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Red Raspberry Virus Disease

Red raspberry (*Rubus idaeus* L.) is the most widely cultivated of the several *Rubus* spp. grown throughout the world, and cultivars developed and selected in one area have been distributed to other areas, inadvertently disseminating any viruses present in the original clone. This practice has dispersed a number of important virus diseases. Fortunately, the viruses involved usually have a unique means of spread, and unless the vectors are also introduced and survive in their new environment, the exotic viruses are perpetuated and propagated only by the actions of man. Thus, despite interchange of planting stock and the possible introduction of exotic viruses, some virus diseases important in Europe have never been recorded in North America and other virus diseases found in North America are not known to occur in Europe.

Recognizing regional variability and the complexity of the Rubus virus situation in different regions, I have opted to restrict the coverage in this article to the virus diseases of major significance in red raspberry in North America. Information on raspberry virus problems in the United Kingdom, New Zealand, and Australia is available in articles by Jones (7), Jones and Wood (8), and Guy et al (6), respectively. In addition, the handbook edited by Frazier (5) and its updated version edited by Converse (3) describe the viruses known to infect the various *Rubus* hosts.

Viruses infecting *Rubus* spp. are frequently referred to as Rubus viruses, although some infect and are important in other plants also. This is particularly the case with all nematode-borne viruses. Probably the most logical and convenient way to group the raspberry viruses and virus diseases is according to mode of transmission (1,2,10), and when control strategies are considered, the mode of natural transmission is of utmost importance. The three categories are aphid-borne, nematode-borne, and pollen-borne (Table 1).

Table 1. Viruses occurring in North American red raspberries and their mode of natural transmission

Virus	Vector
Rubus yellow net	Aphid (*Amphorophora agathonica*)
Black raspberry necrosis	Aphid (*A. agathonica*)
Raspberry leaf curl	Aphid (*Aphis rubicola*)
Tomato ringspot	Nematode (*Xiphinema americanum*)[a]
Tobacco ringspot	Nematode (*X. americanum*)[a]
Cherry raspleaf	Nematode (*X. americanum*)[a]
Raspberry bushy dwarf	Pollen-borne, seedborne
Tobacco streak	Seedborne, pollen-borne? thrips?

[a] The taxonomic status of this nematode species is questionable. Some authorities believe *X. americanum* should be subdivided into several species.

Aphid-Borne Viruses

The involvement of aphids with the spread of some of the important raspberry virus diseases was recognized in the early 1920s, but identification of the vector species and the viruses involved was not clearly resolved. It is now accepted that two species account for most of the aphid spread of viruses in red raspberry. One species, *Amphorophora agathonica* Hottes (formerly *A. rubi* Kalt.), is a relatively large aphid and is commonly called the large raspberry aphid. The other, *Aphis rubicola* Oest., is small and is referred to as the small raspberry aphid. Both species are restricted to a few hosts belonging to the genus *Rubus* and colonize only *Rubus*. Similarly, the viruses they transmit are restricted in nature to a few *Rubus* spp. and can be designated Rubus viruses. Both species occur naturally throughout most of temperate North America, although the small raspberry aphid does not occur west of the Cascade range.

Raspberry mosaic disease. Viruslike diseases were described in red raspberry about a century ago, but it was not until 1922 that the term "raspberry mosaic" was proposed for the disease. The most constant and diagnostic symptoms on plants affected by mosaic are evident in the spring on the leaves of suckers, which show large, irregular green blisters that arch upward. The tissue between the blisters is lighter green than normal, with a yellowish appearance, and the severely blistered leaves curl downward. It soon became apparent that mosaic occurred in varying degrees, and such terms as "mild mosaic" and "yellow mosaic" were introduced into the literature.

I reported in 1956 (9) that mosaic disease of red raspberry resulted from multiple infection with at least two separate viruses, both transmitted in a semipersistent manner by *A. agathonica*. The virus complex was separated by transferring individual aphids from an infected plant to a series of black raspberry seedlings. Both viruses have the same vector relationships and are therefore normally transmitted together, but a single virus is occasionally transmitted by chance to an exposed plant. One of the viruses is readily inactivated by holding an infected plant at 37 C for about a week, whereas the other withstands a higher temperature. The heat-stable virus was designated Rubus yellow net (RYNV) because of the distinctive netlike chlorosis that developed along the leaf veins of infected plants. Red raspberry plants infected with RYNV never show the severe symptoms of mosaic, but when the milder virus is introduced by aphid or graft transmission, mosaic symptoms develop. The milder virus was named black raspberry necrosis (BRNV) because it caused necrosis of the cane tips of black raspberry seedlings. BRNV is latent in some cultivars and induces a mild mottle in others (Fig. 1).

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n North America

Research workers in Europe have separated the mild or latent component of mosaic into three distinct entities based on sap-transmissibility and the differential response of selected red raspberry cultivars. The sap-transmissible entity has been equated with BRNV; the other two, which are not sap-transmissible, are called raspberry leaf mottle (RLMV) and raspberry leaf spot (RLSV). These entities may all be involved in raspberry mosaic disease but it is not known which combinations are required to induce severe symptoms. Further, the differential indicators used in Europe have not been adequately tested in North America to determine whether RLMV and RLSV are common components of raspberry mosaic on this continent. While many questions remain unanswered, all workers agree that mosaic disease of red raspberry is caused by a complex of at least two viruses, both transmitted by *A. agathonica*, and that RYNV is one of the viruses.

Although raspberry mosaic was prevalent and widespread in most of the popular raspberry cultivars in the 1950s, it is debatable today whether it should be considered a significant disease in the Pacific Northwest, where approximately 90% of the commercial production is centered. Raspberry mosaic is rarely encountered in this region. The reason is related to the replacement of aphid-susceptible cultivars with those that are highly resistant to the aphid vector, *A. agathonica*. Older cultivars, such as Newburgh, Latham, Cuthbert, and Taylor, supported high populations of aphids, and even when new plantings are established with virus-free stock, many plants may be infected by viruliferous aphids from nearby plants infected before they reached bearing age. The picture has now changed. Such cultivars as Canby, Haida, Skeena, and Nootka were deliberately selected for aphid resistance, and large plantings of these cultivars have remained free from mosaic after several years of field exposure. Mosaic is now

Fig. 1. Mottle on red raspberry caused by black raspberry necrosis virus.

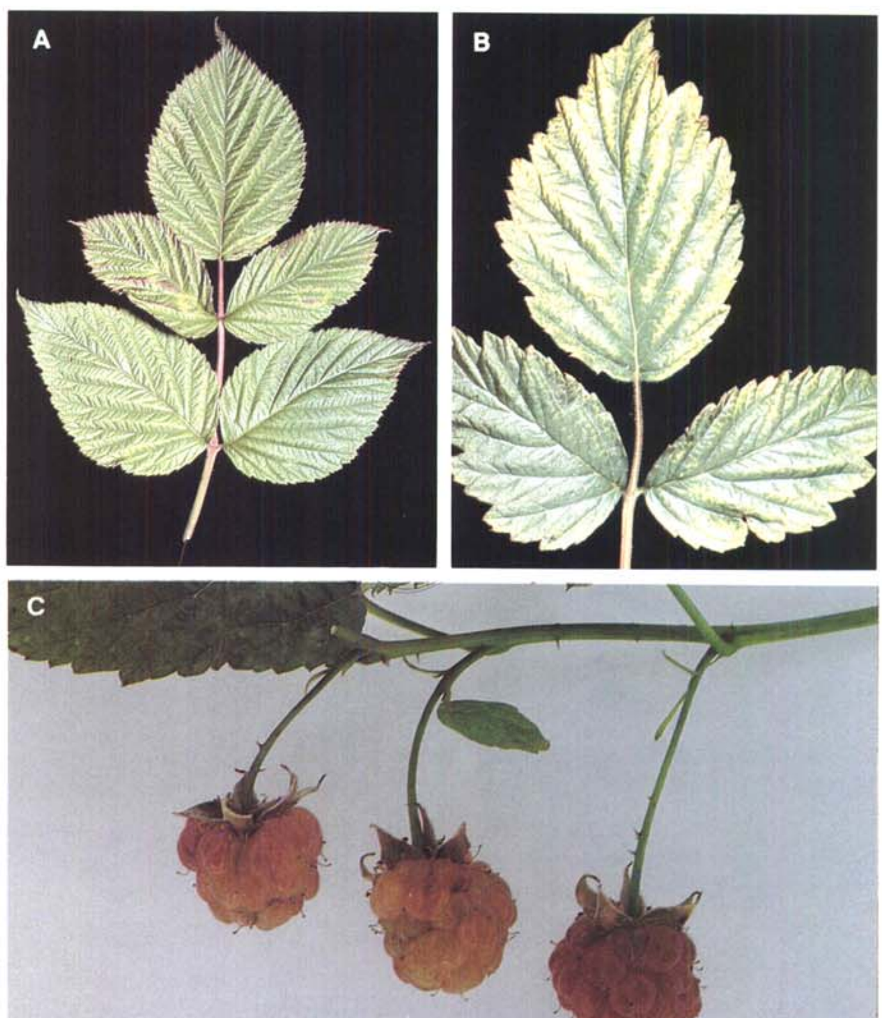

Fig. 2. Symptoms associated with tomato ringspot virus infection of red raspberry: (A) Markings on primocane leaf. (B) Chlorosis on leaf of fruiting cane. (C) Misformed and undersized fruit.

completely absent from large areas, and infected plants can be found only in experimental plots.

Lest I give the wrong impression, I should point out that not all raspberry-growing areas in North America have changed over to aphid-resistant cultivars, and in those areas that have not, mosaic is still prevalent. This fact was clearly drawn to my attention during a survey of raspberry plantings in the Province of Quebec during the summer of 1983. Newburgh is still a popular cultivar in this region, and I observed mosaic, at infection levels approaching 100%, in every planting I visited.

Raspberry leaf curl. Raspberry leaf curl is vectored by the *Rubus*-inhabiting aphid *Aphis rubicola*. The virus evolved with the North American *Rubus* spp. and has not been reported to occur in any other parts of the world. In North America the virus has been recognized as a problem in red raspberry plantings for about a century.

Infected plants would probably not be detected during the growing season in which they acquire the virus, but the following spring, leaves in both fruiting canes and primocanes are curled and slightly yellowed. The fruiting laterals are shortened and shoots may proliferate, producing a rosette of numerous stunted laterals at each leaf axil. Such plants produce little fruit, and the fruit that is produced is small and crumbly. The disease is so severe that infected plants may not survive the winter. Those that do survive have yellow foliage and remain stunted and unproductive.

Because the aphid vector does not occur in the Pacific Northwest, the virus has rarely been observed there. I have never detected infected plants in the Fraser Valley region of British Columbia on the west side of the Cascade range, but the virus is common on the east side. The industry there is confined to backyard plantings, so damage is minimal, but in other raspberry-growing areas east of the Cascades, the disease was prevalent a number of years ago. I recall, for instance, seeing heavy infections in commercial plantings during a survey in Utah in 1954, and I had no difficulty finding infected plants during a survey in the same area in 1980. However, I did not find a single infected plant during a survey of red raspberry plantings near Quebec City in 1983. Indeed, I was wondering whether the virus may actually be extinct in some regions where it was once prevalent. This illusion was shattered when I ceased looking at commercial plantings in Quebec and began examining patches of native red raspberry in nearby forested land. The virus, along with its aphid vector, is still prevalent in the wild red raspberry, waiting for man to decrease his vigilance over his cultivated crops. I can only conclude that the virtual absence of the disease from areas where it was once a problem is related to improved extension services available to growers. Since all commercial cultivars are susceptible and symptoms are obvious, even those extension agents with little knowledge of raspberry viruses recommend that affected plants be removed and destroyed.

Nematode-Borne Viruses

Those plant viruses that are readily transmitted experimentally by mechanical inoculation, in which seed transmission is common, and in which most members are transmitted by soil-inhabiting longidorid nematodes have been classified in the nepovirus group. The occurrence and geographic distribution of individual viruses in this group are correlated with the occurrence and feeding habits of specific vectors. Of the eight nepoviruses known to occur in North America, only three—tomato ringspot, tobacco ringspot, and cherry raspleaf—have been isolated from *Rubus*, although several other nepoviruses commonly infect *Rubus* in Europe. Tobacco ringspot and cherry raspleaf have only rarely been found to infect *Rubus*, and for practical purposes, tomato ringspot (TomRSV) is the only nepovirus of importance in North America. Field spread is restricted to areas where vectors of the genus *Xiphinema* occur. Traditionally, *X. americanum* Cobb. has been considered to be the major vector, but the taxonomic status of this species is now in doubt. The nematode itself causes little damage to red raspberry unless the virus is also present.

There is still much to learn about the distribution and mode of spread of TomRSV, but the limited evidence available suggests that it is the most damaging virus disease of raspberry in North America. In contrast to the aphid-borne diseases of raspberry, whose identity and means of transmission have been known for more than 60 years, TomRSV as a virus problem in raspberries was not identified until 1962. Circumstantial evidence suggests, however, that it has been a factor in raspberry production since the crop was first cultivated. The early extension bulletins on raspberry diseases refer to "running out" or "decline" characterized by the gradual loss of productivity. Some 40 years ago, raspberry decline was recognized in the Pacific Northwest, and it was speculated from the pattern of occurrence that the disease was spread by some underground vector. It is reasonable to assume now that TomRSV was a major contributing factor in the raspberry decline syndrome.

Symptoms of TomRSV in red raspberry vary, depending to a large extent on the cultivar, the duration of infection, and the time of year plantings are examined. Plants normally show no symptoms in the season in which they acquire the virus, but in the spring of the following year, some leaves on the primocanes show yellow rings, line patterns, or vein chlorosis (Fig. 2A). These symptoms may be considered a "shock reaction" because distinctive foliar markings are rare in subsequent years. Chronic symptoms are delayed foliation in the spring, a tendency for leaves in the fruiting canes to develop varying degrees of chlorosis (Fig. 2B), and a higher proportion of misformed or crumbly fruit (Fig. 2C) than on plants that are not infected. All these symptoms are variable and unreliable, however, and can be missed in disease surveys based on field observations. For this reason, field surveys to determine the extent of virus infections are of little value unless visual observations are supplemented with mechanical inoculations to indicator hosts or with serological tests.

Enzyme-linked immunosorbent assay

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(ELISA) is useful for detecting TomRSV infections in raspberry plants. During the 1983 growing season, we used the ELISA technique to index some 2,000 raspberry plants collected from several commercial plantings in Washington and British Columbia. Some of these fields had no history of virus problems and the plants that were indexed appeared to be healthy, yet the ELISA results were positive for TomRSV. When fields with a history of poor productivity indexed positive, it came as no surprise. The survey at least demonstrated that TomRSV is probably the most widespread virus problem in Washington and British Columbia and that visual surveys are of little value in determining whether the plantings are infected. It also justified the recommendation that growers not use suckers from an untested source to establish new plantings, since they could unwittingly contribute to the spread of both the virus and its nematode vectors.

We recommend that ELISA indexing be used in conjunction with other diagnostic procedures, such as sap-transmission tests. The ELISA procedure is highly specific and may fail to detect some aberrant strains of TomRSV. Until recently, I was of the opinion that TomRSV was a homogeneous virus and that antiserum prepared against one isolate would probably detect all isolates. During our 1983 survey, however, we indexed all plants by two techniques, by the ELISA procedure and by mechanical transmission to the herbaceous test plant *Chenopodium quinoa* Willd. With one exception, the results were identical. In the exceptional field, several plants indexed positive for TomRSV on *C. quinoa* but negative on ELISA. The reason for the discrepancy became clear when agar gel serology was used to compare a number of TomRSV isolates with the unusual isolate. The spur formation in agar gel tests demonstrated that the unusual isolate was an aberrant strain of TomRSV, sufficiently close to the common strains to be identified as a strain of TomRSV by agar gel serology but sufficiently distant to be missed by ELISA.

Pollen-Borne Viruses

Two viruses, raspberry bushy dwarf (RBDV) and tobacco streak (TSV), are usually included in the pollen-borne virus category. Transmission of RBDV is unquestionably via pollen, but there is some doubt as to whether TSV is naturally transmitted via pollen. In any event, TSV is rarely found in red raspberry, and if care is taken to establish new plantings with virus-tested clones, the virus apparently will remain rare in red raspberry.

The evidence suggests that RBDV originated in Europe and was introduced into North America in such European cultivars as Lloyd George that were totally infected until steps were taken to establish virus-free clones. Because Lloyd George was a popular gene source in many North American breeding programs, the virus was inadvertently carried over into many of the progeny arising from the breeding programs. However, the cultivar Willamette, which is widely planted in the Pacific Northwest, is immune to RBDV. This, together with the fact that RBDV does not induce distinct symptoms in infected cultivars, has led to the impression that the disease is primarily a plant breeding problem and is of little or no consequence in field plantings. Such a complacent attitude is unfounded. We now know that several plantings of the popular cultivar Meeker are infected, that other promising cultivars are susceptible, that field spread can be rapid, and that infection is associated with deterioration in the useful life of a planting (4,11).

Symptoms of RBDV vary with the cultivar and the season. Some cultivars, such as Creston and Canby, produce leaves with varying degrees of interveinal chlorosis (Fig. 3A), whereas others develop an irregular line or oak leaf pattern in the foliage (Fig. 3B). Infected Meeker plants, for instance, do not show interveinal chlorosis but some do show the line pattern symptom. Plants that show symptoms one year will not necessarily show symptoms in subsequent years. Variations in leaf symptoms are considered to be dependent on genetic resistance to symptom expression and on environmental factors. Crumbly fruit is associated with infections in some clones, but since none of the above symptoms is reliable, diagnosis is usually based on serological tests or sap transmission to *C. quinoa* (Fig. 4).

RBDV is transmitted through pollen, from which infection spreads not only to a proportion of the seeds but also to the pollinated plant. The virus is confined to plants belonging to the genus *Rubus*, and the only means of natural spread is through pollen or seeds. Fortunately, not all cultivars are susceptible and control can be achieved by the use of resistant cultivars. This appears feasible as a long-term solution but is of little help to a grower with a large planting of a susceptible cultivar such as Meeker and virus-infected plants scattered throughout the planting. Under such circumstances, little can be done but accept the fact that the yield will be reduced, and at the point it becomes uneconomical, the planting can be replaced by a resistant cultivar.

This approach has kept the problem under control in Great Britain until recently. Now, some cultivars previously considered to be immune have become infected after graft inoculation with some isolates; even more serious, severe isolates of RBDV have been recovered from field plantings of cultivars that were considered to be immune to the virus (12). The significance of this finding is debatable, but it serves to remind us that a breakdown of the high resistance to pollen-borne infection is possible in the presence of resistance-breaking strains of RBDV. We have no evidence that any severe strains are present in North American cultivars, but more experimental work is required to confirm this. Meanwhile, if adequate precautions are taken and plant breeders restrict their crosses to parent plants known to be virus-free, we should be able to eradicate RBDV from the major raspberry-producing areas.

Control Strategies

Viruses are economically important only in plants in which they become systemic, ie, plants in which the virus moves from a single point of infection to invade all or most of the vegetative parts. Once systemically infected, plants remain so for as long as they live. Consequently, virus diseases are much more important in perennial plants, or those propagated vegetatively, than in crops raised annually from true seed. Although some viruses do enter the seed set by infected plants, most do not, and seedlings usually start life virus-free. By contrast, viruses are consistently transmitted to offspring whenever plants are propagated vegetatively, whether by bulbs, tubers, cuttings, or grafting or budding, and almost all new plants raised by these methods will be infected. Since red raspberry is propagated exclusively by sucker plants or root cuttings, the progeny of an infected mother plant would all be infected. Under good conditions, the infected progeny may grow indefinitely, yielding less each year than they would if they were not infected. Another problem is that red raspberry cultivars do not remain infected with a single virus but, with time and field exposure, tend to acquire a complex of two or more viruses. Such accumulation of viruses was common with many of the popular red raspberry cultivars, and useful clonal material has often become so crippled by viruses that its cultivation has had to be abandoned. The need for virus-free stock is therefore essential.

A

B

Fig. 3. Symptoms associated with raspberry bushy dwarf virus infection of red raspberry: (A) Chlorosis of leaf veins. (B) Line pattern.

Fig. 4. Symptoms of raspberry bushy dwarf virus on *Chenopodium quinoa*, 10 days after sap inoculation from infected raspberry.

The term "virus-free" may be presumptuous and misleading, but terms such as "virus-tested" and "virus-indexed" are equally poor. With all terms, the assumption is that the clonal source used for propagation has been extensively indexed by someone who is knowledgeable about raspberry viruses and has had sufficient indexing experience to judge the adequacy and reliability of the test procedures. Because raspberry is subject to infection by several unrelated viruses, detection and identification of the virus or viruses that may be present in selected clones could prove difficult. Certainly, any certification scheme that involves visual examination with approval based on the absence of symptoms is next to worthless. Several of the raspberry viruses induce either no symptoms or symptoms that are so vague and variable as to be unreliable. Sap inoculation, aphid inoculation, graft inoculation, serology, or electron microscopy or combinations of these techniques are required to detect virus infections. Extensive indexing of an individual plant and subdividing this plant to establish a certification block is preferable to establishing a certification block from several mother plants.

To supply healthy planting material, certification schemes are now operating in provinces or states that have any significant industry. Special stocks that form the basis of certification schemes are built up by propagating from single virus-free plants. Until the 1950s, the production of the virus-free stocks of plants depended on finding an uninfected plant of the cultivar to start the stock. Fortunately, this is no longer so; methods have been found whereby red raspberry plants can be freed from the viruses that commonly infected them. The method with the widest application is shoot tip culture, usually taken from clonal material that has been subjected to a prolonged period of growth at a temperature considerably higher than would normally be used for plant production. Red raspberry shoot tips are fastidious in their rooting requirements, however, and the process of virus eradication may be prolonged and frustrating. For this reason, virus eradication should not be attempted unless a specific virus-free stock cannot be obtained from some other source. Some of the older cultivars were probably infected at the time they were released, so that all clonal material is infected. Cultivars released over the past two or three decades, however, were subjected to indexing for viruses before release, and although some sources have since become infected, others can be found that are still virus-free. Hence, virus eradication is probably unnecessary except in rare instances.

We attempt to convince growers not to use suckers from a producing field to establish new plantings but rather to use certified stock raised under special conditions that assure freedom from viruses and other pests and diseases. To be creditable, the certified stock must be as productive as the best available stock of a particular cultivar and it must be true to the cultivar. This may appear obvious, but I am personally familiar with two red raspberry certification programs where sufficient attention was not given to productivity and trueness to variety. These programs not only were discredited but also eventually ended up with costly litigation. In the first case, the cultivar had a genetic weakness whereby some clones produced crumbly fruit. When this cultivar was included in the certification scheme, a clone with the propensity to produce crumbly berries was inadvertently selected for propagation. To complicate matters, the fruiting canes were pruned, leaving only the current year's shoots, and the problem was not detected until field plantings arising from the certified stock began to bear fruit. In the second case, an error was made in naming the cultivar, and several thousand plants of the wrong cultivar were distributed. These two instances serve to make the point that, in addition to freedom from pests and diseases, the clonal material should be true to variety and genetically equivalent to the best available stock.

In general, the application of insecticides to control spread of aphid-borne viruses is ineffective and the expense is not warranted. Fumigation to eradicate nematode vectors is necessary where viruses are present. Some postplanting treatments can reduce the numbers of *Xiphinema* spp. and slow the rate of spread but are unlikely to eliminate populations from the deep soil layers.

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