

## Viral Diseases of Strawberries<sup>1</sup>

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Viral diseases have not been an issue for strawberries grown in Florida, probably because most viruses are symptomless on commercial cultivars. However, at the end of the 2008–2009 strawberry season, serological tests confirmed the presence of *strawberry necrotic shock virus* (SNSV) (formerly *tobacco streak virus* [TSV]) in research fields at the University of Florida Gulf Coast Research and Education Center (UF GCREC) and some commercial strawberry farms.

This publication provides basic information on viral diseases of strawberries with particular emphasis on the presence of SNSV on Florida strawberry plants.

### Viruses in plants

#### Biology

Viruses are smaller than bacteria or fungi and are not visible by light microscopy. They are submicroscopic parasites whose bodies have a variety of sizes and shapes (spherical, rod-shaped, isometric, etc.), which consist of DNA or RNA and protective proteins. Some viruses are among the most significant plant pathogens, as they reduce plant vigor, yield, and market value, or can even cause plant death. Viruses

multiply by inducing the host cells to produce more virus particles. Plant viruses are obligate parasites that cannot be grown in artificial media and require a host plant for their survival and multiplication.

Virus symptoms may include:

**Necrotic local lesions:** Dead or discolored and localized spots on plant tissue

**Mottle:** Uneven areas of light and dark yellowing (Fig. 1)

**Ringspot:** Circular chlorotic spots

**Mosaic:** Mixed areas of mottled and normal tissue

**Chlorosis or yellowing:** Pale or yellow areas

**Malformation, leaf distortion, and stunting:** Irregular growth, dwarfing, and loss of vigor

#### Detection, dissemination and control

Virus-infected plants may be symptomless, or the symptoms may be confused with those caused by bacteria, viroids, insect feeding, herbicide damage, nutritional deficiencies, high temperatures (Fig. 2),

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**Figure 1.** Leaf of *Fragaria vesca* with mottle symptoms. (Photo: David Moore, UF/IFAS GCREC)

air pollutants, or genetic abnormalities (Converse 1987).



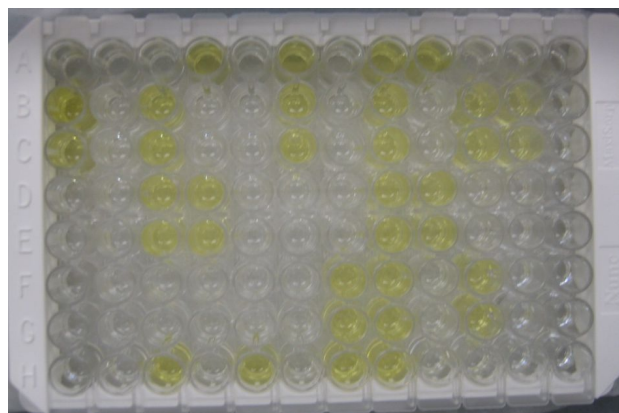
**Figure 2.** Symptoms caused by exposure to high temperatures in *F. vesca*. (Photo: Catalina Moyer, UF/IFAS GCREC)

Detection and diagnosis of viral infections can be achieved by biological assays, such as graft or sap inoculation of species or cultivars that are especially sensitive and show obvious symptoms (Martin 2004). If symptoms develop on these indicator plants, then the plant in question is positive for the virus (Fig. 3). This technique can detect many viruses, although it will not necessarily diagnose a specific virus.



**Figure 3.** *Fragaria virginiana* plants: right, healthy; left, leaf distortion and stunting symptoms after grafting with virus-infected tissue. (Photo: Catalina Moyer, UF/IFAS GCREC)

Enzyme-linked immunosorbent assay (ELISA) is a serological test that detects the presence of a specific antigen in plant tissue. Leaf sap from virus-infected plants (containing the antigen) is mixed with the antibody for the specific virus on a microplate. If the antigens and antibody match, a color develops, indicating the presence of the virus in the sample (Fig. 4). The wells in the microplate can be read visually as positive or negative or quantified with a colorimeter.



**Figure 4.** ELISA microplate showing colorimetric reaction of virus-infected samples. (Photo: Catalina Moyer, UF/IFAS GCREC)

Nucleic acid analysis is another technique to confirm viral infection. Small amounts of DNA or RNA are amplified many times using polymerase chain reaction (PCR) and then detected with agarose gel electrophoresis. Nucleic acid analyses are very useful in detecting organisms that cannot be cultured or that are present in very small amounts.

Plant viruses are disseminated through seed or pollen, vegetative propagation, vectors (aphids, thrips, mites, whiteflies, leafhoppers, plant hoppers, beetles, and nematodes), and/or mechanically (Converse 1987).

Control of plant viruses is often difficult because viruses move readily between plants without detection and because there are no "viricides" or cures for virus-infected plants. Depending on the disease, proper management may include: chemical or biological control of vectors, use of resistant cultivars, use of clean propagation material (i.e., plant material cleaned by meristem culture and heat treatment), and exclusion (plant quarantine).

### Viruses in strawberries

Identification of viruses in strawberries began in the 1930s. Currently, more than 30 viruses are reported to infect strawberries worldwide (Table 1) (Converse 1987; Martin and Tzanetakis 2006). Viral infections of commercially grown strawberries have the potential for economic impact, but growers usually do not observe problems because most viruses are eliminated during the early stages of propagation. New cultivars go through a clean propagation program where meristem culture, heat treatment, and disease testing are conducted before plants are delivered to registered nurseries for mass propagation. Therefore, strawberry nurseries start with clean plants; consequently, viruses are not easily spread into fruiting fields. Unfortunately, fruiting fields may become infected with viruses that have hosts other than strawberries, such as bean, clover, tomato, and weed species, among others (Cupertino et al. 1984; Klose et al. 1996).

Heat treatment and meristem culture are frequently combined for virus elimination in strawberries (Biswas, Hossain, and Islam 2007; Mullin et al. 1974). Well-rooted plants and daughter plants (stolons) are grown at 37°C for four to six weeks, followed by meristem removal and culture in vitro (Converse 1987). Heat treatment can also be applied to in vitro plantlets. After heat treatment and meristem culture, plants need to be tested again since some viruses may survive these procedures.

The main viruses affecting strawberries include *strawberry veinbanding virus* (SVBV), *strawberry crinkle virus* (SCV) (Fig. 5), *strawberry mottle virus* (SMoV), and *strawberry mild yellow edge* (SMYEV). Numerous other viruses affect strawberries (Table 1) (Converse 1987), and probably many more remain to be discovered (Diekmann, Frison, and Putter 1994; Martin and Tzanetakis 2006).



**Figure 5.** *F. vesca* infected with *strawberry crinkle virus* and exhibiting symptoms of leaf deformation. (Photo: Catalina Moyer, UF/IFAS GCREC)

Virus testing has not been customary during strawberry fruit production, as it is assumed that plants come "virus free" from the nursery. One could assume that if viruses do not cause symptoms and/or reduce yield, then there is no need for concern. However, viruses that seem harmless by themselves may become problematic in combination with others (Martin and Tzanetakis 2006). Plants with these mixed infections may develop more severe symptoms than those harboring a single viral pathogen.

Virus testing is, then, an important step in the plant propagation process. Negative results for diagnostic tests only indicate that plants are free of those viruses for which they have been tested. Therefore, plants that had tested negative should be called "virus tested" and not "virus free." Nonetheless, the term "virus free" is widely misused.

## SNSV in strawberries

For many years, strawberry necrotic shock disease was thought to be caused by a strain of *tobacco streak virus* (TSV). Tzanetakis, Mackey, and Martin (2004) found that strawberry necrotic shock disease is caused by a different virus and not by a strain of TSV. The name *strawberry necrotic shock virus* (SNSV) was then suggested for this virus instead of TSV.

SNSV causes no symptoms in commercial cultivars. Grafted susceptible indicator strawberry plants (*Fragaria vesca*) may show a severe necrotic reaction in new leaves; however, these symptoms are temporary, and the new growth appears normal and healthy (Frazier et al. 1962). Depending on the virus isolate, symptoms may also include chlorosis, stunting, and leaf malformation.

SNSV has been reported in the U.S., Australia, and Israel. Although commercial cultivars are symptomless, reduction of yield and runner production has been reported. Dissemination of this virus occurs through seed, pollen, or thrips (Klose et al. 1996). This virus has a wide host range, and host plant species near strawberry fields can serve as sources of inoculum. The most practical way to minimize the risk of infection on commercial fields is to use clean plant material (tissue cultured and virus tested) and to follow best management practices for insect and weed control (Biswas, Hossain, and Islam 2007; Martin and Tzanetakis 2006).

SNSV was detected in Florida at the end of the 2008–2009 strawberry season in research fields at UF GCREC. Cultivars that tested positive for SNSV included 'Strawberry Festival', 'Sweet Charlie', 'Florida Radiance', and 'Florida Elyana'. However, yields were not noticeably different than those from previous years. Thus, it was assumed these were new infections of SNSV that were transmitted in Florida; however, the hypotheses that the plants were infected in the nurseries could not be dismissed because plants were not tested early in the season.

During the 2009–2010 strawberry season, leaf samples were collected from seven cultivars and eleven nursery sources at three times during the season. Samples from the UF GCREC research fields

in Wimauma and from a selected grower's field in Dover were tested for SNSV using the ELISA method. The first samples were collected in November and December to determine if plants were already infected upon arrival from the nurseries. Samples of 'Florida Radiance' from all nursery sources grown at both locations tested positive for SNSV. Only one nursery source was tested for the other newly released cultivar, 'Florida Elyana', and it was also found to be positive. SNSV was not detected in samples of the cultivars 'Strawberry Festival', 'Camarosa', 'Treasure', 'Camino Real', or 'Sweet Charlie' from any of the sources tested. Samples from the same plants were collected again during the middle and end of the strawberry season to determine if the virus was spreading through the fields. The last sampling was conducted during the first week of April. SNSV was confirmed in 'Florida Radiance' from all nursery sources and in 'Florida Elyana' from the one source previously noted. In addition, 'Strawberry Festival' plants from two sources were positive in the UF GCREC fields. Despite the presence of SNSV in 'Florida Radiance' in the grower's field since the beginning of the season, yields did not seem to be affected, and SNSV was not detected in other cultivars planted nearby. This indicates that transmission and infection by SNSV does not progress rapidly in strawberry fields. However, it is possible that the colder-than-normal temperatures during the 2009–10 strawberry season may have prevented a more rapid spread of SNSV.

## Conclusion

In general, infection by a single strawberry virus does not significantly reduce strawberry growth and yield. However, mixed infections by multiple viruses have the potential to cause more serious losses. For this reason, it is important to minimize viruses in strawberry propagation through the use of meristem culture, heat treatment, and virus testing.

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Table 1. Viral diseases of strawberries

Virus name (Acronym)	Genus of causal organism	Diagnostic symptoms on commercial cultivars	Detection <sup>a, b</sup>	Geographic distribution	Host range
<b>Aphid transmitted</b>					
Strawberry crinkle (SCV)	<i>Cytorhabdovirus</i>	None or severe in mixed infections	RT-PCR	Worldwide	<i>Fragaria</i> spp.
Strawberry mottle (SMoV)	<i>Sadwavirus</i>	None or severe strains may reduce vigor	RT-PCR	Worldwide	<i>Fragaria</i> spp., <i>Duchesnea</i> spp. (Indian strawberry), <i>Potentilla sundaica</i>
Strawberry mild yellow edge (SMYEV)	<i>Potexvirus</i>	None or present in mixed infections	ELISA, RT-PCR	Worldwide	<i>Fragaria</i> spp., <i>Duchesnea</i> spp. (Indian strawberry)
Strawberry veinbanding (SVBV)	<i>Caulimovirus</i>	Chlorosis in some cultivars	PCR	Australia, Brazil, Europe, Egypt, Japan, U.S.	<i>Fragaria</i> spp.
Strawberry latent C (SLCV)	<i>Nucleorhabdovirus</i>	None or present in mixed infections	Indicators	U.S., Japan	<i>Fragaria</i> spp.
Strawberry pseudo mild yellow edge (SPMYEV)	<i>Carlavirus</i>	None	ELISA	Japan, U.S.	<i>Fragaria</i> spp., <i>Duchesnea</i> spp. (Indian strawberry), <i>Rubus parvifolius</i> (native raspberry)
Strawberry chlorotic fleck (StCFV)	<i>Closterovirus</i>	None	RT-PCR	Louisiana, U.S.	<i>Fragaria</i> spp.
<b>Nematode, pollen, or seed transmitted</b>					
Tomato ringspot (ToRSV)	<i>Nepovirus</i>	None or symptoms similar to <i>Verticillium</i> wilt	ELISA, RT-PCR, indicators	U.S.	Broad
Strawberry latent ringspot (SLRSV)	Listed as <i>Sadwavirus</i>	Severe in mixed infections		Europe	
Arabid mosaic (ArMV)	<i>Nepovirus</i>	Dwarf plant, chlorosis on some cultivars, or none			
Raspberry ringspot (RpRSV)	<i>Nepovirus</i>	Leaf curl, ringspots on leaves, or none			
<b>Thrips, pollen, or seed transmitted</b>					

Table 1. Viral diseases of strawberries

Virus name (Acronym)	Genus of causal organism	Diagnostic symptoms on commercial cultivars	Detection <sup>a, b</sup>	Geographic distribution	Host range
<i>Tobacco streak (TSV)/strawberry necrotic shock (SNSV)</i>	<i>Illvirus</i>	None	ELISA, RT-PCR, indicators	Australia, Israel, U.S. Chile	Broad <i>Fragaria chiloensis</i>
<i>Fragaria chiloensis latent (FCILV)</i>					
<b>Whitefly transmitted</b>					
<i>Strawberry pallidosis associated (SPaV)</i>	<i>Crinivirus</i>	None, mild in some cultivars or in mixed infections. Masked in summer months.	RT-PCR	Australia, Canada, Egypt, U.S.	<i>Fragaria</i> spp.
<b>Oomycete transmitted (funguslike organisms)</b>					
<i>Tobacco necrosis (TNV)</i>	<i>Necrovirus</i>	Dwarf plant with leaf and root necrosis	ELISA, RT-PCR, indicators	U.S., Europe, Japan, Israel	Broad
<b>Unknown</b>					
Strawberry feather leaf	Unknown	None or mild in some cultivars	Indicator	U.S.	<i>Fragaria</i> spp.

<sup>a</sup> RT-PCR Reverse Transcription Polymerase Chain Reaction is a modification of PCR.<sup>b</sup> Indicators are susceptible plants that will show virus symptoms after sap inoculation or graft transmission.