

Cherry green ring mottle virus

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Introduction

The disease, cherry green ring mottle (CGRM), was first reported in 1937 affecting sour cherry (*Prunus cerasus*) trees in Michigan, U.S.A. and described as a viral disease in 1951 (Rasmussen et al., 1951). Rough bark and cherry vein yellow spot diseases, are known to be caused by the same viral agent (Kegler, 1968; Milbrath, 1960). *Cherry green ring mottle virus* (CGRMV) infects several *Prunus* species, including sweet cherry (*P. avium* L.), sour cherry (*P. cerasus* L.), oriental flowering cherry (*P. serrulata* L.), peach (*P. persica* (L.) Batsch), nectarine (*P. persica* var. *nectarine*), and apricot (*P. armeniaca* L.). The disease has been found after indexing of symptomless sweet cherry trees in New York, Wisconsin, Oregon, and Washington in the United States and in British Columbia and Ontario, Canada as well as in several European countries, Africa, New Zealand, and Japan (Gentit et al., 2002; Isogai et al., 2004; Parker et al., 1976; Rasmussen et al., 1951; Rouag et al., 2008; Sipahioglu et al., 2008). The complete nucleotide sequence of the CGRMV genome was determined after cDNA cloning of double-stranded RNA template extracted from infected peach leaves. Sequence and genome structure comparisons with other filamentous viruses showed that CGRMV was most similar to *Apple stem pitting virus* (Zhang et al., 1998). CGRMV is an unassigned species in the family *Betaflexiviridae* (Adams et al., 2005). Serological assays were developed using antisera raised from purified virus particles (Zagula et al., 1989) or chimeric fusion coat protein expressed in *E. coli* (Zhang et al., 1998). However, due to the limited performance of the obtained antisera in ELISA assays, virus detection by RT-PCR is currently the laboratory method of choice for routine purposes (Zhang and Uyemoto, 2008).

Taxonomic Position and Nucleotide Sequence

The complete genomic sequence of CGRMV was determined from an isolate originating from 'Shirofugen' flowering cherry in California, and inducing typical green ring mottle symptoms on 'Kawazan' flowering cherry (Zhang et al., 1998). The complete sequence of a European isolate of CGRMV, identified in a cherry tree displaying symptoms of the cherry necrotic mottle leaf disease, was determined by Gentit et al. (2002). The two CGRMV sequences share an overall nucleotide sequence similarity of 83%. The genome of CGRMV is 8,372 nt in length, excluding a 3' poly (A) tail, and harbors open reading frames (ORFs) coding for a replicase protein, a triple gene block (TGB), the coat protein (CP), and two small additional proteins of unknown function. Currently, CGRMV is

listed as an unclassified species of the Family *Betaflexiviridae* (Adams et al., 2004, 2005) and is most closely related to *Cherry necrotic rusty mottle virus* (CNRMV) and *Cherry rusty mottle virus* (CRMV), which are also unclassified species of the *Betaflexiviridae*. The genomic organization of the three viruses is unique compared with other members of the *Betaflexiviridae*, with ORFs nested and in alternative reading frames within the first TGB and CP ORFs, and may represent ultimately a new genus. Overall nucleotide sequence identity to CNRMV and CRMV is about 67%. Additional phylogenetic analysis indicates that these viruses are most similar to *Apple stem pitting virus* (genus *Foveavirus*) and close to members of the *Carlavirus* and *Potexvirus* genera (Adams et al., 2004; Martelli and Jelkmann, 1998).

Economic Impact and Disease Symptoms

Among cultivated cherry trees, green ring mottle disease is most important in the sour cherry cultivar 'Montmorency,' with infected fruit often not marketable. The virus can be found in several commercially grown cultivars of sweet cherry, but whether there are any associated economic losses is still unknown. However, there is some suggestion that infected sweet cherry trees are slightly stunted compared to healthy ones (Parker et al., 1976). Leaf symptoms, consisting of green islands and rings cast against a yellow mottle background, are ephemeral in nature, appearing 4–6 weeks after petal fall and lasting 2–3 weeks. Leaf lamina develops a chlorotic constriction along midribs and major veins. Fruits are misshapen and fruit flesh is pitted, necrotic, and bitter in taste. The green and yellow leaf symptoms appear in the 'Duke' cherry (*P. cerasus* × *P. avium*) cultivars 'Late Duke,' 'May Duke,' and 'Reine Hortense' (Parker et al., 1976). Symptoms also occur in English 'Morello' sour cherry (leaves with chlorotic constrictions) and in Japanese flowering cherry (*P. serrulata* cvs. 'Kwanzan' and 'Shirofugen'), which show characteristic twisting and curling of leaves (Fig. 23.1) with mild to severe leaf vein necrosis and epinasty, shoot stunting, roughen and cracked bark, stem pitting, and terminal dieback of shoots, and in mazzard cherry trees as stem pitting (J. Uyemoto, unpublished data). Overall, latent infections of CGRMV are the norm in most *Prunus* species. For example, the virus is symptomless in several cultivars of sweet cherry (*P. avium* cvs. 'Bing,' 'Lambert,' 'Black Republican,' 'Napoleon,' and 'Deacon'); in peach ('Halehaven,' 'Sunhaven,' 'Richaven,' 'Glohaven,' 'Suncling,' and 'Rio Oso Gem'); nectarine; apricot; Nanking cherry (*P. tomentosa*); and the cherry rootstock, *P. mahaleb*. Moreover, infections in sweet



Fig. 23.1. Oriental flowering cherry 'Kwanzan' infected with *Cherry green ring mottle virus* (CGRMV) showing characteristic twisting and curling of leaves; healthy leaves on the left.

cherry and peach trees are reported as commonplace. Several unpublished observations by P. R. Fridlund on the influence of virus strains, environmental conditions, and different cherry cultivars on symptom expression have been reviewed by Parker et al. (1976).

Host Range

Natural infections of CGRMV occur on sour cherry (*Prunus cerasus*), oriental flowering cherry (*P. serrulata* L.), sweet cherry (*P. avium* L.), and several other *Prunus* species, including peach (*P. persica*), nectarine (*P. persica* var. *nectarine*), and apricot (*P. armeniaca* L.). The virus has been transmitted experimentally to *P. mahaleb* and *P. tomentosa* but could not be graft transmitted to almond (*Prunus amygdalus*) (Parker et al., 1976; Zhang et al., 1998). CGRMV was not mechanically transmitted to any of the herbaceous plants tested, including *Nicotiana tabacum* L. cvs. Xanthi n, Xanthi nc, Turkish, and Havana 425; *N. benthamiana* Domin.; *N. glutinosa*; *N. clelandii*; *N. occidentalis* Wheeler 'No.1' and '37B'; *Chenopodium quinoa* Willd.; *C. amaranticolor* Coste & Reyn.; *Cucumis sativus* L. cv. National Pickling; *Lycopersicon esculentum* Mill.; *Gomphrena globosa* L.; and *Cucurbita maxima* cvs. Buttercup and Butternut squash.

Transmission

CGRMV has no known vector and is not seed-transmitted (Fridlund, 1966; Gilmer and Brase, 1962). The virus is transmitted by common vegetative propagation practices, such as grafting and by root grafts between healthy and diseased trees (Barksdale, 1959; Milbrath, 1966). Attempts to transmit the virus mechanically to herbaceous hosts have been unsuccessful in different studies (Parker et al., 1976; Zhang et al., 1998).

Geographical Distribution and Epidemiology

It has been suggested that CGRMV originated from western North America, since CGRMV was initially associated almost exclusively with cherry clones originating from this area. Many cultivars of sweet cherry and peach are universally infected, which would indicate that the original sources were infected or that these cultivars were grafted onto infected rootstocks. CGRMV infects cultivated *Prunus* species worldwide. The virus is introduced onto new sites solely by planting infected

stocks. From there, secondary spread, involving healthy trees in closest proximity to infected ones, may occur through root graft transmissions.

Detection

In biological assays, CGRMV is reliably detected on 'Kwanzan' and 'Shirofugen' flowering cherry trees. Diagnostic leaf, shoot, bark, and stem symptoms develop in 2–3 months post-inoculation. Serology-based detection assays have been developed by Haufler and Ramsdell (1989), Zagula et al. (1989), and Zhang et al. (1998), but are limited in scope due to paucity of commercially available antiserum.

More recently, molecular assays have been developed (Li and Mock, 2005; Rott and Jelkmann, 2001; Zhang et al., 2000; Zhang and Uyemoto, 2008) for large scale assays. The RT-PCR assays are sensitive, rapid, and reliable. General and specific primer pairs have been developed to detect and identify various CGRMV strains. There is no indication of irregular distribution of the virus in the tree. Different protocols have been published for effective extraction of total RNAs prior to PCR avoiding inhibition of enzyme reactions by high levels of phenolic or polysaccharide compounds (Rott and Jelkmann, 2001; Zhang and Uyemoto, 2008). Such extractions can also be achieved by commercially available RNA extraction kits.

Control

Use of virus-free propagation material and prompt removal of infected trees are the most effective means of limiting spread (EPPO, 2001). Infected trees in production orchards may be destroyed to avoid spread via root contact.

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- Rebenstorf et al. (2006). Since then numerous hosts have been recorded including its wide natural host range which includes 17 genera of woody plants and a variety of herbaceous plants. Some of the most reported and common natural hosts of CLRV are common hick species (*Betula* sp.), black elderberry (*Sambucus nigra* L.), English walnut, and sweet cherry. The virus has been detected worldwide for example, throughout Europe, the former USSR, North America, Chile, New Zealand, and Japan (Jones 1986).
- CLR V belongs to the *Nepovirus* genus within the family *Secoviridae* (Wellink et al., 2000). Unlike the majority of other members of this genus, CLR V is not considered to be transmitted by nematodes. However, reliable investigations on nematode transmission are still lacking. CLR V belongs to the subgroup C of the nepoviruses, which are characterized by a large RNA-2 with a long (1.3-1.6 kb) 3' non-coding region (3' NCR) which is almost identical to that of RNA-1 (Borge et al., 1995). The bipartite, single-stranded, positive-sense RNA genome is estimated to be about 13 kb, with RNA-1 and RNA-2 at about 5 and 7 kb, respectively. Both RNAs are encapsidated separately in isometric particles 28 nm in diameter (Jones, 1986). Rebenstorf et al. (2006) assessed the serological and molecular diversity of CLR V using a collection of isolates and samples recovered from woody and herbaceous host plants from different geographical origins. Serological and molecular phylogenetic reconstructions were strongly correlated. Remarkably, the diversity of CLR V is defined, to a large extent, by the host plant from which the viral samples were originally obtained.
- Taxonomic Position and Nucleotide Sequence**
- Family: *Secoviridae*; genus: *Nepovirus*; species: *Cherry leaf-roll virus* (CLR V). CLR V is an established species within the genus *Nepovirus* that belongs to the family *Secoviridae*. In accordance with the currently acknowledged criteria for classification within this family, CLR V consists of two single-stranded RNAs, encapsidated separately in isometric, non-enveloped particles, measuring 28 nm in diameter (Wellink et al., 2000). Both particles are required for infectivity (Jones and Dunstan, 1980). Genomic RNAs, each coding for a polyprotein, have a genome encoded protein (VPg) covalently linked at their 5' end and are polyadenylated at the 3' terminus (Jones and Mayo, 1972; Walkey et al., 1973; Helfen and Cooper, 1987). The full length sequences of the CLR V genomic RNAs
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- Economic Impact and Disease Symptoms**
- As CLR V is transmissible by seed, it is a threat to genebank contamination. Such CLR V-contaminated propagative material is of major importance for human-mediated propagation and dispersal. Latent virus contamination of mother plantations also has to be taken into consideration. Therefore, CLR V is included in the list of plant viruses that should be closely monitored during sanitary production of propagation material, especially for walnut and olive trees (Hass and Martelli, 2003).
- Kegler et al. (1972) reported on crop losses in sour cherry (*Prunus cerasus* L.) of 91 to 98%. In Italy approximately 5% of the tested olive trees grown in areas in which national and local olive tree cultivars and selections are grown were CLR V infected. The percentage of infection by CLR V in olive in Italy was similar to that in Spain (Aggioli et al., 2005). CLR V