Occurrence of Asparagus Virus II in Commercial Asparagus Fields in Michigan

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ABSTRACT

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A mechanically transmissible virus was found in 23 asparagus (*Asparagus officinalis*) fields of varying ages and in seedling nurseries. This survey indicated that virus infection was widespread. In most established fields, more than 50% of the plants were infected. The virus was isolated from four of five seedling nurseries, but incidence was lower than in established fields. Using Ouchterlony gel double-diffusion tests, all isolates tested were found serologically identical to asparagus virus II (AV II) described previously in Washington. This is the first report of AV II occurring in asparagus in Michigan.

Several viruses have been reported to infect asparagus (*Asparagus officinalis* L.) in both Europe and North America (2,6,9–12,14). In Europe during the 1960s, three mechanically transmitted viruses were found in asparagus: asparagus stunt virus (related to tobacco streak virus [TSV]), asparagus latent virus or asparagus virus II (AV II), and asparagus virus I (AV I) (2,6,10,12). Additional work indicated that AV I is a flexuous rod-shaped particle of the Potyvirus group and is aphid-transmitted (G. I. Mink, *personal communication*).

In 1977, three viruses initially designated A, B, and C were isolated in Washington (9). Further investigation revealed virus A was serologically related but not serologically identical to asparagus stunt virus isolates from Europe and TSV isolates from Washington. On the basis of host range and particle morphology, virus B was designated AV I. Virus C was determined to be a member of the Ilarvirus group and the same as AV II isolated in Europe (13).

Little research has been done to determine what effects, if any, viruses may have on commercial asparagus yields. In Europe, Weissenfels and Schmelzer (14) reported yield losses of at least 20% in asparagus fields infected with viruses. In Washington, studies on the effects of asparagus viruses on growth and productivity indicated that when either AV I or AV II was present in 16-mo-old asparagus plants, a mild reduction in growth and vigor was noted. When asparagus plants contained both AV I and AV II, however, severe decline and death of plants resulted after 2 yr in the field (15). The interaction of the TSV-related isolate (virus A from Washington) was not investigated.

Michigan asparagus growers have noted an increasing incidence of a syndrome called asparagus decline, which has been defined as a reduction in the profitable life of asparagus plantings (7). Asparagus decline is attributed primarily to the presence of *Fusarium* spp. that cause wilt and root rot (4,5); however, various stress factors are believed to hasten the decline problem (4,5). No research has been done to determine if asparagus viruses stress the asparagus plant sufficiently to increase the incidence and severity of the decline syndrome. Because the literature indicates that asparagus viruses can directly cause decline or death of asparagus plants and also act as a stress factor, it was important to determine if commercial asparagus plantings and asparagus seedling nurseries harbored viruses. This research was initiated to determine if asparagus viruses were present in commercial fields and seedling nurseries in Michigan and to identify the viruses present.

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MATERIALS AND METHODS

Survey for viruses in commercial asparagus fields. During the summer of 1981, 25 samples of actively growing asparagus spears or flower buds were taken from each of five locations: an experimental plot at the Sodus Experimental Farm, Sodus, MI, and four commercial fields in Oceana County, MI. Twenty-five asparagus plants (cultivar Mary Washington or Viking) from each location were sampled. Samples were maintained at 4 C until they were processed 24 hr later. Each sample was ground in 0.01 M phosphate buffer + 0.1% 2-mercaptoethanol (pH 6.8) and rub-inoculated on a limited herbaceous host range first dusted with Carborundum (320 grit). Plant species included *Vigna unguiculata* (L.) Walp. 'SR', *Chenopodium quinoa* Willd., *C. amaranticolor* Coste & Reyn., *Lycopersicon esculentum* Mill. 'Marglobe,' *Phaseolus vulgaris* L. 'Bountiful,' *Nicotiana tabacum* L. 'Xanthi' and 'Turkish,' *Gomphrena globosa* Murr., and *Cucumis sativus* L. 'National Pickling.' Plants were visually assessed for symptoms after 10–14 days. Plants showing symptoms were back-inoculated to *C. quinoa, G. globosa,* and *N. glutinosa* L. Single yellow lesions on *C. quinoa* were transferred sequentially three times to ensure that symptom development was due to only one virus. Plants were maintained in a growth chamber at 21–24 C with a 16-hr photoperiod.

In June 1982, 23 commercial asparagus fields were surveyed, including 12 fields in Oceana County and 11 in Van Buren County. Also, six seedling nurseries in Oceana County were surveyed. In each field, an experimental plot (100 × 100 m) was established at a location 100 m diagonally from a corner of each field. Tips 1–2 cm long of 20–30 spears from each plot were picked and kept at 4 C until ground and rub-inoculated on *C. quinoa* as described previously. Indicator plants were assessed for symptoms 1–2 wk after inoculation and percent infection from each field was determined by the number of plants found to contain virus.

Serology. Several isolates from commercial asparagus fields were maintained in *C. quinoa* plants and used for agar gel double-diffusion tests against antisera prepared against TSV isolates D, BRN, and HF, tomato ringspot virus, tobacco ringspot virus (blueberry isolate), peach rosette mosaic virus, tobacco ringspot virus (tobacco isolate, originally from G. Gooding), and AV I and AV II. Antisera for AV I and AV II were provided by G. I. Mink, Washington State University, Prosser, WA. All others were provided by D. C. Ramsdell, Michigan State University, East Lansing. All double-diffusion serology tests included known positive controls for the respective virus and a healthy sap control. Serology plates were prepared by pouring 15 ml of agarose (4 g of agarose/500 ml of glass-distilled water [GDW], autoclaved 10 min at 120 lb of pressure, plus 4.25 g of sodium chloride and 0.5 g of sodium azide) into 9-cm petri plates. Agar was

Table 1. Percentage of sap-transmissible virus infection as determined with *Chenopodium quinoa* indicator plants for 12 asparagus fields in Oceana County and 11 fields in Van Buren County, MI[a]

Age of plants (yr)	Van Buren County: Fields assayed (no.)	Van Buren County: Total plants assayed (no.)	Van Buren County: Incidence for individual fields (%)	Oceana County: Fields assayed (no.)	Oceana County: Total plants assayed (no.)	Oceana County: Incidence for individual fields (%)	Mean infection for all fields (%)
0–2	3	57	60,11,0	3	68	0,71,67	35
3–10	3	61	15,0,0	3	62	60,5,70	25
11–15	3	60	50,25,71	3	60	75,60,80	60
16–20	2	41	55,55	3	65	90,70,76	69

[a] Each sample was ground in 0.01 M phosphate buffer + 0.1% 2-mercaptoethanol (pH 6.8) and rub-inoculated on separate indicator plants that had first been dusted with 320-grit Carborundum. Asparagus cultivars assayed were Mary Washington and Viking. Samples were kept at 4 C after collection until processed 24 hr later. Indicator plants were viewed for virus symptoms 1–2 wk after inoculation.

Table 2. Percentage of sap-transmissible virus infection as determined using *Chenopodium quinoa* indicator plants for five Oceana County asparagus seedling nurseries in Michigan[a]

Age of plants (yr)	Fields assayed (no.)	Total plants tested (no.)	Incidence for individual fields (%)
1.0	2	24	17,17
1.5	2	30	0,42
2.0	1	21	33

[a] Each sample was ground in 0.01 M phosphate buffer + 0.01% 2-mercaptoethanol (pH 6.8) and rub-inoculated on separate indicator plants that had first been dusted with 320-grit Carborundum. Asparagus cultivars assayed were Mary Washington and Viking. Samples were kept at 4 C after collection until processed 24 hr later. Indicator plants were viewed for virus symptoms 1–2 wk after inoculation.

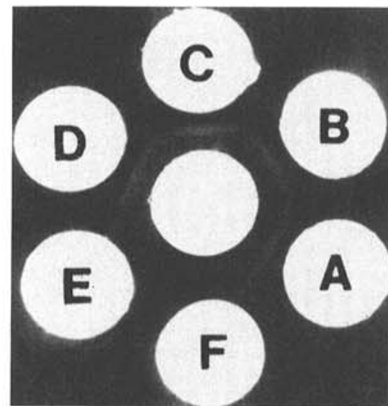

Fig. 1. Ouchterlony gel double-diffusion test. Center well was charged with asparagus virus II antiserum (dilution end point = 1/64, dilutions = 1:1 or 1:2), peripheral wells were charged with asparagus virus II from Washington State (A), Michigan isolate I (B), Michigan isolate II (C and D), asparagus virus I (E), and virus-free sap of *Chenopodium quinoa* (F).

allowed to solidify and wells were cut in plates using a Grafar auto gel cutter (Grafar Corporation, Detroit, MI).

Physical properties of viral isolates. Isolates from several fields were characterized after single-lesion transfer to *C. quinoa* by determining dilution end point, thermal inactivation, and longevity in vitro. All samples were kept at 4 C until rub-inoculated onto two *C. quinoa* plants. Inoculated plants were assessed for symptom development 1–2 wk after inoculation. For dilution end point tests, 5 g of infected leaves of *C. quinoa* was ground in 0.01 M phosphate buffer (pH 7.0) and strained through two layers of cheesecloth. Dilutions were made in GDW to 10^{-7} and each sample rub-inoculated to *C. quinoa* plants. For thermal inactivation point tests, 5 g of infected tissue of *C. quinoa* was ground in 0.01 M phosphate buffer (pH 6.8) and strained through two layers of cheesecloth. One milliliter of infected sap was placed in a test tube heated in a water bath for 10 min at temperatures ranging from 50 to 80 C at 10-degree increments, then plunged into ice. Samples were rub-inoculated on two *C. quinoa* plants. For longevity in vitro tests, each sample of 15 g of infected *C. quinoa* tissue was ground in GDW and the extract kept at 21–24 C. Plants were inoculated at intervals up to 14 days after initial preparation of the extract.

Partial purification and host range. One Michigan isolate was partially purified using the technique of Brunt and Stace-Smith (3). Further purification was done using a linear-log sucrose density gradient (1), centrifuged 90 min in a Beckman SW 41 rotor at 38,000 rpm at 4 C, then fractionated with an ISCO gradient fractionator. Two peaks were identified by ultraviolet absorption, collected, and each assayed for infectivity on *C. quinoa*.

The host range of one Michigan isolate (AV II-M) was determined using purified virus preparations. Eleven plant species previously shown to produce symptoms in response to AV II (13) were first dusted with 320-grit Carborundum and then rub-inoculated with AV II at 0.5–1.0 mg/ml in 0.01 M phosphate buffer + 0.1% 2-mercaptoethanol (pH 6.8). Plants were visually assessed for symptoms after 5–10 days.

Electron microscopy. Partially purified virus was prepared for electron microscopic observations by first fixing in 1% glutaraldehyde for 1 min. The preparation was then placed on to Formvar-coated copper grids and negatively stained with 2% ammonium molybdate (pH 7.0). The grids were observed under a Philips 201 electron microscope for the presence of virus particles.

RESULTS

Survey for virus in commercial asparagus fields. In 1981, the preliminary survey revealed the presence of a virus or viruses in all five fields surveyed. A limited herbaceous host range study using infected asparagus as source tissue revealed that four of 11 plant species tested were susceptible to the virus isolated: *C. quinoa, C. amaranticolor, N. tabacum* 'Turkish,' and *G. globosa*. After three successive single-lesion transfers to *C. quinoa*, chlorotic ring lesions developed after 3 days on inoculated leaves followed by light, systemic mottling on uninoculated leaves within 1–2 wk.

The 1982 survey revealed widespread virus infection in commercial asparagus fields in Oceana and Van Buren counties (Table 1). All four fields in which virus was not found were less than 10 yr old; three were located in Van Buren County. A survey of the seedling nurseries showed the plantings were infected with virus but at a much lower incidence than commercial fields (Table 2).

Serology. Infected *C. quinoa* plant sap diluted 1:1 produced a single precipitin line in gel double-diffusion tests with antiserum to AV II diluted 1:3 (v/v) but did not react with other antisera tested (Fig. 1). No precipitin line was formed with healthy plant sap. Sap from *C. quinoa* infected with a Washington isolate of AV II also produced a precipitin line.

Physical properties of viral isolates. Three isolates from different asparagus fields yielded similar physical characteristics including a dilution end point of

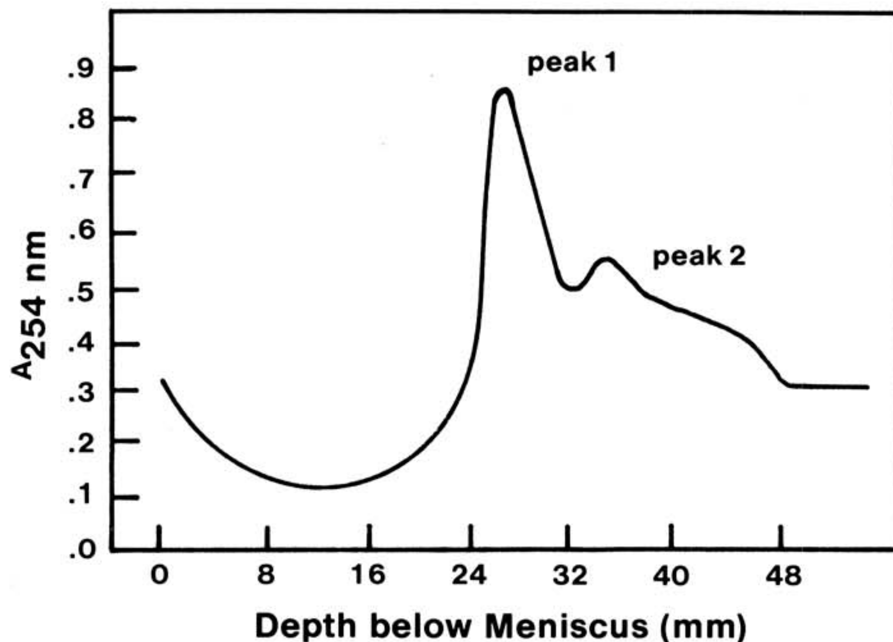

Fig. 2. Ultraviolet absorption profile of a Michigan isolate of asparagus virus II sedimented in a 0–30% linear-log sucrose density gradient. Direction of sedimentation is from left to right. A Beckman SW-41 rotor was used at 38,000 rpm for 90 min at 4 C.

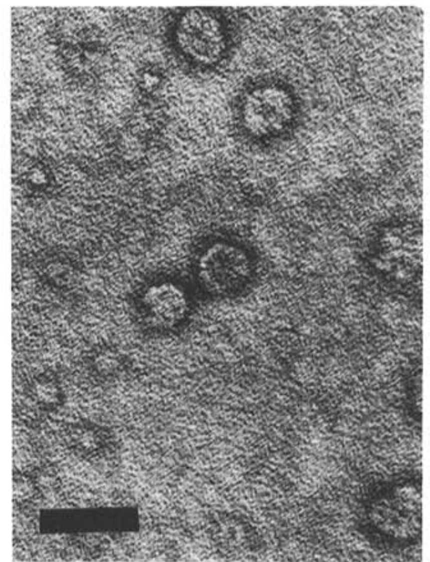

Fig. 3. Electron micrograph of a Michigan isolate of asparagus virus II. Isometric virus particles were first fixed with 1% glutaraldehyde for 10 min, then negatively stained with 2% ammonium molybdate (pH 7.0). Scale bar = 50 nm.

10^{-2}–10^{-3}, thermal inactivation point of 50–60 C, and longevity in vitro of less than 10 days. These general characteristics agree well with results obtained with AV II isolates from Washington.

Partial purification and host range. After two cycles of sucrose density gradients, two major peaks were determined to be infective on *C. quinoa* plants (Fig. 2). The host range of one Michigan isolate, AV II-M, was determined to be the same as reported for AV II in Washington (13).

Electron microscopy. Observations of negatively stained, purified virus revealed isometric virus particles with diameters of 23–30 nm (Fig. 3).

DISCUSSION

In Michigan, our survey revealed that at least one virus was widespread in almost all commercial asparagus fields tested. This virus was also present in four of five seedling nurseries but generally at a lower level than in the older fields. Virus incidence in fields of varying ages was generally the same among age groups, particularly in Oceana County. Mean percent infection data indicated that younger fields had less virus present than older fields. However, there was considerable variation in virus infection among the young fields (0–2 and 3–10 yr old) assayed. For example, in the 0- to 2-yr-old fields, two sites were assayed at 71 and 67% infection, whereas the other site showed no infection by our assay methods.

Our isolates reacted serologically only with antisera against AV II from Washington State. Serological precipitin lines coalesced, indicating the isolates were serologically identical. No spur formation was noted. Serology has not been performed with isolates of European strains. Dilution end point, thermal inactivation, longevity in vitro, and host range results agree with those obtained from isolates in Washington State.

Serologically specific electron microscopy (SSEM) (8) was employed to search for AV I in the samples because this virus is known to occur at low concentrations in asparagus in Washington and the antisera available was of low titer. Throughout all our indexing survey, host range studies, and sequential transfers to *C. quinoa*, no AV I-type local lesions were observed. Although AV I was not observed by these methods and was not detected by serology, we cannot say for certain that this virus is not present in Michigan. Further studies are being done to confirm this negative result.

At present, we can conclusively identify one virus in commercial asparagus fields and seedling nurseries in Michigan. Although it has been reported that the presence of one virus in asparagus causes only a mild reduction in vigor, to date no research has been done to determine what role this stress may play in combination with root and crown rot caused by *Fusarium* spp. We are now in the process of determining if AV II predisposes the asparagus plant to Fusarium root and crown rot. Because AV II is seedborne, we are also investigating how prevalent the virus is in asparagus seed. Should AV II weaken the plant to the degree that susceptibility to Fusarium crown and root rot is increased, removal of this pathogen may help reduce the asparagus decline problem in Michigan.

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