Cherry virus A: cDNA cloning of dsRNA, nucleotide sequence analysis and serology reveal a new plant capillovirus in sweet cherry

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The nucleotide sequence (7383 nucleotides) of a newly identified member of the genus Capillovirus, cherry virus A (CVA), was obtained from cDNA clones. The cDNA was generated from dsRNA extracted from plant tissue infected with little cherry virus (LCV). Small amounts of LCV dsRNA served as template nucleic acid and enabled the construction of a library of which, unexpectedly, 7.5% of the recombinant plasmids were specific for CVA. The genome organization of CVA resembles that of apple stem grooving virus (ASGV), the type member of the genus Capillovirus and is composed of a 266 kDa polyprotein (ORF1), a 52 kDa ORF2 located within ORF1 and a poly(A) tail. The 266 kDa ORF1 contains all the elements of a replication-related protein and has high identity with 'Sindbis-like' viruses. The ORF encodes the coat protein (CP) in the C-terminal region. The 52 kDa ORF2 has high identities with the putative viral cell-to-cell movement proteins of capillo- and trichoviruses. The CP was identified in immunoblot analysis and estimated to have a molecular mass of 24 kDa. Antiserum was obtained by expression of antigens as fusion proteins in Escherichia coli. There is significant sequence identity between CVA CP and the corresponding proteins of other capillo- and trichoviruses. However, no serological cross-reaction was obtained in immunoblot analysis with ASGV, apple chlorotic leafspot trichovirus (ACLSV), apple stem pitting virus (ASPV) and cherry mottle leaf virus (CMLV) antisera. Flexuous filamentous CVA virions were identified in extracts of sweet cherry by immunosorbent electron microscopy (ISEM) and decorated with the antiserum to the fusion protein. CVA was identified in three cherry sources of different disease status by ISEM, immunoblot analysis and hybridization to dsRNA. CVA is not closely related to any of the currently described diseases in cherry but it has all the properties of a capillovirus. It is suggested that CVA should be classified as a new member of the genus Capillovirus.

Introduction

Capilloviruses possess flexuous filamentous particles of ca. 640 × 12 nm, with obvious cross-banding (Bar-Joseph & Martelli, 1991). Apple stem grooving virus (ASGV) is the type member. The ASGV particles contain an ssRNA of 6496 nucleotides, excluding the poly(A) tail, and a coat protein of 27 kDa (Yoshikawa et al., 1992). The only other definitive member is citrus tatter leaf virus (CTLV), but recent data suggest that CTLV should be regarded as a closely related strain of ASGV rather than as a different virus (Ohi et al., 1994). Capilloviruses have morphologically comparable particles to the newly established genus Trichovirus of which apple chlorotic leafspot virus (ACLSV) is the type member. These viruses have been distinguished from closteroviruses (Candresse, 1994; Dolja et al., 1994) by differences in particle length, biological, molecular, physiochemical and cytopathological properties (Martelli et al., 1994). In addition to the complete nucleotide sequences of two ACLSV isolates, three 3'-terminal sequences are available for the definitive trichovirus potato virus T (PVT; Ohi et al., 1992) and the two tentative species grapevine viruses A and B (GVA, GVB; Minafra et al., 1994) respectively. Although capilloviruses and trichoviruses appear to be closely related, the separation into two different genera is supported by their difference in genome organization and strategy of expression (Martelli et al., 1994).

A wide range of virus and virus-like diseases is known to occur in sweet and sour cherry (Gilmer et al., 1976; Németh, 1986). Several of these diseases can be related to formally described viruses. Others are either described only by their symptomatology in cherry or are charac-
alyzed by very little information. The closterovirus-like pathogen associated with little cherry disease belongs to the latter class. Flexuous, virus-like rods 12 nm wide and hexagonal tubules were observed in infected plants by electron microscopy (Raine et al., 1975, 1979) and a high molecular mass dsRNA was extracted from symptom-bearing leaf tissue (Hamilton et al., 1980; Eastwell & Bernardy, 1993). However, currently no antisera to the virus particles or nucleic acid sequence data for the dsRNA are available.

Extraction of dsRNA from plant tissue infected with viruses with ssRNA genomes and the subsequent use of this nucleic acid template for cDNA cloning has been demonstrated to be a useful alternative to the use of ssRNA templates when virion RNA is unavailable (Jelkmann et al., 1989; Coffin & Coutts, 1992). This method has been used to obtain partial (Pappu et al., 1994) or complete virus genomes (Jelkmann et al., 1992b; Jelkmann, 1994) of recalcitrant fruit viruses.

The initial objective of this study was to extract little cherry disease-related dsRNA from plant tissue to enable cDNA cloning. While sequencing the cDNA library, a new capillovirus was detected for which the provisional name of cherry virus A (CVA) is proposed. This paper describes its nucleotide sequence, immunoblot analysis of the coat protein, and particle morphology by immunosorbent electron microscopy. The genome organization and encoded proteins are compared to those of other filamentous plant viruses. The role of the virus as a disease causing agent is discussed in relation to other diseases in cherry.

Methods

Plant material. A 20 g leaf tissue sample from sweet cherry cv. ‘Sam’ grafted onto the rootstock Prunus serrulata ‘Weiroot’ was the original source of material infected with CVA for dsRNA extraction. This material was collected from an orchard in southern Germany and analysed because it displayed symptoms typical of little cherry disease (Welsh & Cheney, 1976). Buds of scions taken from the same tree were grafted onto the rootstock Prunus avium F 12/1 but the material was lost during propagation. The original plant could not be traced back in the orchard. Diseased plants were rogued in the year after samples had been taken.

Other sweet cherry material used in the experiments was from the following: ‘Sam’ on F 12/1 infected with an isolate of little cherry disease and an agent causing stunting of Prunus serulata ‘Shirofugen’ (no. 119/86) (Kunze, 1982). ‘Sam’ on F 12/1 infected with an isolate of necrotic rusty mottle (no. 120/86) (Wadley & Nyland, 1976). The rootstock F 12/1 infected with an isolate of rusty mottle (Wadley & Nyland, 1976) and an agent causing stunting of ‘Shirofugen’ (no. 28/88). All diseases were evaluated by woody indexing on ‘Sam’, ‘Canindex’, ‘Shirofugen’ and F 12/1. Material of cv. ‘Sam’ originating from Switzerland (Wädenswil) was used for negative controls and was confirmed as virus-free on the results of woody indicator tests and the absence of detectable dsRNAs.

Preparation of viral dsRNA and cDNA cloning. The isolation of dsRNA from cherry leaf tissue was as described in detail by Jelkmann et al. (1992a). Synthesis of cDNA from the original CVA dsRNA source material was done as outlined by Jelkmann et al. (1989) but omitting re-extraction from low melting point agarose. Blunt-ended cDNA fragments were cloned into the EcoRV site of Bluescript M13+ (Stratagene). E. coli DH5α-cells were transformed and selected cDNA clones were analysed by small-scale isolation of plasmid DNA and digestion with PstI for determination of insert length followed by nucleotide sequence analysis. A proportion of the genome that was missing after all available cDNA clones had been analysed (nt 4929-5401) was amplified by PCR from dsRNA extracted from source no. 119/86. The dsRNA template, which was invisible in an ethidium bromide-stained agarose gel, was used for cDNA synthesis with oligonucleotide (1) 5’-TTGATTCTGTCCTGCGTACT 3’; nt 5435-5454. An 834 bp PCR product was obtained by amplification using primer (1) and oligonucleotide (2) 5’-GAATCTCAAGCTT-ACTGAG 3’; nt 4621-4641). It was purified by electrophoresis in low melting point agarose, subcloned into the EcoRV site of Bluescript M13+, and designated p834.

Nucleotide sequence analysis. The 14 cDNA clones representing parts of the CVA genome and cDNA clone p834 were sequenced from a total of 185 recombinant plasmids analysed. The sequence reactions were either with dsDNA and a T7 DNA polymerase sequencing kit (Pharmacia) or with Taq DNA polymerase using the Promega fmol cycle sequencing system. To obtain the sequence information from both strands, where convenient, subclones were generated by restriction enzyme digestion and subcloned into Bluescript M13+. Additionally, four virus specific oligonucleotides were used for cycle sequencing. Sequence data were collected, assembled and analysed using the GCG program package version Unix-7.3 (Devereux et al., 1984) and databases of the Heidelberg Unix Sequence Analysis Resources (HUSAR 3.0) (German Cancer Research Centre, Heidelberg). Multiple alignments of amino acid sequences were generated using CLUSTALV (Higgins & Sharp, 1988).

Slot-blot hybridization. dsRNA samples in 50 μl of water were boiled for 5 min and spotted on a nylon membrane. cDNA clone p39 was labelled with [α-32P]dATP (3000 Ci/mmol) using a commercial random prime labelling system (Amersham). Blotting and hybridization details were performed as described in Sambrook et al. (1989).

Expression and purification of viral coat protein. For coat protein expression in E. coli, two constructs were prepared using the prekaryotic pQE Type IV vector (Quiggin). A protein with an Ms of 38214 was obtained after digestion of cDNA clone p39 with HindII (nt 6116) and CloI (MCS) and subcloning of this fragment into Bluescript M13+ which had been cut with Smal and Clal. Subcloning into pQE-31 was then performed by using the BamHI and SalI sites. A protein with an Ms of 29217 was expressed after subcloning of an EcoRI (nt 6333) and SalI (MCS) fragment of cDNA clone p39 into Bluescript M13+ followed by a subcloning into pQE-31 as above. Fusion protein expression including a tag of six histidines and purification on nickel-charged nitrilotriacetic acid agarose (Ni-NTA) were done according to the manufacturer’s instructions.

Antiserum production and serological tests. A rabbit was immunized with ca. 1 mg of a mixture of both chimeric proteins emulsified with Freund’s complete adjuvant. A second and third injection with ca. 0.5 mg protein emulsified in incomplete adjuvant were given 1 week and 2 weeks later. The antiserum was collected 4 weeks after the last injection and designated no. 655. Other antisera used for electroblot immunooassays were: apple stem grooving virus (ASGV) kindly provided by E. Fuchs, Halle, Germany; apple chlorotic leaf spot virus (ACLSV) kindly provided by T. Malinowski, Saanichton BC, Canada; apple stem pitting virus (ASPV) fusion protein antiserum no. 647 (W. Jelkmann, unpublished). Sample preparation of total phenol-soluble proteins.
from cherry leaf tissue and electroblot immunoassay was as previously described (Jelkmann, 1994).

For immunosorbent electron microscopy (ISEM) the crude antiserum no. 655 was diluted 1:1000 in 0.1 M-sodium/potassium phosphate, pH 7.0. Pioloform-carbon coated nickel grids were floated for 5 min on diluted antiserum, rinsed with 2 ml dilution buffer and transferred to leaf samples homogenized in 0.1 M-sodium/potassium phosphate, pH 7.0, 2% PVP 10000, 0.2% sodium sulphite and 0.05% sodium azide and incubated overnight at room temperature. Decoration of particles was for 30 min with antiserum diluted 1:50. The grids were washed with water and stained with five drops of 2% uranyl acetate. The samples were examined using a Zeiss EM 109 T electron microscope.

Results

DsRNA extraction and cDNA cloning

In sweet cherry leaf tissue samples displaying symptoms typical of little cherry disease a prominent dsRNA band of ca. 15 kbp and three faint bands of ca. 9, 8 and 5-5 kbp were detected in agarose gel electrophoresis (data not shown). The dsRNA loaded on the gel was equivalent to 5 g of leaf tissue. The remaining nucleic acid was used for cDNA synthesis and colony filter hybridization. A library of 185 cDNA clones with inserts ranging from 300-3200 bp was obtained. After analysis of all recombinant plasmids by a combination of nucleotide sequencing, restriction enzyme digestion and Southern blot analysis, 14 clones (7.5%) appeared to be specific for the CVA genome (Fig. 1). The results for little cherry disease virus-specific cDNA clones will be presented elsewhere.

After it became apparent that a proportion of the genome was not represented among the cDNA library (nt 4929-5401) cDNA clone p834 was generated using PCR and subcloning of the 834 bp product. As the original source material was unavailable a slot-blot hybridization with cDNA clone p39 (Fig. 1) was carried out to dsRNA samples extracted from the plant sources 119/86, 120/86 and 28/88. One out of the two 28/88 samples and both of source 119/86 gave a clear hybridization signal (data not shown). The latter dsRNA was selected as template for PCR amplification of the 834 bp product.

Nucleotide sequence of CVA RNA and coding regions

The nucleotide sequence of the CVA RNA was determined from the 14 available cDNA clones and the cloned PCR fragment p834. Their position relative to the genome is shown in Fig. 1. Six inserts were identified starting with the same base at the 3' end and containing a poly(A) stretch of between 9–20 nucleotides. The sequence determined totalled 7383 nucleotides, excluding the 3' poly(A) tail (Fig. 2). The sequence has a base composition of 30.8% A, 18.7% C, 20.7% G and 29.8% T. Two one-base ambiguities, which were resolved by sequencing at least one other clone, were found in the original 14 CVA cDNA clones and are denoted with lower-case letters above the sequence. Both have no effect on the amino acid composition. In the 362 overlapping nucleotides of cDNA clone p834 four one-base differences (99% identity), denoted by capital
Fig. 2. For legend see opposite.
letters above the sequence, were identified. Two of them are silent mutations; the other two result in amino acid substitutions indicated by the single-letter code below the letters above the sequence. Two of them are silent mutations; the other two result in amino acid substitutions indicated by the single-letter code below the sequence. Note that the sequence from the 3' end was obtained from a second isolate.

Fig. 2. Nucleotide sequence of CVA cDNA. A(n) indicates the 3' poly(A) tail. The predicted amino acid sequences of the two putative ORFs are shown below the nucleotide sequence. Two one-base ambiguities in the original cDNA clone are denoted by lower-case letters above the sequence. Four one-base differences in cDNA clone p834, obtained from a PCR fragment, are denoted by capital letters above the sequence. The two resulting amino acid substitutions are indicated below the sequence. Note that the sequence from nt 4929–5401 (part of cDNA clone p834) had to be obtained from a second isolate.

CVA (2185–2342) / ASGV (1930–2105) / CTLV (762–937) / ACLSV (98–250)

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Fig. 3. Partial alignment of capilloviruses and ACLSV trichovirus coat proteins. For CVA, ASGV (accession no. D14995) and CTLV (accession no. M13174) the sequence represents 250 amino acids at the C terminus of ORF1. ORF3 of ACLSV (accession no. M31714) encodes the coat protein. Identical residues are indicated by asterisks and those with similar properties by dots. Conserved Arg and Asp residues putatively forming a salt bridge are in bold type.

Amino acid sequence comparisons

Elements typical of viral specific RNA replicases were apparent in alignments of the 266 kDa ORF1 with the respective ORFs of ASGV and ACLSV (alignments not shown). The sequence motifs I–IV of the putative methyltransferase domain of ‘Sindbis-like’ viruses (Rozanov et al., 1992) are located in the N-terminal region (nt 241–798). The six reported motifs in nucleic acid helicases (Habili & Symons, 1989) are located in the central part of ORF1 (nt 5400–6790) encoding a polyprotein of M, 52175 (52 kDa) is located in the —1 frame. The correct location of both initiation codons is supported by a multiple alignment of CVA, ASGV and ACLSV ORFs 1 and CVA, ASGV and citrus tatter leaf (CTLV) ORFs 2 (data not shown). Moreover, both ATG codons are in a favourable context for functional initiation sites according to Kozak (1987).

Using NALIGN (Myers & Miller, 1988) the overall nucleotide sequence identity of both ASGV and ACLSV was 57.6%. The open reading frame 1 (ORF1) (nt 55–7081) encodes a polyprotein of M, 266410 (266 kDa). Within the C-terminal part of this gene ORF2 (nt 5400–6790) encoding a polyprotein of M, 52175 (52 kDa) is located in the —1 frame. The correct location of both initiation codons is supported by a multiple alignment of CVA, ASGV and ACLSV ORFs 1 and CVA, ASGV and citrus tatter leaf (CTLV) ORFs 2 (data not shown). Moreover, both ATG codons are in a favourable context for functional initiation sites according to Kozak (1987).

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Table 1. Relationship between the amino acid capsid sequences of definitive and tentative members of the Capillovirus and Trichovirus genera*

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* Percentages of identical amino acids are above the diagonal; percentages of similar amino acids are below the diagonal. Accession numbers: ASGV, D14995; CTLV-J(apan), D16368; CTLV-L(ily), D14455; ACLSV-M(alus), D14996; ACLSV-P(rinus), M31714; PVT, D10172; GVA, X75433; GVB, X75448.

Table 2. Relationship between the amino acid sequences of putative movement proteins of definitive and tentative members of the Capillovirus and Trichovirus genera*

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* Percentages of identical amino acids are above the diagonal; percentages of similar amino acids are below the diagonal. Database accession numbers are as in Table 1.

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**Fig. 4.** Immunoblot analysis of total phenol-soluble proteins extracted from healthy (lane 1 and 2) and infected (lanes 3–5) sweet cherries of the cv. 'Sam'. Lane 6 was loaded with the chimeric protein of Mr 38,214 expressed in *E. coli*. The blot was probed with CVA 655 chimeric protein antiserum.
Cherry virus A

Fig. 5. (a, b) Flexuous filamentous particles trapped from CVA-infected sweet cherry of the variety ‘Sam’ with a polyclonal antiserum prepared against a chimeric protein of the capillovirus coat protein. (c) Decoration of a particle with the same antiserum diluted 1:50. Bars represent 100 nm.

(Koonin, 1991) are in the region between nt 4081–4776. The highly conserved GDD motif at nt 4579–4587 is indicated in bold type in Fig. 2.

Amino acid alignments with the C-terminal region of ORF1 showed significant identity with the capsid proteins of definitive or tentative members of the Capillovirus and Trichovirus genera. For clarity, Fig. 3 is limited to a partial alignment including the type members of the two genera, ASGV and ACLSV (for genome organization see Fig. 1), respectively and CTLV, which was reported to be a closely related strain of ASGV (Ohira et al., 1994). The Arg and Asp residues conserved in rod-shaped viruses and putatively involved in salt bridge formation (Dolja et al., 1991) are in bold type. In comparisons with eight available coat protein cistrons of viruses and virus strains belonging to the above genera, CVA showed the highest percentage of identical and similar amino acids to capilloviruses (27%) (Table 1). As for all capilloviruses the correct start of the coat protein is currently unknown, the 250 C-terminal residues of ORF1 were included in the alignment. This size was chosen because the $M_t$ of the translation product is in good agreement with the apparent size in SDS-gel electrophoresis of 27 kDa for ASGV and CTLV respectively. When the polyprotein of the capilloviruses was enlarged to 300 amino acids CVA did not cluster differently in a dendrogram of the alignment (data not shown).

The CVA ORF2 protein showed significant identity to the putative movement proteins of ASGV (21.6%) and ACLSV (18.3%) (Table 2). In a dendrogram of the respective proteins of definitive or tentative members of the Capillivirus and Trichovirus genera CVA is most closely related to ASGV and CTLV (data not shown). The alignments did not reveal the Gly-Asp-Ser-Gly sequence found in ASGV (Yoshikawa et al., 1992) that is suggested to indicate a function as a protease.

Antiserum production and immunoblot analysis

As alignments of the C terminus of ORF1 indicated a function as the viral coat protein, two chimeric proteins of $M_t$ 29217 and 38214 were expressed in E. coli. Both proteins were used to produce antiserum CVA 655 which was used in immunoblot analysis. Fig. 4 shows an immunoblot probed with CVA 655 antiserum. Lanes 1 and 2 were loaded with total phenol-soluble proteins extracted from two sources of the cv. ‘Sam’ which were regarded as virus-free and lanes 3–5 with virus sources 28/88, 119/86 and 120/86 respectively. Lane 6 was loaded with the prominent fusion protein band of $M_t$ 38214 and proteins of lower $M_t$ resulting from degradation products or of bacterial origin. A band estimated at 24 kDa was obtained with the three different virus sources but no comparable band was visible with the healthy samples. In repeated experiments the 24 kDa band was not clearly detectable every time but another two sources of the cv. ‘Sam’, also regarded as virus-free, did not show the 24 kDa band. Molecular mass estimation of the 24 kDa band was performed by an immunoblot including the two chimeric proteins of $M_t$ 29217 and 38214 and SDS-6H $M_t$ markers (Sigma) (data not shown). To exclude non-specific reactions and to test
for cross-reactions, parallel blots to those presented in Fig. 4 were probed with polyclonal antisera of ASGV, ACLSV and ASPV, and monoclonal antibodies to CMLV. None of the antisera detected the 24 kDa band and the expression protein of Mr 38214.

**Electron microscopy**

Extremely low numbers of flexuous filamentous particles were trapped from leaf extracts of source 120/86 on grids coated with CVA antiserum 655 (Fig. 5a, b). Subsequent experiments were carried out with this source only, although CVA was identified from other sources by dsRNA hybridization and immunoblot analysis. As the number of trapped particles could not be improved particle length measurements were not done. The particles had a diameter of ca. 12 nm and showed an obvious cross-banding. In decoration tests with the hybrid protein antiserum a moderate reaction was observed (Fig. 5c). All 15 particles observed in this experiment showed a similar degree of decoration.

**Discussion**

A new capillovirus identified in sweet cherry is described by its nucleotide sequence, immunoblot analysis of the coat protein (CP), and particle morphology in immunosorbent electron microscopy (ISEM). Owing to the uncertainty as to whether this virus is related to a disease in cherry it is provisionally named cherry virus A (CVA).

DsRNA templates have been used for cDNA cloning as an alternative to genomic RNA template (Pappu et al., 1994), particularly when virion RNA is unavailable (Coffin & Coutts, 1992; Jelkmann et al., 1992b; Jelkmann, 1994). Pappu et al. (1994) obtained nearly identical results by nucleotide sequencing two sets of cDNA clones generated from genomic RNA and dsRNA of a large portion of citrus tristeza closterovirus.

The identification of CVA as a capillovirus was based on analysis of the virus genome and particle morphology. In genome organization, CVA resembles ASGV, the type member of the genus *Capillovirus* (Yoshikawa et al., 1992). The 266 kDa ORF1 revealed all elements of a replication-related protein and high identity with ‘Sindbis-like’ viruses (Habili & Symons, 1989; Koonin, 1991; Rozanov et al., 1992). As with ASGV, the CP of CVA is found in the C-terminal region of the ORF1 polyprotein. Two isolates of CTLV, the only other definitive member of the capilloviruses (Bar-Joseph & Martelli, 1991), have been partially sequenced (Yoshikawa et al., 1993; Ohira et al., 1994). Owing to the high degree of similarity to ASGV, Ohira et al. (1994) suggested that CTLV should be regarded as an isolate of ASGV rather than as a separate virus. This suggestion is supported by the amino acid sequence identities of the CPs (Table 1) and the putative movement proteins (Table 2). In contrast, CVA shows a more distant relationship. The 250 amino acid section of CVA included in the CP alignment gave similar values for nucleotide sequence identity to ASGV (27%) and ACLSV (24%). However, there is a substantial difference between CVA genome organization and that of the trichoviruses ACLSV (Fig. 1) and PVT, which consist of three slightly overlapping ORFs (Martelli et al., 1994). Grapevine viruses A (GVA) and B (GVB) are currently classified as tentative members of the genus *Trichovirus* but they have an additional open reading frame (ORF4) at the 3' end of the viral genome (Minafra et al., 1994).

For technical reasons 472 nucleotides of the CVA genome had to be obtained from a second virus source by generating cDNA clone p834. The 99 % identity of the overlapping 362 nucleotides with the original source reflects very low heterogeneity in comparison to the two sequenced isolates of ACLSV (German et al., 1990; Sato et al., 1993) which have 80 % nucleotide identity.

The function of the C-terminal region of the ORF1 polyprotein was ascertained by expression as a chimeric protein in *E. coli* and the preparation of antiserum which reacted with the capsid protein in immunoblot analysis and ISEM. In contrast, no cross-reactions were observed in immunoblot analysis with ASGV, ACLSV, ASPV and CMLV antisera.

In ISEM the filamentous virus particles showed the obvious cross-banding typical for capilloviruses and trichoviruses (Bar-Joseph & Martelli, 1991; Martelli et al., 1994) but too few particles were seen to enable their length to be estimated. The low trapping of CVA particles may simply reflect the low concentration and, perhaps, the uneven distribution of virions in infected trees, as reported for other fruit tree viruses, e.g. carnation Italian ringspot (CIRV) and petunia asteroid mosaic (PAMV) tombusviruses (Pfeilstetter et al., 1992).

At present no sweet cherry disease can be related to CVA. The virus was identified in the little cherry diseased source 119/86 by hybridization to dsRNA and immunoblot analysis. But as it was also identified by two different tests in sources 120/86 and 28/88, both of which have not displayed little cherry symptoms on woody indicators, a connection with this disease seems unlikely. This reasoning also suggests that CVA is not related to rusty mottle and necrotic rusty mottle diseases which are present in sources 28/88 and 120/86 respectively, but are absent from source 119/86. Furthermore, a relationship to the ‘Shirofugen’ stunt disease seems unlikely as this symptom was caused by sources 119/86 and 28/88 but not found in source 120/86. These conclusions are made on the assumption that a single virus is responsible for symptom expression in indicator hosts of the diseases discussed. Moreover, it should be noted that a biological
test does not always provide a clear-cut result. A relation to cherry mottle leaf virus (CMLV) is unlikely because a monoclonal antibody to CMLV did not react with CVA in immunoblot analysis. Furthermore, the CP size of 20.5 kDa for CMLV (James & Mukerji, 1993) is not in agreement with the 24 kDa CP estimated for CVA and the 27 kDa CP of ASGV, the type member of the genus Capillovirus (Yoshikawa & Takahashi, 1988). There are several other virus diseases or disorders of cherry with an unknown aetiology (Gilmer et al., 1976; Németh, 1986).

Experiments to index the CVA sources used in this study on a wider selection of woody indicators have been initiated. This may eventually establish an association of CVA with a new or already described disease in sweet cherry.

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