Distribution of Cactus Viruses in Wild Plants

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ABSTRACT

Flat-padded *Opuntia* species (prickly pears) were sampled in Arizona, Nevada, and Utah for the presence of elongated viruses. The TMV-like Sammons' Opuntia virus and a flexible rod of the cactus virus X and/or Zygocactus virus type were found throughout the collecting area. These were detected, singly or as mixtures, in at least some specimens of all species sampled. A striking disjunct distribution of virus was observed, particularly in *Opuntia basilaris*, with no virus being found north of a line drawn from Searchlight, Nev., to Grasshopper Junction, Ariz. Possible reasons for this absence of virus are discussed.
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Since the original demonstration by Rosenzopf of the presence of a graft-transmissible disease agent in the cactus *Epiphyllum* (9), five viruses have been reported to occur naturally in Cactaceae. These include Sammons' Opuntia virus (SOV) (10), cactus virus X (CaVX), cactus virus II (4), Zygocactus virus (ZyV) (5), and a virus of the giant saguaro *Carnegiea gigantea* (M. R. Nelson, *personal communication*). Only the *Saguaro* virus and Sammons' *Opuntia* virus have been demonstrated in cacti in their native habitats in Arizona (6).

There are few detailed epidemiological studies of viruses in wild plants in the literature of phytopathology. This is unfortunate, as such studies could contribute to an understanding of the evolution of plant viruses and the role of wild plants as reservoirs of viruses of direct importance to man.

Possibly the most detailed study of this kind was made by MacClement & Richards (7), using an 1,800-acre reserve at the Royal Botanical Gardens at Hamilton, Ontario, Canada. They sampled plants in both terrestrial and aquatic environments, and reported seasonal fluctuations in virus concentrations and occurrence. Less virus was found in aquatic than in terrestrial plants, and the presence of viruses resembling sugar beet curly top, cucumber mosaic, potato and tobacco ringspots, and spotted wilt, based on symptoms produced on several assay hosts, was reported (7).

Eastern Canada is, of course, an area of considerable urbanization and intensive agriculture. On the other hand, much of the southwestern portion of the United States is still in a relatively undisturbed condition, although there are a few large urban centers and some areas of intensive cultivation.

The cacti are the most distinctive feature of the vegetation of this area. Although they manifest certain obvious disadvantages for virus work (spines and bristles, thick wax coat, and mucilaginous sap), they exhibit some positive features as well. Their widespread distribution, distinctive appearance, and perennial habit; the longevity, ease of shipping, and rooting make them ideal material for long-term studies on the origin and spread of viruses in wild plant populations.

M. R. Nelson (*personal communication*) has undertaken a distributional study in Arizona of the only cactus virus with spherical particles, the Saguaro virus, while we have been making similar studies of the viruses occurring naturally in the flat-padded *Opuntias*.

To determine how many of the other cactus viruses have wild reservoirs, to ascertain the probable modes of virus spread under natural conditions, and to discover whether new viruses or virus strains appear spontaneously in such wild plant populations, we undertook a more detailed study of the distribution of virus in several *Opuntia* species collected in the field in Arizona and neighboring states. Specimens of the following species were collected: *O. basilaris, O. engelmannii, O. macrocentra, O. phaecantha,* and *O. chlorotica*. The nomenclature of the *Opuntias* has recently been revamped (1). In view of the widespread hybridization in the prickly pears, and because our identifications were generally made on the basis of vegetative characteristics, we found it convenient to record our data on the basis of three species groups: *O. basilaris, O. chlorotica* Engelm. & Bigelow, and the other three species combined as *Opuntia* spp. Miller.

MATERIALS AND METHODS.—Three collecting trips were made to the southwestern United States during the early spring seasons of 1968, 1969, and 1970. Included in these trips were the southwestern portion of Utah, the southern "wedge" of Nevada, including Clark County, and most of Arizona except for the extreme northeastern and southwestern portions of that state. Some samples from Texas were also examined. Arizona material was also supplied by Jane Sauck and Robert Boulton, formerly Departments of Botany and Plant Pathology, respectively, University of Arizona, Tucson, and Robert McKittrick, Boyce Thompson Arboretum, Superior, Ariz. The Texas material was collected by Robert Irving, Botany Department, University of Montana, Missoula.

Individual pads (cladodes) of the plants sampled were removed from the plants by means of crucible tongs, and wrapped in newspaper so as to avoid

contact between the cut surfaces. Plants sampled were along major highway routes, generally at least 100 ft from the highway, and, in some cases, in mountain canyons up to several miles from the highway.

The pads were placed in cardboard cartons and shipped to Missoula, Mont., by air or rail. Generally, no more than 1 week ensued between collection and planting in the greenhouse. The pads were maintained in greenhouses until assays were complete.

The presence of virus was tested in several ways. Electron microscopy was performed at the Biologische Bundesanstalt, Braunschweig, Germany, on grids prepared in Missoula. This was done by scraping away most of the wax from a small portion of the cactus pad and removing a small wedge of tissue which was placed in contact for 2-3 sec with a drop of distilled water on an electron microscope grid. The grids were shadowed with palladium at Braunschweig prior to examination in the electron microscope (Siemens Elmiskop IA). The length of viruslike particles was compared at a calibrated magnification of X 40,000 with a standard on the fluorescent screen of the electron microscope. Viruslike particles were either of the TMV (including SOV) or X-virus (including CaVX and ZyV) types, and were so recorded.

Free-hand sections of all pads were made to determine whether the spindle- or cigar-shaped cell inclusions characteristic of infection with cactus virus were present. At least two sections from each pad were examined microscopically before report of a negative finding.

Several bits of pad tissue with much of the wax removed were ground in small amounts of 0.1 M, pH 7 phosphate buffer, and rubbed on leaves of *Chenopodium quinoa* Willd. which had been dusted with Carborundum powder. Local lesions are produced on this plant only after leaves are inoculated with SOV (3), local lesions on inoculated leaves and a systemic mottle when plants are infected with CaVX (2), and a systemic mottle only when they are infected with ZyV (5). Plants were kept under observation for at least 1 month after inoculation.

External symptoms were also noted. Cacti are subject to attack by some insects which produce puncture scars and small, radiate chlorotic areas around the puncture as a result of feeding. Generally, these can be readily distinguished from the more distinctive chlorotic ringspotting which frequently indicates the presence of virus. However, virus may still occur in the complete absence of external symptoms (6).

RESULTS.—Three hundred and forty plants were sampled. Of these, one hundred and seventy-four showed no evidence of virus. The remaining 166 gave positive results for at least one of the four criteria of virus presence. However, because external symptoms on the original cactus pad and on inoculated *C. quinoa* are not unequivocal indications of virus, those samples which were positive for either of these criteria alone (50 specimens) were considered negative with respect to virus occurrence, leaving 116 plants remaining which we considered infected with virus.

Of the 57 plants which showed virus particles by electron microscopy, 43 had SOV-like particles alone; six, only XV-like particles; and eight, at least some of both. Both groups of viruses were found throughout most of the area studied, and in all species or species groups sampled. The southernmost collections of *O. chlorotica* and *Opuntia* spp. southwest of Tucson to the Pena Blanca Recreational Area near Nogales, and southeast of Tucson to the Empire and Santa Rita Mountains, and the extreme northwesterly collections of *O. basilaris* north of a line drawn from Searchlight, Nev., to Grasshopper Junction, Ariz. were devoid of virus. The latter is of particular significance because of the large number of samples collected. In the Texas collections, the significance of the occurrence of SOV in one *Opuntia* plant out of the nine sampled cannot be determined until a much more complete study is made. The central Texas collections were made in an area roughly bounded by Kerrville and Del Rio on the Devil's River to Fort Stockton on the north, and terminated in the region of the Pena Blanca Mountains on the west.

Our results suggest a widespread distribution of virus extending throughout most of the area in which the collections were made, including at least one with elongated, flexible particles previously unreported in wild cacti. In addition, a disjunct distribution of virus in *O. basilaris* suggests the operation of a factor or factors limiting the spread of virus in this species.

DISCUSSION.—Previously, SOV was the only virus reported from the flat-padded *Opuntias*, in the wild (6). We can now report the strong likelihood that cactus virus X, Zygocactus virus, or both also occur in uncultivated *Opuntias* in the field. This leaves cactus virus II as the only virus occurring naturally in cacti for which there is no evidence for its presence in the wild.

Our experiments were essentially complete when the *Zygocactus* virus was first described in print (5). Because this virus normally occurs in very low concentrations, and occurs in mixed infections with CaVX, and because its normal length is nearly the same as that of CaVX (580 and 520 picometers, respectively), it is very difficult to distinguish between the two viruses in natural infections. The most distinctive difference is the reported infection of *Nicotiana glutinosa* by the *Zygocactus* virus, but not by CaVX.

Although it is theoretically possible to distinguish between CaVX and ZyV on the basis of external symptoms on *C. quinoa*, it is difficult to do so because the latter is particularly susceptible to spotting, marking, and mild mottling which are unrelated to virus infection. Thus, we are unable to state which of the two viruses was present in our samples.

The absence of virus in *O. basilaris* from northwestern Arizona and southern Nevada, particularly since so many samples were represented,

presents an intriguing problem. Whether the disjunct distribution of virus in *O. basilaris* is due to the relatively recent introduction of virus into this species in the wild or to the operation of certain biotic or climatic factors deserves additional study. The absence of virus in *O. chlorotica* and *Opuntia* spp. collected south of Tucson is also a matter of concern, although considerably fewer specimens were collected in those areas. More intensive collecting needs to be made throughout the ranges of the species studied. Moreover, other cactus species whose ranges coincide or overlap with those of the prickly pears should also be investigated.

The role of insects, man, and mammalian herbivores in the dissemination of cactus viruses cannot be overlooked (8). One would, however, anticipate a slow spread of viruses within prickly pear populations merely on the basis of the importance of vegetative propagation in plants of this type, but animal vectors could be expected to greatly speed up virus spread.

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