

## Viroids

Growers today, have to face the problem of viroids in *Argyranthemum*, *Chrysanthemums*, and in other ornamental crops. Danziger - 'Dan' Flower Farm's professional staff would like to share with you, the grower, basic information, which might help you, cope with this challenge.

A viroid is a cell-invading plant pathogen 80 times smaller than a virus. An ARS plant pathologist Theodor O. Diener discovered it in 1971 and named it a 'viroid', since it acts "like a virus". Up until then, the scientific dogma was that an organism with no protein (such as a viroid) was not supposed to be able to replicate itself, even with the host's help.

Viroids are the smallest-known agents of infectious diseases, highly structured, single-stranded ribonucleic acids (RNA). RNA and DNA are nucleic acids, the molecules of heredity; with the exception of viroids and some viruses; all genes are made of deoxyribonucleic acid (DNA). Both viruses and viroids are obligate parasites - they are able to multiply or replicate only within a living cell of a particular host. Like a virus, the viroid invades a cell, and takes over its reproductive mechanisms, forcing the cell to duplicate the viroid's RNA instead of its own. Viroids differ from the viruses in that they have no protective protein coat that is typical for viruses. In 1971, the first viroid to be identified was the Potato spindle tuber viroid (PSTVd) of the genus *Pospiviroid*. Since then, some 33 species have been identified in plants. Among those identified is the *Chrysanthemum stunt viroid* (CSVd), found within the same genus as the PSTVd. CSVd is one of the most important diseases of florist's chrysanthemums (*Dendranthema morifolium*). The main hosts of CSVd are chrysanthemums and other ornamentals such as *Argyranthemum*. The symptoms of the disease include a decrease in the height of the plant (stunting); anthocyanin on stems disappears; the stems become brittle; the leaves and flowers become small and rooting ability decreases.

Susceptibility to stunt viroid varies between cultivars; in florists' chrysanthemums, about 30% of the plants are symptomless carriers. Due to the fact that plants are sometimes symptomless, a systematic approach is required to determine the extent of viroid infection. This is one of the reasons why molecular biology is used for the identification of plant diseases.

The use of molecular biology techniques is spreading widely around the world. It makes large body of information accessible to researches; leading to increasingly disease causal-agent's identification.

The polymerase chain-reaction (PCR) is a widely used technique in medical and biological research labs for a variety of applications. PCR is used to exponentially amplify specific regions of a DNA strand. With PCR, it is possible to amplify from a single copy or a few copies of a piece of DNA, into millions or more copies of the same DNA.

Primers are used to selectively complement the specific DNA strand of the viroid; where it undergoes replication via the PCR machine. These primers

are strands of nucleic acid, which serve as a starting point for DNA replication.

Since the viroid has no DNA its detection and the screening for viroid-free plants is carried out through extraction and purification of viroid-RNAs from the plant tissue and its subsequent conversion to DNA. This process is known as 'reverse transcription polymerase chain reaction (RT-PCR)'. Upon completion of the DNA replication, it is then run on the gel. This gel (gel electrophoresis) is a technique used for the separation of DNA using an electric current applied to a gel matrix. Once the gel electrophoresis is complete, the molecules in the gel can be stained to make them visible. The visibility is seen as 'bands'. The presence of the bands on the gel means a definite presence of the viroid in the plant, regardless whether it is symptomless or not.

It is very difficult to produce viroid-free chrysanthemum plants, and considerable effort has been invested in producing them.

At Danziger's lab RT-PCR detection methods are used.

In the case of chrysanthemum plants, the preparation of RT-PCR templates requires laborious steps, because chrysanthemum plants contain several PCR inhibitors, which makes the extraction and purification of viroid RNAs more difficult. Nevertheless, the methods we use are highly sensitive and extremely successful in producing viroid-free plants.