

Viroids and other sub-viral pathogens of plants: the smallest living fossils?

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Viroids and satellite RNAs are the smallest and simplest replicating molecules known. Possibly 'living fossils' they are the cause of a wide variety of infectious diseases in plants.

Contrary to earlier belief, viruses are not the smallest causative agents of infectious diseases in plants. Single-stranded RNAs, termed viroids, exist in many higher plants, causing a variety of virus-like diseases. Viroids can be as small as 246 nucleotides or as large as 400, but their RNA is not organized into codons and they are not translated. This is in contrast to plant viruses where translation of viral genetic information is essential for virus replication. The physical structure of viroids was first shown directly by electron microscopy; under non-denaturing conditions viroids appear as small rods of approx. 37 nm, but under denaturing conditions they can be seen to be covalently

rolling circle mechanism, using either just plant or plant and viral components. Satellite RNAs and just two viroids are able to self-cleave from the concatameric RNA but most viroid RNAs are enzymatically cleaved.

● Plant diseases caused by viroids

Crop diseases caused by viroids probably originated by chance transfer from endemically infected wild plants or by use of viroid-infected germplasm during plant breeding. Despite their extreme simplicity, viroids can cause syndromes in plants that are as varied as those caused by plant viruses but others, such as coleus latent viroid (CLVd), are totally symptomless and occur at extremely high rates of infection (up to 100%). Because viroids are not translated, their effects on plants must be a consequence of direct interactions of the viroid with the host and its environment. Although the molecular mechanisms of viroid pathogenesis are still unknown, analysis of molecular chimeras has revealed that the severity of symptoms is the result of complex interactions among three of the five viroid domains. As a group, there is nothing that distinguishes the disease symptoms produced by viroids from those induced by viruses – these include stunting, mottling, leaf distortion and necrosis. Diseases cover a wide range from the slowly developing lethal disease in coconut palms caused by coconut cadang-cadang viroid (CCCVd) to the usually symptomless hop latent viroid (HLVd; Fig. 2), and the latent viroids which are always symptomless. It is possible that many more mild or symptomless viroid infections remain to be discovered.

● Viroid epidemiology

The main methods by which viroids are spread are mechanical transmission, vegetative propagation and through pollen and seed. The relative importance of these methods varies with different viroids and hosts. For example, vegetative propagation is dominant for chrysanthemum stunt viroid (CSVd). Mechanical transmission is a significant factor for others such as citrus exocortis viroid (CEVd; Fig. 3) and hop stunt viroid (HSVd; Fig. 4). Seed and pollen transmission are factors in the spread of avocado sunblotch viroid (ASBVd); seed is particularly important for some of the latent viroids. Potato spindle tuber viroid (PSTVd) is transmitted at low frequency in a non-persistent manner by the aphid *Macrosiphum euphorbiae*. However, it is doubtful if aphid transmission is of any significance in the field.



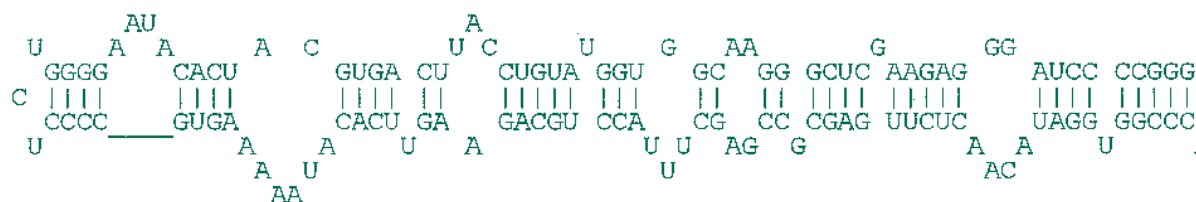
ABOVE:
Fig. 2. Commercial hops (*Humulus lupulus*), variety Omega, with symptoms of HLVd. Omega is the only known hop variety which shows visible symptoms of this viroid.

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BELOW RIGHT:
Fig. 1. The sequence of hop latent viroid (HLVd) arranged in a thermodynamically optimized secondary structure.

AFTER PUCHTA ET AL. (1988)

closed circular molecules. Due to their high degree of internal base pairing, viroids have a characteristic rod-like secondary structure in which short helical regions are interrupted by internal and bulging loops (Fig. 1). In the majority of cases there are five structural domains in the viroid genome. Many satellite RNAs resemble viroids in size and molecular structure, but are associated with specific helper viruses on which they depend for their replication and are encapsidated with, or instead of, host viral genome components into the virus particle. However, the satellite RNA is not part of the helper virus genome and usually has no sequence similarity to it. Both types of small RNA replicate by a





ABOVE:
Fig. 3. CEVd symptoms in a citrus species.
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Viroids move rapidly through a host plant in the manner of competent viruses, almost certainly through the phloem. The relative resistance of viroid RNA to nuclease degradation (which arises from their internally base-paired structure) probably facilitates their long-distance movement. It is also possible that viroid particles are translocated while bound to some host protein. For most viroid diseases the reservoir of inoculum appears to be within the crop itself, raising the question as to where they came from originally. A viroid present in a natural host, possibly causing no disease, might 'escape' into a nearby susceptible commercial crop and spread rapidly within it. Modern agricultural practices, such as widespread monoculture of genetically identical plants, and worldwide distribution of planting material, have probably made possible the sudden appearance and rapid spread of new viroid diseases. For example, a study of HLVD in the UK suggests that the current prevalence of this viroid in hops is a consequence of infection becoming established in the hop propagation system during the late 1970s.

● Viroid detection and diagnosis

Serological tests are widely used for the detection and diagnosis of viruses, but these cannot be used for viroids as they are not immunogenic. Besides indicator plants, biophysical techniques such as two-dimensional gel electrophoresis, return gel electrophoresis, hybridization and RT-PCR of nucleic acid extracts are used for more rapid testing. Viroid identification can be difficult. In the search for viroids and viroid-like RNAs in oil palms from Central and South America affected by a fatal yellowing disease, RNAs showing viroid-like gel-electrophoretic properties were detected. However, the presence of known viroids was excluded by hybridization experiments using viroid-specific probes and the use of double-stranded RNA (dsRNA)-specific monoclonal antibodies (which do not react with viroid RNA), showed the oil palm RNAs to be dsRNA species and not circular single-stranded ones. Furthermore, since the same dsRNA pattern was found in extracts from healthy as well as from diseased oil palms, it was concluded that the dsRNAs were not associated with the disease.

● Viroid disease control

Disinfection of cutting tools is recommended to prevent viroid transmission, especially in nursery production. Heat treatment is effective in eliminating various pathogens from infected plants, but usually not viroids.

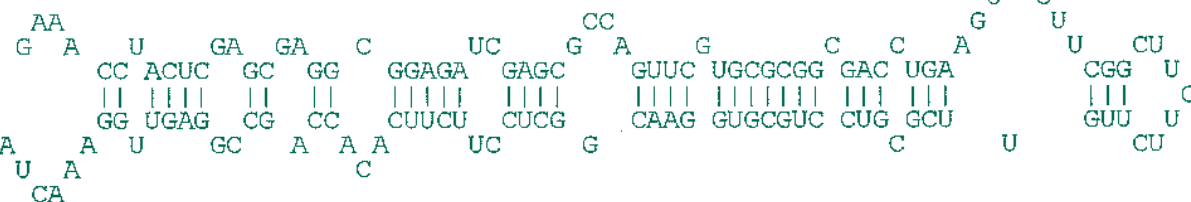
However, cold treatment can be effective, e.g. storage at 4 °C for 6 months or more, followed by apical shoot-tip-culture grafting, can be used to eliminate CSVd and HLVD. Pre-inoculation with protective mild strains of viroid has proved effective to control PSTVd. It has also been shown that interference occurs not only between strains of a particular viroid but also between different viroids. In experiments in which mild and severe strains of PSTVd were inoculated to a host simultaneously, the severe strain dominated and most plants developed severe disease, even when the mild strain was in 100-fold excess in the inoculum. In other experiments, PSTVd RNA transcripts from a cloned PSTVd DNA were inoculated together with HSVd RNA. PSTVd reduced the level of HSVd RNA in infected plants. Plants inoculated with dual transcripts – two copies of a severe PSTVd strain linked to two of HSVd – developed PSTVd symptoms and only PSTVd progeny RNA could be detected. The molecular basis for this interference is not yet understood.

● Recombination between viroids

Nucleotide sequence data shows that it is highly probable that recombination has taken place between different viroids, presumably during replication in mixed infections. For example, tomato apical stunt viroid (TASVd) appears to be a recombinant viroid comprised mostly of the sequence of a CEVd-like viroid but with the T2 domain replaced by the equivalent domain from a PSTVd-like viroid. Australian grapevine viroid (AGVd) appears to have originated by extensive RNA recombination as its sequence can be divided into regions each with high sequence similarity with parts of CEVd, PSTVd, apple scar skin viroid (ASSVd) and grapevine yellow speckle viroid (GYSVd).

● Satellite viruses and satellite RNAs

Purified virus preparations isolated from infected plants may contain a variety of small RNAs other than the genomic RNAs. In addition, some isolates of certain plant viruses may also contain satellite viruses. The two



RIGHT:
Fig. 4. HSVd symptoms in
Humulus japonicus.
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classes of agents can be distinguished according to the source of the coat protein encapsidating the RNA and the sizes of the RNAs. In satellite viruses the RNA encodes its own coat protein whilst small satellite RNAs become packaged in protein shells made from coat protein of the helper virus. Also, the RNAs in satellite viruses have to be large enough to encode the coat protein as opposed to the viroid-like size of the satellite RNAs. Like viroids, satellite RNAs may be responsible for serious disease despite their minimal size. In 1972, for example, a devastating outbreak of a lethal



necrotic disease of field-grown tomatoes occurred in the Alsace region of France. It was quickly realized that cucumber mosaic virus (CMV) was involved, but it was not clear why necrosis occurred instead of the usual fern-leaf symptoms. However, a small RNA component was found to be present in some isolates of CMV in addition to the three genomic RNAs and the subgenomic coat protein mRNA, and this fifth RNA was not part of the viral genome. The additional small RNA (called CARNA5) present in cultures of CMV strain S was found to cause lethal necrotic disease in tomatoes when added to the CMV genomic RNAs and its presence was thought to have been responsible for the Alsace lethal necrotic disease. Similar recent outbreaks in tomatoes in southern Italy have been shown to be due to a necrogenic isolate of CARNA5. Conversely, some isolated types of CARNA5 attenuate symptoms in tomato and have been used in China for pre-inoculation as protective mild strains for the control of CMV.

● Living fossils?

Viroids and satellite RNAs are of great interest because they are the smallest and simplest replicating molecules known and they may represent living fossils of pre-cellular evolution. Phylogenetic analysis of their nucleotide sequences indicates that viroids and satellite RNAs represent a monophyletic group, with all but the two self-cleaving viroids forming one cluster and the satellite RNAs another. The two self-cleaving viroids are phylogenetically distant from either cluster

and may represent ancestral forms. Site-directed mutagenesis experiments indicate that viroids can evolve extremely rapidly in response to selective pressures, with fitter components of the quasi-species often becoming dominant within days or weeks. This extreme plasticity of their nucleotide sequences establishes viroids as the most rapidly evolving biological system known.

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Further reading

Diener, T.O. (1999). Viroids and the nature of viroid diseases. *Arch Viral Suppl.* **15**, 203–220.

Diener, T.O., Owens, R.A. & Hammond, R.W. (1993). Viroids – the smallest and simplest agents of infectious disease – how do they make plants sick? *Intervirology* **35**, 186–195.

Puchta, H., Ramm, K. & Sanger, H. (1988). The molecular structure of hop latent viroid (HLVd), a new viroid occurring worldwide in hops. *Nucleic Acids Res* **16**, 4197–4216.