV, it now appears to be the result of a complex of RBDV and one or more aphid-transmitted viruses, including RLMV and RYNV described above and Raspberry latent virus (RpLV) (90). A recent virus survey conducted in northern Washington revealed the presence of both RLMV and RpLV. RLMV incidence ranged from 4 to 30% in 2-year-old plantings and 50 to 100% in 5-year-old plantings; whereas RpLV incidence ranged from 6 to 40% in 5-year-old plantings (89). Infected 'Meeker' plants under field conditions showed a ~400-fold higher RBDV concentration in mixed infections with RLMV compared to RBDV single-infections (92). Furthermore, plants infected with RBDV, RLMV, and RpLV exhibited a 70% reduction in cane growth during the first year of planting compared to plants free of the viruses (89). The role of each of the viruses in crumbly fruit disease is being evaluated, but raspberry plants in areas with low populations of *A. agathonica* have relatively little crumbly fruit compared to plants of the same cultivars grown in areas with high populations of the same aphid. In addition, it was found that fields with severe crumbly fruit (Fig. 5) had a very high >90% incidence of RBDV, RLMV, and ~50% RpLV (89). By comparison, a field with similar levels of RBDV and low incidences of RLMV and RpLV had very little crumbly fruit. RLMV was described above, RBDV and RpLV will be described below.

***Raspberry bushy dwarf virus* (RBDV).** RBDV is the sole member of the genus *Idaeovirus*, a genus closely related to members of the family *Bromoviridae*. Its bipartite RNA genome is encapsidated in quasi-isometric particles of 33 nm. A virus from citrus that has been partially sequenced showed significant sequence identity with RBDV (24), and thus, the genus *Idaeovirus* may soon expand. Antisera against red raspberry isolates of RBDV recognize this virus in all hosts tested, but isolates from black raspberry form a distinct serotype, a feature that may be representative of North American isolates rather than be specific to black raspberry (R. R. Martin, *unpublished data*). ELISA is the method of choice for the detection of the virus. Although serologically indistinguishable from standard isolates of RBDV, resistance-breaking (RB) isolates of the virus have been found in Europe. These isolates are capable of infecting the raspberry cvs. Glen Clova, Malling Admiral, Malling Delight, Malling Jewel, Willamette, and Haida, which are immune to the common strain of RBDV (52,57). The RB isolates have not been reported from North America.

RBDV is pollen-borne, does not cause pollen abortion but can cause drupelet abortion, which in turn leads to crumbly fruit in some red raspberry cultivars. RBDV is symptomless in many North American red raspberry cultivars in single infections, but in mixed infections can lead to severe crumbly fruit disease. In combination with BRNV, RBDV causes dwarfing and shoot proliferation in red raspberry, a typical bushy dwarf condition. The name raspberry bushy dwarf is derived from plants that we now know were co-infected with RBDV and BRNV (45).

RBDV occurs naturally worldwide in many *Rubus* species and cultivars. In North America, it naturally infects blackberry, red raspberry, black raspberry, and blackberry-raspberry hybrid cultivars. RBDV also occurs in wild *R. idaeus* var. *strigosus*, *R. occidentalis*, *R. parviflorus*, and *R. leucodermis*. It has also been reported from *R. multibracteatus* grown from seed collected in China (16) and from arctic bramble (*Rubus arcticus*; 58). *R. molaccanus* L. and *C. oblonga* 'C7/l' develop diagnostic symptoms when graft-inoculated with RBDV. *Chenopodium quinoa* is often used for the detection of RBDV by sap inoculation. Until recently, RBDV was known to naturally infect *Rubus* spp. but was recently discovered in grapevine in Slovenia (85).

RBDV has been reported to occur in a wide range of blackberry cultivars in areas including: eastern and western United States, Europe, Chile, and New Zealand (55,68). In blackberry, a less common host than raspberry, RBDV can cause severe drupelet

Fig. 5. Raspberry cv. Meeker infected with *Raspberry bushy dwarf virus*, *Raspberry latent virus,* and *Raspberry leaf mottle virus* showing severe crumbly fruit symptoms.

abortion leading to misshapen fruit (116) and leaf chlorosis in some cultivars (Fig. 6). The drupelet abortion does not lead to crumbly fruit since the receptacle stays with the fruit at harvest in contrast to raspberry. RBDV occurred in mixed infections in those studies. In the United States, it is common in some blackberry-raspberry hybrids, such as 'Boysen' in California.

Raspberry latent virus (RpLV). RpLV was detected initially in British Columbia in 1988 (40) and provisionally called Raspberry leaf spot virus since it was isolated from red raspberry plants cv. Glen Prosen exhibiting typical leaf spot symptoms. Later, in characterizing dsRNA from plants exhibiting leaf mottling symptoms in northern Washington, this virus was detected along with RLMV and in further studies found to be widespread in that area (Fig. 7). The RpLV genome consists of 10 dsRNA segments, with organization similar to several plant reoviruses (90). Interestingly, RpLV is transmitted by *A. agathonica*, whereas all other known plant reoviruses are transmitted by leafhoppers. It has also been shown that RpLV replicates in the aphid vector (91).

It is obvious that virus biology is very important when it comes to controlling the disease. RBDV is transmitted by pollen; thus, bees play an important role in spreading the virus. RLMV and RpLV, on the other hand, are transmitted by aphids. RLMV is transmitted in a semi-persistent manner (114). For RpLV, acquisition takes ~24 h and transmission also ~24 h; however, there is a 6-day latent period after acquisition before transmission can occur (91). This characteristic in the virus transmission makes RpLV less of a problem in terms of developing an effective control strategy and may explain its lower incidence in the field. RpLV transmission highlights the importance of validating the "best guess" of a vector based on sequence data as this is not always correct; other members of the *Reoviridae* that infect plants are transmitted by leafhoppers or planthoppers (5).

Fig. 6. Blackberry cv. Nightfall showing severe yellowing symptoms, infected with at least *Raspberry bushy dwarf virus.*

Fig. 7. Symptomatic black raspberry cv. Black Mac infected with *Raspberry latent virus* and *Raspberry leaf mottle virus.*

Nematode-Transmitted Viruses

There are eight nematode-transmitted viruses known to infect *Rubus* (*Arabis mosaic virus* [ArMV], *Cherry leafroll virus* [CLRV], *Cherry rasp leaf virus* [CRLV], *Raspberry ringspot virus* [RpRSV], *Strawberry latent ringspot virus* [SLRSV], *Tobacco ringspot virus* [TRSV], *Tomato black ring virus* [TBRV], and *Tomato ringspot virus* [ToRSV]). All are members of the family *Secoviridae* with six of them (ArMV, CLRV, RpRSV, TRSV, ToRSV, TBRV) belonging to the genus *Nepovirus*, subfamily *Comovirinae*; one (CRLV) to the genus *Cheravirus*; and one (SLRSV) not assigned to a genus (103). These viruses have spherical (icosahedral) particles of ~28 to 30 nm in diameter and are efficiently transmitted by members of the nematode genera *Xiphinema* and *Longidorus* as well as via pollen and seed. The nematode-transmitted viruses have wide host ranges and can cause significant losses in multiple crops (15,32,64,74,93,112), especially when present in mixed infections with other viruses. Many of these viruses are quite diverse at the nucleotide level, possibly a result of their adaptation to a diversity of hosts (43). This genetic diversity makes detection based on RT-PCR a challenging task, as oligonucleotide primers may not detect all strains. Antisera are available for each of these viruses and detection by ELISA is possible, but again strain diversity can be a problem with this assay. Even though the use of methyl bromide and other soil fumigants has reduced the importance of the nematode-borne viruses in strawberry, these treatments are less effective in *Rubus* crops for which the fields are maintained for many years. Even after an "effective" soil fumigation, some nematodes survive below the depth of the fumigation, and after 2 to 5 years foci of infected plants are observed in replanted fields. However, the restrictions on methyl bromide and pressure to reduce the use of other chemical fumigants may result in an increased importance of these viruses in *Rubus* production in the future. This brief review will focus on the data obtained recently on nematode-transmitted viruses, whereas extended reviews of the biological properties of the viruses in blackberry and raspberry can be found elsewhere (114).

***Arabis mosaic virus* (ArMV).** ArMV was first described in the 1940s (109). It infects almost 100 plant species belonging to about 30 families, causing significant losses in many crops (76,77). The virus was reported to be widespread prior to the introduction of chemical control of the nematode vector. In mixed infections, ArMV and SLRSV caused raspberry yellow dwarf disease, which has not been a problem in recent years due to chemical control. In a study focusing on the presence of strawberry viruses in the United States, ArMV and SLRSV were never detected in mixed infections (I. E. Tzanetakis and R. R. Martin, *unpublished data*).

ArMV is a member of subgroup A of the genus *Nepovirus* and is transmitted in nature mainly by *Xiphinema diversicaudatum* (12), although there are reports of other *Xiphinema* species that can transmit the virus (122). The efficiency of transmission by the vectors is highly strain-dependent (13). The complete nucleotide sequence of ArMV (135,136) confirmed the close relationship of ArMV with *Grapevine fanleaf virus* (GFLV), another member of subgroup A. There are sources of genetic resistance to ArMV in raspberry cultivars (114).

***Cherry leaf roll virus* (CLRV).** CLRV has many serologically distinct isolates and belongs to subgroup C of the genus *Nepovirus,* but its phylogenetic placement depends on the genomic area used in the analysis (134). It was first reported from blackberry in England (21) in *Rubus armeniacus* 'Himalaya Giant,' in which it was sometimes lethal. Subsequently, it was reported in cultivated red raspberry in New Zealand (54), where infected red raspberry plants were depressed in vigor and exhibited severe leaf symptoms. The virus has not been reported on wild or cultivated *Rubus* in North America, but it has a wide natural host range in wild and

cultivated trees and shrubs in Europe, North America, and other areas around the world (3). In the blackberry cv. Himalaya Giant naturally infected in England, CLRV is reported to cause chlorotic mottling and line patterning in leaves. In three red raspberry cultivars naturally infected by CLRV in New Zealand, infected plants had stunted, distorted leaves with severe chlorotic mottle and ring and line patterns. No vector of this virus has been identified to date. In walnut, CLRV spreads via pollen and causes black line disease in California, characterized by graft incompatibility reaction (75). Resistance or immunity to CLRV in *Rubus* has not been reported.

***Cherry rasp leaf virus* (CRLV).** Thompson et al. (121) showed that CRLV shares characteristics with *Apple latent spherical virus* and is now the type member of the genus *Cheravirus*. Members of the *Cheravirus* genus have many similarities with other members of the *Secoviridae* family in RNA 1, but there are three mature coat proteins that correspond to the three domains of members of the genus *Nepovirus* (103).

The virus naturally occurs in western North America in cherry trees and in weeds such as dandelion (*Taraxacum officinale* Wigg.), and it has been reported from potato in New York (121). It has been found in *Rubus* only in a few red raspberries exported from Quebec, Canada to Scotland (46). CRLV is readily mechanically transmitted to a number of common greenhouse herbaceous test plants, including *Chenopodium quinoa* Willd., *C. amaranticolor* Coste & Reyn., *Cucumis sativus* L., and *Phaseolus vulgaris* L. and has been experimentally graft-transmitted to cultivated and wild *Rubus* hosts (114). The economic importance of the virus in commercial *Rubus* crops is not known.

***Raspberry ringspot virus* (RpRSV).** RpRSV was first identified in the 1950s as the putative causal agent of the raspberry leaf curl disease (15). The virus has been found throughout Europe and infects dicotyledonous and monocotyledonous plants belonging to at least 14 families (80). RpRSV belongs to subgroup A of the genus *Nepovirus* (26) and is transmitted by members of the genus *Longidorus*. There is also a report of RpRSV transmission by members of the genera *Paratrichodorus* and *Xiphinema* (122). Sources of resistance to RpRSV have been reported in red raspberry (114).

***Strawberry latent ringspot virus* (SLRSV).** SLRSV has a host range that exceeds 125 plant species belonging to 27 families of both monocots and dicots, and it is transmitted by nematodes of the genus *Xiphinema* (79). The virus is currently an unassigned species in the family *Secoviridae* (103). Phylogenetic analysis of the RNA-dependent RNA polymerase and helicase motifs indicate that SLRSV is closely related to *Apple spherical latent virus* and *Cherry rasp leaf virus*, members of the genus *Cheravirus* (129); whereas analysis of the coat protein gene sequence shows it is also related to members of the genus *Sadwavirus*. Unlike the nepoviruses, which encode a single coat protein, SLRSV has two coat proteins, a feature found in the *Sadwavirus* genus.

SLRSV is often found in association with ArMV in Europe, where they are transmitted by the same nematode vector, *Xiphinema diversicaudatum* (13). The symptoms on blackberry and raspberry include yellowing and stunting. This virus has only been detected in *Rubus* plants in Europe, although it has been reported in North America in strawberry and an ornamental mint (66,86) and in several other hosts around the world (1,30,34). There is no evidence of spread from infected mint in North America, likely due to the lack of a vector for this virus.

Because of the previous quarantine status of SLRSV, it was not included in virus surveys until recently. The wide host range of the virus and its wide geographical distribution in mint in the United States suggest it should be included in certification programs of *Rubus* spp. and possibly of other vegetatively propagated crops. On the other hand, the ornamental mint that was found infected with SLRSV is widely distributed and probably has been in the ornamental trade in the United States for many years. Alternate vectors for SLRSV should be further investigated, based on its unusual sequence compared to the nepoviruses. SLRSV also can be pollen- and seed-borne, thus care should be taken by *Rubus* breeders that the virus is not introduced into their programs via germplasm. There are sources of resistance to SLRSV in blackberry and raspberry cultivars (114).

***Tomato black ring virus* (TBRV).** TBRV was identified in 1946 (78) and is widespread in Europe, where the virus is often found together with RpRSV because they are both vectored by the nematode *Longidorus elongates.* There are also reports of the virus in India (65). TBRV tends to spread more slowly in the field than RpRSV, possibly reflecting differences in transmission efficiency. In mixed infection, these viruses cause diseases referred to as raspberry leaf curl, or raspberry ringspot, depending on cultivar. The host range of the virus is as wide as those of the other nematode-transmitted viruses. TBRV belongs to subgroup B of the genus *Nepovirus*. The complete nucleotide sequence of the virus genome has been determined and confirms a close relationship of TBRV with *Grapevine chrome mosaic virus* and *Cycas necrotic stunt virus* (45). Resistance to TBRV has been identified in blackberry and raspberry cultivars (114).

***Tomato ringspot virus* (ToRSV).** ToRSV was first identified in *Rubus* in 1938 from Ontario, Canada (114). The virus has a broad host range that includes 35 families of both dicotyledonous and monocotyledonous plants (112). It can be a serious problem in raspberry production in the Pacific Northwest of the United States but has not been reported in blackberry in the same region (84). ToRSV has been reported to infect blackberry in Chile (68) and several other locations (4). The virus can be easily detected by mechanical transmission to *Chenopodium quinoa* and *Nicotiana clevelandii*, which avoids the potential problem of strain variation in detection.

The genome of ToRSV is typical of nepoviruses and picorna-like plant viruses, divided between two polyadenylated monocistronic genomic RNA molecules that encode polyproteins that are processed to mature proteins by a cysteine protease encoded by RNA1 (95). The virus belongs to subgroup C of the *Nepovirus* genus and is transmitted by *Xiphinema* spp. ToRSV can be detected serologically by ELISA and by RT-PCR; however, there are significant strain differences, and one must take care to ensure that an appropriate test is used.

Other Important Viruses

Raspberry leaf blotch virus (RLBV). RLBV, a new putative member of the plant virus genus *Emaravirus*, has been identified recently in field- and tunnel-grown red raspberry cv. Glen Ample plants in Scotland and Serbia (73). Emaraviruses have multiple negative-strand genomic RNAs that form thread-like ribonucleoprotein complexes, and are enclosed in membranous structures in the host cell. RLBV is associated with leaf blotch disorder, a disease that was identified previously in Tayberry and linked with infestation of plants with the raspberry leaf and bud mite (*Phyllocoptes gracilis*) (47). This mite is widespread in Europe and North America (where it is also known as the dryberry mite), and currently leaf blotch disease is increasing in prevalence and severity in the UK. In the earlier studies, the leaf blotch disease symptoms of leaf discoloration, leaf malformation, and necrosis were ascribed to toxins produced by the mite (Fig. 8). It appears, however, that RLBV plays a significant role in disease symptom expression.

Raspberry vein chlorosis virus (RVCV). RVCV is a plant rhabdovirus, having bacilliform (bullet-shaped) virus particles and containing a negative-strand RNA genome. Recently, part of the RVCV RNA was sequenced, enabling the development of an RT-PCR test to detect the virus in infected plants (69).

RVCV was first described in 1952, is widespread in the UK, in Europe, and in the former USSR, causing reductions in plant vigor and fruit yield, particularly when occurring in combination with other viruses. Symptoms include a characteristic chlorosis of the minor leaf veins, which is especially noticeable in field-grown red raspberry plants (Fig. 9). Grafting experiments revealed that black raspberry and blackberry were resistant to the virus (42). RVCV is transmitted by the small raspberry aphid (*Aphis idaei*) but not by the large raspberry aphid (*Amphorophora idaei*), the vector of

RMD-associated viruses. Although not proven for RVCV, rhabdoviruses are known to multiply in their insect vectors, ensuring that the insect remains infectious over a large part of its lifetime.

***Strawberry necrotic shock virus/Tobacco streak virus* (SNSV/TSV).** SNSV was discovered in commercial strawberry (*Fragaria* × *ananassa*), and it causes necrosis on the newly emerging leaves upon grafting onto *F. vesca* clones (31). In the mid-1960s, a similar, symptomless virus was discovered in *Rubus* and was named Black raspberry latent virus (BRLV; 20). In later studies, it was suggested that both SNSV and BRLV are isolates of *Tobacco steak virus* as antisera made against each of the viruses cross-reacted strongly with the others (48). Given that TSV was discovered first, its name remained to characterize these isolates/strains. There was strong evidence that SNSV and TSV were significantly different, as Northern hybridization using SNSV probes did not detect the white clover or tobacco isolates of TSV (115). It was not until 2004 when several 'TSV' isolates from *Fragaria* and *Rubus* were sequenced, that it was determined that SNSV and BRLV belong to a new virus species. Thus, the name SNSV was revived to characterize those isolates (126). The virus has since been found in China and Australia (62,106). Hundreds of *Rubus* and *Fragaria* accessions were tested for the presence of SNSV or TSV. More than 100 plants tested positive for SNSV, but only two strawberry accessions were verified to be TSV positive (128). To this date, no *Rubus* plants have been identified as TSV positive, and it may be that the virus does not infect plants in this genus.

Like BCRV, both SNSV and TSV belong to *Ilarvirus* subgroup 1 and are very similar at the molecular level as their genomes share about 70% nucleotide identities. Despite high similarities at the molecular level, BCRV can be serologically differentiated from the other two viruses, as it does not cross react to antisera developed against SNSV or TSV (50). Other than the obvious application of differentiating viruses using immunological techniques, this has an additional importance. SNSV and BCRV were found in a clone of the plant used in the characterization of BRLV. Given that TSV was never found in that clone and the lack of cross-reactivity between SNSV and BCRV, it is now proven beyond doubt that BRLV is actually a strain of SNSV.

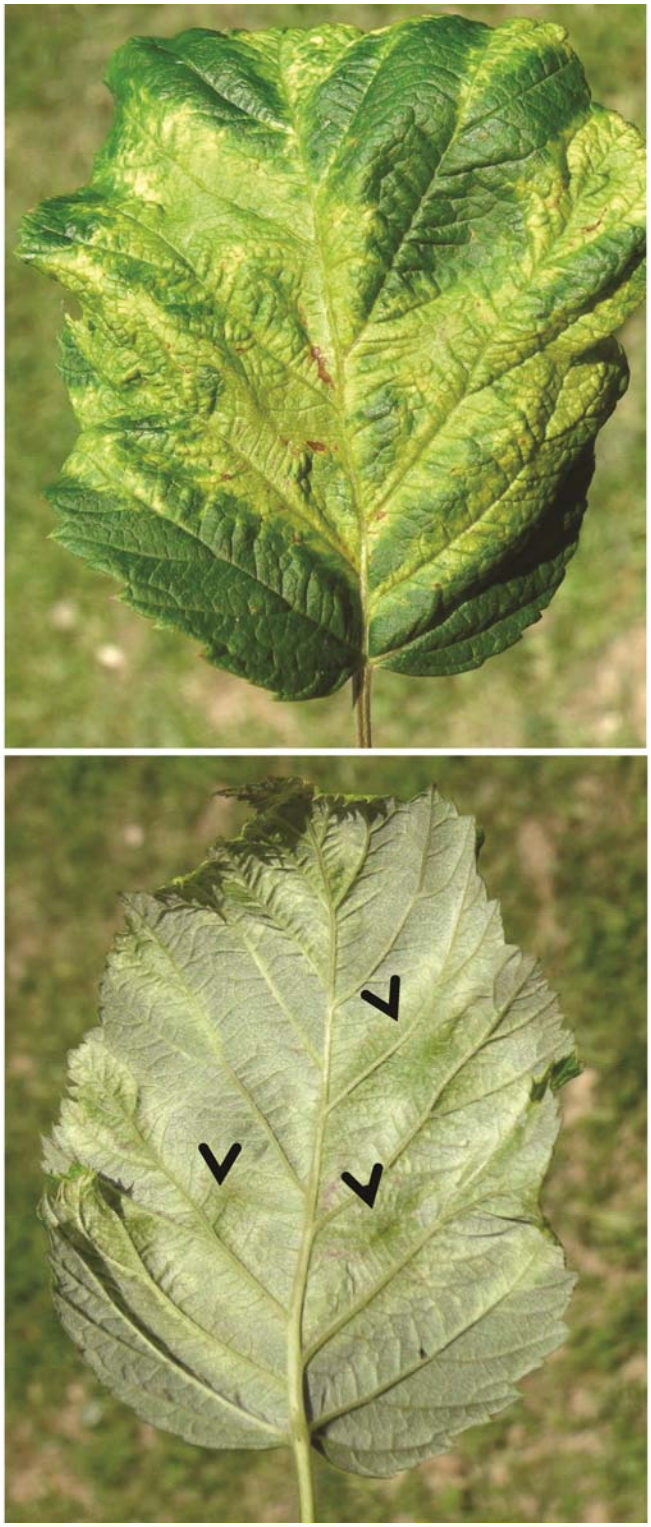

Fig. 8. Top: Raspberry with symptoms of virus infection caused by *Raspberry leaf blotch virus* (yellowing and distortion) and mite feeding. Bottom: Abaxial view with eriophyid mite feeding sites (arrows).

Less Common Viruses

***Apple mosaic virus* (ApMV).** ApMV belongs to subgroup 3 of the genus *Ilarvirus* (107,108). The virus is found around the world

Fig. 9. Symptomatic red raspberry cv. Malling Landmark infected with *Raspberry vein clearing virus.*

(35), as it has a broad host range that exceeds 65 species in 19 families, with many diverse strains. ApMV has been reported in symptomless plants of native and cultivated *Rubus* in eastern and western United States and from Germany (6,114). In cv. Schoenemann in Germany, ApMV caused a yellow mottling and line pattern (6). ApMV is not known to impact cane growth or fruit yield. In field settings, the virus has been reported to spread quickly in red raspberry with up to 92% incidence in plants just 2 years after planting (19).

Grapevine Syrah virus 1 (GSyV-1). This virus was independently reported from cultivated and wild grapevines under different names: GSyV-1 (2) and Grapevine virus Q (100). It was found, along with BlVS, in a sample of wild blackberry collected in the Great Smoky Mountains National Park in the southeast United States. It has also been found in grape in Chile (27).

GSyV-1 is currently a recognized species in the genus *Marafivirus* characterized by circular permutation of the RNA-dependent RNA polymerase motifs, a phenomenon not reported in other plant viruses (100). The importance and distribution of this virus is yet to be understood. Partial data from on-going research indicate the presence of additional members of the family *Tymoviridae* in wild and cultivated blackberries (S. Sabanadzovic and N. Abou Ghanem-Sabanadzovic, *unpublished*).

***Raspberry leaf curl virus* (RpLCV).** RpLCV, first reported in the 1920s (7), is limited to hosts in the genus *Rubus.* The uncharacterized virus is transmitted persistently by the small raspberry aphid, *Aphis rubicola*. It is found only in North America, principally in the northeastern United States and southeastern Canada and in the Rocky Mountain regions of both countries, but not west of the Rocky Mountains. On red raspberry, characteristic severe symptoms usually appear in the growing season following infection where leaves of primocanes as well as floricanes are severely curled, distorted, and chlorotic (Fig. 10). Shoots are dwarfed and may branch excessively at the nodes. Fruit of infected plants is small and crumbly or seedy. Chronically infected raspberry plants have severely reduced yields of poor quality fruit and are susceptible to winter injury. Infected plants have no economic value; however, significant losses from this disease are rare, because RpLCV spreads slowly in the field (10,114).

Rubus canadensis virus 1 (RuCV-1). This virus was recently identified in a few samples of smooth blackberry (*R. canadensis*) collected in the Great Smoky Mountains of North Carolina. The samples showed mild mosaic/vein chlorosis symptoms, but samples were co-infected with other viruses (i.e., BlVS and BYVaV) (102).

The 8.3-kb-long, polyadenylated RuCV-1 genome resembles those of flexiviruses and phylogenetic analyses using the entire replication associated protein grouped RuCV-1 with members of the genus *Foveavirus*, family *Betaflexiviridae*. Pairwise comparisons with known foveaviruses strongly suggest that RuCV-1 is a new member of the genus. Results of a virus survey using BYVD plants indicate that RuCV-1 has limited geographic distribution and is probably not associated with the disease in cultivated blackberries.

Fig. 10. Raspberry cv. Canby exhibiting severe leaf distortion, a symptom of *Raspberry leaf curl virus*.

***Sowbane mosaic virus*-Rubus strain (SoMV-R).** A virus from the genus *Sobemovirus*, with a small, single-RNA genome contained within a 30-nm-diameter spherical particle, was recently identified in glasshouse-grown red raspberry plants and in wild blackberry (*Rubus fruticosus*) in Scotland (70). Symptoms in *R. idaeus* 'Gaia' were a transient downward curling of tip leaves, whereas blackberry plants had small numbers of diffuse, chlorotic spots on infected leaves. This virus was initially named Rubus chlorotic mottle virus (RuCMV); however, it was then found to have a very similar sequence to the partially characterized sobemovirus *Sowbane mosaic virus* (SoMV), a widespread virus around the world (56). Consequently, RuCMV is now classified as a Rubus strain of SoMV (SoMV-R). Different isolates of SoMV have been found to infect a wide range of plant species, and to be seed transmissible in some. This has not been determined for SoMV-R. Several insects have been proposed as vectors for this virus, including leafminer fly, beet leafhopper, and green peach aphid; however, experimental evidence is lacking. SoMV has also recently been found in Scotland and Switzerland to infect cultivated blackcurrant.

***Wineberry latent virus* (WLV) and *Blackberry calico virus* (BCV).** Both WLV and BCV are not characterized at the molecular level, although elongated particles have been observed by electron microscopy. WLV was discovered in a single symptomless plant of wineberry, *Rubus phoenicolasius* Maxim., which was growing in an experimental planting in Scotland. The plant originated in the United States, where wineberry, a species native to Japan, is established in the wild in the Northeast. Experimentally, WLV can be graft-transmitted to some wild *Rubus* spp. and to some blackberry, raspberry, and blackberry–raspberry hybrid cultivars. Other blackberry and red raspberry cultivars appeared to be immune to the virus. A number of commonly used herbaceous virus indicator plants, including *Catharanthus roseus*, several *Chenopodium* spp., *Gomphrena globosa*, and *Lycopersicon esculentum*, can be successfully sap-inoculated with the virus by using a buffer containing 2% nicotine alkaloid. Recent studies in Scotland have suggested that WLV might be the cause of blackberry calico disease; however, plants infected with blackberry calico reacted with antisera to several carlaviruses, whereas plants infected with WLV did not (51). Further work on the relationship, if any, between these two viruses needs to be conducted.

BCV occurs in wild Pacific Coast trailing blackberry *(Rubus ursinus*) and several Pacific Coast trailing blackberry cultivars, such as Chehalem, Marion, Olallie, and Waldo, and blackberry–raspberry hybrids, such as the cultivars Boysen, Logan, and the mutant Thornless Logan. Infected plants appear to flower and fruit normally. BCV has occurred naturally in the blackberry *(R. laciniatus*) cv. Thornless Evergreen. It has been transmitted mechanically to *Nicotiana occidentalis* subsp. *oblique,* which developed small chlorotic–necrotic lesions on the inoculated leaves (133).

Implication of Virus and Virus-like Diseases in Certification and Quarantine

What if there are no known isolates of an agent? There are several virus or virus-like diseases that have been reported in the literature, but for which there are no type isolates available and the corresponding disease has not been observed for many years. It is recommended that the latter category of "virus" diseases be removed from quarantine lists. In each case, there is not a type isolate available and often there is only a single report of the disease. If a similar disease were encountered and a causal virus characterized, it would be impossible to determine whether this disease was the same as a previously reported symptom for which there is no known isolate for comparison. In these cases, the newly

identified causal agent would be given a new name and then be included in certification and quarantine programs.

Alpine mosaic agent. This agent originally graft transmitted from blackberry to *Fragaria vesca* 'Alpine' (18) may represent one or more of the many viruses identified recently in blackberry that are described above. No type isolate of the agent is available, and thus further characterization and comparison to these recently characterized viruses is not possible.

***Bean yellow mosaic virus* (BYMV).** BYMV, a potyvirus, was isolated once from two red raspberry plants in New York State (17). No information is available on the symptoms it causes in red raspberry. Unless the virus can be shown to infect raspberry through re-inoculation, or by another test such as ELISA or RT-PCR, it should not be listed as a pathogen of red raspberry, as the positive testing may be an artifact of insects that have fed on infected material and just landed on raspberry.

Black raspberry latent virus. Sequence data of Black raspberry latent virus showed that this virus is the same as SNSV, which has also been mistakenly referred to as TSV in *Rubus* spp. (126). Thus, any further communications should refer to this virus as SNSV.

Black raspberry streak. Without a type isolate to work with, one cannot determine whether a new virus or any of the viruses recently characterized from *Rubus* spp. may be involved in this disease.

Boysenberry decline. It has been determined that this disease is caused by *Cercosporella rubi*, rather than by a phytoplasma or a virus (60), and the disease should not be included in certification programs.

Bramble yellow mosaic virus (BrYMV). Bramble yellow mosaic disease, caused by BrYMV, was described in one wild blackberry species (*Rubus rigidus*) in one location in South Africa (28). It has no known economic importance. This is a case where no type isolate is available and therefore it is impossible to determine the identity of the virus.

Raspberry yellow spot. Based on symptoms and vector, this reported virus cannot be distinguished from RLMV. Without a type isolate, it is impossible to identify and characterize the agent, and thus keeping the name in quarantine lists is impractical.

***Thimbleberry ring spot virus* (ThRSV).** ThRSV is aphid transmitted, albeit inefficiently. It has only been observed in *Rubus parviflorus* (thimbleberry) at a single site near Vancouver, British Columbia (111). There is no isolate available and therefore it is impossible to identify the virus.

***Tobacco necrosis virus* (TNV).** TNV is transmitted by motile spores of the chytrid fungus *Olpidium brassicae* (Woron.). It occurs in the roots of a very large number of flowering plant species, but no pathogenicity studies have been performed on *Rubus* spp. For this reason, TNV should not be listed as a *Rubus* pathogen.

***Tobacco rattle virus* (TRV).** TRV is transmitted by several species of the stubby-root nematode *Trichodorus.* It has a wide host range in flowering plant species. It has been isolated once from raspberry roots in Scotland, but the transmission was not repeatable and it has not been isolated from leaves of *Rubus* spp. It has not been possible to detect TRV by ELISA in *Rubus* spp. collected from sites where TRV was present in weeds. At this time, the best guess is that TRV does not infect *Rubus* spp. and the transmission from roots of field grown plants was due to the presence of viruliferous nematodes or roots of a host plant species in the root samples collected.

Control

Preplant. Virus control is based on understanding the biology and ecology of the virus vectors and knowing the vector/virus pressure in the region. Prior to planting, it is important to test for potential nematode vectors in the field, and ideally to know whether any of the nematode-borne viruses have been reported in that field previously. If a virus is present in the area there needs to be control measures for the vector; whereas if there is no such report the nematode population threshold can be much higher without any effects to the plant (84).

Given the complexity of *Rubus* diseases caused by virus complexes, it is often impossible to eliminate all viruses from the system. In order to minimize the impact of these diseases, efforts should focus on the identification of the weakest link(s) in a particular environment, viruses with vectors that are easy to control or have a prolonged latent period before transmission. This approach will minimize disease impact and prolong plant longevity in the field, even though the plantings may be infected with some viruses.

As a working hypothesis, we assume that one virus in the complex is transmitted by nematodes, whereas the others are transmitted by whiteflies and hoppers. Nematodes move very slowly in the soil, whereas whiteflies and hoppers can fly long distances. Therefore, it is easier to control nematodes when compared to whiteflies or hoppers (84). Another example deals with the transmission mode of viruses. If a virus is transmitted in a persistent, propagative manner, the latent period between virus acquisition and transmission can be days or even weeks. In the case of a nonpersistent virus, transmission can occur in a matter of seconds. It is obvious that it is easier to minimize transmission by a vector of the persistent, propagative virus than transmission of a nonpersistent virus. This is why it is necessary to know the vector biology, the virus–vector interactions, the specific virus pressures in a given region, and which viruses in a complex are critical for disease development so as to implement an effective disease control strategy for a fruit production system.

Planting stock. It would be difficult to overstate the importance of using planting stock propagated from virus-tested sources to reduce *Rubus* crop losses by many virus diseases, and by other important systemic pathogens. State or government-supervised *Rubus* certification programs exist in several countries that produce commercial *Rubus* planting stock that is essentially free from known viruses, other systemic pathogens, harmful insects, and plant-pathogenic nematodes. In the United States, as a result of the Farm Bill of 2008, the U.S. Department of Agriculture has developed the National Clean Plant Network (NCPN) to provide stability to the programs that carry out virus therapy, testing, and maintenance of G1 blocks. G1 refers to the first generation of plants produced after virus elimination and testing. The term G1 is used to provide consistency across crops and borders for this top tier of plants in clean plant systems, previously referred to as foundation, elite, mother block, etc. The G1 plants developed in the NCPN will feed into certification programs in the United States. Additionally, there is an effort underway to harmonize the certification guidelines across states that produce certified *Rubus* plants. In the United States, certification programs are managed by each state, and currently the guidelines vary by state, creating confusion as to what certified means. Harmonization of the state programs will essentially result in a national certification program that aims to facilitate plant movement between states and internationally.

Few *Rubus* viruses produce characteristic symptoms in nursery plantings that would enable them to be eliminated from stock by rogueing. The temptation of many growers to purchase inexpensive, symptomless, field-run stock is strong, but experience has shown that such stock is generally infected with one or more latent viruses. When planted, it usually lacks the vigor of certified stock and the planting gets off to a poor start. It is prone to rapid decline in fruit productivity and quality, as additional viruses are vectored into the planting and interact deleteriously with those already present in the planting stock. In the case of nursery production, efforts should be made to control all viruses.

Resistance. RBDV resistance is the best studied example of virus resistance in *Rubus* and is thought to be controlled by a single dominant gene. However, the resistance gene appears to be linked to negative horticultural traits since very few selections from a resistant × susceptible cross have RBDV resistance.

Effective control of RMD has been achieved by the use of vector-resistant cultivars, virus-tolerant cultivars, certified planting

stocks known to be free of these viruses, management methods to restrict the spread of RMD, and aphicides to control vector populations. Aphid resistance in North American germplasm was derived from cv. Lloyd George (Ag_1) and has been effective for over 40 years. Recently, a biotype of *A. agathonica*, the major virus vector in North America, has been reported that can survive on raspberry plants containing the Ag_1 gene (22). There is one dominant biotype of *A. agathonica* in North America; several other biotypes have been described but they do not appear to colonize plants with the Ag_1 gene for resistance or have remained very minor components in *A. agathonica* populations.

In the UK, the major aphid vector of RMD is *A. idaei,* and breeding for resistance to this aphid was begun in the 1950s. Control of RMD was enhanced by the deployment of cultivars carrying dominant genes; genes A_1 and A_{10} for resistance to the aphid vector *A. idaei* have been deployed most widely. More recently, biotypes of aphids capable of overcoming these resistances have emerged, such that by 1993 more than 75% of aphids sampled in the UK were able to overcome A_1 resistance, and some A_{10}-resistance-breaking individuals were identified in England by 1997 and in Scotland by 2004. Genetic studies showed that major gene resistance was influenced by the presence or absence of additional, minor genes as well as by environmental conditions. However, it is clear that new sources of genetic resistance, as well as the development of new approaches, are required if RMD is to be controlled effectively into the future as aphid biotypes have evolved to overcome the resistance genes currently used in the UK (9).

Management methods to restrict the spread of diseases caused by viruses. Decisions on where to plant, how much area to plant, and how long to retain a *Rubus* field are relevant to the control of viruses. The further away (isolation) a planting can be located from cultivated or wild *Rubus* infected with any of the above-listed viruses, but especially those with aerial vectors, the longer the planting is likely to escape damaging virus diseases. Isolation from virus-infected *Rubus* is most important for black raspberries because they are the most severely damaged by the aphid-transmitted viruses. A commercial *Rubus* planting should not be kept beyond its useful economic life. If the market demands the production of fruit of a given susceptible cultivar in a field where virus pressure is high and infection is rapid and severe, frequent replanting may be an economic necessity to maintain adequate fruit yield and quality of that cultivar. This is the current situation with growing cv. Meeker red raspberry in northern Washington and British Columbia.

When an old planting located near younger ones is removed, the older planting should first be sprayed with an effective insecticide to prevent viruliferous vectors from moving into the younger plantings (38). An alternative would be to remove the older planting during the dormancy season when the vectors are not present on the plants or not active. Many aphicides give very good control of *Rubus* aphids but must only be applied as permitted by the pesticide regulations currently in effect in the area of use.

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