# The Distribution and Electron Microscopy of Viruses of Cacti in Southern Arizona

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## ABSTRACT

Extensive surveys for naturally occurring, virus-infected cacti within the region of southern Arizona resulted in the detection of only two of the five viruses reported to infect members of the Cactaceae. Symptomatology, host assays, light and electron microscopy, and subsequent analyses of particle morphology revealed the presence of the Sammons' Opuntia virus (a rod-shaped particle with a normal length of 321 nm and a width of 18 ± 1 nm) and the saguaro virus (a spherical particle with a diam of 32 ± 1 nm). Both viruses were found to occur naturally.

Examination of a cultivated specimen of *Platyopuntia monacantha* was the single occasion in which an elongated virus with a size identical to that reported for cactus virus X was detected. A mixed infection (consisting of the Sammons' Opuntia virus and cactus virus X) was noted only once, and this was in a cultivar of *Platyopuntia chlorotica*. Neither of the remaining two filamentous viruses (cactus virus 2 and zygocactus virus) was encountered during this study.

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Little is known about the viruses of cacti in their native habitat, although for many years they have been studied extensively in cactus collections in Europe. The unusual anatomical structure of cacti led to early cytological studies and reports of spindle-like, proteinaceous structures (11). Subsequently, these inclusions of "protein bodies" were found in several species of cacti and were considered by many early botanists to be food reserve material. In 1920, Weingart (15) demonstrated that the "agent" responsible for the formation of these bodies was graft transmissible; and this "agent" was ultimately shown to be viral in nature (1, 12).

Five viruses have hitherto been reported to occur in the Cactaceae: Sammons' Opuntia virus (SOV) (13); cactus virus X (CVX) and cactus virus 2 (CV2) (2, 4), the zygocactus virus (ZV) (5); and the saguaro virus (SV) (9).

The first report on the presence of virus-infected cactus species native to Arizona was made by Chessin in 1965 (6); and a general distributional study of cactus viruses in portions of Arizona, Nevada and Utah was published in 1972 by Chessin & Lesemann (7).

The following study, initiated in 1966, is concerned with the identification and distribution of cactus viruses in the southern portion of Arizona.

MATERIALS AND METHODS.—Each of the viruses which have thus far been isolated from cacti will multiply in, and generate distinctive symptoms on, the leaves of certain members of the Chenopodiaceae. The virus receptors most commonly used are *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., and *C. capitatum* (L.) Asch. Cactus samples collected from the field for virus assaying were macerated with mortar and

pestle in 0.1 M Na-K phosphate buffer, pH 7.0; and the resulting extract, combined with a small amount of 600-mesh Carborundum, was brushed onto the leaves of test plants. Light microscopy afforded an additional means of diagnosing for the presence of SOV, by the rapid detection of the spindle-shaped, intracellular inclusions.

Field samples consisted of flattened pads of the *Platyopuntia* spp., cylindrical joints of the *Cylindropuntia* spp., reproductive organs of the saguaro, and small pieces of tissue of species, where no discrete organ or plant part was available for convenient collection. Three to 10 samples of each species were collected at each site depending upon availability. Each of the samples was kept separately in a thick plastic bag; and those collections requiring refrigeration were immediately transferred to insulated containers filled with ice. The collections were then either maintained under greenhouse conditions or kept refrigerated until they could be assayed.

Collections were made in the vicinity of Phoenix, Tucson, and Ajo, with surveys also being made in terrain far removed from urbanized and agricultural areas. Specific locations of collection included the Desert Botanical Garden in Tempe, both the East and the West sections of the Saguaro National Monument near Tucson, an area south of Picacho Peak, Mt. Lemmon (at an elevation of approximately 1,067 m), and The Organ Pipe National Monument. One collection of *Platyopuntia* spp. was made at Palm Canyon, near Quartzsite. In addition, a few samples were obtained from private cactus collections, maintained in southern Arizona.

Electron microscopic examinations were conducted with an Hitachi HS-7S electron microscope. Most crude-sap preparations were negatively stained with 2% phosphotungstic acid (PTA), pH 7.0; whereas 1% uranyl acetate (UA), pH 4.0, was the negative stain most frequently used for purified preparations. The microscope was calibrated by the use of a carbon grating replica of 2,160 lines per millimeter (Fullam No. 1002), and the resulting magnifications were frequently checked against such internal standards as tobacco mosaic virus (TMV) and fraction-1-protein (18-S). Based upon the calibration, and with 1% UA (pH 4.0) used as the negative stain, TMV showed a normal length (4) of 300 nanometers (nm) and a width of $18 \pm 1$ nm: these are the classic dimensions of TMV, generally agreed upon. The diam of fraction-1-protein was found to be $11 \pm 1$ nm, which is in excellent agreement with previously reported sizes for this particle (8, 14). All sample numbers utilized in these statistical checks on the reliability of the diffraction grating were in excess of 500. When 2% PTA (pH 7.0) was used with the above standards, there was in every case perfect agreement as to particle dimensions—with the exception of TMV-width, which consistently measured $15 \pm 1$ nm. Notwithstanding this single disagreement, the magnification calibration was deemed accurate; the particle morphology for each of the

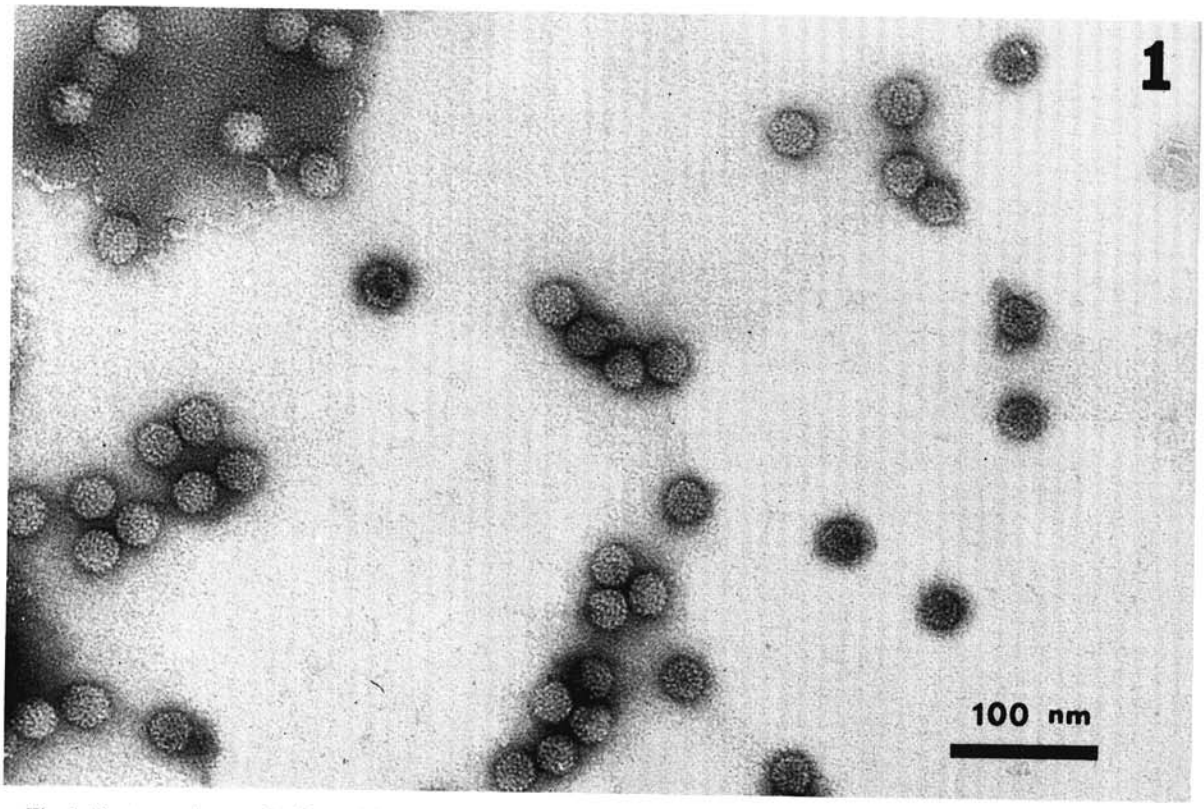


**Fig. 1.** Electron micrograph of a purified preparation of the saguaro virus mounted in 1% uranyl acetate, pH 4.0. With a sample number of 1,387 (obtained from 40 individual micrographs) the diam of this virus is shown to be $32 \pm 1$ nm (× 186,000).

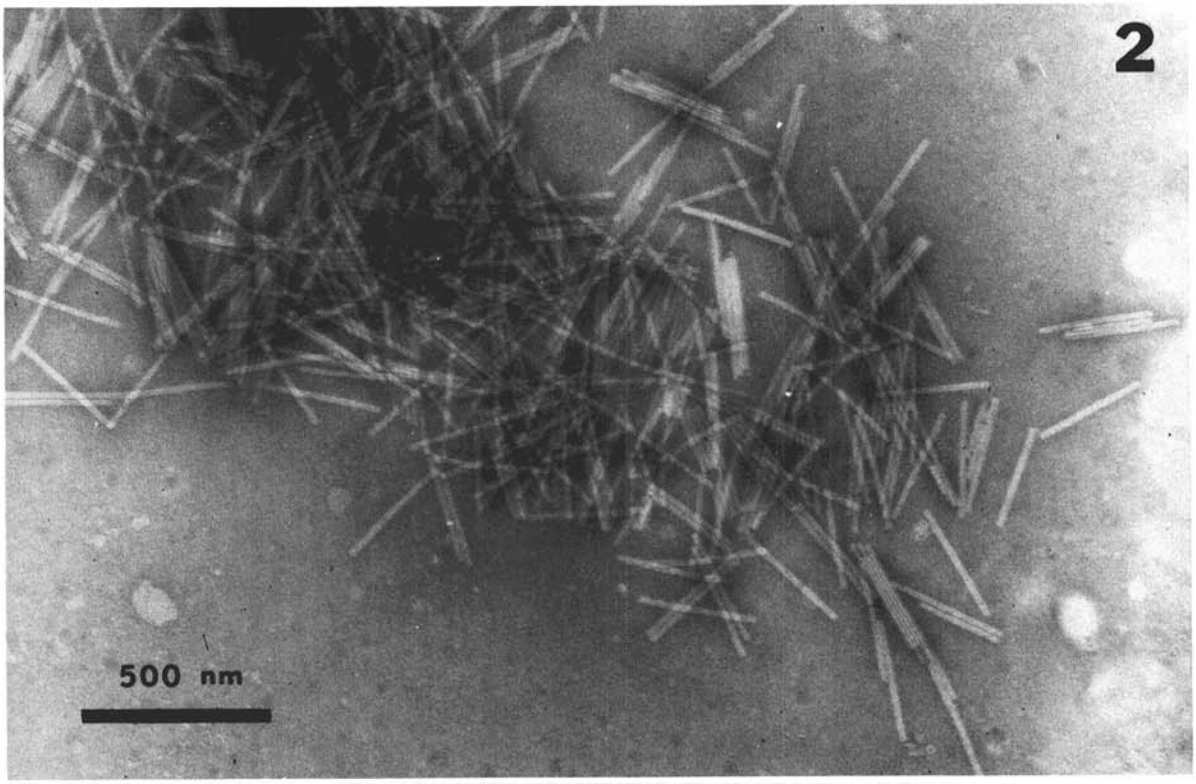


**Fig. 2.** Electron micrograph of negatively stained crude extract from *Platyopuntia engelmannii*, showing a portion of a disintegrating, spindle-shaped crystal of Sammons' Opuntia virus. Stain: 2% phosphotungstic acid, pH 7.0 (× 48,100).

viruses herein reported was based upon this calibration; and all sample numbers used in the determination of particle morphology were drawn from a minimum of 25 individual micrographs at magnifications ranging from ×30,500 to ×226,000.

The most intensive laboratory work was with the saguaro virus. This included physical, chemical, and serological studies which have been reported elsewhere (10).

RESULTS.—The following cacti were assayed but not found infected, judged upon the absence of external symptoms, inclusion bodies, and negative infectivity tests: cholla (*Cylindropuntia arbuscula* Eng., *C. bigelovii* Eng., *C. fulgida* Eng., *C. leptocaulis* Eng., *C. versicolor* Eng.), robust hedgehog (*Echinocereus robustus* Peebles), barrel (*Ferocactus acanthodes* Britt. & Rose, *F. wislizenii* Britt. & Rose), organ pipe (*Lemaireocereus thuberi* Britt. & Rose), senita (*Lophocereus schottii* Britt. & Rose), and pincushion (*Mammillaria microcarpa* Eng.).

Saguaro (*Carnegiea gigantea* Britt. & Rose) and prickly pear (*Platyopuntia* spp.) were the only genera found to be infected by viruses, and three different viruses were detected: an icosahedral-shaped virus infecting the saguaro, and two different rod-shaped viruses infecting the *Platyopuntia* species.

The virus in the saguaro was most easily isolated from the reproductive portions of the plant. Whenever portions of virus-infected floral tissue (petals, sepals, and fruit) were excised with a razor blade, homogenized with mortar and pestle, and rubbed onto Carborundum-dusted leaves of *C. amaranticolor*, the leaves developed discrete, local lesions 3-4 days after inoculation. When the virus was purified from systemically infected *C. capitatum* and examined in the electron microscope, spherical virus particles with a diam of 32 ± 1 nm were observed (Fig. 1).

Insofar as has been determined, SV is a new, previously unreported virus. The physical, chemical, and serological qualities of SV distinguish it from any other isometric virus common to Arizona (10). It is the only virus ever detected in the giant saguaro and is the first spherical virus ever reported to infect any member of the Cactaceae. No evident symptoms have yet been associated with the virus-infected saguaro.

The distribution of SV appears to be limited to several localized areas near Tucson. No virus-infected saguaros were found in regions distant from either urbanization or agriculture. Where SV does occur, up to 40% of the saguaros were found to be infected. In one portion of the Rincon Division of the Saguaro National Monument, 48 of 107 saguaro plants (45%) were infected. Such localized infection sites indicate that the virus spreads at a very low rate. Field plots have been established to monitor the progression of natural infection.

Insect vectors for SV have not as yet been determined; but a variety of insects, each in abundance, do visit the saguaro during the flowering season and could easily carry nectar and pollen, contaminated with the virus,

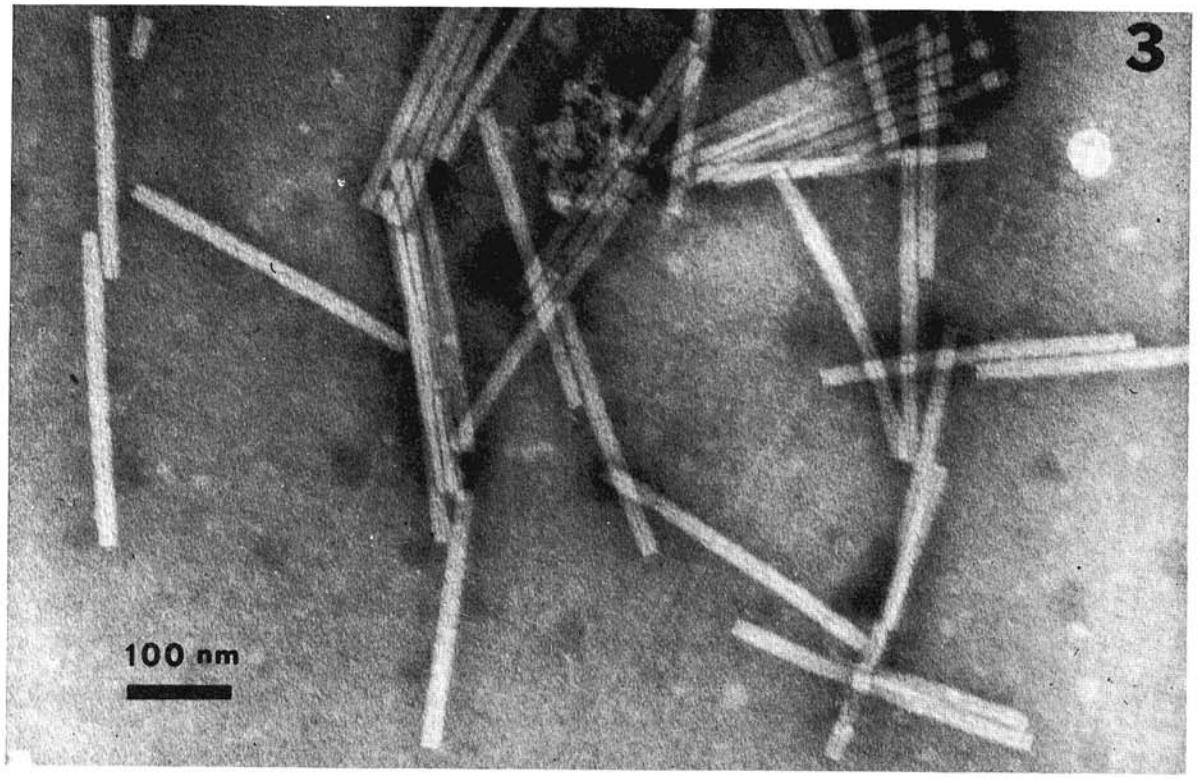


**Fig. 3.** Electron micrograph of Sammons' Opuntia virus (321 × 18 nm), affixed with 2% phosphotungstic acid, pH 7.0. Note the darker core of the individual particles, reminiscent of TMV (× 132,100).

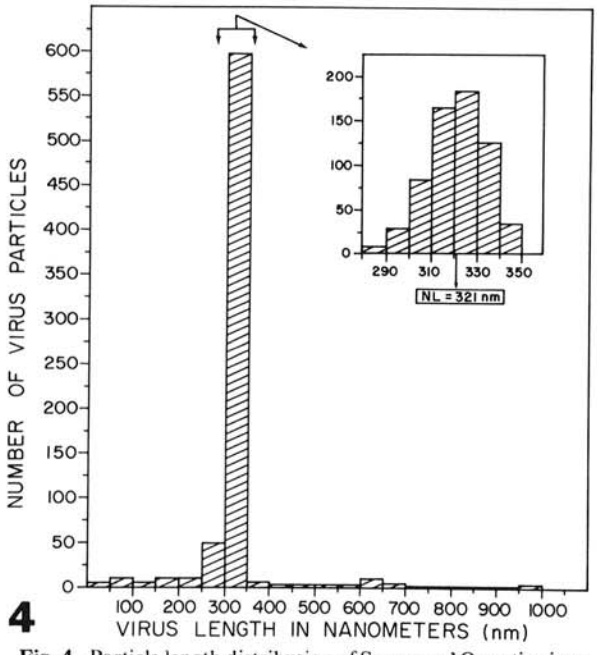


**Fig. 4.** Particle length distribution of Sammons' Opuntia virus extracted from *Platyopuntia engelmannii*. The larger histogram has a class interval of 50 nm and includes a sample number (n) of 723 particles. The insert, showing a class interval of 10 nm, is represented by 88% of the total sample. The normal length (NL) of this virus is 321 nm and the width is 18 ± nm (where n = 3 observations/particle).

from infected to noninfected plants. The saguaro virus has not yet been isolated from any other native plant in the areas thus far surveyed.

Specimens of *Platyopuntia basilaris* Eng. & Big., *P. chlorotica* Eng. & Big., *P. engelmannii* Salm Dyck, *P. gosseliniana* Weber, and *P. phaeacantha* Eng. were also found to be infected with virus. While an infected saguaro cactus neither exhibits external symptoms nor contains spindle-shaped, inclusion bodies, all species of virus-infected prickly pear, found in their native habitat, exhibited striking external symptoms; and exclusive of *P. basilaris*, all *Platyopuntia* spp., showing such symptoms, contained spindle-shaped, crystalline structures (Fig. 2).

A rigid, rod-shaped virus (Fig. 3), with a particle morphology identical to that reported for the Sammons' Opuntia virus (3), was found in those *Platyopuntia* spp. showing chlorotic ring symptoms. The normal length of SOV (Fig. 4) is slightly greater than that reported for tobacco mosaic virus; the widths of the two viruses are identical (18 ± 1 nm); both viruses have a similarity in structure, where the central portion of the particle assumes a greater density; and both have a distant serological relationship (16).

All naturally occurring *Platyopuntia* spp. showing chlorotic, concentric, and interlocking rings on the pads were found to be infected with SOV. Instances were encountered in which an individual plant in the field showed symptoms on some of the pads, while other pads were symptomless; yet all the pads of this plant contained inclusion bodies and virus particles. Symptoms on the pad can be mild enough to cause only a chlorotic flecking

or severe enough so the pad can be depressed where the chlorotic rings occur. The rings are structural entities that extend through the palisade layer of the pad; and where this occurs, the areoles protrude above the surface of the pad, giving it a rough, uneven appearance. Based upon electron microscopic examinations and the host response of *C. quinoa*, the SOV is believed to be the incitant of this ringed symptom.

The opuntia joint bug (*Chelinidea vittiger* Uhler) causes chlorotic markings similar to those caused by virus infection (Alcorn, Nelson & Olin, *unpublished*), and a feeding period of 15 min is sufficient to cause such markings. However, an exudate plug can usually be found in the center of the chlorotic ring to distinguish these markings from virus symptoms. Such markings have been observed on many different cacti, including prickly pear, cholla, barrel, and saguaro and have been noted also by other workers (7, 9).

The distribution of SOV in populations of prickly pear appears to be similar to that of SV in the saguaro. In areas where the desert has been relatively undisturbed and somewhat isolated there is very low incidence of virus infection in prickly pear. This suggests that there may be some relationship between man's disruption of the natural biome and the presence of SOV. For example, prickly pear in the metropolitan areas of Tucson and Phoenix and contiguous areas have a relatively high incidence of virus infection. On the other hand, only transplanted prickly pears at park headquarters and some native plants at the ancient Indian agricultural area near Quitobaquito Springs of the vast Organ Pipe National Monument show virus infection. Prickly pears in other areas of the Monument are free of virus infection. In addition, none of the prickly pears collected near isolated Palm Canyon (70 miles north of Yuma) in western Arizona contained virus. While these observations point toward some cultivated plant species as a possible source of SOV, no direct evidence has been obtained to substantiate this.

A specimen of *Platyopuntia monacantha*, obtained from a private collection and showing variegated symptoms, contained only flexuous particles (Fig. 5), identical in size to that reported for CVX by Brandes & Bercks in 1962 (2) (Fig. 6). Neither the zygocactus virus nor cactus virus 2 was detected during any of these studies; and mixed infections were not found in any of the uncultivated collections. The single encounter with two types of virus particles in the same plant was from one specimen of *P. chlorotica,* obtained from the Desert Botanical Garden, Phoenix, in which SOV and CVX were found (Fig. 7).

DISCUSSION.—Virus-infected cacti studied in Europe generally have not shown morphological symptoms. Indications are that, in Arizona, symptoms are governed by environmental conditions. As stated earlier, an individual, virus-infected plant in its natural

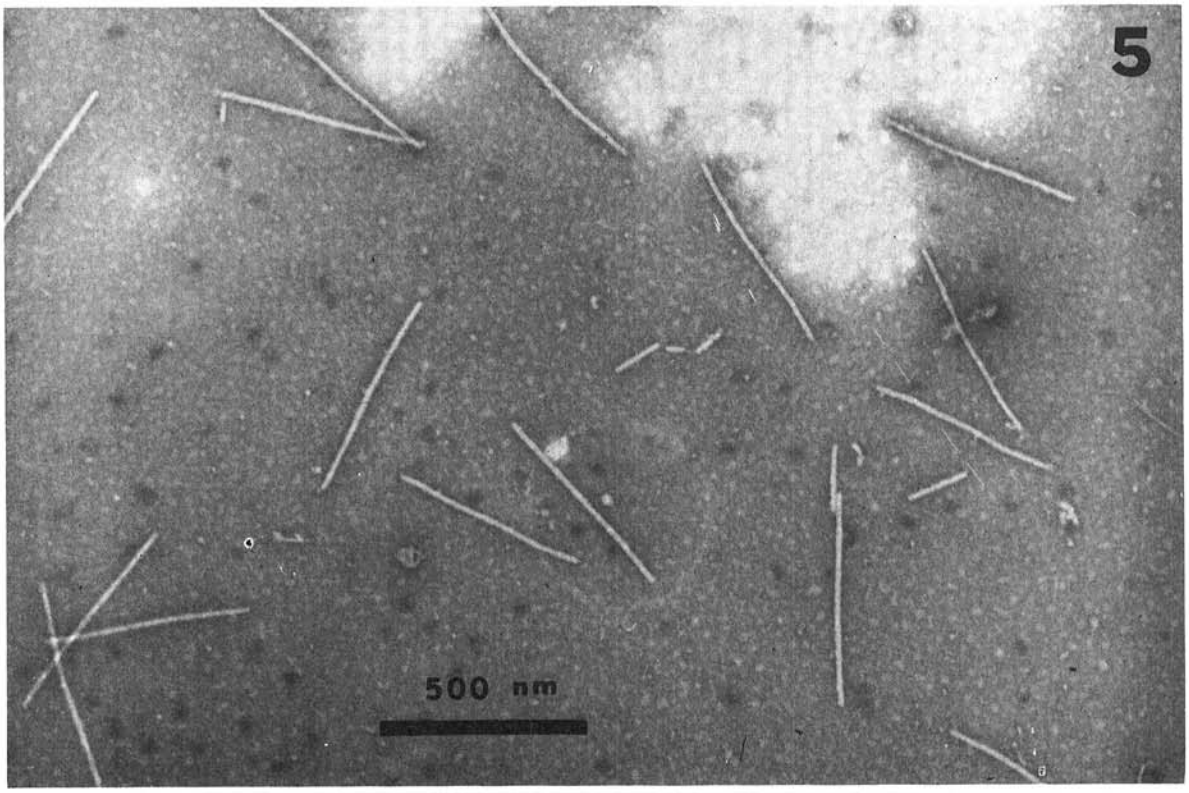


**Fig. 5.** Electron micrograph of negatively stained crude extract from *Platyopuntia monacantha*, showing the more flexuous cactus virus × particles (511 × 13 nm). Stain: 2% phosphotungstic acid, pH 7.0 (× 53,000).

environment can have pads with severe symptoms, while other pads may show only mild symptoms or none at all, even though all pads on the plant contain virus. When pads with severe symptoms were collected in the field and maintained under greenhouse conditions for several years, all growth that developed from the original pads contained virus particles but none of the new growth showed morphological symptoms. Thus, virus-infected cacti, which may not exhibit symptoms under artificial conditions, may show symptoms in their natural habitat as a consequence of an enhanced sensitivity to the "micro" environment.

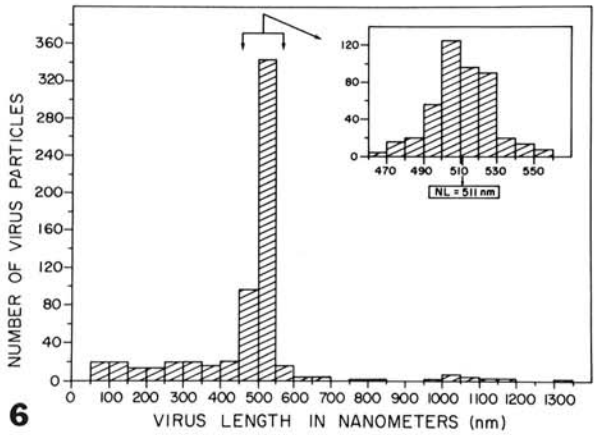


**Fig. 6.** Particle length distribution of cactus virus X extracted from *Platyopuntia monacantha*. The larger histogram has a class interval of 50 nm and includes a sample number (n) of 618. The insert, showing a class interval of 10 nm, is represented by 72% of the total sample. The normal length (NL) of this virus is 511 nm and the width is 13 ± 1 nm (where n = 3 observations/particle).

A major contribution of this study was the discovery of the virus infecting the saguaro cactus, a virus distinct from other isometric viruses found in Arizona. SV is latent in the saguaro; hence, what effect the virus may have on the cactus is currently open to speculation. The slow growth-rate of the plant, along with the extreme variability in size, flowering, and growth among the plants, will render the evaluation of virus effects difficult and will necessitate long-range studies. The saguaro virus is widespread in localized areas contiguous with urbanized and agricultural regions. The scattered, localized virus infection is also common among the native *Platyopuntia* spp. SOV was found to be the most prevalent virus.

In part, these investigations offer the first confirmation of: (i) Chessin's report in 1965 (6) on the presence of SOV-infected *Platyopuntia* spp. in southern Arizona; (ii) the particle morphology of SOV, determined by Brandes & Chessin in 1965 (3); and (iii) the 1972 survey by Chessin & Lesemann (7), in which neither CV2 nor ZV was detected

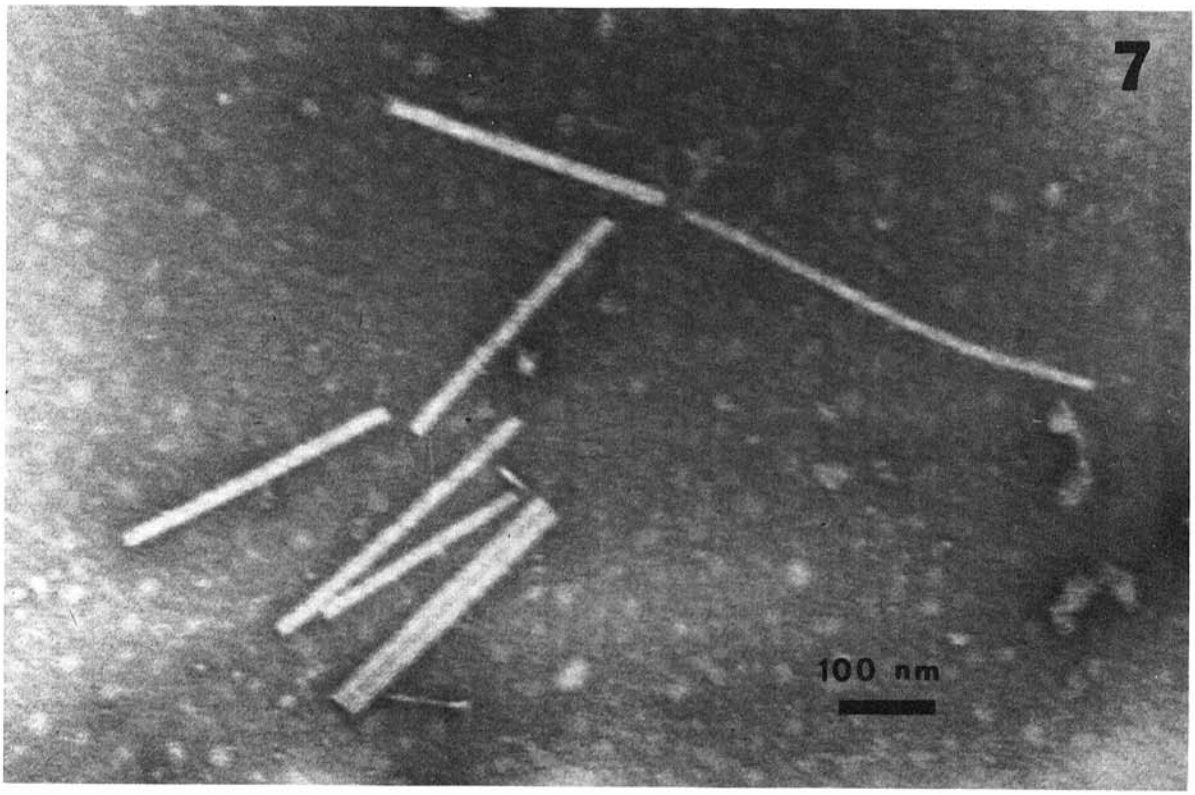


**Fig. 7.** Electron micrograph of negatively stained (2% PTA, pH 7.0) crude extract from *Platyopuntia chlorotica*, showing a mixture of two viruses. Statistical analyses of particle morphology show the wider, more rigid particles to be identical in size to that reported for SOV; and the physical proportions of the longer particles were shown to be identical with those reported for CVX (× 122,300).

in those collections obtained in southern Arizona.

This report should provide background information, useful for the establishment of epidemiological and ecological studies of viruses of desert species, with regard to the detection, rate, and method of virus infection. The large areas of undisturbed cacti provide an ideal system for such long-range studies.

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