Virus and Virus-Like Diseases of Pome and Stone Fruits

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CHAPTER 24

Cherry leaf roll virus

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Introduction

Cherry leaf roll virus (CLRV) was reported for the first time in 1933 in English walnut (*Juglans regia* L., Schuster and Miller, 1933) and sweet cherry (*Prunus avium* L.; Posnette and Cropley, 1955). Since then numerous hosts have been recorded revealing 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 birch 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).

CLRV belongs to the *Nepovirus* genus within the family *Secoviridae* (Wellink et al., 2000). Unlike the majority of other members of this genus, CLRV is not considered to be transmitted by nematodes. However, reliable investigations on nematode transmission are still lacking. CLRV belongs to the subgroup C of the nepoviruses, which are characterized by a large RNA-2 with a long (1.2–1.6 kb) 3′ non-coding region (3′ NCR), which is almost identical to that of RNA-1 (Borja et al., 1995). The bipartite, single-stranded, positive-sense RNA genome is estimated to be about 15 kb, with RNA-1 and RNA-2 at about 8 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 CLRV 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 CLRV 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* (CLRV). CLRV 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, CLRV consists of two single-stranded RNAs, encapsidated separately in icosahedral, non-enveloped particles, measuring 28 nm in diameter (Wellink et al., 2000). Both particles are required for infectivity (Jones and Duncan, 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; Hellen and Cooper, 1987). The full length sequences of the CLRV genomic RNAs have not yet been determined. The sizes of RNA-1 and RNA-2, estimated by denaturing electrophoresis of viral RNA preparations, ranged between 7.02–8.2 (RNA-1) and 6.33–6.8 (RNA-2) kilobases (Murant et al., 1981, Pallas et al., 1991). CLRV belongs to the subgroup C of nepoviruses. Species within this cluster are characterized by a large RNA-2 with a 3′ NCR which is identical or nearly identical to that of RNA-1 (Scott et al., 1992; Borja et al., 1995). The 3′ NCR region is among the longest known for nepoviruses as estimated for six CLRV isolates exhibiting lengths between 1,557 and 1,602 nucleotides (Langer et al., 2010). Although no evidence of transmission by nematodes or other animal vectors has been found to date, genome organization of CLRV seems to be in accordance with that of *Tobacco ringspot virus* (TRSV), the type species of the nepoviruses. The CLRV genome codes for a single coat protein (CP), the coding sequence of which is located 3′ proximal of the putative movement protein sequence on genomic RNA-2. Coat protein sequences for one birch isolate and for four walnut isolates of CLRV, respectively, have been published (Scott et al., 1993, Zhou et al., 1998). Comparison of this CP coding region with additional sequences obtained from seven CLRV isolates varied between 1,539 and 1,542 nucleotides in length (Langer et al. 2007), but substantiated the serological relationships found by Rebenstorf et al. (2006). Additionally, a 719 bp fragment of the coding region of viral RNA-1 for a virus isolate originating from ash (*Fraxinus excelsior*) has been sequenced by Maliogka et al. (2004). Phylogenetic comparison of the derived amino acid sequence, corresponding to part of the viral RNA-dependent RNA polymerase (RdRP) with other taxa of the picorna-like plant viruses, supported the taxonomic classification of CLRV within the genus *Nepovirus.*

Economic Impact and Disease Symptoms

As CLRV is transmissible by seed, it is a threat to genebank contamination. Such CLRV-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, CLRV 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 (Bassi 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 CLRV infected. The percentage of infection by CLRV in olive in Italy was similar to that in Spain (Faggioli et al., 2005). CLRV

infection correlates with the death of grafted English walnut scions (*Juglans regia*) propagated on rootstocks of Northern California black walnut (*J. hindsii*), 'Paradox' hybrids (*J. hindsii* × *J. regia*), Chinese wingnut (*Pteriocarya stenoptera*) or other *Juglans* spp. (Mircetich and Rowhani, 1984; Németh et al., 1990; Grant and McGranahan, 2005). The infection remains symptomless in seedlings of many cultivars of *J. regia*. However, if the virus invades the graft union of suitable graft combinations, it induces tissue necrosis at the scion-rootstock junction, known as blackline or brownline, which eventually girdles and kills the tree (Mircetich et al., 1980; Rowhani and Mircetich, 1988). The potential monetary value of developing effective measures of CLRV in walnut is indicated by annual losses due to blackline disease of 3% of the total California crop, as well as a 13% loss in coastal orchards in this state (Brooks and Bruening, 1995).

A rapid decline over a one or two year period was described by Németh (1986) when CLRV-infected cherry trees were mix-infected with *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV). Furthermore, the germination rate of seeds obtained from those trees was reduced by 20 to 50%, when compared with seeds from healthy trees (Löw, 1995). According to Cooper and Massalski (1984), seedlings and cuttings from naturally infected birch trees grow less rapidly than their healthy counterparts. They also reported that 35% of the hardwood cuttings from healthy trees became established under mist propagation whereas only 20% of the cuttings from CLRV-infected trees survived. The annual increment of CLRV-infected seedlings cultivated under greenhouse conditions was half that of healthy birch seedlings.

Referring to the production of wood as raw material and to the ecological value of forests, the extent of losses due to virus infection is difficult to calculate as there is a strong impact of other stress factors in forest stands in a long period between sowing in nurseries and wood harvesting. In any case, it is suggested to prevent virus dispersal by using clean planting material for the production of wood and to sustain the function of forest as recreational, cultural forest parks and suburban forests.

CLRV symptoms vary according to plant species, virus strain, and season, and have been summarized by Bandte and Büttner (2001). For instance, CLRV-infected birch, elderberry, dogwood, and blackberry show yellow vein netting, chlorotic ringspots, mottling, and leaf roll (Fig. 24.1). Dieback is often observed in CLRV-infected sweet cherry, birch, and blackberry. Susceptible walnut leaves may develop chlorosis, discolored rings, or arabesks. Blackline disease affects only English walnuts grafted onto non-*regia* rootstocks, and causes terminal shoot dieback in some cases. Chlorotic leaf mottling and spotting is associated with CLRV infection in European beech (*Fagus sylvativca* L.) whereas chlorotic spots, ringspots, and line patterns are induced in European ash (*Fraxinus excelsior*

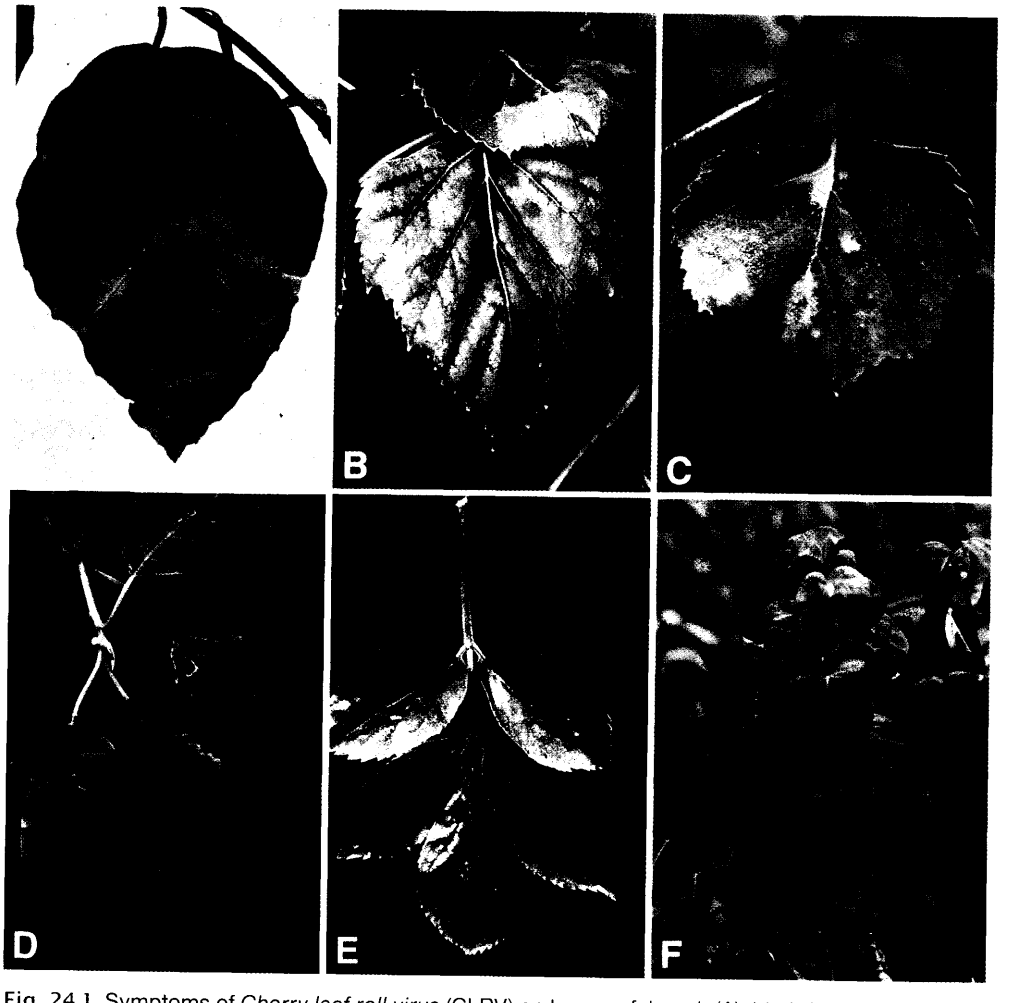

Fig. 24.1. Symptoms of *Cherry leaf roll virus* (CLRV) on leaves of: beech (A), birch (B and C) showing chlorosis and ringspots, respectively, buckthorn (D), elderberry (E), and dogwood (F).

...). CLRV-infected rhubarb (*Rheum rhaponticum* L.), hoary allison (*Berteroa incana* L.), delphinium (*Delphinium elatum* ...), and bitter dock (*Rumex obtusifolius* L.) remain symptomless (Harris et al., 2002).

Investigations over the last four years confirm the countrywide presence of virus-like symptoms in birch species in Finland (Büttner, *unpublished data*). Leaves exhibited chlorotic ringspots, leaf roll, and a loss of vigor. Initial tests confirmed CLRV infection in 17 out of 20 samples taken in the Rovaniemi region close to the Arctic Circle (Jalkanen et al., 2007). It was shown that CLRV is widely distributed in *B. pendula* and *B. pubescens* throughout the country. Furthermore, dwarf birch, mountain birch, Kiilopää birch, and curly birch were confirmed to be previously unknown hosts of CLRV (von Bargen et al., 2009).

Host Range

Table 24.1 shows the wide natural host range of CLRV, which includes 17 genera of woody plants and a range of herbaceous hosts.

Natural herbaceous hosts include rhubarb (*Rheum rhaponticum* L.; Tomlinson and Walkey, 1967) garlic (*Allium tuberosum* Rottler ex. Spreng; Yamashita and Fukui, 2004), delphinium (*Delphinum elatum;* Ahmed and Bailiss, 1975), broad-leaved dock (*Rumex obtusifolius* L.), and hoary allison (*Berteroa incana* (L.) DC.; Jones, 1985).

The experimental host range includes more than 36 plant families (Jones, 1985).

Table 24.1. Host range of CLRV.

Host range (species)	Reference
Birch (*Betula* spp.)	Schmelzer, 1972a; Cooper and Atkinson, 1975; Rebenstorf et al., 2006; Jalkanen et al., 2007; von Bargen et al., 2009; Buchhop et al., 2009; Bandte et al., 2009
Hornbeam (*Carpinus betulus* L.)	Rebenstorf et al., 2006
Dogwood (*Cornus florida* L.)	Waterworth and Lawson, 1973
Spindle (*Euonymus europaeus*)	Larsen et al., 1990
Beech (*Fagus sylvatica* L.)	Winter and Nienhaus 1989
Ash (*Fraxinus excelsior* L.)	Nienhaus and Hamacher, 1990, Ford et al., 1972
Walnut (*Juglans* spp.)	Cooper and Edwards, 1980; Mircetich et al., 1980; De Zoten et al., 1982; Rowhani et al., 1985; Buchhop et al., 2009
Privet (*Ligustrum vulgare* L.)	Schmelzer, 1972b; Bandte and Buttner, 2001; Obermeier et al., 2003
Olive (*Olea europaea* L.)	Savino and Gallitelli, 1981
Cherry (*Prunus* spp.)	Posnette and Cropley, 1955, Cropley, 1960; Cropley, 1961, Schimanski et al., 1975a; Schimanski et al., 1975b
Hoptree (*Ptelea trifoliata* L.)	Schmelzer, 1972b
Buckthorn (*Rhamnus frangula* L.)	Werner et al., 1997
Blackberry and raspberry (*Rubus* spp.)	Cropley, 1961; Cropley and Tomlinson, 1971; Jones and Murant, 1971; Jones and Wood, 1978
Elderberry (*Sambucus* spp.)	Schmelzer, 1966; Schmelzer, 1972b; Jones and Murant, 1971, Buchhop et al., 2009
Mountain ash (*Sorbus aucuparia* L.)	Rebenstorf et al., 2006
Lilac (*Syringa vulgaris* L.)	Novak and Lanzova, 1975
Elm (*Ulmus americana* L.)	Varney and Moore, 1952; Jones and Murant, 1971, Schmelzer, 1972b
Grape vine (*Vitis vinifera* L.)	Herrera and Madariaga, 2001

Transmission

Not all modes of CLRV transmission have been investigated and described in the same detail. Most studies have been on the seed and pollen transmission of CLRV. The seed transmission rate of CLRV is highly variable and depends on whether male or female gametophytes originate from a CLRV-infected tree (Cooper, 1993). CLRV adheres loosely to the surface of anemophilous pollen such as that of birch or walnut and strongly to entomophilous pollen such as that of cherry (Massalski and Cooper, 1984). Up to 22% of seeds collected from open pollinated and naturally infected birch trees carried virus that was transmitted to progeny seedlings (Cooper 1976). Pollen germination is presumably required for virus transmission. Furthermore, CLRV can be introduced into the embryo, multiplied within the embryo, and distributed through seeds from infected birch trees (Cooper et al., 1984). In olive, CLRV was detectable in 90% of the seeds obtained from virus-infected trees, and the rate of seedling infection was 41% (Saponari et al., 2002).

CLRV is transmissible by mechanical inoculation (Nienhaus et al., 1990) and by grafting (Jones, 1986). Schmelzer (1966) showed that a CLRV isolate from *Sambucus racemosa* was pathogenic to 62 species in 24 families. The author found most of the virus-susceptible plants in the families *Chenopodiaceae, Compositae,* and *Solanaceae.* Plants known to be susceptible to many different plant virus species, such as *Amaranthus caudatus, Datura stramonium,* and *Lycopersicum esculentum,* were shown to be resistant to the virus. Horvath (1979) added 34 other plant species susceptible to the virus and 23 plants in eight families which seemed to be resistant.

Rumbou et al. (2009) provided a model system to study infectivity and seed transmission of CLRV in *Arabidopsis thaliana* (L.) Heynh. Transmissibility of CLRV by seed in *A. thaliana* was shown by virus detection in two consecutive generations grown from seeds of infected plants. The results indicated that genetically diverse CLRV isolates have different capability to be vertically transmitted in *A. thaliana.*

Detailed information on transmission by nematodes and insects is missing. Although nematode transmission has been postulated for CLRV due to its taxonomic status in the *Nepovirus* genus, it has not yet been confirmed (Wang et al., 2002). CLRV has been detected by RT-PCR in the seed-feeding bug *Kleidocerys resedae* (Werner et al., 1997). Potentially, insects may therefore occasionally contribute to the infection of plants through wounds via contaminated pollen.

Investigations by Bandte et al. (2007) showed that CLRV is easily transmitted by water. The results were obtained when pots with CLRV-infected *Chenopodium quinoa* were grown in hydroculture. Virions were released from roots of infected plants and transmitted through the nutrient solution. Healthy plants were infected within three weeks. Under natural conditions, this type of transmission has not been reported for CLRV and, if it happens, it would probably be less efficient than in greenhouse experiments, due to an abated infection pressure.

The recent detection of CLRV in birch trees above the Arctic Circle (latitude 66° 34′ N) brings up new questions on the transmission and fast spread. The contaminated pollen by melting water has to be verified and further evaluated.

Geographical Distribution and Epidemiology

CLRV occurs throughout Europe, North America, Chile, the former USSR, China, Lebanon, Syria, Australia, and New Zealand (Jones, 1985; Herrera and Madariaga, 2001; Fadel et al., 2005; Al Abdullah et al., 2005; Jalkanen et al. 2007). By

analyzing the genetic diversity of CLRV isolates Rebenstorf et al. (2006) reported a strong relationship between CLRV genetic diversity and the original host plant species. The geographical origin of the isolates was found to be of minor influence on their phylogenetic affinities. This grouping is explainable by either host specialization of the CLRV isolates or by the existence of ecological transmission barriers which limit or altogether prevent host change. Pollen transmission could potentially represent such a barrier, which could result in rapid genetic isolation of viral variants within given host populations and, over time, result in evolutionary divergence of these separate virus populations. By comparing host plant origin and phylogenetic affinities of a range of isolates, these authors showed that the genetic isolation of host-specific CLRV variants is partial and not complete with some CLRV isolates appearing to have the ability to infect a broad range of potential hosts. Such isolates might be transmitted with contaminated pollen through wounds by means of insect vectors. Milder winter climates due to climate change will enable a longer period in which viruses may spread and infect plants.

The distribution of CLRV is mainly driven by pollen and by human movement of infected seeds or plants. To date, CLRV, a quarantine pest in *Rubus* in the EPPO region, has only been found in a few plants in England, the Czech Republic, and Slovenia in these hosts (CABI/EPPO, 1997). In the case of *Rubus*, crops, transmission by pollen from imported *Rubus* to local plants or propagation of imported *Rubus* would be the practical means of establishment of CLRV in the EPPO region.

Detection

CLRV can be detected by biological assays, either in the form of mechanical transmission to herbaceous indicator plants (such as *Chenopodium quinoa, Cucumis sativus,* and *Nicotiana* spp.), or in some cases to woody plants in their early physiological stages (such as diverse birch species, sweet cherry, and blackberry) by graft transmission. Attention should be paid to the long latent period of several months to a few years that may be needed in some hosts for symptom development (Nienhaus and Castello, 1989). For indexing of *Prunus* material, the peach seedling indicator GF305 shows rosetting and slight leaf rolling after being chip-budded with CLRV-infected *Prunus* spp. (Diekmann and Putter, 1996).

Electron microscopy has been applied for studies of size and structure of the particles, detection and identification of the virus in infected plants, and investigations on cellular changes caused by virus infection (Rubio Huertos et al., 1985; Nilsson and Tomenius, 1987). Histological investigations are useful to understand alterations due to the CLRV-infection process and in virus replication, but cannot be applied to virus diagnosis. A number of different techniques have been established to detect and identify virus particles in suspensions or in thin sections (Dijkstra and de Jager, 1998a, b). Lesemann (1982) reported that the detection by immunosorbent electron microscopy (ISEM) is in many cases as sensitive as the enzyme-linked-immunosorbent assay (ELISA), which is limited to 0.1–10 ng/mL. The detection of CLRV in leaf tissue of woody plants by this approach may, however, be hampered by a low virus titer.

The reliability of enzyme-linked immunosorbent assays depends on the homogenous virus titer in the tested plant. For instance, in cherry, the period of reliable serological testing in routine surveys is extendable by using the emergent flower and leaf tissue (Torrance, 1981). Therefore, dormant budsticks can be cut in midwinter, stored at 4°C, and forced to sprout just before starting the assays. In walnut, reliable detection of CLRV requires a sample size of at least 80 g of male catkins from each tree to balance the uneven and erratic distribution of the pathogen within infected trees (Rowhani et al., 1985).

Studies by Jones et al. (1990) and Rebenstorf et al. (2006) have demonstrated significant serological variation between CLRV isolates. Depending on the serological assay and antiserum used, not all CLRV isolates are detectable using a given antiserum in DAS-ELISA or related methods. This is evident in the investigations of Gentkow et al. (2007), who showed that only 11 out of 19 CLRV isolates characterized by Rebenstorf et al. (2006) were detectable by a polyclonal antiserum raised against an elderberry CLRV isolate, whereas all isolates were detected by molecular methods.

The high sensitivity of molecular methods in comparison with serological techniques facilitates the detection of viruses in soil, water, vectors, mixed samples, and samples taken during early stages of infection, even with a small amount of sample material or a low virus titer. To date, several polymerase chain reaction (PCR)-based protocols have been described for the detection of CLRV in woody hosts of economic importance. Reverse transcription PCR is applied either after extraction of total RNAs from plant tissue (Fadel et al., 2005; Faggioli et al., 2005) or after direct binding of viral particles to reaction tubes (Rowhani et al., 1995). Nested PCR can also be used to increase sensitivity (Pantaleo et al., 2001). Furthermore, serological methods can be combined with molecular techniques, such as in the Immunocapture-RT-PCR developed by Werner et al. (1997), which was further optimized for the screening of large numbers of samples by Gentkow et al. (2007) and Jalkanen et al. (2007). This protocol is suitable for the detection of the virus in leaves, buds, or fruit tissues and can be successfully applied to isolates originating from walnut, birch, and black elderberry. It can also be used for all other characterized CLRV isolates, including strains from sweet cherry and from herbaceous plants. According to phylogenetic clades, a method for differentiation of CLRV isolates by a RFLP assay was developed by examining restriction patterns of the partial 3′ NCR (approx. 420 bp) genomic fragments. The method was successfully applied in an IC-RT-PCR-RFLP assay to differentiate samples from walnut, black elderberry, and birch and determine their genetic relations. In the future, this method will facilitate quick estimation of phylogenetic cluster of CLRV isolates detected in certain host plants by the universal IC-RT-PCR (Gentkow, 2010). It is also suitable for study of CLRV population diversity as well as investigation of genetic drift within virus populations (Buchhop et al., 2009).

Although more cumbersome and probably not as sensitive, dot-blot-hybridization (Borja and Ponz, 1992; Mas et al., 1993) has also been applied to CLRV detection, particularly when a fast and simple test is desired. Simple detection tools are desirable to support breeders with fast and cheap means of detection for selection of plant and seed material. Such a tool may be achieved by developing a lateral flow test.

Control

CLRV seems to be distributed mainly by movement of infected plants and seeds, or by pollen. Pollen transmission gives the virus the potential for rapid dissemination, making its control difficult. Water transmission of CLRV in ecosystems also has to be taken into consideration as a potential cause of spread.

Regularly spaced pollenizer rows of walnut trees are recommended by Polito et al. (2005) to supply uninfected pollen at elevated levels, in order to out compete potentially infected pollen from outside the cropping system. This strategy is based on

their investigations showing an influx of walnut pollen from sources outside the orchard. Therefore, an infection by CLRV-inducing blackline disease may occur even in orchards that are free of the virus if the virus is prevalent in neighboring orchards or in the surrounding environment.

Early detection followed by seed eradication, sanitary selection, and the use of virus-free certified planting material is the most important integrated strategy used to prevent the spread of the disease. An example of a virus-free certification scheme was presented recently for olive trees and rootstocks by EPPO (EPPO, 2006). The scheme provides detailed guidance on the production of propagated cultivars to be grown on their own roots, of vegetatively propagated or seedling rootstocks and of grafted trees.

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