Survey for Asparagus Viruses I and II in New Jersey

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ABSTRACT

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During a 4-yr study of virus diseases of asparagus (*Asparagus officinalis*) in New Jersey, both asparagus viruses I and II (AV-I and AV-II, respectively) were detected in asparagus clones and cultivars growing in the field and greenhouse, with AV-I occurring more frequently than AV-II. The detection of these viruses by ELISA and bioassay in 15 plants of one clone of particular value to the breeding program at Rutgers University varied with the month of testing. *A. officinalis* var. *pseudoscaber* was found as a new host naturally infected with both AV-I and AV-II in the field.

Asparagus viruses I and II (AV-I and AV-II) were first reported in Germany (10), then in the United States (12) and Japan (7). Asparagus (*Asparagus officinalis* L.) plants infected with these viruses alone or in combination remain symptomless but suffer some reduction in vigor and productivity (15,16). In addition to these direct effects, infection by AV-II reportedly predisposes asparagus to fungal root and crown rot caused by *Fusarium* sp. (5). Horticulturalists at the New Jersey Agricultural Experiment Station, Rutgers University, have been involved in breeding asparagus to develop superior cultivars for about 30 yr. Recently, tissue-culture techniques have been implemented in their program. AV-I and AV-II have been detected in these tissue-culture lines, greenhouse clones, and field-grown asparagus plants (4). Because these viruses may pose a threat to the asparagus either directly or by predisposing plants to *Fusarium* infection, we examined the breeding material for virus infection.

We report results of a preliminary study to determine the occurrence and incidence of AV-I and AV-II in asparagus produced in New Jersey. Portions of this work were presented previously (4).

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MATERIALS AND METHODS

Virus isolates and field survey. Samples of actively growing asparagus spears or stem brushes were collected from Rutgers Horticulture Research Farm in New Brunswick, Rutgers Vegetable Research Center in Centerton, and commercial fields in Elmer, NJ, in the spring during 1982–1985. Asparagus material sampled included tissue-culture clones, greenhouse clones, field clones, and hybrids. The samples were stored in polyethylene bags at 4 C or −20 C until indexed individually by bioassay on *Chenopodium quinoa* Willd. or *C. amaranticolor* L. Samples were also triturated in 1:10 (w/v) coating buffer (2) and stored at −20 C for enzyme-linked immunosorbent assay (ELISA).

Mechanical transmission. Tissue samples were triturated in a sterilized mortar with buffer and rub-inoculated with a cotton swab or a forefinger on the leaves of young test plants that had been previously dusted with 600-mesh Carborundum. Immediately after inoculation, the leaves of test plants were rinsed with distilled water. The grinding buffer for AV-I was 0.01 M sodium phosphate, pH 7.0, and for AV-II, it was 0.02 M sodium phosphate, pH 6.5. The test plants were grown and maintained in a screened greenhouse. Supplemental lighting was supplied, and temperatures ranged between about 22 and 35 C.

Purification. AV-I was purified from locally infected *C. quinoa* leaves about 14–21 days after inoculation. Tissue was blended 1:10 (w/v) with 0.1 M citrate, 0.01 M disodium ethylenediaminetetraacetate buffer, pH 7.0. The mixture was stirred for 1 hr at room temperature and centrifuged for 10 min at 10,000 × *g* (LS spin). The supernatant was centrifuged for 2 hr at 64,000 × *g* in a Beckman 30 rotor (HS spin), and pellets were resuspended overnight at 4 C in 0.01 M phosphate buffer, pH 7.0 (G. I. Mink, *personal communication*). The supernatant from the first HS spin was centrifuged at high speed again, and the pellets were resuspended. The resuspended solutions were combined and stirred vigorously for 10 min with one volume of chloroform and subjected to an LS spin. The clarified solutions were layered onto 10–40% sucrose density gradients in phosphate buffer and centrifuged for 2.5 hr at 97,000 × g in a Beckman SW 28 rotor. Bands were monitored and collected on an ISCO density gradient fractionator.

AV-II was purified from fresh locally and systemically infected *C. quinoa* leaf tissues by the method of Uyeda and Mink (14) with slight modifications (M. S. Montasser and R. F. Davis, *unpublished*). Virus concentration was estimated using extinction coefficients E(mg/ml)/1 cm (260 nm) of 5.3 as determined for citrus leaf rugose virus (8) and 2.4 as typical for potyviruses (11) for AV-II and AV-I, respectively.

Antisera production and serology. Antibodies to AV-I and AV-II were produced in Swiss Webster mice by methods described previously (3), using 25 μg of purified virus per injection. Immunogammaglobulins (IgG) were purified from murine ascites fluid by affinity chromatography on a 3-cm column of protein A-sepharose, CL-4B (Pharmacia Fine Chemicals, Piscataway, NJ). Ouchterlony plates were prepared by pouring 10 ml of a medium consisting of 0.6% purified agar (Code L 28, Oxoid Limited, England), 0.85% sodium chloride, and 0.04% sodium azide in 0.01 M phosphate buffer, pH 7.0. For detection of AV-I, the medium consisted of 0.5% agar, 1.0% sodium azide, and 0.25% sodium dodecyl sulfate (SDS) in distilled water. Wells were cut 7 mm in diameter, and distance between wells was 5 mm. Simple indirect ELISA was performed as described previously (3), using unfractionated and fractionated polyclonal antibodies prepared against a New Jersey isolate of AV-I. Each sample was tested in three wells, and reaction was rated positive if the mean absorbance at 405 nm exceeded twice the mean absorbance of the healthy sample. ELISA was also developed for AV-II, but unsatisfactory results precluded its use. AV-II was detected by bioassay on *C. quinoa*.

RESULTS

Detection by bioassay. Two distinct reactions were observed on *C. quinoa* plants. The first was characterized by local necrotic lesions, typically about 2–3 mm wide, which appeared within 10 days. These necrotic lesions and the absence of systemic symptoms indicated the presence of AV-I; results of ELISA later confirmed this. The second reaction type involved diffuse chlorotic spots appearing within 10 days that grew to about 5 mm followed by systemic chlorotic spots and mosaic and slight necrosis on young leaves. These symptom types are characteristic of AV-II. The appearance of these symptoms and their severity varied with the environmental conditions. *C. quinoa* was a much more sensitive and reliable indicator than *C. amaranticolor*.

Antisera production and detection by ELISA. Murine ascites produced to AV-I was capable of specifically detecting AV-I in infected asparagus and produced absorbance values (405 nm) in ELISA up to 0.78 with infected compared with 0.04–0.15 with healthy tissue after a substrate incubation time of 30–60 min. Although unfractionated ascites fluid was used in this study, recent results indicate greater differences in absorbance values between healthy and infected samples using IgG purified from ascites and a modification of the procedure in which reagent incubation times were shortened to 1 hr and conducted at 20 C. With this modified procedure, the absorbance values at 405 nm were up to 2.6 for AV-I infected and 0.09 for healthy asparagus tissue. AV-I ascites fluid did not yield satisfactory results in ELISA using *C. quinoa* tissue because of a high background reaction with healthy sap. High background reactions have also precluded the use of AV-II ascites against either *C. quinoa* or asparagus tissue, but AV-II was detected by bioassay on *C. quinoa*.

Field and greenhouse survey. From 1982 to 1984, 65% (51/78) of the samples tested from greenhouse and field clones were infected with AV-I and 6% (5/78) were infected with AV-II (Table 1). In 1985, AV-I was detected in 49% (57/117), AV-II in 10% (12/117), and AV-I and AV-II mixed in 5% (6/117) of random field samples (Table 2). Of the 17 clones or cultivars tested during this survey, 13 (D-1, D-2, Jersey Giant, Greenwich, 22-8, 51, 53, 55, 56, 277C, 277E, 314C, and 382B) were infected and four (Md-10, 61, 299E, and 362M) were not infected with AV-I, whereas four (Md-10, 53, 56, and 61) were infected and 13 (D-1, D-2, Jersey Giant, Greenwich, 22-8, 51, 55, 277C, 277E, 299E, 314C, 362M, and 382B) were not infected with AV-II. AV-I and AV-II were also detected in three field-grown samples of *A. officinalis* L. var. *pseudoscaber* (Grec.) Asch. & Graebn. but not in greenhouse-grown *A. sprengeri* Regel. Greenwich and Jersey Giant are male hybrids, 22-8 is a selected male hybrid seedling, *A. sprengeri* is dioecious, and all others are selected female hybrid seedlings reproduced through vegetative propagation.

Table 1. Detection of mechanically transmitted viruses similar to asparagus viruses I and II (AV-I and AV-II, respectively)[a] in asparagus samples collected from 1982 to 1984

Sample type	Location[b]	Number of samples	AV-I	AV-II	AV-I and AV-II
Tissue-culture clones	1	11	9	0	0
Greenhouse clones	1	46	27	0	0
Field clones	1	15	13	1	3
Field clones and hybrids	2	6	2	1	0
Hybrid seedlings	3	400	0	1	0

[a] AV-I identification based on production of local necrotic lesions on *Chenopodium quinoa*. AV-II identification based on production of local chlorotic lesions and systemic mosaic in *C. quinoa*.
[b] 1 = Hort Farm, New Brunswick, NJ; 2 = Rutgers Vegetable Research Center, Centerton, NJ; and 3 = greenhouse grown.

Table 2. Detection of mechanically transmitted viruses similar to asparagus viruses I and II (AV-I and AV-II, respectively)[a] in field-grown asparagus samples collected in May 1985

Location[b]	Field	Number of samples	AV-I	AV-II	AV-I and AV-II
1	1	8	2	2	1
	2	5	4	0	0
	3	9	3	1	2
2	1	6	3	0	0
	2	8	6	0	0
	3	12	2	3	3
	4	5	2	1	0
	5	5	4	0	0
3	1	59	31	5	0
Total	9	117	57	12	6

[a] AV-I identification based on production of necrotic local lesions on *Chenopodium quinoa*. AV-II identification based on chlorotic local lesions and systemic mosaic on *C. quinoa*.
[b] 1 = Hort Farm, New Brunswick, NJ; 2 = Rutgers Vegetable Research Center, Centerton, NJ; and 3 = home garden.

One of the most useful clones in the tissue-culture breeding program is asparagus clone 56. To determine the incidence of virus in clone 56 and to compare bioassay and ELISA for detection at different times of the year, 15 plants vegetatively propagated and growing in the greenhouse for 3 yr were tested by bioassay and ELISA for AV-I and by bioassay alone for AV-II in August 1985, October 1985, and again in January 1986. The results are presented in Table 3. The bioassay and ELISA tests for AV-I provided identical results, except in October, when AV-I was detected by bioassay in two more samples than by ELISA. The number of samples in which AV-I was detected was greater in October and January than in August. AV-II was detected by bioassay in January but not in August or October.

Table 3. Detection of asparagus viruses I and II (AV-I and AV-II, respectively) by bioassay and enzyme-linked immunosorbent assay (ELISA) in 15 asparagus samples collected from tissue-culture clone 56 grown in the greenhouse

	AV-I							AV-II		
	Bioassay[a]			ELISA[b]				Bioassay[a]		
Sample	Aug. 1984	Oct. 1984	Jan. 1985	Aug. 1984	Oct. 1984	Jan. 1985	June 1985	Aug. 1984	Oct. 1984	Jan. 1985
1	0/3	4/4	3/3	0.19	0.49	0.14	1.30	0/3	0/4	1/3
2	3/3	4/4	3/3	0.45	0.32	0.11	1.50	0/3	0/4	0/3
3	1/3	4/4	3/3	0.37	0.35	0.13	2.40	0/3	0/4	0/3
4	0/3	4/4	3/3	0.19	0.61	0.12	0.60	0/3	0/4	0/3
5	1/3	2/4	3/3	0.39	0.28	0.18	1.10	0/3	0/4	0/3
6	3/3	4/4	3/3	0.43	0.78	0.17	2.60	0/3	0/4	0/3
7	1/3	2/4	3/3	0.33	0.39	0.15	2.50	0/3	0/4	1/3
8	0/3	0/4	3/3	0.22	0.13	0.14	0.42	0/3	0/4	2/3
9	0/3	4/4	3/3	0.14	0.51	0.21	2.10	0/3	0/4	1/3
10	3/3	4/4	1/3	0.48	0.32	0.07	1.50	0/3	0/4	1/3
11	0/3	4/4	3/3	0.17	0.59	0.15	1.80	0/3	0/4	0/3
12	0/3	4/4	3/3	0.20	0.42	0.21	0.80	0/3	0/4	1/3
13	0/3	4/4	3/3	0.18	0.19	0.18	0.70	0/3	0/4	0/3
14	1/3	0/4	3/3	0.33	0.14	0.09	0.42	0/3	0/4	2/3
15	0/3	2/4	3/3	0.19	0.23	0.12	0.90	0/3	0/4	1/3
Healthy	0/3	0/4	0/3	0.15	0.12	0.04	0.09	0/3	0/4	0/3
Total infected	7	13	14	7	11	14	15	0	0	8

[a] Number of assay plants infected with necrotic local lesion (AV-I) or systemic mosaic (AV-II)/number inoculated; bioassay host = *Chenopodium quinoa*.

[b] Absorbance at 405 nm in indirect ELISA (see text for details). Positive reaction is defined as an absorbance greater than twice that of the healthy tissue.

DISCUSSION

AV-I was first detected in New Jersey in 1982 (4) from asparagus plants growing in tissue culture. Since then, other sources of asparagus have been tested, and both AV-I and AV-II have been detected in greenhouse clones (produced asexually from field plants or plants produced in tissue culture), field clones (also originally from field or tissue culture), and hybrids. AV-I, however, was detected more frequently than AV-II in these samples.

Variation in symptomatology produced by AV-II was observed in *C. quinoa*, ranging locally from faint chlorotic local lesions and general chlorosis to semi-necrotic local ringspots and oak leaf patterns and ranging systemically from mild mottling to severe chlorotic spots and mosaic with slight top necrosis. These results of symptomatology on *C. quinoa* are similar, though not identical, to those of other strains of AV-II (1,7,9,13,14); however, the production of systemic symptoms on *C. quinoa* plants by AV-II was unreliable and seemed to depend on the environmental conditions. Systemic symptoms were most often noted between January and March. The lack of systemic symptoms during certain times of the year could reflect variations in virus titer or susceptibility and sensitivity of *C. quinoa*. The results of AV-I detection show fair agreement between ELISA and bioassay and suggest that for AV-I either virus titer was too low in infected plants for detection in August or significant spread occurred between August and October, possibly via aphids or during pruning.

From this study, plants produced in tissue culture have been found to be infected with AV-I and AV-II at all stages, from seedlings growing in tissue-culture tubes for only a few weeks to plants transferred to pots and grown in the greenhouse for 3 yr. Other researchers have reported that tissue-culture techniques were successful in eliminating AV-I and AV-II from asparagus (6,17). In our experience, shoot-tip tissue culture used for propagating asparagus in New Jersey, without any other treatment such as chemotherapy or thermotherapy, was not sufficient to eliminate viruses in all cases.

In this report, *A. officinalis* var. *pseudoscaber* was found to be naturally infected with AV-I and AV-II. This host is not a commercial cultivar, but it could play a role in virus transmission and distribution to commercial cultivars under field conditions.

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