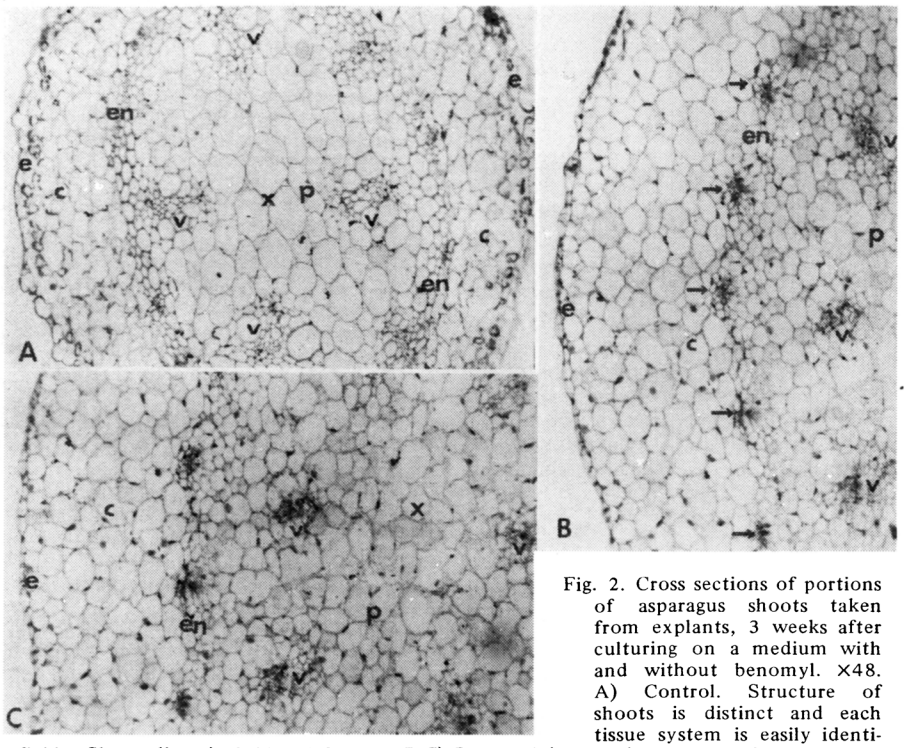

Fig. 2. Cross sections of portions of asparagus shoots taken from explants, 3 weeks after culturing on a medium with and without benomyl. ×48. A) Control. Structure of shoots is distinct and each tissue system is easily identifiable. Shoot diam is 0.68 x 0.97 mm. B,C) Benomyl (50 ppm) treatment. Shoot diam is 1.12 x 1.70 mm. B) The cells surrounding the endodermis became more active (arrow) and more vascular bundles formed. C) More cells formed causing enlargement of the cortex and the portion between the endodermis and the pith. c = cortex; e = epidermis; en = endodermis; p = pith; v = vascular bundle; x = center of pith.

The results show that 10 to 50 ppm of benomyl significantly promoted shoot development on asparagus explants in culture media. This suggests that benomyl may be used advantageously in asparagus nurseries or fields not only for controlling fungus diseases, but also to promote shoot development and to improve yield. Further studies on effect of benomyl on shoot growth and yield of asparagus and fungitoxicity in the field are required to determine any potential usefulness of this fungicide in commercial field and nursery practice.

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HortScience 11(5):474–475. 1976.

Obtaining Virus-free Plants of *Asparagus officinalis* L. by Culturing Shoot Tips and Apical Meristems[1]

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Additional index words. tissue culture, asparagus

Abstract. **Asparagus plants freed of 3 viruses were obtained by aseptic culture of shoot tips and apical meristems. More plantlets developed from shoot tip cultures than from apical-meristem cultures, but a much larger proportion of the meristem cultures were virus free. Consequently, the number of virus-free plants obtained by these 2 methods were approximately equal. The ease of excising and culturing shoot tips makes this the preferred method. The aseptic stock plants obtained are being used as the source of propagants for mass production of virus-free asparagus plants.**

Although several viruses have been reported to infect asparagus plants in Europe (1, 3, 12, 13, 14), little attention has been paid to virus infection of asparagus in North America. Recently, Mink and Uyeda (6) found 3 viruses, tentatively designated A-, B-, and C-type viruses, in commercial and experimental fields of asparagus in Washington. C type was also detected in asparagus seed obtained from California and New Jersey (Mink and Uyeda, personal communication). Asparagus latent virus is seed transmitted, and up to 65% infection in seed has been reported (12). It is probable that such viruses are widely disseminated throughout the asparagus growing areas of the world.

As expected we found that plants of several asparagus cultivars used for breeding and tissue culture studies were infected by these 3 viruses, separately or in combination. Therefore, we decided to make a major change in our asparagus research programs. We are attempting to produce large quantities of virus-free plants by tissue culture. These will be used to determine the effects of viruses on growth, yield and quality of asparagus, and to establish virus-free plantings of outstanding selections for breeding studies and seed production.

Virus-free plants of many species have been obtained by tissue culture of apical meristems and shoot tips (4, 5, 7, 8, 10). Asparagus plants have been produced by culturing shoot tips (2, 11) and apical meristems (9), but this procedure has not been used heretofore to obtain virus-free plants. Preliminary studies indicated that asparagus plants obtained from shoot tips 0.5 to 1.0 mm in length often were virus in-

[1]Received for publication May 25, 1976. Scientific Paper No. 4602. Washington State University College of Agriculture Research Center, Project No. 0173.

[2]The authors are grateful to the Washington Asparagus Growers Association for grant funds supporting these studies, and Mr. I. Uyeda for helping inoculation and identifying the viruses.

fected. However, we report here that virus-free plants can be obtained by culturing shorter shoot tips and apical meristems. We also describe a procedure for developing aseptic virus-free stock plants for use as the source of propagants for mass production of asparagus plants by tissue culture.

Explants for tissue culture were obtained from spears about 20 cm long and 1.7 cm diam from asparagus plants growing in the field. These plants were naturally infected by A-, B- and C-type viruses alone or in combination that were identified by a previously reported procedure (6). The surface of each spear was washed with water, and the scales on the tip of each spear were removed. Two types of explants were used: 1) apical meristem domes less than 0.1 mm in height and free of leaf primordia; 2) shoot tips 0.1 to 0.3 mm in length with 1 to 3 leaf primordia. The meristems and shoot tips were excised under a binocular dissecting microscope at 27× magnification with a knife made from a razor blade chip attached to a handle. The isolated meristems and shoot tips were placed singly in Pyrex test tubes (10 × 2.5 cm) containing 10 ml of modified Murashige and Skoog's medium (MMS) (15) with 0.1 ppm α-naphthaleneacetic acid (NAA) and 0.1 ppm kinetin (6-furfurylamino purine). The medium was adjusted to pH 5.7 with 1N NaOH and HCl and autoclaved at 121°C and 1 kg cm^2 (15 psi) for 15 min. The tubes were placed in a growth chamber and maintained at 27 ± 1°C under 1600 lux from 20-W Gro-Lux fluorescent lamps with a 16-hr photoperiod. Ninety cultures of each type of explant were made.

After 7 weeks the plantlets were transferred to 125-ml flasks containing 50 ml MMS medium with 0.1 ppm NAA and 0.3 ppm kinetin, one plantlet per flask. Four weeks after transfer the plantlets were removed from the flask and portions of the shoots and roots of each plantlet were collected for virus indexing. The plantlets were resterilized with 10% commercial Clorox (5.25% sodium hypochlorite) for 4 to 5 min followed by 2 rinses with sterile water and placed on fresh medium.

The virus-indexing procedure of Mink and Uyeda (6) was used. Inoculum was prepared by grinding shoot and root tissues of each plantlet in an aqueous solution of 0.01M sodium diethyldithiocarbamate and 0.01M cysteine hydrochloride. A cotton swab was dipped into this suspension and rubbed on carborundum-dusted mature leaves of *Chenopodium quinoa* Willd. The inoculated plants were maintained in a Corrulux greenhouse at 22 to 28°C. Local lesions appeared 5 days after inoculation, and systemic symptoms appeared in young leaves and shoot tips after 7 to 10 days. The specific symptoms caused in *C. quinoa* by the 3 viruses are summarized in Table 1.

Table 1. Symptoms produced on *Chenopodium quinoa* by 3 asparagus viruses 2 weeks after inoculation.

Virus	Necrotic or chlorotic local lesions	Systemic symptoms: Necrosis or mottle	Systemic symptoms: Shoot tip wilt
A	+[z]	+	+
B	+	–	–
C	+	+	–

[z]+ Distinct symptoms; – no symptoms.

The no. of plantlets that developed in culture and their virus status were influenced by the size of the explants (Table 2). More plantlets developed from shoot-tip cultures than from meristem cultures. However, virus indexing indicated that only 24 of 56 plantlets which developed from shoot-tip cultures were virus free. The other 32 plantlets still contained one or more of the 3 viruses. In contrast, 21 of 23 plantlets raised from meristem cultures were virus free. The remaining 2 carried the C-type virus only.

The 45 plantlets that indexed virus free from the 2 groups were reindexed after 4 to 6 weeks. Virus was detected in 2 more plantlets from the shoot-tip cultures. The plantlets were again resterilized and placed on fresh medium. Virus-free staminate and pistillate plants of asparagus cultivars UC-66, 500, 500W, 711, and 873 were obtained.

Six weeks after the second indexing and at 5- to 6-week intervals thereafter these plants were transferred to fresh medium in 125-ml flasks and eventually were transferred to 500-ml flasks. When these plants had developed sufficient size, they became stock plants for the production of virus-free propagants. Large-scale vegetative propagation of virus-free asparagus plants is now being carried out using previously described procedures (15, 16).

Excising and culturing apical meristems is much more difficult than excising and culturing shoot tips. Since approx equal no. of virus-free plants were obtained by the 2 methods, we recommend shoot-tip culture to obtain virus-free asparagus plants. However, if shoot-tip culture fails to eliminate certain viruses, then apical-meristem culture may be successful.

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Table 2. Effect of explant size on culturing success and on the virus status of the asparagus plantlets obtained.

Explants[y]	Total no. cultured	No. plantlets	Virus free	Plantlets with virus: A	B	C	B+C
Meristem	90	23	21	0	0	2	0
Shoot tip	90	56	22	3	12	15	4

[y]Meristem: Apical meristem domes less than 0.1 mm in height and free of leaf primordia. Shoot tip: Shoot tips 0.1 to 0.3 mm in length with 1 to 3 leaf primordia.