Chrysanthemum stunt viroid

R. A. Mumford
Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK

Introduction

Chrysanthemum stunt was first identified in the USA in 1945 (Dimond, 1947) and the causal agent was subsequently identified as a viroid in 1973 (Hollings & Stone, 1973; Diener & Lawson, 1973).

Synonyms:

Chrysanthemum stunt virus (Brierley, 1953)
American stunt virus (Hollings, 1960)

A viroid consisting of a single-stranded, circular RNA molecule, of 354-356 nucleotides. Transmitted mechanically and by vegetative propagation. Found in florists' chrysanthemum (*Dendranthema x grandiflorum* Kitam) crops worldwide.

Main Diseases

Causal agent of the disease 'chrysanthemum stunt' (Hollings & Stone, 1973; Diener & Lawson, 1973). Symptoms can be variable and are highly dependent on both cultivar and environmental conditions, especially temperature and light (Handley & Horst, 1988). The main symptom is stunting, with a reduction of between 30 and 50% in overall height of mature plants (Hollings, 1960) (Fig.1 & Fig.3). The other common symptoms are floral, with infected plants having reduced flower size (Fig.2) and demonstrating premature flowering (Fig.3), which can be up to ten days early (Hollings & Stone, 1973). In certain varieties, especially red, pink or bronze-pigmented ones, symptoms can also include 'bleaching' (a reduction in colour intensity) (Hollings, 1960) (Fig.2 & Fig.3). Foliar symptoms are less common and often are only seen as smaller, pale leaves (Brierley & Smith, 1949). Leaf spots or flecks are also sometimes observed (Hollings & Stone, 1973). The most extreme example of this is the symptom described as `measles', which appears as large, yellow leaf blotches (Keller, 1951) (Fig.4). However, this symptom is restricted to certain old varieties and is rarely seen in naturally-infected modern varieties (Horst & Nelson, 1997). In the presence of Chrysanthemum virus B (syn. Chrysanthemum virus Q), certain varieties (e.g. Blanche) can show 'crinkle' symptoms, consisting of severe leaf distortion accompanied by white streaks or flecks (Keller, 1951). In many chrysanthemum cultivars, up to 30% of infected plants are symptomless (EPPO, 1989). The rooting efficiency of CSVd-infected cuttings is also adversely affected (Hollings & Stone, 1973).

Geographical Distribution

Widely distributed, with records from Europe (Austria, Belgium, Czech Republic, Denmark, France, Netherlands, Germany, Spain, Italy, UK, Norway, Sweden, Poland), North America (USA, Canada), South America (Brazil), Africa (South Africa), Asia (Japan, India, China) and Oceania (Australia, New Zealand) (CABI/EPPO, 1997; 1998). Probably found in all countries where chrysanthemums are grown.

Host Range and Symptomatology

In addition to *Dendranthema x grandiflorum*, natural CSVd infections have also been reported for *Argyranthemum* (syn. *Chrysanthemum*) *frutescens* (Menzel & Mais, 2000) and *Petunia hybrida* Surfinia-type (Verhoeven *et al*., 1998). The symptoms of CSVd in *Argyranthemum frutescens* are similar to those in *Dendranthema*, with stunting and premature flowering observed in some cultivars. Infected *Petunia hybrida* Surfinia are generally symptomless, although stunting is seen in some varieties (W. Menzel, University of Hannover, Germany, personal communication). An isolate from *Ageratum* sp. has been sequenced (unpublished; Acc. No. Z68201) but further details regarding this host are unavailable.

A wide range of other plant species from the *Compositae* can be infected experimentally, by sap inoculation or grafting (Brierley, 1953). However, most of these show no symptoms. *Lycopersicon esculentum* has also been described as an experimental host of CSVd (Kryczynski & Paduch-Cichal, 1987), although it had been reported earlier as a non-host (Hollings & Stone, 1973).

Diagnostic species:

> The most reliable diagnostic host is the chrysanthemum cultivar 'Mistletoe'. Following chip-budding, measles symptoms (Fig.4) should appear within 6-8 weeks, provided environmental conditions are correct (Hollings & Stone, 1973; EPPO, 1989). The cultivars 'Bonnie Jean' and 'Blazing Gold' can also be used (EPPO, 1989; Brieley & Smith, 1951), the latter giving a distinct yellow vein banding symptom As a simpler but less reliable alternative, *Senecio cruentus* can be mechanically inoculated with extracts from infected plants and local lesions should appear within 30 days (Brierley, 1953). Bio-assay symptoms are less distinct during winter months (Hollings & Stone, 1973; Handley & Horst, 1988). Neither bio-assay is quantitative.

Propagation species:

> Both *Dendranthema x grandiflorum* and *Senecio cruentus* have been used to maintain CSVd and as purification hosts (Diener & Lawson, 1973; Hollings & Stone, 1973).

Strains

None reported. All isolates sequenced are very similar, sharing over 94% sequence identity.

Transmission by Vectors

Despite initial reports of aphid transmission (Brierley & Smith, 1949), no transmission was detected in later studies using four different aphid species (Hollings & Stone, 1973). No transmission was observed using the red spider mite (*Tetranychus urticae*) (Hollings & Stone, 1973).

Transmission through Seed

The situation regarding seed transmission is unclear. Whereas both Hollings & Stone (1973) and Brierley (1953) found no transmission through the seeds of CSVd-infected

chrysanthemum plants, Monsion *et al*. (1973) reported an 11% transmission rate. Transmission via seed and pollen has been reported in tomato (Kryczyski *et al*., 1988).

Transmission by Dodder

Data are contradictory, with transmission reported by Keller (1953) but not by Hollings & Stone (1973).

Relationships

Nucleotide sequence data indicate that CSVd is distinct from all other viroids, including Chrysanthemum chlorotic mottle viroid, the other viroid that naturally infects chrysanthemum (Navarro & Flores, 1997). However, CSVd does possess a high degree of sequence similarity with other members of the Genus *Pospiviroid*, sharing 73 and 67% sequence identity with Potato spindle tuber viroid and Citrus exocortis viroid, respectively (Gross *et al*., 1982).

Stability in Sap

In common with other viroids, CSVd is very stable, with chrysanthemum sap retaining infectivity in dilutions down to 10^{-4} and after 10 minutes at 95-98 °C, 2-3 months at 18°C and at least 5 years at about 2°C (Hollings & Stone, 1973). Dried leaf material can remain infective at 18°C for at least 8 weeks (Hollings & Stone, 1973).

Purification

A detailed viroid extraction protocol is given by Niblett *et al*. (1980). This method is based upon deproteinisation using phenol, followed by DNase treatment and subsequent fractionation of the extracted RNA by LiCl precipitation and CF-11 cellulose chromatography. Further purification is achieved by using polyacrylamide gel electrophoresis. Viroid yields in the range of 1.2 - 1.7 mg/kg can be expected from CSVd-infected chrysanthemum (Niblett *et al*., 1980; Palukaitis & Symons, 1979).

Properties of Infective Nucleic Acid

A covalently closed, circular, single-stranded RNA, with a calculated molecular weight of 111-114 kDa. In common with other viroids, the genomic RNA contains no open reading frames and does not encode any proteins. Under native conditions, CSVd is predicted to have a rod-shaped secondary structure (Gross *et al*., 1982; Haseloff & Symons, 1981). All isolates sequenced contain between 354 and 356 nt. Several full-length sequences are available, including ones for isolates from chrysanthemum (NC_002015=V01107, E13156, AB006737, AB055974, AJ000046, AJ001849, AJ001850, AJ001851, AJ001852, AJ001853, D88895, M19506), petunia (U82445) and ageratum (Z68201).

Relations with Cells and Tissues

Can be detected in both the leaves and stems of infected chrysanthemum plants.

Ecology and Control

The only effective means of control is through the use of viroid-free propagation material. Meristem-tip culture and subsequent heat treatment failed to eliminate CSVd from infected cuttings (Hollings & Stone, 1970). Spread occurs readily by mechanical means, including plant-to-plant contact, handling during cultivation and contaminated cutting tools (Brierley & Smith, 1951; Keller, 1953). Good plant hygiene measures (e.g. use of disposable blades and gloves when taking cuttings, removal of dead material etc) can help to limit spread (Horst & Nelson, 1997).

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Chrysanthemum plants infected with CSVd, showing severe (left) and mild (centre) stunting symptoms. The plants on the right are uninfected. (Picture courtesy of Dr Yukimasa Hirata, Plant Biocenter, The Federation of Wakayama Prefectural Agricultural Cooperative Associations, Japan).

CSVd-induced floral symptoms in the chrysanthemum variety Princess Anne Regal. The infected flower (right) is reduced in size and shows marked colour bleaching. The other flower (left) is from an uninfected plant. (Picture courtesy of Prof. Alan Brunt, UK).

CSVd symptoms in a standing chrysanthemum crop, with plants showing varying degrees of stunting. As a result the height of the crop is very uneven. In addition, some plants (in the right foreground) are showing premature flowering and colour bleaching. (Picture courtesy of Central Science Laboratory, UK).

Large, yellow spots or 'measles' on a leaf taken from an infected indicator plant of chrysanthemum variety Bonnie Jean. (Picture courtesy of the Department of Plant Pathology, Cornell University, Ithaca, NY, USA).