Properties of Asparagus Virus 1 Isolated from Washington State Asparagus

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ABSTRACT

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A flexuous rod-shaped virus isolated from asparagus (*Asparagus officinalis*) in Washington State was identified as asparagus virus 1 (AV-1). Antiserum was prepared that reacted readily with filamentous particles from extracts of AV-1-infected asparagus in "decoration" serology. The virus also reacted strongly against antisera of AV-1 from Japan and Europe, but in cross-reactivity tests, it was serologically distinct from iris mild mosaic virus. The virus induced pinwheel inclusions. Filamentous particles from purified preparations had a modal length of 741 nm, a nucleic acid content of 6% (as estimated from a 260/280 ratio of 1.2), and a sedimentation constant of 1.46. AV-1 spread rapidly in the field, with most new infections occurring in either mid-June or early September during both 1979 and 1980.

In 1977, Mink and Uyeda (11) described the occurrence of a flexuous rod-shaped virus in asparagus (*Asparagus officinalis* L.) fields of Washington State. The virus produced no symptoms in asparagus but caused local lesions on various *Chenopodium* species. Particle morphology and symtomatology were similar to those reported for asparagus virus 1 (AV-1) in Germany (7) and to a subsequent description of AV-1 from Japan (5). We report results of studies on properties of the AV-1 isolate from Washington State and of its serological relationship to AV-1 from both Europe and Japan. Also described is the variation in the amount of spread of AV-1 in the field in relation to distance from inoculum source and time of the year.

MATERIALS AND METHODS

Virus source. The virus isolate used in this study was obtained from meristem culture of naturally infected asparagus growing near Prosser, WA. The isolate was passed through three single local lesion transfers on *Chenopodium quinoa* (Willd.) and maintained by frequent mechanical transfers on *C. quinoa*.

Purification. Virus was purified from inoculated leaves of *C. quinoa* plants 10 days after being rubbed with crude virus extracts. The inoculated leaves, which were held at 4 C after harvest, were triturated in 0.1 M sodium citrate and 0.01 M sodium ethylenediaminetetraacetic acid, pH 7.0, and centrifuged at 8,000 *g* for 10 min followed by 66,000 *g* for 1.5 hr. Buffered suspensions (potassium phosphate, 0.01 M, pH 7.0) of the high-speed pellet were clarified by emulsification in chloroform (1:1, v/v). Virus in the aqueous phase was precipitated through a solution containing 30% sucrose and 4% polyethylene glycol (mol wt 6,000) in 0.12 M sodium chloride by centrifugation at 51,000 *g* for 2.5 hr. The pellets were suspended in 0.01 M potassium phosphate and centrifuged on rate-zonal sucrose-density gradients (10–40%) in a Beckman SW25.1 rotor at 4 C for 2.5 hr at 51,000 *g*. The gradients were fractionated into 2-ml aliquots on an ISCO density-gradient fractionator, and the fractions were assayed on *C. quinoa* and examined by electron microscopy.

Serology. An antiserum against AV-1 was prepared by injecting rabbits intravenously four times with 0.1 mg of virus. After the first dosage, injections were given on days 4, 7, and 12. Rabbits were bled twice a week beginning on day 14. Antisera against the European and Japanese isolates of AV-1 were provided by A. A. Brunt and I. Fujisawa, respectively. A. A. Brunt also provided antiserum to iris mild mosaic virus (IMMV). John Hammond, USDA, Beltsville, MD, provided IMMV and antiserum to IMMV that was prepared by Miriam Alper and G. Loebenstein of Israel. "Decoration" and serologically specific electron microscopy (SSEM) were performed as described by Milne and Luisoni (10) and by Derrick (3). Degree of reaction in SSEM was determined by recording the number of virus particles observed in a 5-min scan of the grids.

Electron microscopy. Virus from plant juice diluted in 0.1 M potassium phosphate buffer, pH 7 (1 g/10 ml), and from purified samples taken directly from gradient fractions was viewed on a Zeiss EM-9 electron microscope. Formvar-coated (0.25%) 300-mesh copper grids were pretreated with 95% ethanol, floated on drops of tissue extract or purified samples for 30 min, washed with a stream of water, and stained with six drops of 2% uranyl acetate. Measurements were calibrated with standard 463-nm grids enlarged to the same magnification.

Local lesions from *C. quinoa* leaves were cut into 2-mm squares fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.2, for 2 hr at 21 C, rinsed in the same buffer, and postfixed with 1% osmuim tetroxide in 0.1 M buffer for 1 hr at 21 C. After rinsing and ethanol series dehydration, the specimens were embedded in Epon 812. Ultrathin sections were stained with 5% uranyl acetate (in 50% ethanol) and Reynold's lead citrate.

Sedimentation coefficient. Sedimentation coefficients were determined by using linear-log sucrose density-gradient centrifugation (1). Sowbane mosaic virus (SMV) purified from *C. quinoa* by acidification and centrifugation (differential and rate-zonal sucrose density gradient) was used as the sedimentation standard. The sedimentation constant used for SMV was 1.04.

Natural spread. Both seasonal and distance spread of AV-1 in the field were monitored during the 1979 and 1980 growing seasons. For both measurements, asparagus seedlings (cultivar 500-W) indexed free of AV-1 were used. To determine the distances over which AV-1 might spread, 80 seedlings were planted during the spring of 1979 in each of three linear rows located 3, 210, and 390 m downwind (prevailing westerly winds) from a field of asparagus known to be heavily infested with both AV-1 and asparagus virus 2 (AV-2). Spread was monitored by indexing newly emerging spears during the springs of 1980 and 1981. Asparagus in the source fields was not harvested during these studies.

To study seasonal movements of AV-1, 20 pots of asparagus seedlings were placed for 2-wk periods in a field of asparagus heavily infected with both AV-1 and AV-2. Before exposure, they were transferred from the greenhouse to a lathhouse for 2 days for acclimatization. In the field, the 20 plants were scattered evenly along an open 200-ft row in the middle of the field. After exposure, they were fumigated with nicotine sulfate, held in the greenhouse, and indexed the following winter on *C. quinoa*. Local lesion development

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characteristic of AV-1 was considered a positive reaction.

RESULTS

Purification. A clear visible zone 27 mm below the meniscus of density-gradient tubes was infectious and contained viruslike particles. The gradients displayed a general opalescence throughout the lower portions of the tube, including a faint zone 33 mm below the meniscus. Infective virus aggregates were associated with this second zone and all of the opalescent region. No visible zone was observed in gradients layered with material from healthy plants similarly prepared.

Light absorbance properties. Aliquots that contained infectious virus from the 26- to 28-mm region of rate-zonal density-gradient tubes had ultraviolet absorption spectra typical of nucleoproteins with low nucleic acid content. These solutions showed low light scattering above 320 nm and had 260/280 ratios of 1.2, which suggests a nucleic acid content of about 6%.

Serology. Antisera obtained after intravenous injection reacted in decoration electron microscope serology against virus from crude extracts of infected asparagus or *C. quinoa* and against virus particles from either the 27- or 33-mm density-gradient zones.

The Washington AV-1 isolate reacted strongly with Japanese, European, or homologous antisera in SSEM (Table 1). The density of particles on electron microscope grids presensitized with any of these antisera was eightfold greater or more than that of the controls (sensitized with normal serum or not presensitized) or of grids treated with either of the IMMV antisera. IMMV failed to react with any of the three AV-1 antisera but reacted strongly against the IMMV sera from Europe or Israel.

Electron microscopy. One hundred ten AV-1 particles from the 27-mm zone of rate-zonal density gradients were measured. Thirty-eight percent of the particles had lengths of 710–772 nm (Fig. 1). The modal length was 741 nm.

Abundant pinwheel inclusion bodies with laminate aggregates similar to those of cylindrical inclusion subdivision II (4) were found in ultrathin sections of *C. quinoa* local lesions infected with AV-1 (Fig. 2). These bodies were observed in cytoplasm, often adjacent to chloroplasts.

Sedimentation coefficient. The sedimentation constant for AV-1 was calculated to be 1.46.

Natural spread. Under central Washington field conditions, AV-1 spread rapidly into nearby young asparagus seedlings during 1979 and 1980. Ninety percent of seedlings transplanted adjacent to infected plants were infected during the first season. Seedlings 210 and 390 m from this source were only 36 and 30% infected, respectively. After the second season, more than 90% of the plants in the two most proximal treatments were infected as were 68% of those in the most distant row. Movement of AV-1, as monitored by the biweekly field exposure of potted asparagus seedlings, peaked during late June and again in early September during both 1979 and 1980 (Fig. 3). Little if any spread occurred in early May, late July, or late September in either year. This bimodal distribution of spread corresponds to the seasonal occurrence of alate aphids (9). During these studies, no field spread of AV-2 was detected.

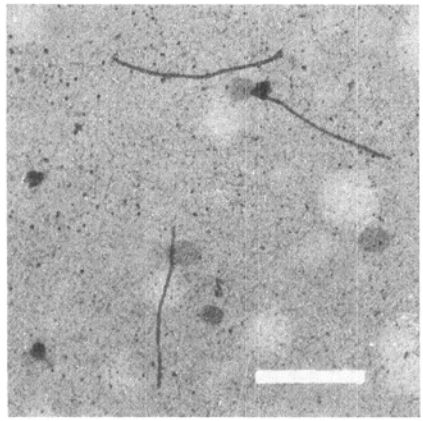

Fig. 1. Electron micrograph of flexuous rod-shaped particles from the main zone of rate-zonal density-gradient centrifugation of a preparation from *Chenopodium quinoa* infected with asparagus virus 1. Scale bar = 463 nm.

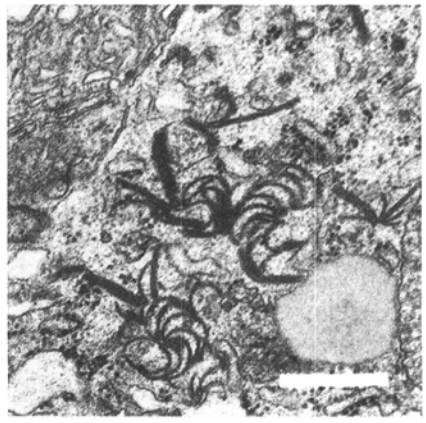

Fig. 2. Electron micrograph of an ultrathin cross section of *Chenopodium quinoa* local lesions infected with asparagus virus 1 showing pinwheel inclusion bodies. Scale bar = 463 nm.

DISCUSSION

AV-1-infected asparagus was originally observed in 1960 by Hein (7) in Germany. A particle length estimate (763 nm) (2), pinwheel inclusion bodies associated with

Table 1. Serologically specific electron microscopy: cross-reactivity tests between the Washsington isolate of asparagus virus 1 (AV-1), the Beltsville isolate of iris mild mosiac virus (IMMV), and their various antisera from around the world

	AV-1		IMMV	
Antiserum	Particle density	Interpretation	Particle density	Interpretation
None	7[a]	...	19	...
Normal sera	1	−	5	−
AV-1 Washington	73	+	3	−
AV-1 Europe	103	+	3	−
AV-1 Japan	61	+	3	−
IMMV Israel	2	−	1,154	+
IMMV Europe	1	−	437	+

[a] Average number of filamentous particles observed during a 5-min scan of each of two to four prepared grids.

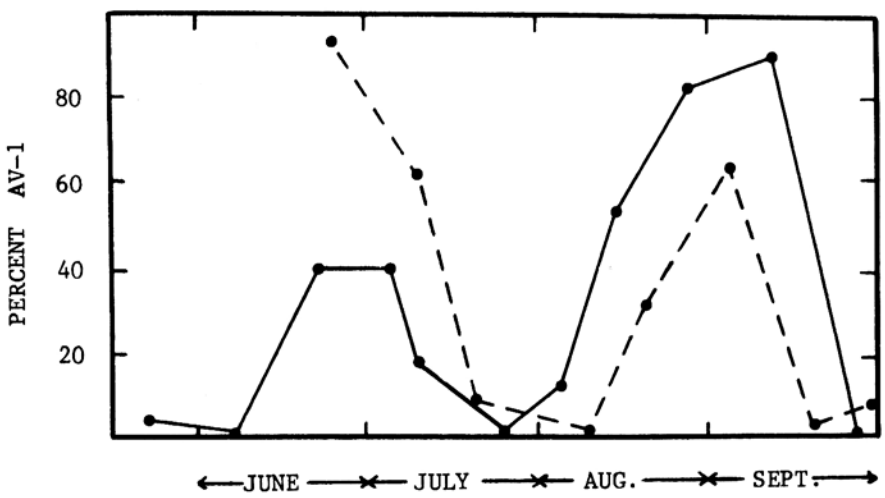

Fig. 3. Incidence of asparagus virus 1 in groups of 20 potted asparagus seedlings subjected to natural field infections for 2-wk periods during the 1979 (——) and 1980 (- - - -) growing seasons.

infection (6), and probable aphid transmission (7,8,12) have been reported in Europe. In 1983, Fujisawa et al (5) identified and purified AV-1 from asparagus in Japan. The virus was aphidborne, and except for having a slightly wider host range, properties of the Japanese AV-1 were similar to those of the European isolates. The Washington AV-1 isolate is transmitted by the aphids *Myzus persicae* Sulz. and *Aphis craccivora* Koch (W. E. Howell and G. I. Mink, *unpublished*). Because our AV-1 isolate has properties similar to isolates from Europe and Japan and reacted readily with their antisera, strains of AV-1 that occur worldwide uniformly appear to have properties typical of the potyvirus group.

In 1979, Yang (13) demonstrated severe decline and mortality of young asparagus infected with both AV-1 and AV-2. Effects of either virus alone were minimal, thus disease control measures could be based on the control of only one virus. Control of both would not be necessary. Because AV-1 spread rapidly in fields of central Washington, control efforts might more aptly be applied to the slow-spreading, seedborne AV-2. The use of virus-indexed nursery stock could effectively lessen the incidence of AV-2 in commercial fields (13).

In 1969, Hein (8) suggested that AV-1 and IMMV might be the same virus. In our SSEM cross-reactivity tests with AV-1 and IMMV antisera prepared worldwide, these two viruses were distinct in their serological activity.

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