Isolation and Characterization of a Virus from Saguaro Cactus

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ABSTRACT

An isometric virus was isolated from saguaro cactus (*Carnegiea gigantea*) in Arizona. This is the first virus found in this plant, and the first isometric virus to be isolated from the family Cactaceae. The virus is most easily isolated from the floral parts of the saguaro. The virion is ca. 35 nm in diam, contains less than 20% RNA, sediments as a single band in density-gradient centrifugation, and appears as a single component in the analytical ultracentrifuge. Antiserum to the saguaro virus antigen failed to show any serological relationship with 7 other isometric viruses tested. *Chenopodium amaranticolor* is a good local lesion host, whereas *Chenopodium capitatum*, a good systemic host, is useful as a source of virus for purification.

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Additional key words: electron microscopy, nucleic acid, purification, serology.

Virus diseases of *Opuntia* sp. have been reported in the USA and other parts of the world (1, 4, 7, 8, 18). Investigation of other cactus species for possible virus infection was initiated in 1966. Of particular interest in Arizona was the giant saguaro (*Carnegiea gigantea* Britt & Rose), a prominent member of the plant community of the Sonoran Desert of southern Arizona and northern Sonora, Mexico.

Saguaros were sampled in connection with a general cactus virus survey. It was during such a survey that the saguaro virus was first discovered. This study reports on certain properties of the virus.

MATERIALS, METHODS, AND RESULTS.—*Isolation.*—Comparison of extracts from various parts of the saguaro revealed that reproductive tissues (buds, flowers, fruit) were superior to vegetative tissues as sources of the virus. The reproductive parts from 131 saguaro plants were collected within the boundaries of the Saguaro National Monument near Tucson, Ariz., and used for virus isolation. The fresh tissue was ground in 0.1 M phosphate buffer, pH 7.0, by mortar and pestle. Inoculations were made by stroking Carborundum-dusted leaves with stiff-bristled plumbers' acid brushes dipped in virus containing extracts and rinsing the leaves with distilled water. *Chenopodium amaranticolor* Coste & Reyn. was the main index host; it produced local lesions 4 to 5 days after inoculation. Each sample tested was assayed by the whole leaf method.

Of the 131 saguaros sampled, 52 (40%) were infected with saguaro virus (SV). Lesions on *C. amaranticolor* inoculated with saguaro extracts resembled those caused by Sammons' opuntia virus (SOV). Inoculum prepared from leaves of *C. amaranticolor* infected with SV were highly infectious as contrasted with poor infectivity of similar extracts containing SOV.

Host range.—The following plants were inoculated with sap from *C. amaranticolor* infected plants: *Chenopodium capitatum* (L.) Asch.; *C. quinoa* Willd.; *Carthamus tinctorius* L.; *Datura metel* L.; *Gomphrena*

globosa L.; *Nicotiana glutinosa* L.; *N. rustica* L.; *N. sylvestris* Speg & Comes; *N. tabacum* L. 'Havana', 'Burley', 'Hicks', 'Samsun', and 'Xanthi'; *Phaseolus vulgaris* L. 'Pinto'; *Triticum vulgare* L.; and *Vigna sinensis* (Torner) Savi 'Blackeye'. The plants were observed for symptom development for 30 days, after which back-inoculations were made to *C. amaranticolor* from inoculated plants and controls. Only *Chenopodium* spp. and *G. globosa* proved to be susceptible. *C. amaranticolor* and *C. quinoa* reacted with the production of local lesions only, whereas *C. capitatum* reacted with a systemic mottle. Four to 8 weeks after infection, *C. capitatum* wilted and died.

Gomphrena globosa produced lesions on the inoculated leaves followed by the development of local lesions on the noninoculated leaves.

Purification.—Infected leaf tissue of *C. capitatum* was triturated in a mortar and pestle and diluted with 2.5 vol of cold 0.05 M K_2HPO_4-NaH_2PO_4 buffer, pH 7.0. The virus was purified by Steere's method (20), except that 1% Triton X-100 (alkyl polyethoxyethanol, Rohm & Haas, Philadelphia, Pa.) was substituted for chloroform-butanol. Two cycles of differential centrifugation followed by sucrose density-gradient centrifugation (3) were sufficient to purify the virus. Final suspensions (2.5 ml) in 0.05 M phosphate buffer, pH 7.0, were floated on rate sucrose-density gradient tubes which consisted of 4, 7, 7, 7 ml of 100, 200, 300, 400 g sucrose/liter prepared 12 hr previously and centrifuged for 2.5 hr at 64,000 *g* (24,000 rpm) in a Spinco SW 25.1 rotor. The tubes were fractionated with an ISCO density-gradient fractionator and analyzed in an ISCO UA-2 absorbance analyzer.

Density-gradient centrifugation of purified infective *C. capitatum* extract resulted in a single light-scattering band (Fig. 1-G). When the tube was fractionated, the presence of only a single component was confirmed (Fig. 1-F, G).

The optical density of virus preparations was read after density-gradient centrifugation. The ratio of the 280:260 nm readings was ca. 0.700, whereas the 260:244 ratio was 1.25. No significant ultraviolet absorbing material was present in the preparations from healthy controls.

Analytical ultracentrifugation.—Sedimentation rates of purified preparations from both SV-infected and healthy *C. capitatum* were determined with a Spinco Model E analytical centrifuge (Beckman Instruments). The sedimentation pattern was observed with Schlieren optics, and pictures taken at 4-min intervals once the programmed speed of 22,000 rpm was reached (Fig. 1-E). An $S_{20,w}$ of 107 was estimated for the virus by the graphical method and corrected to standard conditions (16).

Nucleic acid.—Nucleic acid was prepared from purified SV by the phenol method (11) and by degradation of the purified virus with lithium chloride (9). Intact SV, phenol-extracted SV-RNA, and lithium-chloride prepared SV-RNA were tested by the diphenylamine method (6) for the presence of DNA and by the orcinol test for the presence of RNA (15). Deoxyadenosine was used as a standard for the diphenylamine test; and TMV-RNA, as a standard for the orcinol test.

Nucleic acid of SV was successfully prepared by both methods employed. The diphenylamine test gave a positive reaction with deoxyadenosine but was negative with SV-NA. The orcinol test gave a positive reaction with both TMV-RNA and SV-RNA prepared by either method. The SV-RNA preparation gave a 260:230 ratio of 2.1.

The saguaro virus nucleic acid preparation proved to be moderately infectious (7-20 lesions/leaf) when mixed with 50 mg/ml bentonite and rubbed on leaves of *C. amaranticolor.*

The 280:260 ratio of optical density readings of purified virus was used to estimate the proportion of nucleic acid in the virus particle (14). For the saguaro virus, this ratio averaged approximately 0.700, which, by the method used, represents 10% nucleic acid. A purified preparation of tobacco mosaic virus had a ratio of 0.843 which represents 5.5% nucleic acid. The total phosphorus content of SV has been determined subsequently, and the proportion of nucleic acid was calculated to be 17.2% (Nelson & Tremaine, *unpublished data*). The differences between the two methods are not explained at present. The latter method seems to be more widely used and probably more accurate.

Electron microscopy.—Both 2% phosphotungistic acid, pH 7.0 (5), and 1% uranyl acetate, pH 4.0 (12), were used to stain purified virus and epidermal dip preparations of SV infected *C. capitatum* for electron microscopy. In both cases, excess stain was removed with filter paper and grids immediately viewed in a Hitachi HS-7 electron microscope.

Both purified preparations and leaf dip preparations of *C. capitatum* infected with SV showed isometric particles ca. 35 nm in diam, based on the calibration figures for the microscope (Fig. 1-D).

Serology.—Antiserum to SV was prepared in rabbits by three weekly intravenous injections of 2 mg of purified SV. One week after the third injection, the rabbit was bled and the serum fraction separated. The resulting serum was tested in gel diffusion plates (2) against SV and healthy plant sap for antibodies specific to SV. Also tested against purified SV were antisera to cherry necrotic ringspot virus, apple mosaic virus, rose mosaic virus, plum line pattern virus, tobacco ringspot virus, cucumber mosaic virus, and squash mosaic virus. Antiserum with a reciprocal titer of 600 (determined by gel diffusion) was obtained from rabbits injected as described. The SV antiserum and its homologous antigen reacted in gel double diffusion by the formation of an arced band nearest the antigen well. The SV antigen did not react with antisera to any of the other viruses mentioned above.

Saguaro inoculations.—Since nothing was known about the method or speed of spread of SV in the field, the utilization of large field plants for infectivity tests was deemed impractical. Instead, attempts to infect saguaro were done with small seedlings in the greenhouse. Seedlings, 15 months of

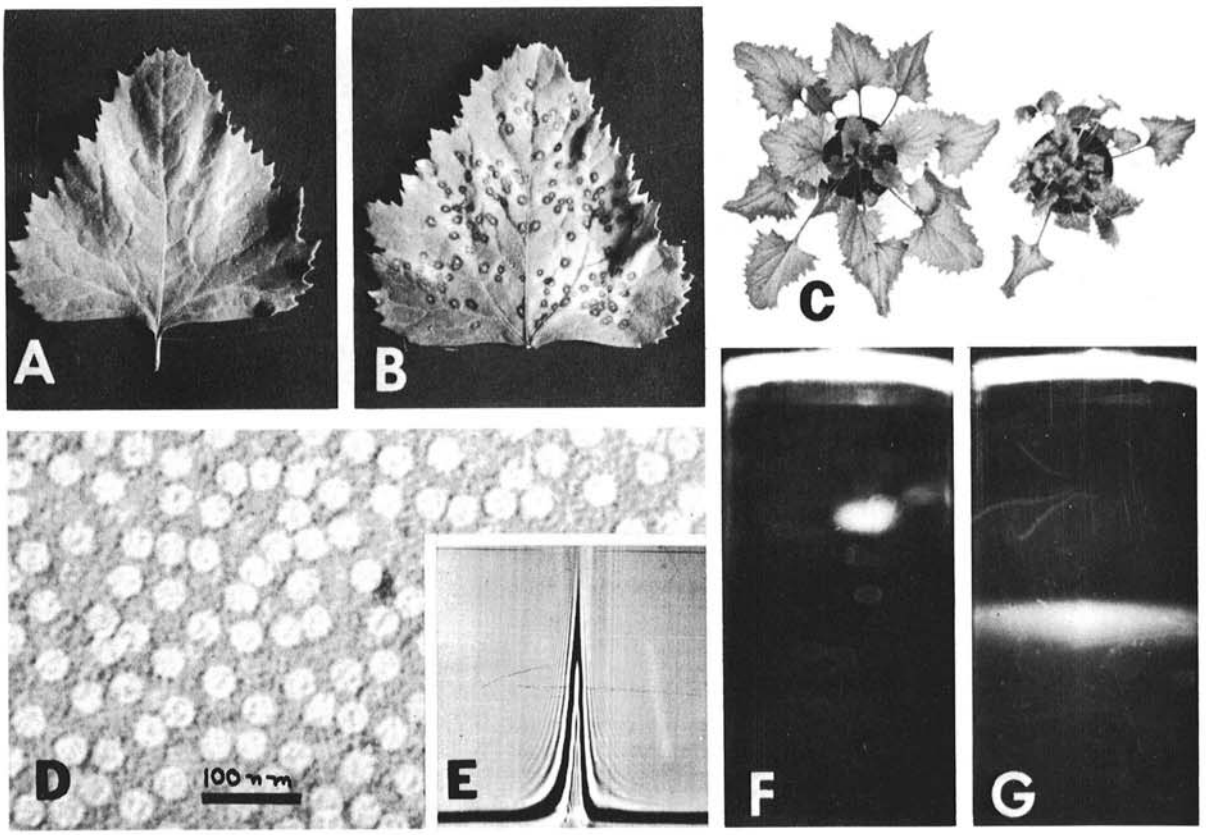

Fig. 1. Saguaro virus properties: **A)** Healthy *Chenopodium amaranticolor* leaf. **B)** *C. amaranticolor* leaf with local lesions from the saguaro virus. **C)** Healthy (left) and infected (right) *C. capitatum*. **D)** Electron micrograph of negatively stained saguaro virus. **E)** Single peak typical of the saguaro virus 8 min after reaching speed of 22,000 rpm in Model E analytical ultracentrifuge. **F)** Purified extract of healthy *C. capitatum* after centrifugation in density gradient. **G)** Purified extract from saguaro virus-infected *C. capitatum* after centrifugation in density gradient.

age, plus the only 5-year-old plant available were used. Inoculations were accomplished by injected 0.02 ml of purified virus at the base of each plant. At 30, 120, and 180 days, whole plant homogenates were rubbed on *C. amaranticolor*. No virus was recovered from any of the 15-month-old seedlings.

The 5-year-old plant was inoculated in the same fashion. Small pieces of tissue were removed and homogenized, and the resulting extract was rubbed on *C. amaranticolor* at 30, 120, 180, and 395 days. This plant was positive only at 395 days both by infectivity and serology.

Effects of in vitro dilution, aging, and heating on infectivity of crude extracts of SV.—Crude extracts of infective *C. capitatum* sap diluted in 0.05 M phosphate buffer, pH 7.0, were used in these tests. The assay host used in this work, *C. quinoa*, produced on the average maximum of 100 lesions/leaf when inoculated with concentrated virus solutions. When crude sap of *C. capitatum* was diluted 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, etc, the maximum lesion number was maintained to a dilution of 10^{-3}. Between dilutions of 10^{-3} and 10^{-4}, there was a 95% drop in lesions produced. A few scattered lesions continued to appear on plants through several subsequent tenfold dilutions.

A crude sap dilution of 10^{-1} was used in aging experiments. This preparation was left at room temperature (20 C), and portions were used periodically for assay. Maximum lesion counts (average $\approx$ 100) occurred to 16 days. At 22 days, lesion counts dropped 90% to 10. After 34 days, infectivity was completely lost.

Since dilutions of 10^{-1} and 10^{-2} apparently contained far more virus than was needed to saturate all infectible sites on *C. quinoa* leaves, it was decided that dilutions of 10^{-2} and 10^{-3} should be used for temperature inactivation tests. Each dilution was heated at 50, 55, 60, 65, 70, and 75 C for exactly 10 min. No reduction in infectivity of 10^{-2} dilution (as compared to unheated control) occurred until exposed to 65 C (50% reduction in lesion numbers). At 75 C, all infection in 10^{-2} dilution was lost. The 10^{-3} dilution of crude sap showed a reduction in infectivity of 50% at 55 C; at 65 C, all infectivity was lost. Thus, the two dilutions showed similar inactivation curves but 10 C apart.

DISCUSSION.—This is the first virus to be found

in saguaro and the first isometric virus reported from any member of the family Cactaceae. Its properties are distinct from those of other isometric viruses such as cucumber mosaic and squash mosaic commonly found in Arizona.

The isometric viruses have been divided into several groups on the basis of proportion of nucleic acid, presence or absence of accessory particles, and type of nucleic acid (10). Based on properties thus far determined for the saguaro virus, it belongs in the 20% nucleic acid group. The basic characteristics involved are its proportion of NA (17%), type of NA (RNA), and finally, the lack of discernible accessory particles. None of the other viruses of this group was available during this study for comparison except cucumber mosaic virus.

Sowbane mosaic virus was considered as a possible contaminant, but was ruled out because of differences in symptomatology (13), and the lack of a history of seed-transmitted virus in our *C. amaranticolor*.

Cocksfoot mottle virus isolated from *Dactylis glomerata* by Serjeant (19) was used in a comparative study by Paul & Huth (17). In this study, sowbane mosaic, cocksfoot mottle, and cocksfoot mild mottle were compared by host range, physical properties, and serological cross reaction. No relationship was found between these viruses despite their similarities. Saguaro virus with a $S_{20,w}$ of 107 and with no accessory particle might be another member of this group of viruses that have no intimate relationships. These viruses may be transmitted by aphids or beetles; and for some, no vectors are known.

The difficulty of recovering the viruses from plants inoculated in the greenhouse may be related to the growth habits of the saguaro cactus. The slow growth of the plant and the slow translocation of materials in the cells might retard the systemic spread of the virus so that infection would be difficult to detect in a time period adequate for most other virus-host systems.

The widespread occurrence of the SV within the native stands is significant. The SV has probably been present for a long time, and has been unnoticed because the cactus is a latent carrier of the virus. Studies are underway to determine the spread of the virus in Arizona. Comparison of SV with more members of the 20% NA group of viruses is in progress to determine whether there is any relationship.

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