Successful juice inoculation of the aphid-vectored strawberry crinkle virus

Jean Richardson ❑ Edward S. Sylvester

May lead to a rapid method for detecting the disease in commercial strawberries

California's multimillion dollar strawberry industry is based on a certification program that, in part, attempts to provide virus-free plants to growers. Certification depends on the ability to determine whether or not the plants supplied to nurseries are virus-free. Strawberry crinkle virus is one of several that infect commercial strawberries and can cause reduced vigor, resulting in substantial yield reduction. It is a rhabdovirus (a rod- or bullet-shaped virus) that multiplies in plants and in its aphid vectors.

Infected commercial strawberries normally are tested for the presence of strawberry crinkle virus by attempting to transmit the virus to an indicator host, such as Alpine strawberry, *Fragaria vesca* L. var. *semperflorens* (Duch.) (Ser.), that will develop characteristic symptoms of crinkle disease. Transmission to the indicator plants can be done only by grafting or by using the natural vectors—that is, aphid species of the genus *Chaetosiphon* that feed only on strawberry plants. Since it has not been possible to concentrate or purify strawberry crinkle virus directly from infected strawberries, other, more efficient and rapid diagnostic methods employing modern biotechnological approaches have not been available for use in the strawberry certification program. Such methods include serological tests and the use of nucleic acid probes.

Recently we found that although the pink and green potato aphid, *Macrosiphum euphorbiae* (Ashmead), would not acquire strawberry crinkle virus by feeding on diseased Alpine strawberry, it could be infected with the virus if injected with extracts obtained from infected *Chaetosiphon* aphids. Once infected, the potato aphid would transmit the virus to Alpine strawberry with a high rate of efficiency.

Since the potato aphid feeds on other plants as well as strawberry, we were able to use it as a surrogate vector to transmit the virus to plants in the Solanaceae family—ground cherry (*Physalis*) and tobacco (*Nicotiana*). We now have demonstrated that, once in the ground cherry species *Physalis floridana*, strawberry crinkle virus can be moved to other *Physalis* and *Nicotiana* species by juice inoculation.

Methods

Our isolate of strawberry crinkle virus originally came from an infected commercial strawberry cultivar. We currently maintain the virus in infected *Chaetosiphon* aphids frozen at -65°C. As needed, the virus is retrieved from the frozen aphids by grinding the head of an aphid in a small amount of distilled water, checking the extract for virus particles with an electron microscope, and if positive, using the extract to inject healthy recipient aphids. Following injection, the aphids are transferred to a sequence of test plants. The test plants, after the aphids have been removed, are fumigated and put in the greenhouse to await symptom development.

By this procedure, young adult potato aphids reared under controlled conditions in a growth chamber were infected with strawberry crinkle virus and then transferred to a series of test plants—first to Alpine strawberries to check on their infectivity and then to ground cherry. Once symptoms developed on the ground cherry plants, leaves were ground in 0.5 percent sodium sulfite solution and the suspension rubbed on carborundum-dusted leaves of healthy test plants. The plants had been placed in the dark for at least six hours before inoculation and were rinsed with water shortly after inoculation to remove excess inoculum and carborundum.

Results

The first juice transmission occurred from *P. floridana* to *P. floridana*. Five plants were inoculated, and one developed symptoms about two weeks later. In an attempt to improve the efficiency of juice transmission, selected isolates (lines) of the virus have been repeatedly transmitted by juice inoculation over a period of 15 months. The rate of transmission now averages 60 to 80 percent, and a recent strain from *N. glutinosa* has proved to be both efficiently transmitted and quite destructive in the symptoms it produces in ground cherry.

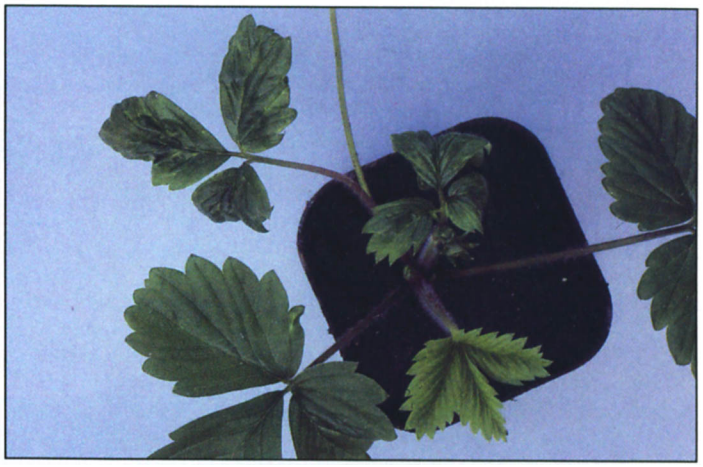
After the original juice-inoculated plants developed symptoms, they were tested in three ways to determine if the virus infecting these plants was in fact strawberry crinkle. First, extracts from some of the plants were negatively stained and examined with the electron microscope. Typical rhabdovirus-like particles were found. Second, extracts from the plants were injected into both aphid species—*Chaetosiphon fragaefolii* and the potato aphid (*Macrosiphum euphorbiae*). Some of the test Alpine strawberry plants to which the injected aphids were transferred developed typical symptoms of strawberry crinkle disease, and rhabdovirus-like particles were confirmed as being present in samples from petals of these symptomatic plants. Finally, *C. fragaefolii* fed on a sample of the infected Alpine strawberry plants, successfully acquired the virus, and then transmitted it to fresh Alpine test plants. Several lines of evidence thus suggest that strawberry crinkle virus, transferred by juice inoculation, was responsible for the symptoms produced in the recipient ground cherry plants.

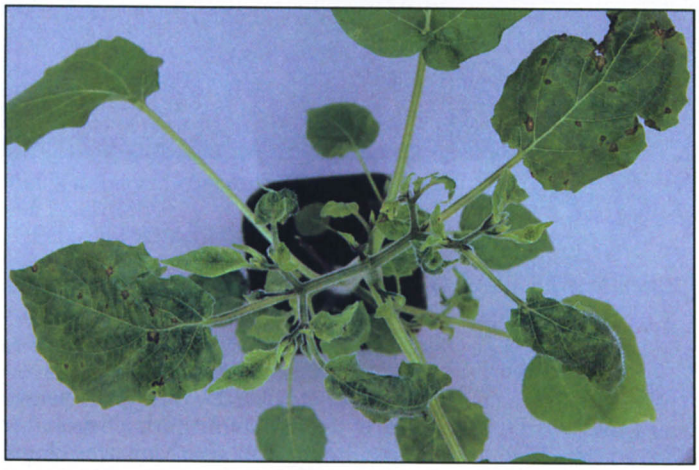
Since the initial experiments, juice transmission of strawberry crinkle virus has been

TABLE 1. Juice transmission of strawberry crinkle virus

Test species	Virus source species*				
	Physalis (ground cherry)			*Nicotiana* (tobacco)	
	floridana	*ixocarpa*	*pubescens*	X *edwardsonii*	*glutinosa*
Physalis					
floridana	+	+	+	+	+
ixocarpa	–	+	–	nt	nt
pubescens	+	+	+	+	+
Nicotiana					
clevelandii	+	nt	–	nt	–
X *edwardsonii*	+	nt	+	+	+
glutinosa	+	nt	+	+	+

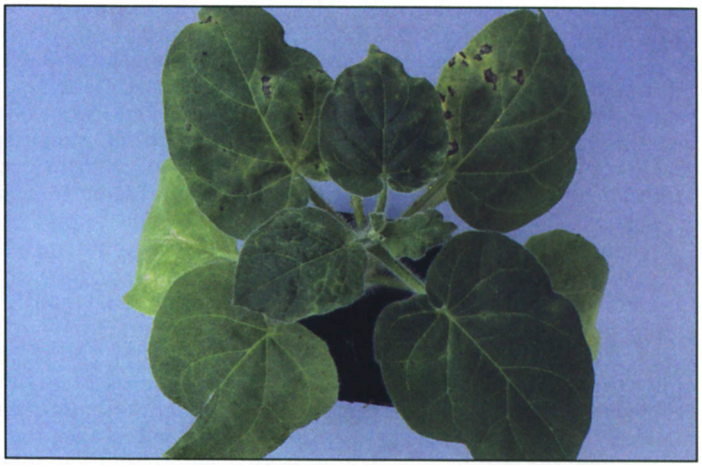
* The plus and minus signs, respectively, indicate that transmission did or did not occur. The "nt" means the combination was not tested.





Infections of strawberry crinkle virus in commercial strawberries can only be detected by graft transmission (above left) or aphid transmission (above) to *Fragaria vesca* indicator plants. Plant above left is a runnering clone; above is a nonrunnering Alpine strawberry seedling.

Juice transmission of SCV to ground cherry, *Physalis floridana* (left), and tobacco, *Nicotiana* X *edwardsonii* (lower left) and *N. glutinosa* (lower right) was accomplished in a two-stage process. Virus from infected strawberry aphids, which tend to feed only on strawberry, was injected into pink and green potato aphids, which will feed on a wide variety of plants. These aphids then were used as surrogate vectors to transmit the virus to ground cherry. Once in this plant, the virus was juice-transmissible to other species. It still is not possible to infect strawberry directly by juice transmission.



successful from the ground cherry *P. floridana* to three *Nicotiana* species (table 1). The virus also has been juice-transmitted within *Nicotiana* species and from *Nicotiana* to ground cherry.

Conclusions

We have been able to transmit strawberry crinkle virus from strawberries to solanaceous plants in the genera of *Physalis* and *Nicotiana*, first by using injected individuals of the pink and green potato aphid as surrogate vectors, and then by juice inoculation from these new plant hosts.

Strawberry is a notoriously difficult plant from which to isolate virus. The identification of the many viruses infecting this important commercial crop has been a slow and involved process coupling studies of transmission by aphids with those involving graft transmission to special *Fragaria* indicator plants. These indicator plants are selected for their ability to express definitive symptoms of specific viruses.

Strawberry crinkle virus has been one of the more difficult viruses to work with, but now it is possible to transmit it in juice to host plants that previously have been used successfully in many virological studies—*Nicotiana* species. This development may lead to rapid and specific diagnostic methods for detecting the strawberry crinkle virus in infected commercial strawberries.

Jean Richardson is Staff Research Associate, and Edward S. Sylvester is Professor of Entomology, Department of Entomological Sciences, University of California, Berkeley.

The authors recently learned through personal communication that a Dutch researcher (S. A. van der Meer) may have transmitted an isolate of strawberry crinkle virus by juice inoculation in a species of tobacco.