Viruses Isolated from Wild Carrot and Poison Hemlock

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ABSTRACT

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During a survey of poison hemlock (*Conium maculatum* L.) and wild carrots (*Daucus carota* L.) in southeastern Washington in 1975 and 1979, carrot thin leaf virus, celery mosaic virus, and alfalfa mosaic virus were isolated from poison hemlock and from wild carrot. Clover yellow vein virus was isolated from wild carrot and also found in commercial carrots (*Daucus carota* L. ssp. *sativus* DC.). This is the first report of alfalfa mosaic virus in poison hemlock and and wild carrot and also of clover yellow vein virus in wild and domestic carrots.

In studies to identify natural reservoirs of carrot thin leaf virus (CTLV) during 1975 (7), poison hemlock (*Conium maculatum* L.) and wild carrot (*Daucus carota* L.) were indexed on *Chenopodium quinoa* Willd. The results suggested that several other mechanically transmissible viruses were present besides CTLV.

The identity and incidence of these viruses in wild carrot and poison hemlock of southeastern Washington are reported here.

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MATERIALS AND METHODS

Survey. Poison hemlock and wild carrots tend to grow in thick stands near rivers and in ditches of southeastern Washington. Leaves from 25 plants in each of 26 poison hemlock and seven wild carrot stands in this area were collected during 1975 or 1979. The leaves were indexed on *C. quinoa*. The symptoms on the original host and on *C. quinoa* were recorded and grouped by type.

Serial local lesion transfers of the isolates were made. The resultant cultures were then studied on a larger host range, observed for particle structure with an electron microscope, and serologically tested against antiserum to suspected viruses. During 1979, the results of the *C. quinoa* indexing were verified by electron microscopy decoration serology.

Plant culture. The test plants were grown as described previously (5).

Inoculations. Plants were inoculated with aphids or by rubbing. The aphid inoculations were accomplished with either *Myzus persicae* (Sulzer) or *Cavariella aegopodii* (Scopoli). The aphids were starved for 15 min, given acquisition feedings of 5–15 min on infected *Coriandrum sativum* L. (coriander), and placed in groups of six or more on individual test plants (six plants for each plant species), which were fumigated with nicotine sulfate 24 hr later.

Rub-inoculations for all isolates were usually made by triturating infected coriander leaves with a mortar and pestle in neutral 0.01 M potassium phosphate buffer. This extract was then rubbed with the pestle onto previously dusted (Carborundum, 600 mesh) leaves.

Electron microscopy. Virus for electron microscopy with a Zeiss EM-9 electron microscope was obtained from crude extracts of infected plants or from infectious density gradient zones. An electron microscope grid covered with a thin film of Formvar (0.25%) was floated on the virus suspension (in 0.1 M potassium phosphate buffer, pH 7) for 15

Table 1. Host and serologic reactions and electron microscopy of viruses isolated from *Conium maculatum* (poison hemlock [PH]) and *Daucus carota* (wild carrot [WC]) in southeastern Washington

Property	Virus isolate 1	2	3	4	5	6	7
Original host/symptom[a]	WC-s	PH-s	WC-s	PH-s	WC-s	WC-CM	PH-Ca
Symptoms on							
Apium graveolens L. var. *dulce* DC[b]	–	–	–	–	···	···	···
Chenopodium quinoa Willd.	CL	CL	C&NL	C&NL	C&NL,CM	CM	NL,CM
Coriandrum sativum L.	t,Vc,CM	t,Vc,CM	t,CM	t,Vc,CM	CM&N	···	···
Daucus carota L. ssp. *sativus* DC[b]	t,Vc,CM	t,Vc,CM	–	–	CM	···	···
Glycine max (L.) Merr. 'Bragg'	–	–	···	···	CL,CM	···	CL,SN,CM
Gomphrena globosa	–	–	–	–	–	···	RL,CM
Nicotiana clevelandii Gray	CM	CM	–	–	NL,SN	···	···
Pisum sativum L. 'Perfected Whales'	–	–	–	–	VcWVb,D	···	···
Electron microscopy, filament length (nm)	750	750	750	750–775	750	No rods	No rods
Serology[c]							
Virus antiserum							
Alfalfa mosaic virus	···	···	···	···	–	+	+
Bean yellow mosaic virus	–	–	–	···	+	···	···
Carrot thin leaf	+	+	–	–	–	···	···
Celery mosaic virus	–	–	+	+	–	···	···
Clover yellow vein virus	–	–	···	···	+	···	···
Parsnip mosaic virus	–	–	···	–	–	···	···

[a] C = chlorotic, Ca = calico, D = death, L = local or primary lesion, M = mottle, N = necrosis, R = ring, S = systemic, s = symptomless, t = thin leaf, Vc = vein clearing, WVb = white vein banding, ··· = not tested.
[b] Aphid inoculated. All other species were rub-inoculated.
[c] Decoration serologic tests, except for double diffusion gel tests of alfalfa mosaic virus.

min. The grid was then washed with 20 drops of buffer and 30 drops of distilled water before being stained with 5 drops of 2% uranyl acetate, pH 4.5. Approximately 50 virus particles were measured by comparing electron micrographs of particles with a carbon grating replica grid (22,835 lines per centimeter) enlarged to the same magnification.

Serology. Serologic reactions were conducted with double gel diffusion or ring interface precipitation tests as described by Ball (1) or by the electron microscopy decoration technique as described by Milne and Luisoni (10). Antisera to celery mosaic virus (type, poison hemlock and parsley strains) were provided by R. N. Campbell, to parsnip mosaic virus by A. F. Murant, to clover yellow vein virus by K. Lindsten, and to bean yellow mosaic virus by I. Uyeda. The other antisera had been produced here.

Purification. The celery mosaic virus (CeMV) purification procedure described previously (5) was modified as follows: Tissues were triturated (1 g/5 ml) in solutions containing 0.01 M sodium diethyldithiocarbamate and 0.01 M cysteine · HCl that were adjusted to pH 8 with sodium hydroxide. After trituration, the pH was readjusted to 6.7 and stored at 20 C for 30 min. After ultracentrifugation, the pellets were suspended in 0.5 M urea plus 0.1% 2-mercaptoethanol, pH 7.0. The low-speed centrifugation step preceding density gradient ultracentrifugation was eliminated.

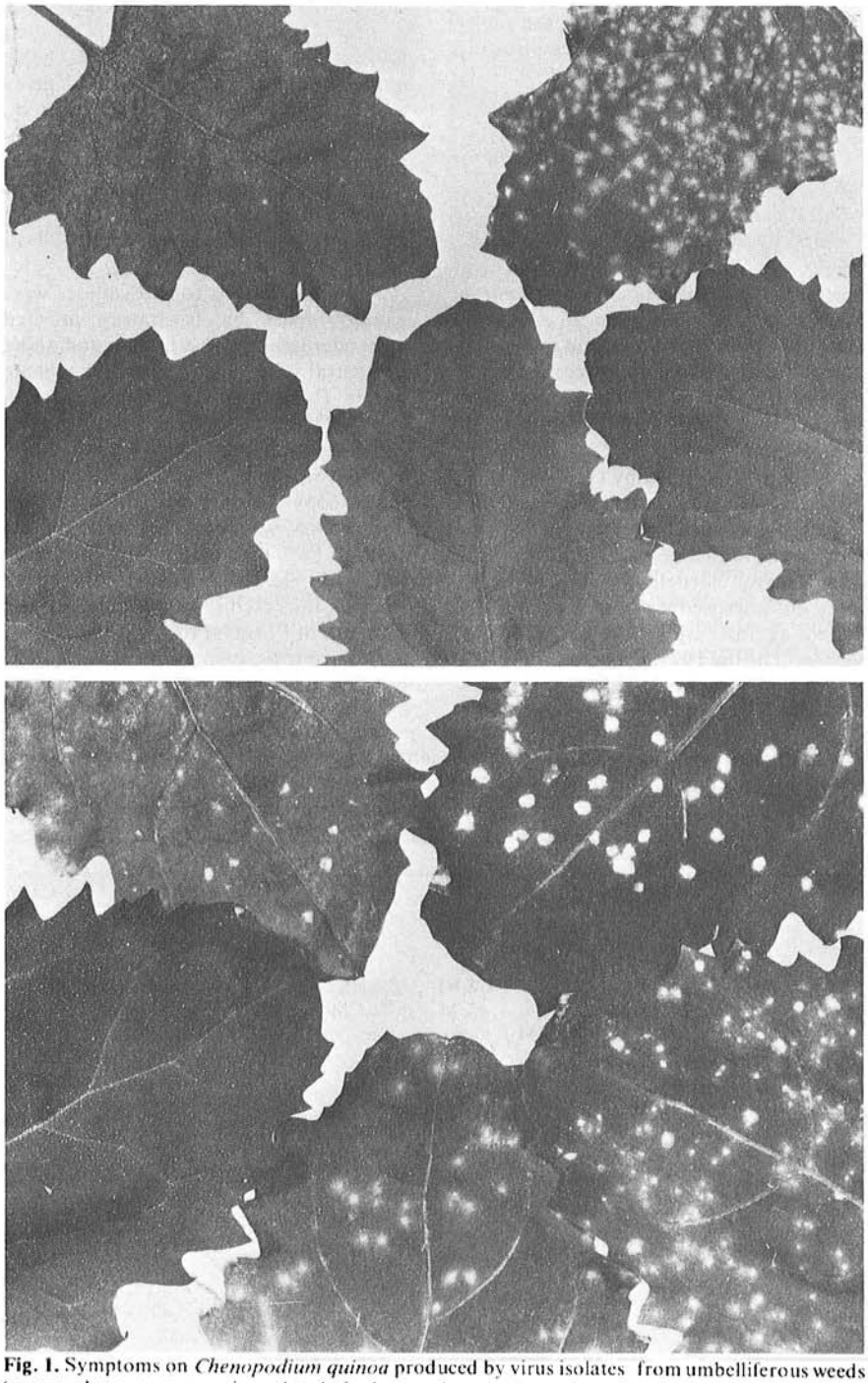

Fig. 1. Symptoms on *Chenopodium quinoa* produced by virus isolates from umbelliferous weeds (upper photo, young uninoculated tip leaves; lower photo, inoculated leaves). The isolates producing these symptoms were identified as (upper leaf on the left, proceeding clockwise): alfalfa mosaic virus—few necrotic primary lesions and faint systemic mottle, clover yellow vein virus—necrotic primary lesions and severe systemic mottle, celery mosaic virus—necrotic and chlorotic local lesions, and carrot thin leaf virus—chlorotic local lesions; healthy leaf at lower left.

RESULTS

Identification of isolates. Isolates comprising four symptomatological types were obtained from wild carrot and three types of isolates were procured from poison hemlock. Bases for their initial distinction were symptoms observed on the weed host and on *C. quinoa* (Table 1, Fig. 1). Subsequent host range, electron microscopy, and serology tests identified the viruses from poison hemlock as alfalfa mosaic virus (AMV), CeMV, and CTLV (Table 1). Three of the four viruses from wild carrot were identified as AMV, CeMV, and CTLV.

The remaining isolate from wild carrot (Table 1, isolate 5) appeared to be either bean yellow mosaic virus or clover yellow vein virus (CYVV). This isolate reacted with antisera against both viruses in electron microscope decoration serology, and it infected hosts diagnostic for each virus (4). In enzyme-linked immunosorbant assays performed by O. W. Barnett, Clemson University, this isolate reacted strongly with antiserum to CYVV and weakly, if at all, with antiserum to bean yellow mosaic virus.

The procedure used for purification of CeMV yielded approximately 20 mg of virus per 1 kg of tissue. The resultant density gradient tubes had a single infectious zone approximately 25–28 mm below the meniscus. ISCO ultraviolet (254 nm) scanning patterns of density gradient tubes containing healthy and virus preparations indicated that the virus was relatively free of healthy material. Light absorbance was maximum at 258 nm and minimum at 248 nm, and the $A_{260/280}$ ratio was 1.15 (corrected for light scattering). The modal particle length was between 750 and 775 nm. These properties are typical of CeMV (13).

In ring interface precipitin tests, the purified virus reacted with homologous and CeMV-type strain antisera diluted 1:16 and 1:32, respectively. It did not react with normal sera or with antisera against CTLV or healthy *C. quinoa* protein. Enough serum to conduct similar tests with antisera of the poison hemlock and parsley strains of CeMV was not available, but in electron microscopy decoration serology, the purified virus reacted with each of the three CeMV antisera.

Incidence. CTLV and CeMV were the

Table 2. Incidence of virus in stands of *Daucus carota* (wild carrot [WC]) and *Conium maculatum* (poison hemlock [PH]) in southeastern Washington

Species	No. of sites	Total plants indexed	Percent infected with[a]				
			CTLV	CeMV	CTLV + CeMV	AMV	CYVV
WC	7	175	9	9	7	1	1
PH	26	650	22	21	11	1	0

[a] CTLV = carrot thin leaf virus, CeMV = celery mosaic virus, AMV = alfalfa mosaic virus, CYVV = clover yellow vein virus.

most prevalent viruses in wild carrot and poison hemlock of southeastern Washington (Table 2). CTLV and CeMV were each recovered from 9% of the wild carrots and from slightly more than 20% of the poison hemlock with 7 and 11%, respectively, infected with both CTLV and CeMV.

AMV was found only at two locations. Four poison hemlock at one site and one wild carrot at another contained the virus (Table 2).

CYVV was found at one 1975 survey site in two wild carrot plants (Table 2). In 1975, this virus was also found in two commercial carrot plants.

The incidence of CTLV and CeMV ranged from 0 to more than 80%. This variation appeared related to moisture availability. Where water was short through the summer, many of the second-year biennials matured and died before new plants emerged, thus decreasing the probability of virus transmission from the older to the younger weeds.

DISCUSSION

Although the CeMV reacted serologically with all three strains of CeMV described by Sutabutra and Campbell (15), its host range varied in some aspects from all of them. However, since it occurs in great abundance here in poison hemlock and its host range most closely resembles that of the poison hemlock strain, we have identified it as CeMV-PHV.

Poison hemlock, which is abundant in southeastern Washington, is considered a natural reservoir for CeMV in England (11), Argentina (3) and California (15). During our survey of commercial carrots in Washington (6), however, CeMV was not observed in carrot, which is a host to the type strain of CeMV (12). Apparently most, if not all, of the CeMV in the poison hemlock here is of the CeMV-PHV type and is incapable of infecting carrots.

Isolates of CeMV that produce local lesions on *Chenopodium* sp. have been found in Japan (8), California (15), and now in Washington. Purification of CeMV from this host appears to have two advantages: Inoculated tissue can be harvested nearly 2 wk before that from Umbelliferous hosts (9,13,15,17), and apparently more virus can be obtained per gram of tissue (9).

Although CYVV has previously been found in coriander (14), this is the first report of it occurring naturally or artificially in either commercial or wild carrots. We would not have found it if we had not been indexing for CTLV on *C. quinoa*, because the original wild carrot host was symptomless, and even under optimum growth conditions, infected carrots only produce a faint mottle.

AMV has been found in several members of the Umbelliferae such as chervil, celery, and carrot (16) and parsley (2), but this is the first report of it in poison hemlock and wild carrot. The calico symptom observed on the AMV-infected poison hemlock suggests that this isolate might be similar to the calico isolate from parsley (2).

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