CHAPTER 22

Cherry Detrimental Canker

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Introduction

Viral twig necrosis of sweet cherry was discovered in the former Czechoslovakia in 1955 and first described by Blattny (1962) under the name "cherry detrimental canker." The disease is characterized by necrosis along the shoot tip, which develops later into bark splits, stunting of the shoot, and sideways bending of the tip. Leaf twisting is also caused by necrosis of the midvein and of some lateral veins. Fruits are misshapen with sunken, target-like, necrotic spots (Kunze et al., 1983).

Tombusviruses were isolated regularly from diseased trees and were first described as *Tomato bushy stunt virus* (TBSV) or as strains of this virus (Albrechtova et al., 1975). Similar symptoms in cherry associated with TBSV were observed in Canada by Allen and Davidson (1967) and described as "cherry fruit pitting" disease. In more detailed serological studies, all cherry isolates investigated were identified as *Petunia asteroid mosaic virus* (PeAMV) (Koenig and Kunze, 1982). This included isolates from former Czechoslovakia, Canada (Hollings and Stone, 1975), the former German Democratic Republic (Gruentzig et al., 1989), and a Swiss isolate. Similarly, viral isolates detected in apple, pear, plum, and sour and sweet cherry that originally were described as TBSV were later identified as PeAMV (Kegler and Kontzog, 1990). Thus, all TBSV isolates from *Prunus avium* investigated in detail have been shown to be PeAMV. Within the infected sweet cherry trees the virus is unevenly distributed and mainly found in plant parts with symptoms (Albrechtova et al., 1980; Allen and Davidson, 1967; Fuchs et al., 1988). Back transmission experiments (Albrechtova and Kudela, 1982) were used to fulfil Koch's postulates by inducing symptoms of viral twig necrosis in cherry seedlings inoculated with purified virus suspensions. In diseased sweet cherry trees in northern Bavaria (Germany) where PeAMV had been isolated, later research identified a second tombusvirus, *Carnation Italian ringspot virus* (CIRV) (Lesemann et al., 1989; Pfeilstetter et al., 1992).

Taxonomic Position and Nucleotide Sequence

The three tombusviruses associated with cherry detrimental canker were originally members of the "tombusvirus group" (Harrison et al., 1971) and belong now to the genus *Tombusvirus* in the familiy *Tombusviridae* (Lommel et al., 2005). *Tomato bushy stunt virus* is the type species of the genus *Tombusvirus*. The genomic RNA consists of 4,776 nucleotides and contains four ORFs (Szittya et al., 2000; Hearne et al., 1990). ORF1 encodes a 32–36 kDa protein. By readthrough of the ORF1 termination codon, a 92–95 kDa protein is expressed (ORF1-RT), which is the viral RNA polymerase. The internal ORF2 encodes the coat protein. Two nested ORFs (ORF3 and ORF4) located at the 3′ terminus of the genome encode the p22 and p19 kDa proteins, respectively. The p22 protein has a role in symptom induction and is required for cell-to-cell movement. The p19 protein is a suppressor of post-transcriptional gene silencing. It has a role in the systemic spread of the virus, and is involved in the development of necrotic host response to infection (Lommel et al., 2005).

The genomic RNA of CIRV consists of 4,760 nucleotides and has an organization identical to that reported for other tombusviruses (Rubino et al., 1995). Only partial genomic sequences are reported in databases (NCBI) and the literature for PeAMV (Choi et al., 1998; Koenig et al., 2004).

Economic Impact and Disease Symptoms

The disease causes heavy damage on affected trees and is therefore regarded of economic significance. Loss in marketable fruit can vary between 5 and 30% (Hansen and Yorston, 1975). Before 1985, viral twig necrosis was observed only sporadically in northern Bavaria and was considered to be unimportant. Since then, severe outbreaks were found in three large cherry orchards with more than 500 trees affected in each. Subsequent surveys identified the disease in 33 cherry orchards with a total number of about 450 diseased trees. Incidence of infected trees in orchards ranged from 3 to more than 50% (Pfeilstetter et al., 1992). In these investigations, diseased trees were always infected with tombusviruses, either PeAMV or CIRV. Mixed infection with these two viruses occurred rarely and can be clearly differentiated serologically. CIRV causes the same symptoms in cherry trees, but the damage induced by this virus is normally less than that caused by PeAMV (Pfeilstetter et al., 1996). It should be noted that the severe detrimental canker symptoms described on cherry cv. 'Kastanka' in the former Czechoslovakia (Blattny, 1962) appears to have been of a complex character and was due to a combined effect of TBSV and of a strain of *Pseudomonas syringae* (Novak and Lanzova, 1977).

Symptoms can be observed in all parts of the tree. The most pronounced symptoms of the disease are bark splitting, stunting of shoots and sideways bending of their tips, and a tight clustering of distorted leaves (Figs. 22.1–22.3). The sharp twist sideward and downward is caused by necrosis of midribs and main vein of leaves (Fig. 22.1). Symptoms appear on leaves usually in the spring, but can also be observed later in the year. Fruit setting is reduced by extensive flower abortion. Most cultivars produce malformed fruit with necrotic spots and sunken and circular pits (Fig. 22.4).

Fig. 22.1. Severe twisting of leaf, necrosis of midvein and of some lateral veins, fruit with necrotic spots caused by PeAMV in cherry; healthy controls are shown on the left.

Fig. 22.2. Bark and shoot necrosis of a cherry twig infected with PeAMV.

Host Range

The three tombusviruses TBSV, PeAMV, and CIRV that infect cherry have a very wide host range extending to more than 20 families. Although not completely up-to-date, one very helpful source listing the host range of the three tombusviruses is the ICTV Descriptions and Lists from the VIDE Database (http://micronet.im.ac.cn/vide/sppindex.htm).

TBSV has *Gomphrena globosa* (L), *Chenopodium amaranticolor* (L), *Nicotiana glutinosa* (L), *N. clevelandii* (W), and *Datura stramonium* (W) as assay hosts showing either local lesions (L) or symptoms on whole plants (W). Susceptible host species of TBSV are: *Antirrhinum majus, Capsicum annuum, C. frutescens, Celosia argentea, Chenopodium album, C. amaranticolor, C. murale, C. quinoa, Cucumis sativus, Cucurbita maxima, C. pepo, Datura stramonium, Dianthus barbatus, Gomphrena globosa, Gypsophila elegans, Hyoscyamus niger, Lavatera trimestris, Lycopersicon esculentum, Malus* spp., *Nicotiana benthamiana, N. bigelovii, N. clevelandii, N. debneyi, N. glutinosa, N. rustica, N. tabacum, Ocimum basilicum, Petunia × hybrida, Phaseolus vulgaris, Physalis floridana, Pyrus* spp., *Solanum melongena, S. nigrum, S. tuberosum, Spinacia oleracea, Tetragonia tetragonioides, Tolmiea menziesii, Tulipa* spp., *Vicia faba, Vigna radiate, Vigna unguiculata,* and *Zinnia elegans.*

Compared to TBSV, the literature reports fewer hosts in several plant families for PeAMV and CIRV. This, however, is expected to be attributed to less research on the host range of these two other viruses.

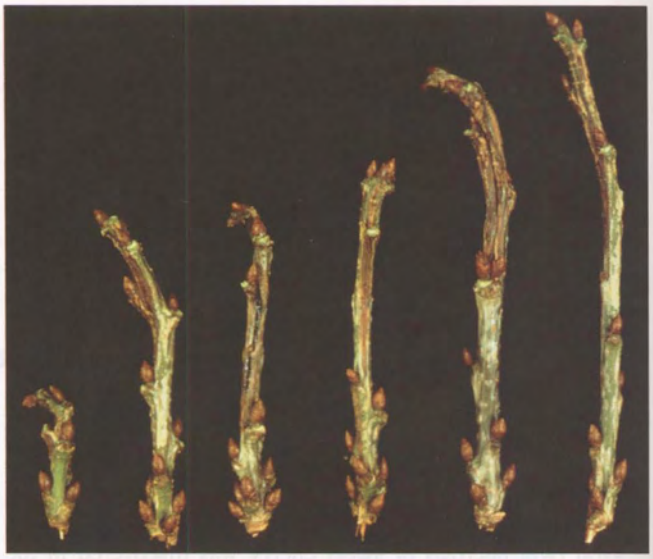

Fig. 22.3. Cherry shoot tips infected with PeAMV and showing necrosis, stunting and sideways bending.

Fig. 22.4. Malformed cherry fruit infected with PeAMV with necrotic spots, sunken and circular pits, and necrosis of stalk; healthy control on the left.

Transmission

All three tombusviruses can be transmitted by mechanical inoculation and grafting. A natural vector for transmission is not known. Seed transmission of TBSV is known to occur at a low rate in apple (Allen, 1969; Kegler and Schimanski, 1982) and at high rate in tomato (Tomlinson and Faithful, 1984), but was not observed in cherry (Kegler and Schimanski, 1982). Although PeAMV was detected by ELISA in *Prunus avium* seeds at high rates, seedlings were not infected with the virus (Pfeilstetter, 1992). Infective tombusviruses can be found in rivers and lakes (Koenig and Lesemann, 1985) and water was suggested to be a source of spread in cherry orchards in northern Bavaria. However, experiments with cherry seedlings planted in soil to which PeAMV was added repeatedly with the irrigation water were inconclusive. Herbaceous plants in virus-infected orchards were tested for the presence of PeAMV and CIRV by ELISA. With PeAMV, the infection rate of *Chenopodium quinoa* was 30% and that of *Lapsana communis* more than 50%. CIRV was identified in samples of *Fragaria vesca, Geranium pusillum, Lapsana communis, Sonchus asper,* and *Taraxum officinale.* In artificial experiments, *Nicotiana clevelandii* seedlings were

infected after planting in sterile soil to which PeAMV was then added with irrigation water. Therefore, it seemed likely that apart from transmission of the viruses by grafting, there is a considerable risk of infection for cherry orchards through the soil and water (Pfeilstetter, 1992).

Geographical Distribution and Epidemiology

Apart from initial reports of viral twig necrosis of sweet cherry in the former Czechoslovakia, other observations of the disease were reported from Canada (Allen and Davidson, 1967; Hansen, 1975), Switzerland (Schmid, 1968), the former German Democratic Republic (Gruentzig et al., 1989; Kegler and Kegler, 1981), and from northern Bavaria in Germany (Kunze et al., 1983).

In Canada, the disease was of limited distribution in orchards of Ontario. The affected trees of the cultivar Windsor were 25–30 years old and were propagated on a mahaleb rootstock. Spread of the disease was not obvious and it remained unknown whether the virus originated from the propagation material or came in through other sources as discussed under transmission. Before severe outbreaks were found in Germany in the mid 1980s, the disease was considered to be unimportant with only a few sporadic reports. Field observations in severely affected orchards in northern Bavaria suggested that there were different means of spread. In some orchards, the disease was limited to a few scattered infected trees, indicating that the virus might have been introduced via infected plant material. Infected rootstocks were also suggested to have contributed to the dissemination of the disease, because older orchards had been completely or partly established on root suckers or seedlings of mazzard (*Prunus avium* L.) collected in neighboring forests. Spread of viral twig necrosis to neighboring trees was observed in some orchards with a high incidence of the disease (Pfeilstetter et al., 1992) indicating natural transmission.

Detection

The tombusviruses TBSV, PeAMV, and CIRV can be detected in ELISA using specific antisera. CIRV is serologically distinct from PeAMV. TBSV and PeAMV are serologically related but classified as distinct species (Koenig et al., 2004). These tombusviruses can be detected reliably in cherry in leaves, fruits, twig-tips, and bark containing symptoms whereas they were found only rarely in samples without symptoms taken from the same tree (Albrechtova and Kudela, 1982; Allen and Davidson, 1967; Fuchs et al., 1988). In leaves with symptoms, the detection of PeAMV was restricted primarily to samples from the necrotic half of the lamina. Uneven distribution of PeAMV was also found in roots and flowers that sometimes exhibit necrotic lesions on the peduncle (Fig. 22.5). In buds of severely affected trees, ELISA positive results were reported between 5 and 58% at bud-burst. ELISA readings were generally high in virus-positive samples (Pfeilstetter et al., 1997). The various results on ELISA detection of the tombusviruses in cherry indicate that, due to the extremely uneven distribution of the viruses in all parts of diseased trees, reliable detection can only be expected from symptomatic tissue.

Recommended woody indicators for the detection of cherry detrimental canker are 'Bing' or 'Sam' with three replications and an observation period of 2 years in field tests (EPPO, 2001). For the same reason as virus detection using ELISA, symptomless infections in cherries cannot be detected reliably by tests with woody indicators. This was confirmed by grafting scions of diseased trees to healthy rootstocks of which only few trees developed symptoms within a period of three years (Pfeilstetter et al., 1996). For detection of PeAMV and CIRV by herbaceous indicators, *Chenopodium amaranticolor* displaying whitish necrotic dots with chlorotic halos or *C. quinoa* showing chlorotic local lesions are recommended. *Cucumis sativus* may also be used (EPPO, 2001). *Nicotiana clevelandii* and *N. glutinosa* show chlorotic or necrotic local lesions and systemic mottle and necrosis.

Fig. 22.5. Sweet cherry flowers infected with PeAMV showing necrosis of pedicels; healthy control on the left.

Although reports on using RT-PCR for the detection of the tombusviruses associated with cherry detrimental canker are lacking, it can be expected that this technology should follow the same principles as for other viruses from cherry tissue. In recent research, RT-PCR was developed for tombusvirus-infected vegetables, fruits, seeds, and seedlings (Chang et al., 2005). Based on multiple alignments of several tombusviruses including TBSV, a primer pair was developed that successfully detected the viruses from total plant RNA extracts. A method to detect TBSV in a multiplex PCR test together with two nepoviruses and a plant internal amplification control has been developed for strawberry tissue (Wei et al., 2008).

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. Because indexing for cherry detrimental canker is not possible at present due to the very uneven distribution of the viruses, plants used for propagation should be regularly examined for disease symptoms. Because the virus distribution is mainly restricted to the symptoms bearing tissues, suspect symptoms can be examined quickly and reliably for the presence of PeAMV and CIRV by means of ELISA.

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