Survey of California Asparagus for Asparagus Virus I, Asparagus Virus II, and Tobacco Streak Virus

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ABSTRACT

Falloon, P. G., Falloon, L. M., and Grogan, R. G. 1986. Survey of California asparagus for asparagus virus I, asparagus virus II, and tobacco streak virus. Plant Disease 70:103-105.

Spear samples were collected from 40 asparagus (*Asparagus officinalis*) fields in California and from 100 female and 100 male asparagus plants in the U.C. 157 Foundation Seed block at Davis. Fern and spear samples were collected from parent plants of the asparagus hybrids U.C. 157 and Ida-Lea. Sap from samples giving positive reactions after mechanical transmission to *Chenopodium quinoa* was tested for asparagus virus I (AV I) with immunosorbent electron microscopy or for asparagus virus II (AV II) or tobacco streak virus (TSV) in Ouchterlony double-diffusion plates. Both AV I and II were found throughout California and in the U.C. 157 Foundation Seed block. TSV was not found. AV II was seed-transmitted in U.C. 157 and Mary Washington. Both male and female parents of U.C. 157 and Ida-Lea were infected with AV II, but tissue-cultured plants derived from them were free of the virus. A stock of parent plants of Ida-Lea free of AV I, AV II, and TSV has been established.

Additional key words: asparagus decline, *Fusarium*

Three mechanically transmissible viruses were first reported in asparagus in North America in 1977 (18). These were asparagus virus I (AV I), a member of the potyvirus group with flexuous rod-shaped particles 700–880 nm long that is transmitted by aphids but not in seed; asparagus virus II (AV II), or asparagus latent virus, a member of the ilarvirus group (19) with quasiisometric particles 26–36 nm in diameter (20) that is seed-transmitted (21,22) and thought to be transmitted by pollen from male plants to seed (T. Evans, *personal communication*); and tobacco streak virus (TSV), a member of the ilarvirus group with an isometric particle 28 nm in diameter (10) that is transmitted by thrips and in seed (16). AV I was subsequently reported in asparagus in New Jersey (3) and AV II was found in asparagus in Michigan (12).

When either AV I or AV II was present in cloned pistillate asparagus plants, there was a slight reduction in vigor, but when both viruses were present in the same plant, there was a serious decline in vigor and survival during the first 16 mo of growth in the field (23). Yang and Clore (24) eliminated AV I, AV II, and TSV from asparagus plants by tissue culture. In 1983 and 1984, the parents of two University of California asparagus hybrids (U.C. 157 and Ida-Lea) were being cloned for the establishment of new seed blocks. This paper reports on 1) the establishment of virus-tested plants for the production of Ida-Lea seed free of AV I, AV II, and TSV; 2) a survey to determine which viruses are present in asparagus in California; 3) the distribution of the viruses in the main production areas of the state; and 4) possible sources of inoculum.

Accepted for publication 22 July 1985 (submitted for electronic processing).

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MATERIALS AND METHODS

Field survey. Spear samples (75–150 mm long) were collected during the 1983 harvest season from 40 production fields throughout California, representing a total area of 724 ha. In each field, two spears were collected from each of 10 plants selected at random. The samples were stored separately at −18 C until indexed individually on *Chenopodium quinoa*.

Mechanical transmission. Asparagus tissue was triturated in an aqueous solution of 0.01 M sodium diethyldithiocarbamate + 0.01 M cysteine hydrochloride (DIECA + cysteine), pH 8.1 (17), and rubbed onto *C. quinoa* leaves previously dusted with 0.22-μm (600-mesh) Carborundum. Immediately after inoculation, the leaves of the young test plants were rinsed with distilled water.

Seed transmission. Seeds of the asparagus cultivars Mary Washington (MW), Brock's Special, U.C. 66, U.C. 72, and two seed lots of U.C. 157 (BR 81-2 and VR 82) were germinated in the greenhouse and grown for 6–8 wk. Two hundred seedlings from each lot were indexed individually on *C. quinoa*.

Two spears from each of 100 male and 100 female plants selected at random in the U.C. 157 Foundation Seed block at Davis were collected in spring 1983. After storage at −18 C, these were indexed separately on *C. quinoa*. During spring 1984, two spears were collected from each plant surrounding plants in the U.C. 157 Foundation Seed block that had been found to be infected with AV II in 1983.

Establishment of plants free of AV I, AV II, and TSV. Fern tissue was collected from the parent plants (F109 and M120, F189 and M138) from which clones were derived to establish new hybrid seed blocks of U.C. 157 and Ida-Lea, respectively.

Explants for tissue culture were obtained by excising shoot tips from fresh field-grown spears of the parent plants of Ida-Lea. The shoot tips were placed on proliferation media in a commercial tissue-culture laboratory (Native Plants, Inc., Salt Lake City, UT). The lower portions of the spears were wrapped in damp paper towels and sent by airfreight to this laboratory. The lower 20–25 mm of each spear was crushed, and a sample of sap was either placed in wells in Ouchterlony plates or indexed on *C. quinoa* after trituration in DIECA + cysteine.

Shoots from tissue-cultured plantlets were removed and transferred to new media in the tissue-culture laboratory. The remaining plantlet bases were placed on fresh media before being sent by air-freight to this laboratory. The bases were allowed to grow for 2 wk at 21 C and 18-hr photoperiod before being triturated in DIECA + cysteine and indexed on *C. quinoa* or placed in wells in Ouchterlony plates.

Serological tests. Ouchterlony plates were prepared by pouring 10 ml of autoclaved 0.7% Difco agar containing 0.85% sodium chloride and 0.04% sodium azide in 0.01 M neutral phosphate buffer into 90-mm-diameter plastic petri plates. Wells were cut in plates immediately before use. Diameters of the center and peripheral wells were 8 and 5 mm, respectively. Distance between edges of the center and peripheral wells

was 5 mm. Healthy and virus-infected tissue from asparagus and *C. quinoa* was tested against antisera of AV II and TSV (asparagus isolate) kindly provided by G. Mink, Irrigated Agriculture Research and Extension Center, Washington State University, Prosser.

Immunosorbent electron microscopy. Single necrotic local lesions on leaves of *C. quinoa* plants showing only this symptom were transferred sequentially two or three times. Carbon-coated copper grids were placed for 30 min on a drop of 1:1 or 1:10 dilution of antisera to AV I (provided by G. Mink) in phosphate-buffered saline (PBS). Either necrotic local lesions were excised and macerated in a drop of PBS or sap from frozen spear tissue was used. After rinsing with PBS, the grids were then placed on the macerate or a drop of sap for 1 hr, rinsed in PBS followed by distilled water, and either placed back on the antisera for 30 min for decoration or shadow-casted with paladium. After the decoration step, grids were washed with water and stained with 2% uranyl acetate, pH 4.2. Purified tobacco mosaic virus (TMV) was used as a control to determine the level of nonspecific binding. Grids were examined with an Associated Electrical Industries EM6B electron microscope.

Host range of AV I. Field-grown asparagus spears and leaves of *C. quinoa* showing local necrotic lesions after two or three sequential lesion transfers were triturated in DIECA + cysteine. The triturate was rub-inoculated onto single leaves of plants that had been dusted with Carborundum as described earlier. The test plants had been grown in a greenhouse and were transferred to a growth room at 21 C and 18-hr photoperiod after inoculation. Plants were observed for 24 days after inoculation, then samples from inoculated and young tip leaves were back-indexed to *C. quinoa*.

Fig. 1. Distribution of asparagus virus II (AV II) in asparagus fields in California during 1983. ● = AV II isolated and □ = AV II not isolated.

Table 1. Relationship between cultivar and presence of asparagus virus II (AV II) in 40 asparagus fields surveyed in California during 1983

Cultivar	No. of fields surveyed	No. of fields with AV II[a]
Mary Washington	2	2
U.C. 72	10	7
U.C. 800	4	0
U.C. 157	19	1
Brock's Special	5	0
Total	40	10

[a] Two spears were collected from each of 10 plants chosen at random in each field. Samples from each plant were stored separately at −18 C until indexed on *Chenopodium quinoa*.

Table 2. Percentage seed transmission of asparagus virus II in five asparagus cultivars

Cultivar	Seed transmission[a] (%)
Mary Washington	21.5
U.C. 66	0.0
U.C. 72	0.0
U.C. 157	
BR 81-2	0.0
VR 82	0.5
Brock's Special	0.0

[a] Two hundred seeds of each seed lot were germinated in the greenhouse and grown for 6–8 wk before being rub-inoculated onto young *Chenopodium quinoa* plants.

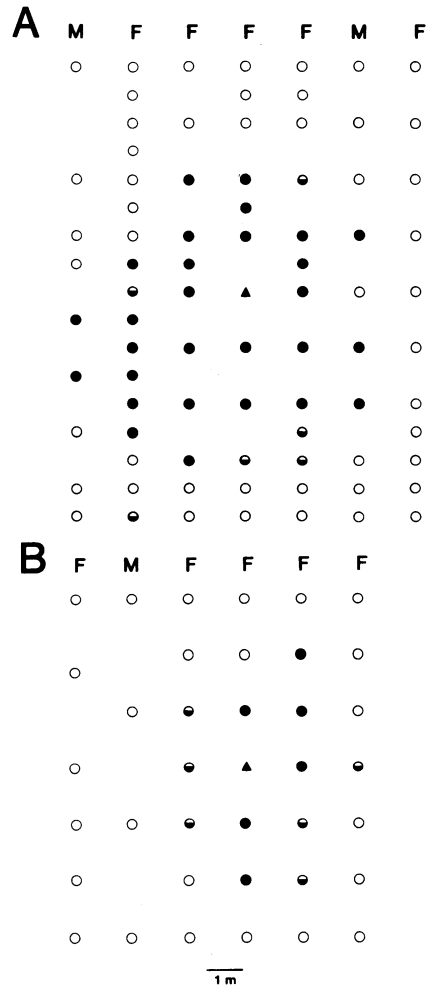

Fig. 2. Distribution of asparagus virus II (AV II) in female (F) and male (M) plants in two sections **(A and B)** of the U.C. 157 Foundation Seed block at Davis. ▲ = Plants found to contain AV II in 1983. Two spears were collected from each plant surrounding ▲ in 1984 and stored at −18 C before being individually indexed against antisera to AV II in Ouchterlony plates. ◒ = AV II-infected plants, one spear in two infected. ● = AV II-infected plants, both spears infected. O = Plants in which AV II was not detected.

RESULTS

Field survey. Local necrotic lesions without a systemic reaction on *C. quinoa* indicated that AV I was present in plants in 31 of the 40 fields surveyed; results of the immunosorbent electron microscopy later confirmed this. Local chlorotic and/or necrotic lesions with systemic mottle, mosaic, or necrosis on *C. quinoa* indicated that AV II was present in plants in 10 of the 40 fields surveyed (Fig. 1). This was confirmed by the results of serological reactions in Ouchterlony plates. TSV was not found in any plants.

AV I was found in all cultivars. AV II was present in cultivars MW, U.C. 72, and U.C. 157 but was not found in U.C. 800 and Brock's Special (Table 1). The percentage of AV II-infected plants, averaged over the number of fields surveyed for each cultivar, was higher in fields of MW (90%) than of U.C. 72 (25%) or U.C. 157 (1%).

Seed transmission. AV II was the only virus found in the seed lots tested. The percentage seed transmission of AV II was 0.5% or less for all cultivars except MW (Table 2).

Symptoms on *C. quinoa* characteristic of AV I were obtained from 29 female and 25 male plants collected from the U.C. 157 Foundation Seed block. AV II was found in only two female plants. In 1984, indexing of plants surrounding these two AV II-infected females showed that they were not isolated plants but instead were part of a group of plants infected with AV II (Fig. 2).

To determine the distribution of AV II among spears of the same plant, five female plants known to be infected with AV II in the U.C. 157 Foundation Seed block were selected in spring 1984. The soil surface above each plant was divided into four quadrats, and spears that emerged in each quadrat were harvested and stored at −18 C before being indexed in Ouchterlony plates. All spears harvested during 1984 of four of the five AV II-infected female plants were infected with AV II. Seventy-five percent of the spears harvested from the fifth plant were infected with AV II.

Establishment of plants free of AV I, AV II, and TSV. Both the female (F109) and male (M120) parents of U.C. 157 and the female (F189) and male (M138) parents of Ida-Lea contained AV II but

not AV I or TSV. However, none of the tissue-cultured plantlets derived from these had AV I, AV II, or TSV. None of the tissue-cultured bases or the field-grown spears of the parents of Ida-Lea from which explants for tissue culture were derived were infected with any of the three viruses.

Electron microscopy. Plants that gave positive reactions on *C. quinoa* for AV I in the U.C. 157 hybrid seed block contained long flexuous rods with a mean length of 848 nm. Comparable particles were not seen in preparations from healthy *C. quinoa* leaf tissue. Many more flexuous rods were trapped on the grids pretreated with 1:1 than 1:10 dilution of AV I antisera, and the rods were decorated by AV I antisera at either dilution. Although some nonspecific binding of TMV was seen, the particles were not decorated by AV I antisera.

Host range. Isolates of AV I produced necrotic lesions on inoculated leaves of *C. quinoa, C. album, C. capitatum,* and *C. amaranticolor* but did not infect *Gomphrena globosa, Nicotiana tabacum* cv. Havana 425, *Phaseolus vulgaris* cv. Bountiful, *Glycine max,* or *Vigna unguiculata* cv. California Blackeye.

DISCUSSION

Two viruses were present in the main production areas of California. Positive serological reactions with antisera to AV I and AV II, the presence of long flexuous rod-shaped particles within the size range of the potyvirus group, and host range reactions indicated that AV I and AV II were present in asparagus throughout California. Although variation in symptomatology produced by AV II was observed in *C. quinoa* ranging from systemic mottle to severe necrosis of the tip leaves, this did not indicate the presence of different serotypes of AV II (19) because there was no evidence of spur formation in Ouchterlony plates.

Despite the presence of TSV in California (2) and the frequent infestation of asparagus by a known thrip vector of TSV (*Frankliniella occidentalis* (Pergande)) (1), this virus was not found.

AV I is transmitted by aphids (13–15,18), and these are its most likely method of spread in California asparagus. AV II was found in seed lots of asparagus cultivars grown in California and in both female and male plants in the U.C. 157 Foundation Seed block at Davis. This seed block has been the main source of asparagus seed for California between 1980 and 1984 and is a likely source of AV II in recent plantings of U.C. 157. When part of the seed block was established in 1974 (Fig. 2A), plants were transplanted in rows 1.5 m apart with plants 1.5 m apart in each row. Subsequently, more plants were transplanted between existing plants in each row. It is unlikely, therefore, that the grouped pattern of infected plants in the seed block is the result of a chance planting of a group of AV II-infected plants. More likely, it is the result of spread of AV II from a few infected plants to previously healthy plants. Seed is harvested from this block by cutting the green fern stalks with either an adapted machete for the female plants or a tractor-mounted slasher for the male plants. Both methods may provide a means of mechanical transmission of AV II. Although Uyeda and Mink (19) were unable to rub-transmit AV II to healthy asparagus seedlings, Fujisawa et al (9) transmitted AV II from infected to healthy asparagus and transmission rates as high as 70% have been found (T. Evans, *personal communication*).

Tissue culture was an effective method of eliminating AV II from the parents of asparagus hybrids, as demonstrated earlier by Yang and Clore (24). Older cultivars, e.g., MW and U.C. 72, which were propagated without tissue culture, had a higher incidence of AV II-infected plants than U.C. 157, which had been cloned in tissue culture. The spears from the parent plants of Ida-Lea used by Native Plants Inc. to establish tissue-cultured plantlets had previously been cloned in tissue culture. This is a plausible explanation for the absence of AV I, AV II, and TSV in these spears. A stock of parent plants of Ida-Lea free of AV I, AV II, and TSV has now been established. This will facilitate production of seed of Ida-Lea free of these three viruses. Should future plantings of this cultivar become infected after aphid transmission of AV I, it is unlikely that a marked reduction in vigor will occur from a synergistic reaction between AV I and AV II in the same plant (23) because AV II is unlikely to be present.

The presence of AV I and AV II in asparagus in California may be partly responsible for the reduction in profitable life of asparagus plantings, i.e., asparagus decline (11). This was previously attributed to the presence of *Fusarium oxysporum* f.sp. *asparagi* Cohen & Heald (11) and *F. moniliforme* (4), but recent work (5) indicates that asparagus seedlings infected with AV II are more susceptible to *F. oxysporum* f.sp. *asparagi* and *F. moniliforme* than seedlings free of AV II. *Phytophthora* species are responsible for reduced yields of asparagus during wet years in California (6–8) and may affect production in later years. It is likely, therefore, that asparagus decline in California is the result of an interaction between various pathogens as well as adverse management practices (4).

ACKNOWLEDGMENTS

This research was funded by a grant from the U.C. Davis Foundation Seed and Plant Materials Service and a New Zealand Department of Scientific and Industrial Research Overseas Study Award to the first author. We thank L. J. Petersen for assistance with electron microscopy and F. H. Takatori, B. L. Benson, and F. F. Laemmlin for provision of asparagus seed.

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