CHAPTER 26

Cherry necrotic rusty mottle and *Cherry rusty mottle viruses*

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Introduction

The diseases cherry necrotic rusty mottle (CNRM) and cherry rusty mottle (CRM), along with cherry green ring mottle (CGRM), were first reported in the western region of North America (Reeves, 1940). Lambert mottle reported from Canada (Lott, 1945; Lott and Keane, 1961), cherry bark blister (Stout, 1948, 1949), and Frogmore virus canker reported from England (Posnette and Cropley, 1957) are all considered synonyms of CNRM. It has been suggested that the cherry necrotic mottle leaf disease may also be a form of CNRM (Gentit et al., 2002). Cherry necrotic rusty mottle was first identified in Utah (Rhoads, 1945; Richards and Rhoads, 1945) and named rusty mottle disease. The name was subsequently changed to necrotic rusty mottle (Reeves and Richards, 1946) when it was found to be clearly distinct from a disease from Washington described by Reeves in 1940 under the same name. In 1947, two forms of rusty mottle were recognized by Zeller and Milbrath (1947) in Oregon, now referred to as cherry rusty mottle (American). Based on symptoms, the disease originally described by Reeves is known as "severe" rusty mottle, to differentiate it from the "mild" rusty mottle disease identified by the authors from Oregon (Zeller and Milbrath, 1947). There is some speculation however that mild rusty mottle disease may in fact be caused by the same virus associated with the CNRM disease (Desvignes, 1999). To complicate matters further, Posnette (1951) identified a rusty mottle disease in England which he initially thought was the same disease as that identified in the United States of America. It was later determined that the two diseases were different (Posnette and Cropley, 1961) and this disease is now referred to as cherry rusty mottle (European). Cherry rusty mottle (European) now encompasses a group of diseases with similar symptomatology. While data are still limited, evidence suggests that CNRM, CRM (American), and CRM (European) are caused by closely related viruses of the family *Betaflexiviridae*.

Taxonomy and Genomics

Molecular data revealed that *Cherry necrotic rusty mottle virus* (CNRMV) and *Cherry rusty mottle virus* (CRMV) are closely related to each other, and most closely related to *Cherry green ring mottle virus* (CGRMV), another unassigned species of the *Betaflexiviridae* (Adams et al., 2004; Martelli et al., 2007). The genome of CNRMV (GER), an isolate that displays classic symptoms on *P. avium* cv. 'Sam,' determined by Rott and Jelkmann (2001b) is 8432 nucleotides in length excluding the 3′ poly(A) sequence. Two CNRMV isolates (FC4 and FC5) from a symptomless flowering cherry (*P. serrulata*) have also been sequenced (Li and Mock, 2008). When grafted to indicator plants, these isolates displayed only mild foliar mottle on cv. 'Canindex' and no symptoms on cv. 'Sam.' They share 96% identity with each other and 86% identity with CNRMV-GER. A partial sequence of CNRMV (P1B), isolated from a tree with cherry necrotic mottle leaf disease (Gentit et al., 2002) has also been determined and shares about 94% identity with CNRMV FC4/FC5 and 83% identity with CNRMV-GER. CNRMV shares about 67–68% identity with CGRMV (Zhang et al., 1998). The genomic sequence of an American isolate of CRMV (WEN) originally obtained from Wenatchee Washington with characteristic leaf symptoms of severe CRMV, has been also fully determined and is 8401 nucleotides in length (Rott et al., 2004). CRMV shares about 70% nucleotide sequence identity with CNRMV and about 68% identity with CGRMV. Up until 2004, CNRMV was considered a tentative member of the foveaviruses along with CGRMV. Both viruses are now unassigned species in the family *Betaflexiviridae* (Adams et al., 2004), and so too by association, CRMV. The genomic organizations of these viruses are identical (Fig. 26.1) with 7 open reading frames (ORFs). ORF1 codes for the replication-associated protein with conserved methyltransferase, helicase and RNA dependent RNA polymerase domains. Three ORFs comprise the conserved triple gene block (TGB), thought to be involved

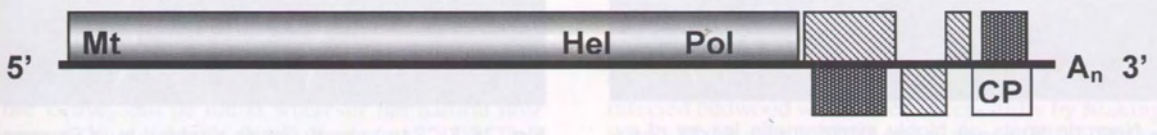

Fig. 26.1. Genomic organization of CNRMV and CRMV. The replication gene codes for a protein with the motifs, Methyltransferase (Mt), Helicase (Hel) and RNA-dependent RNA polymerase (Pol). The triple gene block proteins have a light pattern. Cp, coat protein. Nested, alternative reading frame have a dark pattern.

in viral transport/movement. A fifth ORF codes for the viral coat protein (CP). Unique to CNRMV, CRMV (and CGRMV) are two nested ORFs in alternative reading frames, one within the first TGB ORF, and the second within the CP ORF. The functions of these two ORFs are unknown and are the least conserved between the viruses. This genomic organization is unique within the *Betaflexiviridae* and could perhaps form a new genus of this family.

Economic Impact

There is little recent data on the current economic significance for any of these viral diseases. CNRMV: Virus-infected trees can exhibit reduced growth, significant yield losses and early death of trees (Posnette et al., 1968). Posnette and Cropley (1964) reported the disease to be widespread and prevalent in English orchards with low productivity in the 'Frogmore,' 'Florence,' and 'Noble' cultivars. In Utah it was observed that part of the buds and leaf spurs were killed, resulting in bare, rangy branches that were killed in more advanced stages of the disease (Wadley, 1959). The cultivars 'Lambert,' 'Sam,' 'Seneca,' and 'Hudson' had the most severe reaction and most trees declined.

CRMV (*American*): Virus-infected trees decline, with die-back of main branches and limbs. Fruits are small, flavor is insipid and ripening is delayed. Decline is slower and fruit size is less affected with mild rusty mottle compared to severe rusty mottle (Wadley, 1959). Montmorency sour cherry trees that were graft inoculated were affected by leaf symptoms (Wadley and Nyland, 1976).

CRMV (*European*): Studies from England showed reduced growth on virus-infected Mazzard F 12/1 rootstock by 23% and a reduction in yield of 25% on infected common sweet cherry cultivars (Posnette and Cropley, 1956; Posnette and Cropley, 1961). With high incidence of the disease (35% of sweet cherry cultivars) known to have been present in England (Posnette et al., 1968), potential economic impact is significant.

Disease Symptoms

Leaf symptoms for necrotic rusty mottle and rusty mottle of diseased plants can be highly variable and dependent on climatic conditions, virus isolate and plant cultivar. A cool spring followed by a hot summer can often result in more severe symptom expression. It is likely that the variability in symptoms contributed to the earlier confusion regarding the naming of these diseases, and continues to cause problems with accurate disease identification.

CNRMV: Classic symptoms on virus-infected sensitive cultivars consist of a few to many brown angular necrotic spots on leaves appearing 3–6 weeks after bloom. Highly symptomatic leaves are usually cast early. Centers from remaining leaves with larger spots can drop out giving a shot-hole appearance (Fig. 26.2). Smaller spots can have a purplish coloring. In the fall, leaf senescence occurs earlier with leaves turning yellow along the veins. In later stages of infection, bark symptoms in the form of shallow necrotic areas, general bark necrosis, deep gum pockets and shallow gum blisters can appear. Bare branches can also be observed in older infected trees, due to dying of infected new buds and leaf spurs in the spring or as a result of die back in response to reduced winter hardiness. Infected trees have a greater tendency to be killed by frost damage (Nemeth, 1986; Wadley and Nyland, 1976). After graft inoculation of European strains on the woody indicator 'Sam', the shot-hole symptom often remains localized to some branches (W. Jelkmann, *unpublished observation*).

CRMV (*American*): Virus-infected plants show a light green or yellow mottling first appears on the small basal leaves 4–5 weeks after full bloom, developing into bright yellow, brown or red late-season coloring, with leaves which are soon shed. On remaining leaves, chlorotic mottling soon appears. With mild rusty mottle, some later bronzing of the leaves occurs. With severe rusty mottle, fall senescence coloring and leaf drop occurs early (Nemeth, 1986). In older infected trees decline and die-back occurs and is more pronounced with severe as compared to mild rusty mottle.

CRMV (*European*): On virus-infected sensitive cultivars, symptoms first appear on mature leaves in July, with a clearing followed by a yellowing of the tertiary and quaternary veins. Leaves have a duller, pale green color, which turn yellowish-green later in the year with rusty mottling along yellow veinlets. Extent of mottling is dependent on the disease isolate and cultivar, and can range from a yellow or pale rusty color to dark reddish purple (Fig. 26.3 and 26.4). No conspicuous leaf symptoms are induced on many cultivars (Nemeth, 1986; Posnette et al., 1968).

Host Range

Sweet cherry (*Prunus avium*) is the main natural host, however some other cherry or *Prunus* species can be infected. Sour cherry (*P. cerasus*), *P. mahaleb* seedlings, Manchu

Fig. 26.2. Necrotic spots on highly symptomatic leaves of cv. 'Sam' with some drop out spots giving a shot-hole appearance, caused by *Cherry necrotic rusty mottle virus* (CNRMV); healthy at right.

Fig. 26.3. 'Sam' cherry leaves affected by a European strain of *Cherry rusty mottle virus* (CRMV) showing rusty mottling along chlorotic veinlets; healthy at right.

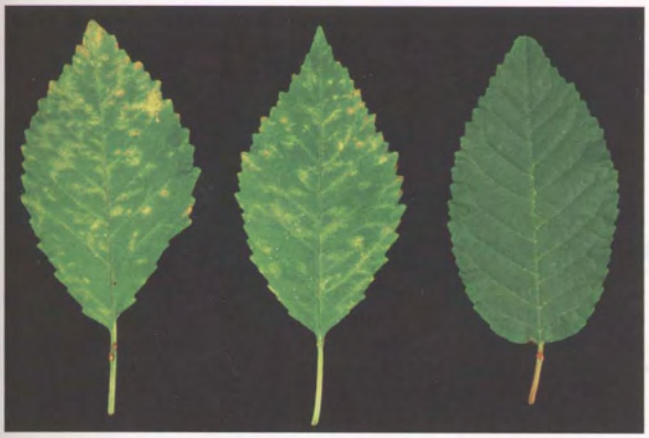

Fig. 26.4. Mazzard F 12/1 rootstock leaves affected by a European strain of *Cherry rusty mottle virus* (CRMV) showing light green or yellow mottling and yellowing of veins; healthy at right.

cherry, peach (*P. persica*), apricot (*P. armeniaca*), prune plum (*P. domestica*), and damson plum are sensitive to rusty mottle virus.

"Lambert mottle virus" could be inoculated to sweet cherry cultivars and *P. serotina*. The disease agent could not be recovered from inoculated trees of Montmorency sour cherry, *P. mahaleb, P. serrulata* Shirofugen flowering cherry, *P. virginiana* choke cherry, *P. serotina* black cherry, and *P. tomentosa* nanking cherry. NRMV caused a symptomless infection on *P. armeniaca* (Nemeth, 1986; Wadley and Nyland, 1976).

In more recent research ten different *Prunus* species were graft inoculated with a strain of *Cherry necrotic rusty mottle virus* (CNRMV). Symptoms were observed on sensitive sweet cherry indicators and on *P. tomentosa*. Virus was recovered by RT-PCR from the following symptomless plant species: *P. armeniaca, P. dulcis* cv. 'Peerless,' *P. mahaleb, P. persica,* and *P. serrulata*. PCR results were negative for *P. cerasifera, P. mandshurica,* and *P. salicina* cv. 'Shiro' (Li and Mock, 2008). None of these viruses has been transferred to herbaceous hosts.

Transmission

There are no known vectors for CRMV or CNRMV and there is no evidence of seed or pollen transmission. Slow spread to nearby trees has been observed in the field for CNRMV and CRMV (American) (Cameron and Moore, 1985; Nemeth, 1986). The diseases are readily transmitted by grafting and budding but not mechanically by sap transmission (Wadley and Nyland, 1976). Susceptible cultivars with mild or no visible symptoms can play an important role in maintenance and spread through grafting and budding.

Geographical Distribution and Epidemiology

For both viruses there have been reports from nearly all continents. Most of the reports were from North America, Europe, Asia and New Zealand (Nemeth, 1986; Wadley and Nyland, 1976). With the availability of complete or partial sequences of viruses associated with the diseases it seems obvious that strains of the viruses can be found wherever the natural host plants are grown (Li and Mock, 2008; Rott et al., 2004; Rott and Jelkmann, 2000, 2001a). Using these sequence data and various primer combinations provided from this research, there has been an increasing amount of reports for these viruses from countries not reported in the literature before 2000.

The closely related viruses CNRMV, CRMV and *Cherry green ring mottle virus* (CGRMV) were all first reported in western North America (Nemeth, 1986). It is possible that cherry breeding programs in this area helped to spread these viruses to other countries, since the viruses can be symptomless on certain cultivars. In this respect, it is interesting to note that many North American isolates maintained at the Sidney Laboratory in Canada, are often mixed infections (*unpublished data*).

In Europe certification programs exist in a number of countries that include production and trade of healthy plant propagating material. The European and Mediterranean Plant Protection Organization (EPPO) has drafted certification schemes recommending the necessary methods to select or generate virus free nuclear stock plants using thermotherapy and to maintain their health status during propagation (EPPO, 2001).

Detection

Most common indicator plants (EPPO, 2001; Jelkmann, 2004; Nemeth, 1986): For CNRMV, *Prunus avium* cv. 'Sam;' for CRMV (*American*): *Prunus avium* cv. 'Bing;' for CRMV (*European*): *Prunus avium* cv. 'Mazzard F12/1.' There are no commercially available antibodies for serologial testing of CNRMV or CRMV. However, more recent molecular data for CNRMV and CRMV has made it possible to develop general and specific reverse transcription polymerase chain reaction (RT-PCR) assays for these viruses. Foissac et al. (2005) developed a polyvalent nested RT-PCR using degenerate primers containing inosine for screening plants for a wide range of viruses including CNRMV. Using this assay the authors were able to detect CNRMV in three French isolates of cherry necrotic mottle leaf disease. These results were confirmed using a more specific set of primers (NEG1U/L) to CNRMV (Rott and Jelkmann, 2001a), which were used also to confirm the presence of CNRMV in a further 6 of 6 isolates of CNRM in Europe. Li and Mock (2005), using primers NCPh/c, were able to detect strains of CNRMV in symptomless flowering cherry from Japan.

Primers to CRMV (ERMUP/LO) could detect CRMV in 6 of 7 European isolates of CRM (Rott and Jelkmann, 2001a), but could also amplify the virus from several isolates of CNRM, CGRM and symptomless control trees. In this study, primers NEG1U/L amplified also isolates of CRM. These results suggested that the viral agents associated with these diseases were closely related, or, that the trees were mix-infected. Subsequent experiments (M. Rott, *unpublished data*) using these and other primer pairs, in combination with sequence analysis of the amplified fragments, showed that many of these and other isolates from America previously identified with the CRM or CNRM disease are mix-infected. As part of this study, no evidence could be found that there were significant differences between viral agents associated with European and American CRM diseases, however, this is based on limited sequence data of only a few hundred base pairs.

Control

Use of virus-free propagation material and prompt removal of infected trees are the most effective means of limiting spread (EPPO, 2001). Heat therapy has given mixed results. CNRMV infected budwood was treated successfully by soaking in warm water 10–13 min at 50°C and 5 min at 52°C (Nyland, 1959). CRMV (American) could not be eliminated after 4 weeks air treatment at 38°C (Wadley and Nyland, 1976).

REFERENCES

Adams, M. J., Antoniw, J. F., Bar-Joseph, M., Brunt, A. A., Candresse, T., Foster, G. D., Martelli, G. P., Milne, R. G., and Fauquet, C. M. 2004. The new plant virus family Flexiviridae and assessment of molecular criteria for species demarcation. Arch. Virol. 149:1045-1060.

Cameron, H. R., and Moore, D. L. 1985. Reduction in spread of necrotic rusty mottle with removal of affected trees. Phytopathology 75:1311.

Desvignes, J. C. 1999. Virus Diseases of Fruit Trees. (Diseases Due to Viroids, Viruses, Phytoplasmas and Other Undetermined Infectious Agents). Centre Technique Interprofessionnel des Fruits et Legumes (CTIFL), Paris.

EPPO. 2001. Certification scheme for cherry. EPPO Bull. 31:447-461.

Foissac, X., Svanella-Dumas, L., Gentit, P., Dulucq, M. J., Marais, A., and Candresse, T. 2005. Polyvalent degenerate oligonucleotides reverse transcription-polymerase chain reaction: A polyvalent detection and characterization tool for trichoviruses, capilloviruses, and foveaviruses. Phytopathology 95:617-625.

Gentit, P., Foissac, X., Svanella-Dumas, L., Peypelut, M., Macquaire, G., and Candresse, T. 2002. Molecular characterization of foveaviruses associated with the cherry necrotic mottle leaf disease and complete sequencing of an European isolate of cherry green ring mottle virus. Arch. Virol. 147:1033-1042.

Jelkmann, W. 2004. International working group on fruit tree viruses - Detection of virus and virus-like diseases of fruit trees - Laboratory assays, bioassays, and indicators. Acta Hortic. 657:575-596.

Li, R., and Mock, R. 2005. An improved reverse transcription-polymerase chain reaction (RT-PCR) assay for the detection of two cherry flexiviruses in *Prunus* spp. J. Virol. Methods 129:162-169.

Li, R., and Mock, R. 2008. Characterization of a flowering cherry strain of Cherry necrotic rusty mottle virus. Arch. Virol. 153:973-978.

Lott, T. 1945. "Lambert Mottle," a transmissible disease of sweet cherry. Sci. Agric. [Ottawa] 25:776-779.

Lott, T. B., and Keane, F. W. L. 1961. Host range of virus of Lambert Mottle of cherry, a progress report. Plant Dis. Rep. 45:204-207.

Martelli, G. P., Adams, M. J., Kreuze, J. F., and Dolja, V. V. 2007. Family Flexiviridae: a case study in virion and genome plasticity. Ann. Rev. Phytopathol. 45:73-100.

Nemeth, M. 1986. Virus, mycoplasma and rickettsia diseases of fruit trees. Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht; Boston; Lancaster.

Nyland, G. 1959. Hot-water treatment of Lambert cherry budsticks infected with Necrotic rusty mottle virus. Phytopathology 49:157-158.

Posnette, A. F. 1951. Virus diseases of sweet cherries. Ann. Rep. E. Mall. Res. St. A 34:209-210.

Posnette, A. F., and Cropley, R. 1956. Virus diseases of cherry trees in England. II. Growth suppression caused by some viruses. J. Hortic. Sci. 31:298-302.

Posnette, A., and Cropley, R. 1957. A canker disease of cherry trees caused by virus infection. Plant Pathol. 6:85-87.

Posnette, A. F., and Cropley, R. 1961. European rusty mottle disease of sweet cherry. Ann. Rep. E. Mall. Res. St. A 44:85-86.

Posnette, A. F., and Cropley, R. 1964. Necrotic rusty mottle virus disease of sweet cherries in Britain. Plant Pathol. 13:20-22.

Posnette, A. F., Cropley, R., and Swait, A. A. J. 1968. Incidence of virus diseases in English sweet Cherry orchards and their effect on yield. Ann. Appl. Biol. 61:351-360.

Reeves, E. 1940. Rusty-mottle, a new virosis of cherry. Phytopathology 30:789.

Reeves, E. L., and Richards, B. L. 1946. A rusty mottle-like virus disease of the sweet cherry in Utah. Phytopathology 36:409.

Rhoads, A. 1945. Symptom expression of rusty mottle in Utah sweet cherry orchards. Plant Dis. Rep. 29:613-614.

Richards, B., and Rhoads, A. S. 1945. Rusty mottle of the sweet cherry in Utah. Farm Home Sci. 6:6-8, 11.

Rott, M. E., and Jelkmann, W. 2000. Complete nucleotide sequence of cherry necrotic mottle virus. Phytopathology 90:67.

Rott, M. E., and Jelkmann, W. 2001a. Characterization and detection of several filamentous viruses of cherry: adaptation of an alternative cloning method (DOP-PCR), and modification of an RNA extraction protocol. Eur. J. Plant Pathol. 107:411-420.

Rott, M. E., and Jelkmann, W. 2001b. Complete nucleotide sequence of cherry necrotic rusty mottle virus. Arch. Virol. 146:395-401.

Rott, M. E., Johnson, V. L., and Belton, M. P. 2004. Characterization of a new foveavirus associated with cherry rusty mottle disease. Phytopathology 94:S89.

Stout, G. 1948. Permanent surveys for plant diseases. Bull. Dep. Agric. Calif. 37:25-28.

Stout, G. 1949. Cherry bark blister. Bull. Dep. Agric. Calif. 38:257-260.

Wadley, B. 1959. Rusty mottle virus complex in Utah. Phytopathology 49:114.

Wadley, B. N., and Nyland, G. 1976. Rusty mottle group. Pages 242-249 in: Virus Diseases and Noninfectious Disorders of Stone Fruits in North America. R. M. Gilmer, ed. USDA, Washington, D.C.

Zeller, S. M., and Milbrath, J. A. 1947. Mild rusty mottle of sweet cherry (*Prunus avium*). Phytopathology 37:77-84.

Zhang, Y. P., Kirkpatrick, B. C., Smart, C. D., and Uyemoto, J. K. 1998. cDNA cloning and molecular characterization of cherry green ring mottle virus. J. Gen. Virol. 79:2275-2281.