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# Natural infections of tomato by *Citrus exocortis viroid, Columnea latent viroid, Potato spindle tuber viroid* and *Tomato chlorotic dwarf viroid*

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**Abstract**

Since 1988, viroids have been occasionally detected in samples of tomato (*Lycopersicon esculentum*) originating both in the Netherlands and other countries. Infected plants showed chlorosis, bronzing, leaf distortion and growth reduction. Initial diagnosis of these viroids was by return-polyacrylamide gel electrophoresis, which did not allow a further identification. This paper reports the identification of these viroids by reverse transcription-polymerase chain reaction and sequence analysis. Three known viroids of tomato, i.e. *Citrus exocortis viroid*, *Potato spindle tuber viroid* and *Tomato chlorotic dwarf viroid* were identified. In addition, six isolates were identified as *Columnea latent viroid*, a viroid so far only detected in some ornamental species. Like the isolates previously isolated from ornamental species, the isolates from tomato share genetic characteristics of both the genera *Hostuviroid* and *Pospiviroid*. The biological characteristics of all four viroids, especially their potential effects on both potato (*Solanum tuberosum*) and tomato, stress the need for reconsideration of their phytosanitary risks.

## Introduction

Viroids are the smallest known pathogens of plants, classified in a distinct group of subviral agents. They consist of a single-stranded circular RNA molecule, whose length varies between 246 and 399 nucleotides. *In vitro* viroids are rod shaped because of internal base pairing between the nucleotides. Viroids differ from viruses as they lack a protein shell; in addition, their small genomes do not encode for any protein. Viroids are classified into two families, the *Avsunviroidae* and the *Pospiviroidae*, consisting of two and five genera, respectively (Flores et al., 2000). Within the latter family five species from the genus *Pospiviroid* have been isolated from naturally infected tomato plants, i.e. *Citrus exocortis viroid* (CEVd; syn. Indian tomato bunchy top viroid, Mishra et al., 1991; Fagoaga and Duran-Vila, 1996), *Potato spindle tuber viroid* (PSTVd, Leontyeva, 1980; Puchta et al., 1990), *Tomato apical stunt viroid* (TASVd, Walter et al., 1980; Walter, 1987), *Tomato chlorotic dwarf viroid* (TCDVd, Singh et al., 1999) and *Tomato planta macho viroid* (TPMVd, Galindo et al., 1982).

Since 1988, the Dutch Plant Protection Service has occasionally detected viroids in diagnostic samples from tomato crops (*Lycopersicon esculentum*) in the Netherlands and from abroad. Although the severity of symptoms varied, infected plants showed chlorosis, bronzing, leaf distortion and growth reduction (Figure 1a). Symptoms were most severe for isolate 89001013, which additionally caused a reddening and purpling of the leaves (Figure 1b). Infection rates varied from only a limited number of plants up to almost 100% infection. Spreading usually occurred along the rows, indicating that contact during crop handling was the main way of transmission. All infections in the Netherlands were subsequently eradicated.


*Figure 1.* Viroid symptoms at naturally infected tomato: (a) chlorosis, leaf distortion and growth reduction in the top of a tomato plant infected by isolate 95001530 and (b) chlorosis, purpling, leaf curl and growth reduction in a plant infected by isolate 89001013.

The presence of the viroids in these symptomatic tomatoes was established by return-polyacrylamide gel electrophoresis (r-PAGE, Huttinga et al., 1987; Roenhorst et al., 2000). However, their identity could not be established by electrophoresis alone. Only the first isolate found in 1988 was additionally sequenced and identified as PSTVd (Puchta et al., 1990). As this isolate (PSTVd-N) differed in sequence from the PSTVd isolates sequenced to date, it was considered a new strain. In 1995 PSTVd-N and eight other viroid isolates from tomato were tested by Agdia (Elkart, IN—USA) by hybridisation with DIG-labelled RNA probes for CEVd, *Chrysanthemum stunt viroid* (CSVd), PSTVd, TASVd and TPMVd. Besides isolate N, only one other isolate from tomato reacted with the PSTVd-specific probe. Four isolates hybridised with a probe for CEVd, and the other isolates did not react with any of the probes. This posed the question whether these isolates belong to another of the known species or represent one or more new viroid species.

This paper describes the characterization of these eight isolates plus five more recently detected isolates by reverse transcription-polymerase chain reaction (RT-PCR) with universal and specific pospiviroid primer sets. Sequence analysis of the amplicons from all thirteen isolates confirmed the positive reactions in the hybridisation tests for PSTVd, but only three of the four positive reactions in the tests for CEVd. Moreover, they revealed the occurrence of TCDVd and *Columnea latent viroid* (CLVd), a viroid not previously reported in tomato.

## Materials and methods

### *Viroid isolates*

Viroid isolates were obtained from tomato samples submitted for diagnosis to the Plant Protection Service in the Netherlands from 1988 up to 2002. Table 1 summarises the tomato varieties affected, the origin of the samples and the estimated infection rates. In addition, PSTVd-H an isolate of PSTVd kindly provided by P. Howell (Scottish Agricultural Science Agency, Edinburgh, Scotland), and PSTVd isolate N (Puchta et al., 1990) were included for reference. After detection by r-PAGE, the isolates were propagated on tomato (see below). Four to five weeks after inoculation young leaves of the inoculated plants were collected and nucleic acids, including viroids, were isolated by a phenol extraction and subsequently stored under ethanol at −20 °C (Roenhorst et al., 2000).

### *Mechanical inoculation and cultivation of tomato and potato plants*

All isolates were mechanically inoculated onto seedlings of tomato 'Money-maker' as soon as the

*Table 1.* Tomato isolates included in this study[a]

| Isolate | Tomato variety | Origin | Infection rate (%) | NCBI accession number |
|---|---|---|---|---|
| 89000808 | Rondella | NL | <1 | AY372390 |
| 89001013 | Blizzard + Turbo | NL | >90 | AY367350 |
| 89002594 | Dombito | NL | <1 | AY372391 |
| 89002600 | Criterium | NL | <1 | AY372393 |
| 93007481 | Cherry Belle | NL | 1–2 | AY372392 |
| 93007908 | Pronto | NL | 1–2 | AY373446 |
| 94005977 | Revido | NL | <1 | AY372394 |
| 95001530 | Trust | NL | <1 | AY372395 |
| 95006685 | Cabrion | NL | not known | AY372396 |
| 96009271 | Durintha | Belgium | <1 | AY365230 |
| 20011470 | Daniela[b] | New Zealand | not known | AY372397 |
| 21008470 | Voyager | NL | <1 | AY372398 |
| 22006456 | Rapsody | USA | 30 | AY372399 |
| H | Potato | – | – | AY372400 |
| N | Not known | NL | – | X17268 |

[a] PSTVd-N and -H were added as controls.
[b] Variety first showing symptoms.

first true leaves appeared. In addition, isolates PSTVd-N, 89000808, 89001013, 93007481, 93007908 and PSTVd-H were transmitted to potato 'Nicola' (*Solanum tuberosum*), by mechanical inoculation of the first leaves emerging after planting of the tubers. The inoculated tomato plants were grown for 4–6 weeks in a greenhouse under quarantine conditions, with a temperature of 25 °C and supplemental illumination for a day length of at least 14 h. Inoculated potato plants were grown under the same conditions until tubers were produced. Plants were inspected visually twice a week. The same six isolates were also used for mechanical inoculation of potato plants cv. Nicola grown in the field under quarantine conditions during two successive years. Ten plants per isolate were inoculated by the end of May and tested by r-PAGE about 2 months after inoculation. In 1994, the tubers formed on the inoculated plants in the greenhouse were planted in the field. For three successive years, the tubers harvested from the field were used for planting in the next season. Planting was by the end of April or the beginning of May, and lifting by the second half of July when the haulms of the certified seed potatoes of grade SE had to be killed. For each viroid isolate, 10 tubers were planted from which the total weight of the newly formed tubers was determined after harvest. Storage during the winter season was at 4 °C.

*RNA isolation, reverse transcription and polymerase chain reaction (RT-PCR)*

For RT-PCR, total RNA was isolated from young tomato leaves (0.5–1.0 g) by using the Pure Script kit (Gentra) according to the manufacturer's instructions. The final RNA pellets were dissolved in 50 µl TE buffer (10 mM Tris, 1 mM $Na_2EDTA$, pH 8.0) of which 1 µl was used in a one-step RT-PCR (Invitrogen). RT-PCR reactions were performed using primers for the detection of CEVd (Önelge et al., 1997), CSVd (Hooftman et al., 1996), CLVd (Spieker, 1996) and PSTVd (Shamloul et al., 1997). In addition, two sets of universal primers were used, designed on the basis of an alignment of pospiviroid sequences, i.e. Pospi1-RE (5′-AGC TTC AGT TGT (T/A)TC CAC CGG GT-3′; complementary to nt 261–283) and Pospi1-FW (5′-GGG ATC CCC GGG GAA AC-3′; identical to nt 86–102), and Vid-RE (5′-CCA ACT GCG GTT CCA AGG G-3′; complementary to nt 336–354) and Vid-FW (5′-TTC CTC GGA ACT AAA CTC GTG-3′; identical to nt 355–16); indicated positions refer to GenBank accession NC_002030 of PSTVd. RT-PCR reactions were performed on a PTC-200 (MJ-Research) thermal cycler programmed for 30 min at 43 °C (cDNA synthesis), 2 min at 94 °C (hot-start activation of Taq polymerase), 30 s at 94 °C (denaturation), 1.5 min at 62 °C (annealing), 45 s at 72 °C

(elongation), for 15 cycles, followed by 30 cycles with an annealing temperature of 59 °C, and a final extension of 7 min at 72 °C. PCR products were analysed by electrophoresis through a 2% agarose gel in TAE buffer containing ethidium bromide (5.0 μg $ml^{-1}$) and visualised on an UV-transilluminator. An 1 kbp ladder (Invitrogen) was used to estimate sizes of the PCR products.

*Sequence and phylogenetic analysis*

Sequence analysis of uncloned PCR products was carried out by Eurogentec DNA-sequencing department. The resulting sequences were compared with viroid sequences in the NBCI Genbank using a BLAST (Altschul et al., 1990), and multiple alignments of related sequences were created using Clustal W (Thompson et al., 1994) as implemented on the GENESTREAM network server (www2.igh.cnrs.fr/bin/align-guess.cgi). Minor adjustments were manually introduced in the final alignment to maximise sequence homology. Phylogenetic relationships among CLVd sequence variants were evaluated using PAUP Version 4.0b10 (Swofford, 1993) and SplitsTree (Huson, 1998). For analyses using PAUP, phylogenetic trees were constructed by exhaustive search. Bootstrap analysis (1000 replicates, Felsenstein, 1987) was performed to estimate support for inferred clades.

## Results

*Mechanical inoculation of tomato and potato*

Under greenhouse conditions, all tomato plants showed growth reduction and distortion and chlorosis of the young leaves 3–5 weeks after inoculation. The severity of the symptoms varied between different isolates, isolate 89001013 inducing most pronounced stunting (Figure 2).

*Figure 2.* Symptoms appearing on tomato after mechanical transmission of two different viroid isolates. Left non-inoculated; middle: PSTVd-N; right: isolate 89001013.

In the greenhouse the inoculated potato plants did not show any leaf symptoms; tubers of the infected plants were smaller and malformed, however. The severity of the symptoms in potato differed between isolates: isolate 89000808 caused rather mild tuber symptoms, while isolates 89001013, 93007481 and 93007908 induced star cracking, severe stunting and malformation (Figure 3). In the field experiments, no infections were detected in the inoculated plants by testing in r-PAGE. Potato plants grown from the infected tubers obtained from the greenhouse experiments were severely stunted and their tubers were malformed. Plants infected by isolate 89001013 died within a couple of weeks after emergence, without producing any tubers. Tubers of the other infected plants were cultivated for three successive years. These plants also showed growth reduction and their tubers

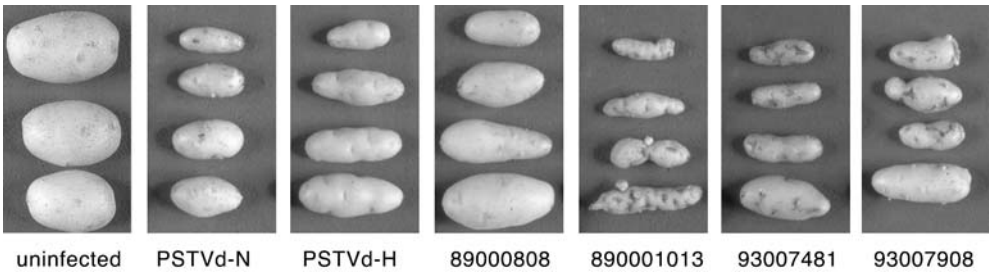


*Figure 3.* Potato tubers showing malformation and various degrees of growth reduction for all six viroid isolates, and additional star cracking for isolates 89001013, 9307481 and 93007908.

remained small and often were malformed. Yield losses varied from ca. 39% for PSTVd-H up to ca. 82% for isolate 93007481 (Table 2).

*RNA isolation, reverse transcription and polymerase chain reaction (RT-PCR)*

Obvious differences in the severity of the symptoms and the rate of spread in the greenhouses of origin made further characterisation of the viroid isolates desirable. Firstly, part of the isolates was tested in RT-PCR by using 'specific' primers for the pospiviroids CEVd, CSVd and PSTVd (Table 3). Isolates 89000808, 89002594 and 89002600 produced amplicons with the CEVd primers; PSTVd-H, PSTVd-N, isolates 9405977, 200011470 and 21008470 produced amplicons with the PSTVd primers. None of the isolates reacted with the CSVd primers. The first set of universal pospiviroid primers (Pospi1RE/FW) designed to detect all pospiviroids reported in tomato so far, only reacted with those isolates also reacting with the CEVd and PSTVd primers (Table 3). Because CLVd was the only pospiviroid not expected to react with the primer set Pospi1RE/FW, tests were performed with two additional sets of primers: one set specific for CLVd and a second set of universal primers designed to recognise several pospiviroids including CLVd (VidRE/FW). In both tests all isolates previously reacting negative, yielded amplicons of the expected size (Table 3, Figure 4). Isolate 22006456 which was only tested by Pospi1RE/FW and VidRE/FW, yielded amplicons with both primer sets.

*Table 2.* Yield of viroid-infected and healthy potato plants cv. Nicola grown in the field for three successive years[a]

| Isolate | 1995 | 1996 | 1997 | Average |
|---|---|---|---|---|
| PSTVd-N | 2.1[b] | 5.0 | 0.6 | 2.6 |
| 89000808 | 5.9 | 11.7 | 2.9 | 6.8 |
| 93007481 | 2.0 | 3.3 | 1.4 | 2.2 |
| 93007908 | 2.6 | 7.0 | 0.6 | 3.4 |
| PSTVd-Howell | 7.0 | 11.2 | 4.3 | 7.5 |
| Healthy | 14.9 | 13.6 | 8.3 | 12.3 |

[a] In 1994 yield of infected plants was extremely low, because of lack of dormancy of the small tubers harvested in the greenhouse. Plants infected by isolate 89001013 even died within a couple of weeks after emergence without producing any tubers.
[b] Total weight (kilograms) of tubers from ten potato plants.

*Table 3.* Summary of RT-PCR results with different primer sets

| Isolate | Primer sets for | | | | Designed primer sets | |
|---|---|---|---|---|---|---|
| | CEVd | CLVd | CSVd | PSTVd | Pospi1RE/FW | VidRE/FW |
| PSTVd-H | – | – | – | + | + | + |
| N | – | nt* | – | + | + | nt |
| 89000808 | + | nt | – | – | + | nt |
| 89001013 | – | + | – | – | – | + |
| 89002594 | + | nt | – | – | + | nt |
| 89002600 | + | nt | – | – | + | nt |
| 93007481 | – | + | – | – | – | + |
| 93007908 | – | + | – | – | – | + |
| 94005977 | – | nt | – | + | + | nt |
| 95001530 | – | + | – | – | – | + |
| 95006685 | – | + | – | – | – | + |
| 96009271 | – | + | – | – | – | + |
| 20011470 | – | nt | nt | + | + | nt |
| 21008470 | – | nt | nt | + | + | nt |
| 22006456 | nt | nt | nt | nt | + | + |

* nt = not tested.

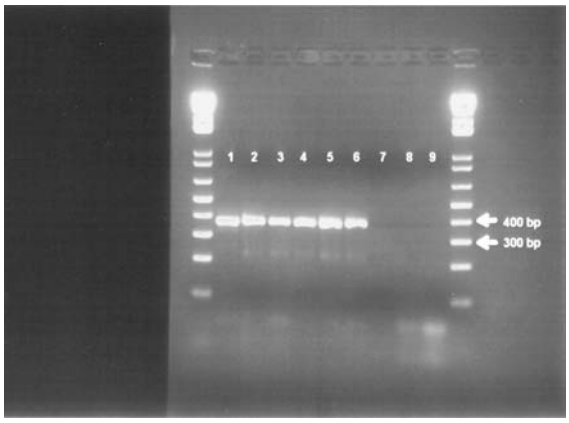


*Figure 4*. Electrophoretic analysis of amplicons yielded by RT-PCR of tomato viroid isolates with CLVd-specific primers: 89001013 (1); 93007481 (2); 93007908 (3); 95001530 (4); 95009685 (5); 96009271 (6); healthy tomato (7); water (8); PSTVd-H (9); 100 bp DNA markers (outer lanes).

### *Sequence and phylogenetic analysis of PCR amplicons*

For all isolates, except PSTVd-N which already had been sequenced, the amplicons obtained by RT-PCR were directly sequenced, and the resulting sequences deposited in NCBI Genbank (see Table 1 for accession numbers). Analysis of the PCR products obtained with the primers for CEVd and PSTVd confirmed their respective identities. The CEVd isolates 89000808, 89002594 and 89002600 showed identities of 92.8–96.0%, 95.8–98.7% and 91.5–93.6% respectively, with the CEVd isolates in the Genbank. In case of PSTVd isolates H, 94005977, 20011470 and 21008470, identities were 93.6–99.4%, 93.7–94.8%, 93.4–99.4% and 91.6–96.4%, respectively, with the PSTVd isolates in the Genbank. Based on its 96.7% identity with the only TCDVd sequence reported to date, the amplicon obtained from isolate 22006456 with the VidRE/FW primers was identified as TCDVd (Singh et al., 1999).

For the remaining six isolates, the amplicons obtained with both the CLVd and the VidRE/FW primers were sequenced. As shown in Table 4, the resulting sequences appeared most similar to CLVd. Overall identity with CLVd varied between 84.0% and 90.3%, and no sequence changes were present in the central conserved region (CCR) and the terminal conserved region (TCR). As shown in Figure 5, phylogenetic analysis of these sequences indicated that CLVd contains two distinct lineages. The first contains CLVd-Col, isolated from *Columnea* (Hammond et al., 1989) plus two sequences previously recovered from other ornamental hosts, i.e. *Brunfelsia* (CLVd-Brun, Spieker, 1996) and *Nematanthus* (CLVd-Nem, Singh et al., 1992); the second lineage contains the six new isolates from tomato. In each lineage the most divergent isolates are slightly less than 90% identical, i.e. 89.9% for the CLVd-Col and CLVd-Brun vs. 88.9% for isolates 89001013 and 93007481. Sequence identity is only 84.0% for the two most divergent isolates from both groups, i.e. the CLVd-Brun and isolate 89001013. Most sequence differences between both lineages were located within the pathogenicity and variable domains.

## Discussion

Molecular analysis of the viroids isolated from tomato showed that at least four different viroid species are able to infect this crop. Seven of the thirteen tomato isolates included in this study belong to viroid species reported in tomato before, i.e. either CEVd (Fagoaga and Duran-Vila, 1996;

*Table 4*. Nucleotide identities among three isolates of *Columnea latent viroid* from ornamentals and six viroid isolates from tomato

| Isolate | CLVd-Col | CLVd-Brun | CLVd-Nem | 89001013 | 93007481[a] | 95001530[b] |
|---|---|---|---|---|---|---|
| CLVd-Col | – | 89.9 | 97.6 | 89.4 | 89.9 | 90.2 |
| CLVd-Brun | | – | 91.0 | 84.0 | 84.5 | 84.7 |
| CLVd-Nem | | | – | 88.7 | 90.0 | 90.3 |
| 89001013 | | | | – | 88.9 | 89.2 |
| 93007481 | | | | | – | 99.7 |
| 95001530 | | | | | | – |

[a] Includes isolates 95006685 and 93007908.
[b] Includes isolate 96009271.

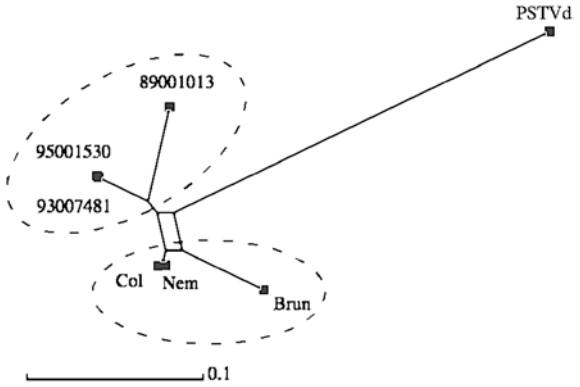


*Figure 5*. Sequence relationships among naturally-occurring isolates of CLVd. Results of SplitsTree analysis, a distance-based method involving Hamming transformation, are shown. Edges of the displayed graph are proportional to the isolation index of the corresponding splits (see scale), and the two lineages are circled. Parsimony and likelihood-based methods predict identical relationships among the six isolates.

Mishra et al., 1991), PSTVd (Leontyeva, 1980; Puchta et al., 1990) or TCDVd (Singh et al., 1999). With regard to CEVd, the results of the nucleic acid hybridisation tests performed by Agdia were confirmed for three isolates; isolate 89001013 showed only 61.8% identity with CEVd, however. For PSTVd, isolates 94005977 and 21008470 grouped with the majority of PSTVd isolates in the NCBI Genbank. Isolate 20011470 from New Zealand, however, was most similar to PSTVd-N and isolates described by Behjatnia et al. (1996) and Elliott et al. (2001). All these isolates either originated in or, like the Dutch isolate N (Puchta et al., 1990), could be related to Oceania (i.e. Australia and New Zealand). Finally, 22006456 is the first isolate of TCDVd from tomatoes growing in the USA. The only previous report of TCDVd was from plants growing in Canada (Singh et al. 1999).

The remaining six isolates were slightly less than 90% identical to two of three published sequences of CLVd, a member of the genus *Pospiviroid* also sharing some characteristics of the genus *Hostuviroid*. Viroids sharing <90% sequence identity have often been considered to be separate viroid species (Flores et al., 2000). For these six remaining isolates, indeed, phylogenetic analysis suggests that CLVd-related sequences form two distinct groups/lineages. One group contains the isolates from three ornamental species, i.e. CLVd-Brun, CLVd-Col and CLVd-Nem; the second group contains the six isolates from tomato. Pair-wise sequence identities within each group are slightly less than 90%. Moreover, pair-wise sequence identities between CLVd-Col and CLVd-Nem isolates from the first group and all six tomato isolates from the other, also reveal identities slightly less than 90%. Therefore, the isolates from tomato have been identified as CLVd. Only pair-wise sequences identities between CLVd-Brun and all tomato isolates, respectively, are clearly below 90% (84.0–84.7). Since phylogenetic analysis suggests that CLVd-related sequences form two distinct groups/lineages, it is proposed to divide the species CLVd into two strains, i.e. one strain containing the isolates from the three ornamental crops and a second strain containing the isolates from tomato. The fact that like CLVd-Col (Hammond et al., 1989), isolates 89001013 and 93007481 were found to infect cucumber (*Cucumis sativus*, results not shown), further supports their identification as CLVd. It might be worthwhile to determine the susceptibility of cucumber to CLVd-Brun, as the susceptibility of this plant species would substantiate the biological identification of this isolate as CLVd.

Very little is known about the origin of the different viroid infections in tomato. Evidence of a common source has only been obtained for the two PSTVd isolates found in the Netherlands in 1988 (Puchta et al., 1990). At each location, the viroid was identified in pepino (*Solanum muricatum*) as well as in tomato. The pepino plants in these greenhouses were grown from seeds imported both from Greece and New Zealand. These seeds might have provided the pathway for introducing the viroid into the Dutch tomato crops. This hypothesis is supported by the fact that the sequence of the Dutch isolate (PSTVd-N, Puchta et al., 1990) is very similar to the sequences of two isolates from New Zealand, i.e. 20011470 and Elliott et al. (2001). All these isolates, in turn, closely resemble another PSTVd isolate from Australia (Behjatnia et al., 1996), one that is quite different in sequence from the majority of North American and European PSTVd isolates. More recently, yet other isolates belonging to this group of divergent sequences were reported from tomato in Australia (Mackie et al., 2002) and the United Kingdom (Mumford et al., 2003). For the UK, this was the first natural infection of tomato by PSTVd, but unfortunately its origin could not be

traced. Taken together, these observations indicate that this group of aberrant PSTVd isolates may have originated in Oceania.

The possible origins of the other viroid isolates from tomatoes grown in the Netherlands is less clear. The ten viroid infections found between 1989 and 2001, all involved different tomato varieties from five different seed companies. Moreover, these infections involved three different viroids i.e., CEVd, CLVd and PSTVd. Tracing back the origins of the particular seed lots failed to reveal additional infections. Therefore, it is likely that hosts other than tomato plants may play an important role as source of infection, especially when they fail to show disease symptoms. Natural infections of PSTVd have been found in the solanaceous crops pepino (Puchta et al., 1990; Shamloul et al., 1997), potato (Diener and Raymer, 1971; Martin, 1922) and tomato (Leontyeva, 1980; Puchta et al., 1990) as well as in avocado (*Persea americana*, Querci et al., 1995). The experimental host range of this viroid, however, is quite extensive and includes many more non-solanaceous plants (Singh, 1973). CEVd has been found to naturally infect several plant species (Fagoaga and Duran-Vila, 1996), but TCDV has only been detected twice in nature — both times in tomato. CLVd infections are symptomless in several ornamental species, i.e. *Brunfelsia undulata* (Spieker, 1996), *Columnea erythrophae* (Hammond et al., 1989) and *Nematanthus wettsteinii* (Singh et al., 1992). Such plants provide a likely reservoir for unnoticed viroid spread.

Although isolates of CEVd, CLVd and PSTVd recovered from tomato could be successfully transmitted to potato by mechanical inoculation in the greenhouse, no evidence was found for transmission under field conditions. Field transmission may have been inhibited by the cool growing conditions in the Netherlands, but two months after mechanical inoculation viroid concentrations may also have been too low to be detected by r-PAGE (Manzer and Merriam, 1961; Pfannenstiel and Slack, 1980).Tuber yields varied considerably, but infected tubers were found to produce infected progeny during four successive years. Since growing conditions were not optimal and the size of the experiment was limited, the data on yield reductions should be considered only indicative.

Irrespective of these limitations, the yield reduction data clearly show that CEVd, CLVd and PSTVd have similar effects on potato. The tomato isolates of CLVd even appeared to be more harmful than PSTVd. Experimental transmission of CEVd to potato also has previously been reported by Semancik et al. (1973). Moreover, successful transmission of TCDVd and TPMVd to potato has been also described (Singh et al., 1999; Galindo et al., 1982). Of these five viroids, however, only PSTVd appears in the quarantine lists of the European Union and the European and Mediterranean Plant Protection Organisation. This quarantine status appears mainly based on the risks that PSTVd poses to the seed and ware potato production within its territories. The fact that other viroids can be at least as harmful to potato and tomato as PSTVd stresses the need to reassess the phytosanitary regulations. Moreover, as testing of potato for PSTVd infection previously often was performed by r-PAGE only, it even is possible that some of these other viroids already occur in potato.

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