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By

Thomas Allen Evans

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ABSTRACT

ASPARAGUS VIRUSES AND THEIR CONTRIBUTIONS TO MICHIGAN ASPARAGUS DECLINE

BY

Thomas Allen Evans

Two viruses known to affect the vigor and productivity of commercial asparagus, *Asparagus officinalis* L., were investigated to determine their incidence and mode of transmission and spread within Michigan asparagus plantings. In addition, the potential of these viruses as biological stress factors leading to an increase in the severity of Fusarium crown and root rot was evaluated in the greenhouse and laboratory.

Asparagus virus II was determined to be widespread within most asparagus plantings in Michigan with as many as 50 to 60% of the plants in most fields being infected. Asparagus virus I was isolated from only 5 of 60 asparagus plantings in Michigan. All five infected fields were in Oceana County and were within one mile of each other.

These studies indicate that AV I can be transmitted within asparagus by the green peach aphid (*Myzus periscae* Sulz.) but not by the European asparagus aphid (*Brachycolus asparagi* Mord.). The distribution of AV I-infected asparagus plants in the field is non-random as determined by ordinary run analysis.

This research indicates that AV II can be mechanically spread within asparagus by sap transmission. Also, the exine of pollen from some AV II-infected asparagus plants was determined to be contaminated with infectious virions of AV II. The distribution of AV II-infected plants within a field was determined by ordinary run analysis to be random.

In greenhouse studies, asparagus seedlings or clones infected with either AV I or AV II became more severely diseased when inoculated by either _Fusarium_ _oxysporum_ f.sp. _asparagi_ (FOA) or _F_. _moniliforme_ (FM) than did virus-free plants. Asparagus plants doubly infected both with AV I and AV II became the most severely diseased when inoculated with FOA.

Infection with AV II was determined to affect the asparagus plant in two ways. First, there is an alteration in the composition of the root exudates of virus-infected asparagus plants. Electrolytes, glucose, total carbohydrates and amino acids were detected in much higher levels in the root exudates of virus-infected asparagus plants. These exudates were determined to positively affect the germination and subsequent germ tube growth of conidia from both FM and FOA. Secondly, AV II-infected asparagus plants were determined to be less able to wall-off and lignify the area surrounding the infection courts of either FM or FOA.

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CHAPTER I

GENERAL INTRODUCTION AND LITERATURE REVIEW

Introduction

Asparagus (Asparagus officinalis L.) is an important commercial vegetable crop in a number of areas in Michigan. Michigan is a major producer of asparagus, ranking third in production of asparagus behind California and Washington. In 1983, 20,000 acres were in production in Michigan and seventeen and one-half million pounds of asparagus were harvested with a value of 10.9 million dollars (H. Foster, personal communication).

Asparagus is a perennial vegetable crop grown on sandy soils in Michigan, and traditionally it has been thought that, if properly managed, a planting should persist for 16 or more years with yields of 2,000 lbs/acre or more. Today, fields are being removed after 12 to 15 years because plantings have become so sparse and yields so low that they are no longer economical to harvest. "Asparagus decline" occurs nationwide and results in a loss of productivity and longevity of established fields. Most fields are planted with approximately 10,000 crowns per acre but a 1978 survey of Michigan asparagus fields indicated that the average crown population was 3,153 crowns per acre (A. Putnam, personal communication). It is the author's hypothesis that no single factor is responsible for this decline in vigor and longevity, but rather, that several cultural, environmental or biological stresses on the asparagus plant may predispose it to infection by the Fusarium crown or root rot organisms. _Fusarium_ _oxysporum_ (Schelcht.) emend. Snyder and Hansen f.sp. _asparagi_ Cohen and _F_. _moniliforme_ (Sheldon) emend. Snyder and Hansen are known to be present in all asparagus fields in Michigan and are believed to be the ultimate cause of "asparagus decline".

Several viruses have been shown to be of importance in asparagus plantings in both Europe and North America (34, 35, 52, 64, 68). Asparagus virus I, a member of the potyvirus group, is aphid transmitted and widespread in Washington (60) and California (23) and has recently been determined to be present in New Jersey (20) and Michigan (Chapter 2). Asparagus virus II, an ilarvirus, is widespread in Michigan (33), California (23) and Washington (60).

These viruses may constitute biological stresses to the asparagus plant. In Europe, Weissenfels and Schmelzer (64) reported a 20% reduction in yield in asparagus plantings infected with these viruses. Researchers in Washington (68) have found that plants infected with either asparagus virus I or asparagus virus II exhibited a mild reduction of vigor and productivity. Plants infected with both viruses showed severe decline and mortality in their second year in the field. It is the purpose of these investigations to evaluate the potential of these viruses as biological stress factors and to elucidate their role and relative importance in "asparagus decline" in Michigan.

Literature Review

Asparagus Viruses

Occurrence and distribution

In 1960, Hein (34) first reported a virus isolated from asparagus which produced necrotic local lesions on several species of *Chenopodium*. This virus, which he designated as asparagus virus I (AV I) (35), was determined to be widespread in asparagus plantings in Germany. Hein (35) reported that asparagus virus I produced no symptoms on asparagus and that the virus was transmitted by several different aphids. Mink and Uyeda (47) in 1977 isolated a long flexous-rod shaped particle, 700 to 880 nm in length from Washington asparagus. Their isolate was mechanically transmissible only to species of *Chenopodium* and symptoms on *C. quinoa* and particle morphology were similar to those reported for asparagus virus I by Hein (34, 35). They reported that nearly every field grown asparagus plant tested contained asparagus virus I. Uyeda (60) reported that asparagus virus I was present in all fields surveyed, except one and that the incidence of infection was related to the age of the planting. In limited attempts Uyeda (60) was unable to transmit asparagus virus I with three aphid species; *Myzus persicae* Sulz. (green peach aphid), *Rhopalosiphum padi* (L.) (oat-bird cherry aphid) and *Cavariella aegopodii* (Scopoli).

Fujisawa et al (25) reported the occurrence of a long flexous rod-shaped virus particle in asparagus in Japan similar to that reported by Hein (34). On the basis of particle morphology and its limited host range and symptomatology in those hosts, it was determined to be asparagus virus I. The Japanese isolate also produced laminate pinwheel inclusions within infected hosts and was transmissible by the green peach aphid. For these reasons, it was considered to be a member of the potyvirus group.

Asparagus virus I was consistently isolated from asparagus in New Jersey by Davis and Garrision (20). They reported that asparagus plants grown in the field or greenhouse were infected without obvious symptoms and there was no apparent correlation between the vigor of asparagus clones in tissue and infection by the virus. In a survey of California asparagus for viruses, Falloon et al (23) found asparagus virus I throughout the state and in all cultivars tested.

The occurrence of asparagus virus II was first reported in Germany by Hein (35) and later by Weissenfels et al (64) and appears to be identical with asparagus latent virus described subsequently in Denmark (49).

Uyeda and Mink (61) first reported the presence of asparagus virus II (AV II) in Washington asparagus and demonstrated that it was an isometric particle with a diameter of 23 to 33 nm and serologically related to citrus variagation virus, citrus leaf-rugose virus and elm mottle virus. Uyeda (60) demonstrated that AV II was widespread in Washington, isolating it from all asparagus plantings surveyed and from more than half of the plants tested in some fields. Uyeda and Mink (61) were unable to infect asparagus seedlings in the greenhouse by manual inoculation. Further, they demonstrated that the virus was readily seed transmitted in asparagus and that it could be separated into three major nucleoprotein components in a rate zonal sucrose density gradient. Each component alone exhibited little or no infectivity and a maximum infectivity was achieved when all three components were present in the inoculation mixture. Also, they identified two serotypes of AV II and designated them AV II-S and AV II-P. In agar gel double diffusion tests, the European isolate of AV II produced a precipitin line that coalesced with that of AV II-P but formed a spur with AV II-S when tested against antiserum to AV II-P and AV II-S. Because of these properties they placed AV II in the ilarvirus (isometric labile ringspot virus) group.

Fujisawa et al (26) isolated AV II from symptomless asparagus plants in Japan and their isolate was determined to be more closely related serologically to AV II-S than AV II-P. Asparagus virus II was found to occur in almost all asparagus plantings in Hokkaido. They were unable to infect asparagus seedlings by manual inoculation with sap from AV II-infected C. quinoa and tobacco but could infect asparagus with concentrated purified AV II. Asparagus shoots infected in this manner sometimes developed a light-green mosaic after about 20 days.

Recent reports from California (23) and Michigan (33) indicate that AV II is widespread in the major asparagus growing regions of the United States and has also been detected at low levels in New Jersey asparagus plantings (R. Davis, personal communication).

Fusarium Crown and Root Rot

History and geographic distribution

The first report of a Fusarium-incited disease of asparagus was made by Stone and Chapman in Massachusetts in 1908 (55). They observed a wilt of young shoots during the harvest season followed by a premature yellowing and rotting of the mature stalks or "ferns" later in the growing season. They consistently isolated an unidentified species of Fusarium from the affected plants. Cook, in 1923, described a similar disorder of asparagus in New Jersey, which he called "dwarf asparagus" (17). He reported a slow dying out of asparagus plants over circular areas and a concomitant stunting and premature yellowing of the affected plants. An unidentified Fusarium was always associated with the diseased plants. Over the next two decades numerous reports of Fusarium root and crown rots and wilts were made from New Jersey (62), Oregon (13), Massachusetts (12), North Carolina (2), South Carolina (4), Pennsylvania (41), Illinois (1), Missouri (55), and Idaho (10).

In 1941, Cohen and Heald (16) observed a wilt and root rot of asparagus in many of the irrigated, sandy soil regions of Washington State. Their investigation demonstrated that the common soil-borne fungus, Fusarium oxysporum Schlecht., was the causal agent of the disease. Pathogenicity tests with their isolate of F. oxysporum from asparagus indicated that it was different from other isolates already described. Their asparagus isolate was unable to produce disease in potato, tomato, carnation or onion and only a slight wilt in Alaskan peas. It was able to "vigorously" infect only asparagus and all asparagus varieties tested were equally susceptible. Cohen (15) distinguished the isolate as F. oxysporum variety asparagi. Some years later Armstrong and Armstrong (5) concluded that this organism was the very specific pathogen of asparagus and called it F. oxysporum f.sp. asparagi.

A Fusarium-incited disease of asparagus seedlings was reported in Canada by Graham in 1955 (30). Seedling nurseries were so severely affected that they were destroyed and the land was used for other purposes. He identified the pathogen as F. oxysporum f.sp. redolens (Wr.) Gordon and first described the infection process in asparagus seedlings. The course of infection was determined by examining sections of several hundred rootlets. Penetration took place between the walls of epidermal cells primarily in the meristematic region of the root tip and through the stomata of the hypocotyl and coleoptile. This was subsequently confirmed by Shoemaker (57). Graham also suggested that the pathogen may take advantage of natural openings on more mature parts of seedlings. In mature crowns he demonstrated that vascular discoloration was associated primarily with a wound, leading him to suggest that this organism was a "wound parasite". Endo (personal communication) later demonstrated that asparagus storage roots were capable of rapidly walling off Fusarium infections and wounds which could serve as potential infection sites. Further, he suggested that stress from overpicking might reduce the ability of these roots to wall off invading pathogens. Graham was also able to isolate F. moniliforme from diseased seedlings and demonstrated that this fungus caused root tip necrosis but also concluded that it was not important in seedling blight. No comparable disease could be found in surrounding production fields, although growers had reported unexplained reductions in yield for years.

Grogan and Kimble in 1959 (31) also reported a slow decline in the productivity of asparagus plantings in California and an inability to re-establish productive plantings in these areas. The asparagus decline and replant problem in California was attributed to the most prevalent pathogenic organism associated with the disorder, F. oxysporum f.sp. asparagi which was determined to be present on the seed. They concluded that the Fusarium wilt of asparagus in California was probably identical with the disease described by Cohen and Heald (16), who, in fact, identified the causal organism from infected asparagus plants received from California.

Lewis and Shoemaker (43) screened a number of plant introductions of the genus Asparagus for resistance to a New

Jersey isolate of *F. oxysporum* f.sp. *asparagi* and found all except *A. sprengeri* Regel to be highly susceptible. *A. sprengeri* was determined to be immune to their isolate.

The first association of *F. moniliforme* with asparagus decline in California was made by Endo and Burkholder (22). *F. moniliforme* was consistently isolated from brown, dry rotting asparagus crowns and Johnston et al in 1979 (39) asserted that *F. moniliforme* should be considered the pathogen of this completely separate disease of asparagus. This disease is characterized by a total collapse of older root tissue and proposed calling this disease Fusarium stem and crown rot.

Asparagus decline was first reported in Michigan by Lacy in 1979 (42) and he also attributed the decline ultimately to the presence of *F. oxysporum* f.sp. *asparagi* and *F. moniliforme*. He suggested that the decline was due to environmental stresses caused primarily by management practices such as overpicking, unsatisfactory weed control, mechanical damage from disking prior to harvesting and lack of irrigation which might predispose crowns to infection by *F. oxysporum* f.sp. *asparagi* and *F. moniliforme*.

Asparagus seed has a very rugged surface and the epidermal cells of its seed coat are tubular in shape and are separated by very deep crevices. Inglis (38) in 1980 demonstrated, using scanning electron microscopy, that *F. oxysporum* f.sp. *asparagi* and *F. moniliforme* were external seed contaminants and that the inablity to surface-disinfest asparagus seed might be due to the rough seed surface and the lodging of fungal spores in these natural crevices and in tunnels produced by insects. She further concluded that asparagus seeds became infested by these fungi during the commercial seed extraction process. Damicone and Cooley (19) developed a reliable method for the production of large numbers of disease-free asparagus seedlings for use in the laboratory and greenhouse. They routinely used 25,000 mg/ml benomyl in acetone for 24 hours to surface-disinfest seed and found that both _Fusarium_ species were eradicated from asparagus seeds with a concomitant increase in seed germination.

Gilbertson and Manning in 1983 (28) confirmed what Booth had suggested in 1971 (4); that wind was an important vehicle for dissemination of _F_. _moniliforme_ spores. They concluded that airborne spores of this fungus were the source of contamination of asparagus flowers, fruit, and ultimately, the seed. They also determined that the sources of airborne inoculum included asparagus stems and corn ears and stalks infected with _F_. _moniliforme_ and Gilbertson (27) reported that this potential inoculum on infected asparagus stems increased with plant age. Manning et al (45) suggested that latent, undetected infections of both _Fusarium_ species may be prevalent in asparagus seedlings and that several environmental stresses may be responsible for expression of latent infections.

The causal agents and symptomatology

According to Messiaen et al (46), _Fusarium_ _oxysporum_

(Schelcht.) emend. Snyder and Hansen and *F. moniliforme* (Sheldon) emend. Snyder and Hansen are ubiquitous pathogens and play a major role in reducing yields and quality of major food crops of the world. *Fusarium* spp. are facultative parasites which colonize living and non-living tissue and may invade non-host tissue (3, 36). This characteristic, along with their ability to form chlamydospores or other resting structures which can survive in soils for many years make these fungi especially persistent once established (32, 48).

Typically, *F. moniliforme* forms sporodochia and macroconidia in small numbers and produce an abundance of microconidia in chains (46). Members of this species do not form chlamydospores, but instead form individual thickened hyphae as survival structures (48). *F. oxysporum* is the most frequently isolated species of *Fusarium* in soils and is characterized by its ability to form chlamydospores, macroconida and microconidia (46).

Both causal agents associated with asparagus decline cause a yellowing, stunting or wilting of mature stalks that may die at various stages of elongation but, they produce this effect by slightly different means (15, 22). *Fusarium oxysporum* f.sp. *asparagi* is known to produce vascular discoloration within stems, roots and crowns as well as reddish eliptical lesions on roots and the basal part of stems (31). This results in a vascular wilt that inhibits the movement of water and carbohydrates within the affected plant. *Fusarium moniliforme* is primarily a root and crown rotting organism that causes extensive dry crown rotting and a brown stem pith discoloration and a total root collapse in some asparagus seedlings (39). Infection of asparagus roots by _F. moniliforme_ is not associated with vascular discoloration (15, 39).

Virus-Fungus Interrelationships

The effects of virus infection on fungus disease has been characterized by numerous researchers. Fungal infection may be increased, decreased or not affected by virus infection of a plant dependent on the system studied. In general, infection by facultative parasites of virus infected plants is increased and infection by obligate parasites, such as the rusts, smuts and powdery mildews, is decreased. The literature concerning virus-fungus interrelationships has been reviewed in detail by Beute (7).

Virus infection was demonstrated to enhance post-emergence damping-off caused by _Rhizoctonia_ sp. in cucumber. Bateman (6) found that the incidence of post-emergence damping-off was 10% to 15% for virus-free cucumber seedlings and 60% to 87% in cucumber seedlings previously inoculated with cucumber mosaic virus. The greatest synergism between pathogens occurred when small quantities of _Rhizoctonia_ inoculum were used. Cotyledons showed an increased rate of respiration 48 hours after inoculation with the virus and Bateman suggested that the movement of materials from the root to the virus-infected cotyledons may increase the susceptibilty of the roots to attack by the fungus.

An increase in the severity of several different root rot diseases in virus-infected plants has been reported for wheat (58), corn (65), red clover (21, 63), white clover (44), white lupine (50) and sugarbeets (18) grown under field conditions. Smith (58) reported that root rots of wheat incited by *Fusarium* and *Rhizoctonia* occurred predominately in plants infected with barley yellow dwarf virus. Corn seedlings infected with maize dwarf mosaic virus were determined to be more susceptible than virus-free seedlings to root rots, stalk rots and seedling blights incited by a number of pathogens (65). Also, *Pythium graminicola* Subram. caused more severe root rot in corn and wheat seedlings infected with a virus resembling wheat streak virus. Similarly, Watson and Guthrie (63) observed a severe root rot, general decline of vigor, and degeneration of the root system of red clover plants under field conditions. They noted that severe root rot developed when plants were infected with clover yellow mosaic virus alone or with white clover mosaic virus and any one of several fungal parasites that were usually only mildly pathogenic to red clover.

In 1964, Farley and Lockwood (24) suggested that viruses may play a role in the development of fungus root rots in peas. They demonstrated that three pea varieties were more susceptible to two common fungus root rot diseases when plants were inoculated with any of four different viruses. Similar results were obtained over a wide range of greenhouse environmental conditions with different strains of two viruses, plants of different ages and different time intervals between virus and fungus inoculations. Beute and Lockwood (9), working with the same system, determined that the increased Fusarium root rot in peas infected with bean yellow mosaic virus was not due to an increased susceptibilty of root tissue but rather that virus infection increased the exudation of nutrients from roots thereby increasing the inoculum potential of the pathogen in the rhizosphere. The roots of virus-infected pea plants released more electrolytes, carbohydrates, amino acids and nucleotides than did the roots of healthy plants. They attributed this increased exudation to an increase in the permeability of cell membranes of the root.

Beute (8) demonstrated that decreased yield of flowers, spikes, corms and cormels and an increase in plant mortality in Gladiolus was a result of the increased susceptibility of virus-infected plants. Chronic infection with cucumber mosaic virus or tobacco ringspot virus increased the prevalence and sometimes the severity of Fusarium and Stromatinia root rot diseases, Curvularia leaf spot disease, and storage rot of corms in Gladiolus. He concluded that even though the extent of virus damage was not immediately apparent, it was without doubt a major factor in the decline of Gladiolus stock.

The first interaction between systemic fungal infection and viral pathogens in crucifers was reported by Reyes and Chadha (54). Their data indicated that the severity of cabbage yellows disease in Brassica campestris var. chinensis L. was increased when plants were inoculated with turnip mosaic virus regardless of the interval between fungus and virus inoculations. Virus-infected plants became more severely stunted when inoculated with the fungus than plants infected with the virus alone. Pieczarka (51) noted a synergistic relationship between Rhizoctonia damping-off in peppers and the concomitant infection with one or more of the prevalent pepper viruses. However, the nature of the relationship varied depending on which pepper variety was used. Infection of pepper variety Early Calwonder with strain P of tobacco mosaic virus (TMV-P) resulted in an increase in susceptibility to Rhizoctonia damping-off while mixed viral infections with TMV-P and pepper mottle virus (PeMV) were no more effective than a single infection in increasing pepper susceptibility to this fungal pathogen.

In contrast to positive interactions discussed above, Wilson (66) noted a cross-protection of bean plants infected with TMV to bean rust disease. Goheen and Schnathorst (29) reported that virus leafroll in affected grapevines had an increased resistance to powdery mildew. Antagonistic relationships between viral infection and fungal disease have been reported by investigators in cucumber (37), tobacco (67), red clover (40), and pigeon pea (14). In all cases described above the cross-protection was afforded by an obligate parasite.

It is clear that infection of some plant species with one or more plant viruses can increase the incidence and severity of certain root rot diseases. Because asparagus is a perennial crop and _F_. _oxysporum_ f.sp. _asparagi_ and _F_.

moniliforme are ubiquitous pathogens, infection with one or more of the asparagus viruses could have severe consequences on the longevity and productivity of asparagus plantings.

Objectives

The objectives of this research were: to determine which asparagus viruses were present in Michigan asparagus plantings (Chapter 2), to determine their distribution and incidence in the asparagus growing regions of the state (Chapter 2), to evaluate possible methods of transmission and spread of these asparagus viruses within asparagus (Chapter 3), to evaluate the effect of virus infection on the severity of Fusarium crown and root rot (Chapter) and to investigate the mechanisms by which asparagus viruses may contribute to "asparagus decline" (Chapter 4).

Literature Cited

1. Anonymous. 1922. Diseases of field and vegetable crops in the U.S. in 1921: Asparagus root rot caused by Fusarium sp.. page 402 in: Plant Dis. Rep. Suppl. 22. G. H. Coons ed.

2. Anonymous. 1927. Diseases of vegetable and field crops other than cereals in the U.S. in 1926. page 325 in: Plant Dis. Rep. Suppl. 54. R. J. Haskell and J. I. Wood, eds.

3. Alexander, M. 1961. Introduction to soil microbiology. John Wiley and Sons, New York. 345 pp.

4. Armstrong, G. M. 1930. Fusarium sp. on asparagus in South Carolina. Plant Dis. Rep. 14:197.

5. Armstrong, G. M., and Armstrong, J. K. 1969. Relationship of F. oxysporum formae speciales apii, asparagi, cassiae, melongenae, and vasinfectum race 3 as revealed by pathogenicity of common hosts. Phytopathology 59:1256-1260.

6. Bateman, D. F. 1961. Synergism between cucumber mosaic virus and Rhizoctonia in relation to rhizoctonia damping-off of cucumber. (Abstr.) Phytopathology 51:574

7. Beute, M. K. 1967. Mechanism of increased root rot in virus-infected peas. Ph.D. Thesis. Michigan State University. 89 pp.

8. Beute, M. K. 1970. Effect of virus infection on susceptiblity to certain fungus diseases and yield of Gladiolus. Phytopathology 60:1809-1813.

9. Beute, M. K., and Lockwood, J. L. 1968. Mechanism of increased root rot in virus-infected peas. Phytopathology 58:1643-1651.

10. Blodgett, E. C. 1945. Diseases of various crops in Idaho in 1943. Miscellaneous crops. Plant Dis. Rep. 29:465.

11. Booth, C. 1971. The Genus Fusarium. Commonwealth Mycological Institute, Kew, Surrey, England. 237 pp.

12. Boyd, O. C. 1942. Recent obserevations on plant disease in Massachusetts. Plant Dis. Rep. 26:334.

13. Boyle, L. W. 1943. Plant disease survey of Oregon in 1943. Plant Dis. Rep. Suppl. 149:386-395.

14. Chadha, K. C., and Raychaudhuri, S. P. 1964. Interaction between sterility virus and *Fusarium udum* Butl in Pigeon-pea. Indian J. of Agric. Sci. 36:133-139.

15. Cohen, S. I. 1946. A wilt and root rot of *Asparagus officinalis* L. var. *atilis* L. (Abstr.) Phytopathology 36:347.

16. Cohen, S. I., and Heald, F. D. 1941. A wilt and root rot of asparagus caused by *Fusarium oxysporum* Schlecht. Plant Dis. Rep. 25:503-509.

17. Cook, M. T. 1923. Dwarf asparagus. Phytopathology 13:284.

18. Crane, G. L., and Calpouzos, L. 1969. Synergism of *Cercospora beticola* and beet yellows virus in killing sugar beet leaves. Phytopathology 57:808-809.

19. Damicone, J. P., Cooley, D. R., and Manning, W. J. 1981. Benomyl in acetone eradicates *Fusarium moniliforme* and *F. oxysporum* from asparagus seed. Plant Dis. 65:892-893.

20. Davis, R. F., and Garrison, S. 1985. First report of a virus present in New Jersey. Plant Dis. 66:628.

21. Denis, S. J., and Elliott, E. S. 1967. Decline of red clover plants infected with red clover vein mosaic virus and *Fusarium* species. (Abstr.) Phyopathology 57:808-809.

22. Endo, R. M., and Burkholder, E. C. 1971. The association of *Fusarium moniliforme* with the crown rot complex of asparagus. Phytopathology 99:122-125.

23. Falloon, P. G., Falloon, L. M., and Grogan, R. G. 1985. A survey of California asparagus for asparagus virus I (AVI), asparagus virus II (AVII) and tobacco streak virus (TSV). Plant Dis. (in press).

24. Farley, J. D., and Lockwood, J. L. 1964. Increased susceptibility of root rots in virus infected peas. Phytopathology 54:1279-1280.

25. Fujisawa, I., Goto, T., Tsuchizaki, T., and Iizuka, N. 1983. Host range and some properties of asparagus virus I isolated from *Asparagus officinalis* in Japan. Ann. Phytopath. Soc. Japan 49:299-307.

26. Fujisawa, I., Goto, T., Tsuchizaki, T., and Iizuka, N. 1983. Some properties of asparagus virus II isolated from *Asparagus officinalis* in Japan. Ann. Phytopath. Soc. Japan 49:683-688.

27. Gilbertson, R. L. 1981. Sources of inoculum and disease increase of stem, crown and root rot of asparagus caused by Fusarium oxysporum and Fusarium moniliforme. M.S. Thesis, University of Massachesuetts. 169 pp.

28. Gilbertson, R. L., and Manning, W. J. 1983. Contamination of asparagus flowers and fruit by airborne spores of Fusarium moniliforme. Plant Dis. 67:1003-1004.

29. Goheen, A. C., and Schnathorst, W. C. 1961. Resistance to powdery mildew in leaf roll-affected grapevines. Plant Dis. Rep. 45:641-643.

30. Graham, K. M. 1955. Seedling blight, a fusarium disease of asparagus. Can. J. Bot. 33:374-400.

31. Grogan, R. G., and Kimble, K. A. 1959. The association of Fusarium wilt with the asparagus decline and replant problem in California. Phytopathology 99:122-125.

32. Hart, L. P., and Endo, R. M. 1976. The reappearance of Fusarium yellows of celery in California. Plant Dis. Rep. 62:138-142.

33. Hartung, A. C., Evans, T. A., and Stephens, C. T. 1985. Occurrence of asparagus virus II in commercial asparagus fields in Michigan. Plant Dis. 69:501-504.

34. Hein, A. 1960. Uber das vorkommen einer virose an spargel. Z. Planzenkr. Pflanzenpathol. Pflanzenschutz 67:217-219.

35. Hein, A. 1963. Viroses an spargel. Mitt. Biol. Bundesanst. Land-Forstwirtsch, Berlin-Dahlem 108:70-74 (Rev. Appl. Mycol, 1964. 43:455).

36. Hendrix, F. F., and Nielson, L. E. 1958. Invasion and infection of crops other than formae suscept by Fusarium oxysporum f. butas and other formae. Phytopathology 48:224-228.

37. Hopen, H. J., and deZeeuw, D. J. 1962. Reduction of susceptibility to cucumber scab by cucumber mosaic virus. Plant Dis. Rep. 46:93-97.

38. Inglis, D. A. 1980. Contamination of asparagus seed by Fusarium oxysporum f.sp. asparagi and Fusarium moniliforme. Plant Dis. 64:74-76.

39. Johnston, S. A., Springer, J. K., and Lewis, G. D. 1979. *Fusarium moniliforme* as a cause of stem and crown rot of asparagus and its association with asparagus decline. Phytopathology 69:778-780.

40. King, L. N., Hamptom, R. E., and Diachun, S. 1964. Resistance to *Erysiphe polygoni* of red clover infected with bean yellow mosaic virus. Science 146:1054-1055.

41. Kirby, R. S. 1943. Reports on diseases of miscellaneous vegetable crops. Plant Dis. Rep. 27:447.

42. Lacy, M. L. 1979. Effects of chemicals on stand establishment and yields of asparagus. Plant Dis. Rep. 63:612-616.

43. Lewis, G. D., and Shoemaker, P. B. 1964. Resistance of asparagus species to *Fusarium oxysporum* f.sp. *asparagi*. Plant Dis. Rep. 48:364-365.

44. McCarter, S. M., and Halpin, J. E. 1961. Studies on the pathogenicity of four species of soil fungi on white clover as affected by the presence of bean yellow mosaic virus under conditions of controlled temperature and light. (Abstr.) Phytopathology 51:644.

45. Manning, W. J., Damicone, J. P., and Gilbertson, R. L. 1979. Asparagus root and crown rot: Sources of inoculum for *Fusarium oxysporum* and *Fusarium moniliforme*. Pages 195-205 in: G. Reuther, ed. Proc. 5th Int. Asparagus Symp., Geisenheim, West Germany.

46. Messiaen. C. M., and Cassini, R. 1981. Taxonomy of *Fusarium*. pages 427-445 in: *Fusarium*: Diseases, Biology and Taxonomy. P. E. Nelson, T. A. Toussoun and R. J. Cook, eds. Pennsylvania State University Press, University Park 457 p.

47. Mink, G. I., and Uyeda, I. 1977. Three mechanically transmissible viruses isolated from asparagus in Washington. Plant Dis. Rep. 61:398-401.

48. Nyvall, R. F., and Kommedahl, T. 1968. Individual thickened hyphae as survival structures of *Fusarium moniliforme* in corn. Phytopathology 58:704-707.

49. Paluden, N. 1964. Virussygdomme hos *Asparagus officinalis* Mannedsovers. Platesygd. 407:11-16.

50. Patil, P. L. 1973 Increased susceptibility to root and stem rot in a virus-infected white lupine (*Lupinus albus* L.) Maharashtra Vidnyas Mandir Patrika 8:24-31. (Rev. Plant Pathol. 54:629).

51. Pieczarka, D. J., and Zitter, T. A. 1981. Effect of interaction between two viruses and Rhizoctonia in pepper. Plant Dis. Rep. 65:404-406.

52. Posnette, A. F. 1969. Nematode transmitted viruses in asparagus. J. Hortica. Sci. 44:403-408.

53. Raju, D. G., Sill, W. H., and Browder, L. E. 1969. The combined effects of two viral diseases and leaf rust on wheat. Phytopathology 59:1488-1492.

54. Reyes, A. A., and Chadha, K. C. 1972. Interaction between Fusarium oxysporum f.sp. conglutinans and turnip mosaic virus in Brassica campestris var chinensis seedlings. Phytopathology 62:1424-1428.

55. Scott, I. T. 1927. Diseases of vegetable and field crops other than cereals in the U.S. in 1927. Plant Dis. Rep. Suppl. 61:294.

56. Schade, C. 1969. Viruskrankheiten des spargels. Nachrichtenbl. Dtsch. Pflanzenschutzdienst. (Berlin), 23:38-40.

57. Shoemaker, P. B. 1965. A comparative histological study of the penetration and infection process in seedlings of Asparagus L. by two isolates of Fusarium oxysporum f. asparagi. Cohen and Heald. M.S. Thesis, Rutgers-The State Univ., N. J. 65 pp.

58. Smith, H. C. 1962. Is barley yellow dwarf a predisposing factor in common root rot of wheat in Canada? Can. J. Plant Dis. Surv. 42:143-148.

59. Stone, G. E., and Chapman, G. H. 1908. Report of the botanist. Massachusetts Agr. Exp. Stat. Rep. 20:127.

60. Uyeda, I. 1978. Identification, characterization, and incidence of viruses isolated from asparagus. Ph.D. thesis, Washington State University. 115 pp.

61. Uyeda, I., and Mink, G. I. 1981. Properties of asparagus virus II, a new member of the Ilarvirus group. Phytopathology 71:1264-1269.

62. Walker, E. A. 1943. Reports of diseases of miscellaneous vegetable crops. Plant Dis. Rep. 27:447.

63. Watson, R. D., and Guthrie, J. W. 1964. Virus-fungus interrelationships in a root rot complex in red clover. Plant Dis. Rep. 48:723-727.

64. Weissenfels, M., and Schmelzer, K. 1976. Untersuchungen ueber das schadausmass durch viren am spargel (Asparagus officinalis L.). Arch. Phytopathol. Pflanzenschutz, Berlin. 12:67-73.

65. Williams, L. E., and Alexander, L. J. 1965. Maize dwarf, a new corn disease. Phytopathology 48:127-128.

66. Wilson, E. M. 1958. Rust-TMV cross protection and necrotic-ring spot reaction in bean. Phytopathology 48:127-128.

67. Wood, H. N., and Braun, A. C. 1961. Studies on the regulation of certain essential biosynthetic systems in normal and crown-gall tumor tissue. Proc. Nat. Acad. Sci. 47:1907-1913.

68. Yang, H. 1979. Early effects of viruses on the growth and productivity of asparagus plants. HortScience 14:734-735.

CHAPTER II

IDENTIFICATION, INCIDENCE AND DISTRIBUTION OF VIRUSES IN MICHIGAN ASPARAGUS PLANTINGS

Introduction

Numerous viruses have been reported to infect asparagus (*Asparagus officinalis* L.) in both Europe and North America (11, 12, 16, 20, 21). In 1960, Hein (12) first reported a virus isolated from asparagus which produced necrotic local lesions on several species of *Chenopodium*. This virus, which he designated as asparagus virus I (AV I) (13), was determined to be widespread in asparagus plantings in Germany. Hein reported that AV I produced no symptoms in asparagus and suggested that it was transmitted by several different species of aphid (14). Mink and Uyeda (17) isolated a long flexous rod-shaped particle, 700 to 880 nm in length, from Washington asparagus in 1977. Based on particle morphology and symptomatology on *C. quinoa* they determined it to be similar to AV I reported in Germany and reported that nearly every field grown asparagus plant in Washington was infected with AV I. In limited attempts, Uyeda (17) was unable to transmit AV I in asparagus by green peach aphid (*Myzus persicae* Sulz.), oat-bird cherry aphid (*Rhopalosiphum padi* (L.)) or *Cavariella aegopodii* (Scopoli).

Fujisawa et al (8) in 1983, reported the occurrence of a long flexous rod-shaped virus particle in asparagus in Japan similar to that reported by Hein (11) in Europe. On the basis of particle morphology, its limited host range and its ability to produce laminate pinwheel inclusions within infected hosts it was determined to be AV I and a member of the potyvirus group (8). Fujisawa et al (8) were able to transmit their isolate of AV I with green peach aphids (Myzus persicae) but not with melon aphids (Aphis gossypii).

Asparagus virus I was consistently isolated from numerous asparagus plantings in New Jersey (4), and detected in asparagus plantings in Michigan (this manuscript) and California (7). Asparagus virus I has also been isolated from asparagus plantings in New Zealand where its incidence has been reported to increase to 90 or 100% of a planting in 3 years (6).

The occurrence of asparagus virus II (AV II) was first reported in 1963 by Hein (12) in Germany and later by Weissenfels and Schmelzer (20) and appears to be identical asparagus latent virus described subsequently in Denmark (15). In 1981, Uyeda and Mink (18) reported that AV II was widespread in asparagus in Washington and demonstrated that it was an isometric particle, 23 to 30 nm in diameter, seed transmitted and belonged to the ilarvirus (isometric labile ringspot virus) group. All attempts by Uyeda (17) to mechanically transmit AV II to asparagus were unsuccessful. Recent reports from California (7), Michigan (10), New Zealand (6), and Japan (9) indicate that AV II is widespread in many of the major asparagus growing regions of the world.

Tobacco streak virus (TSV), another ilarvirus, has been reported to be of importance in the profitable production of asparagus (15). Its occurrence within asparagus has been reported in Denmark (15), England (1) and Washington State (14).

It was the objective of this study to assess the incidence and distribution of AV I, AV II and TSV within Michigan asparagus plantings.

Materials and Methods

Field Survey

During the summers of 1982, 1983, 1984 and 1985, commercial asparagus plantings in Michigan were indexed for the presence of AV I, AV II and TSV. In the 1982 harvest season, 23 production plantings in four different age groupings were surveyed, including 12 plantings in Oceana County and 11 plantings in Van Buren County. In addition, six seedling nurseries in Oceana County were indexed for virus. An experimental plot (100 x 100 m) was established 100 m diagonally in from a corner of each planting and 20 to 30 spears or, in the case of seedlings, ferns were collected at random from each plot. Samples were stored at 4 C until being triturated in 0.02 M sodium phosphate buffer, pH 7.0, and rub-inoculated to Chenopodium quinoa Willd. Indicator plants were maintained in a greenhouse at 21 to 24 C and assessed for symptom development 5 to 14 days after inoculation (17).

During the summers of 1983 and 1984, 20 randomly selected asparagus plants from each of 20 fields in Oceana County were indexed for the presence of AV I and TSV as described above. During the harvest season of 1985, ten asparagus plantings in Oceana County and seven plantings in Van Buren were surveyed for the presence of AV I. One of the plantings in Oceana County suspected of being infected with AV I was intensively surveyed to accurately assess the percent infection of the virus within that field. One hundred plants, in a grid pattern, were selected and two spears were removed from each plant and indexed for virus on C. quinoa and with serologically specific electron microsopy (SSEM) for AV I (5).

Isolates and Serology

Isolates were established from indicator plants exhibiting symptoms typical of AV I or AV II (17). Asparagus virus I typically produces necrotic lesions on inoculated leaves of C quinoa in 10 to 14 days and AV II produces chlorotic ringspots on inoculated leaves of C. quinoa in 5 to 10 days followed the development of a systemic mottle. During the spring of 1982, an isolate was established from single, chlorotic lesions developing on C. quinoa leaves in 8 days and designated isolate A. Lesions were transferred sequentially three times on C. quinoa to assure that symptom development was due to a single virus. In the summer of 1984, an isolate was established from single local necrotic lesions developing on C. quinoa leaves after 12 days. This isolate was transferred three times as previously described and designated isolate B. Plants were maintained in a growth chamber or greenhouse at 21 to 24 C with a 16 hour photoperiod.

Michigan isolate A was used in agar gel double diffusion tests against antisera prepared against tobacco streak virus (strains BRN and HF), tomato ringspot virus, tobacco ringspot virus (blueberry isolate), tobacco ringspot virus (tobacco isolate) [originally from G. Gooding], peach rosette mosaic virus, AV I and AV II [provided by G. Mink]. All other antisera were kindly provided by D. Ramsdell. All double diffusion serology tests included known positive controls for their respective virus and a healthy sap control. Serology plates were prepared by pouring 10 ml of autoclaved 0.8% agarose (w/v) containing 0.85% sodium chloride (w/v) and 0.1% sodium azide (w/v) into petri plates 90 mm in diameter. Wells were cut in agar immediately before use with Grafar auto-gel cutter [Grafar Corporation, Detroit, MI 48232].

Physical Properties of Isolates

Isolates A and B were characterized by determining dilution end-point, thermal inactivation point and longevity *in vitro*. Dilution end-point was carried out on 5 g samples of infected *C quinoa*. Leaves were triturated in 0.01 M sodium phosphate buffer, pH 7.0, and strained through two layers of cheesecloth. Samples were diluted with glass distilled water to 10^{-5} and each dilution was rub-inoculated onto two *C. quinoa* plants at the four to six leaf stage. The thermal inactivation point for each isolate was determined by triturating 5 g of infected *C. quinoa* leaf tissue. The homogenate was strained through two layers of cheesecloth and 1-ml samples placed into test tubes. These samples were heated in a water bath for 10 min at temperatures ranging from 50 to 80 C at 5 degree increments, then quickly cooled in ice water. Each sample was rub-inoculated onto two *C. quinoa* plants. For longevity *in vitro* tests, 15 g samples of infected *C. quinoa* were triturated in glass distilled water and the homogenates were maintained at 21 to 24 C. Subsamples of this preparation were rub-inoculated onto C. quinoa at intervals up to 14 days. Plants used in the determination of physical properties were assessed for symptom development 5 to 14 days following inoculation.

Determination of Host Range

The host range for isolates A and B were determined and compared with those reported by Uyeda (17). Partially purified preparations of isolate A were rub-inoculated onto single leaves of 12 plant species previously demonstrated to produce symptoms in response to mechanical inoculation with the AV II. The host range of isolate B was assessed by first triturating spear tips of Michigan asparagus plants known to be infected only with isolate B and the sap was diluted 1:2 (w/v) with 0.02 M sodium phosphate buffer, pH 7.0. Single leaves of 13 plant species were dusted lightly with carborundum (320 mesh) and rub-inoculated with this preparation. Test plants were maintained in a greenhouse at 21 to 24 C and a 16 hour photoperiod and assessed for symptom development 5 to 14 days after inoculation. Plants suspected of being infected were back inoculated to C. quinoa for confirmation.

Virus Purification

Isolate A, thought to be AV II, was purified from systemically infected C. quinoa leaves using the method described by Brunt and Stace-Smith (2). All purification procedures were carried out at 4 C. One hundred g of tissue was homogenized in a Waring blender in 200 ml of 0.02 M sodium phosphate buffer, pH 6.5, containing 0.01 M MgCl and 0.1% 2-mercaptoethanol. The homogenate was strained through two layers of cheesecloth and adjusted to pH 5.2 with 10% citric acid (w/v) and stirred for 30 min. The homogenate was centrifuged at 10,000 rpm for 10 min in a Beckman No. 40 rotor, the supernatant was decanted and adjusted to pH 6.5 with 1 N NaOH. This solution was centrifuged at 28,000 rpm for 150 min in a Beckman No. 30 rotor and the pellet was resuspended overnight in 2 ml of 0.02 M sodium phosphate buffer, pH 6.5, with 0.01 M MgCl and 0.1% 2-mercaptoethanol. After a second low speed centrifugation, the supernatant was saved and the virus particles were pelleted by high speed centrifugation at 38,000 rpm for 90 min in a Beckman No. 40 rotor. A second round of low and high speed centrifugations was used to further purify the virus.

Final purification was done using 0-30% linear-log sucrose density gradients which were centrifuged in a Beckman SW-41 rotor at 38,000 rpm for 90 min. Gradients were fractionated using an ISCO density gradient fractionator and UV-analyzer [Instrumentation Specialties Co., Lincoln, NE 68505] and the sucrose fractions containing virus were diluted threefold with 0.01 M sodium phosphate buffer, pH 7.0, and centrifuged for 3 hours at 38,000 rpm in a Beckman No. 40 rotor. The pellet was resuspended overnight in 0.01 M sodium phosphate buffer, pH 7.0, and the concentration of the virus preparation was determined using the molar extinction coefficient value $E^{0.1\%}_{280nm} = 5.3$.

Isolate B, suspected to be AV I, was purified from *C. quinoa* leaves using the methods of W. Howell (personal communication). All purification procedures were carried out at 4 C. Leaf tissue (20 g) was triturated in a Waring blender with 200 ml of 0.1 M sodium citrate buffer, pH 7.0, containing 0.01 M sodium ethylenediamine tetraacetate (EDTA).

The homogenate was strained through two layers of cheesecloth and centrifuged at 6,000 rpm for 10 min in a Beckman No. 30 rotor. The supernatant was decanted and centrifuged for 90 min at 28,000 rpm in a Beckman No. 30 rotor and the pellet was resuspended overnight in 2 ml of 0.01 M sodium phosphate buffer, pH 7.0. An equal volume of chloroform was added, shaken vigorously for 10 min and the mixture centrifuged at 6,000 rpm for 10 min in a Beckman No. 30 rotor.

The aqueous phase was layered onto a 5 ml cushion of 4% polyethene glycol (PEG) mol. wt. 6,000 (w/v) and 30% sucrose (w/v) containing 0.12 M sodium chloride in a Beckman SW-41 rotor tube and centrifuged at 28,000 rpm for 90 min. Each pellet was resuspended overnight in 2 ml 0.01 M sodium phosphate buffer, pH 7.0.

Further purification was done using 0-30% linear-log sucrose density gradients as previously described, except that gradients were centrifuged at 15,000 rpm for 60 min. The concentration of the virus preparation was determined using the molar extinction value $E^{0.1\%}_{280nm} = 2.8$.

Serologically Specific Electron Microscopy

The serologically specific electron microscopy (SSEM) or immunosorbent electron microscopy technique of Derrick (5) was used to test asparagus plants for the presence of AV I. Spear tips were triturated in a small volume of 0.01 M sodium phosphate buffer, pH 7.0, and rub-inoculated onto leaves of two C. quinoa plants. Single necrotic local lesions developing after 10 days were excised and triturated in a small volume of 0.01 M phosphate buffer, pH 7.0. Formvar covered copper grids (200 mesh) were first carbon-coated then treated for 1 hour at room temperature with a 1:10 or 1:100 dilution of antiserum to AV I [provided by G.I. Mink], AV I-J [provided by I. Fujisawa], or AV I-M. The grids were placed on a drop of the homogenate overnight at 4 C then rinsed with 0.01 M sodium phosphate buffer, pH 7.0. Degree of reaction in SSEM was determined by recording the number of virus particles observed in a 5 min scan of the grid. Decoration of virus particles was carried out at room temperature using a 1:10 dilution of antiserum prepared against AV I-W, AV I-J or AV I-M. Grids were floated specimen-side down for 1 hour then rinsed with 0.01 M sodium phosphate buffer, pH 7.0, for 3 min. Grids were stained with 2% phosphotungstic acid (w/v), pH 7.0, or 2% ammonium molybdate (w/v), pH 7.0, and observed on a Philips 201 electron microscope.

Electron Microscopy

Partially purified virus preparations of isolate A were first prepared by fixation in 1% glutaraldehye for 1 minute then placed onto grids prepared as before, and negatively stained with 2% ammonium molybdate (w/v), pH 7.0. All grids were examined with a Philips 201 electron microscope for the presence of virus particles.

Antisera Production

Antisera to the Michigan isolates A and B were prepared using the following procedure. The immunogens were prepared for injection by emulsifying 1.0 ml of 250 to 500 ug of the appropriate virus with 1.0 ml of Freund's complete or incomplete adjuvant [Difco Laboratories, Detroit, MI 48232]. Four-month old female New Zealand White rabbits received one intramuscular injection of immunogen in Freund's complete adjuvant followed by four intramuscular injections of immunogen in Freund's incomplete adjuvant at seven day intervals. Blood was collected from marginal ear veins beginning five days after the last injection and continued at weekly intervals for 1 month. Serum was separated from coagulated red blood cells by incubation at room temperature for 3 hours followed by overnight incubation at 4 C. Clear serum was pipetted off and centrifuged at 1,800 rpm for 15 min to remove cellular components and a few crystals of sodium azide were added prior to storage at 4 C or -20 C.

Gamma Globulin Purification

Anti-A or anti-B gamma globulin was purified by the procedure described by Clark and Adams (3). Antiserum was diluted 1:10 (v/v) in distilled water and 10 ml of saturated ammonium sulfate was added drop-wise while stirring. After stirring 30 to 60 min, the mixture was centrifuged for 5 min at 6,000 rpm in an IEC model CL centrifuge [International Equipment Co., Needham Hts., MA 02194]. The precipitate was collected and dissolved in 2 ml half-strength PBS [0.01 M sodium phosphate buffer, pH 7.4, containing 0.8% sodium chloride (w/v), and 0.01% sodium azide (w/v)]. This gamma globulin fraction was dialyzed three times against 500 ml half-strength PBS at 4 C and passed through a 5 cm high bed of DEAE (Whatman DE-23) cellulose in a 10 ml glass pipette. Half-strength PBS was used to pre-equilibrate the column and elute the gamma globulin. Two-ml fractions were collected by monitoring spectrophotometrically at the A280 nm wavelenth for protein. The first protein fractions to elute were collected and adjusted to a 1 mg/ml concentration and stored at 4 C.

ELISA Conditions

Indirect enzyme-linked immunosorbent assay (ELISA) was carried out using the method of Voller and Bidwell (19). Samples were triturated in a 0.05 M carbonate buffer, pH 9.6, with 2% polyvinyl pyrrolidone (w/v) (PVP) mol. wt. 40,000 [Sigma Chemical Co., St. Louis, MO 63178] and 0.45% sodium diethydithiocarbamate (w/v). Samples were diluted

1:10 or 1:50 (v/v) in the grinding buffer, filtered through two layers of cheesecloth and 200 µl of the dilutant added to the wells of flat bottomed polystyrene microtiter plates [Dynatech Laboratories Inc., Alexandria, VA 22314]. Plates were incubated overnight at 4 C and washed three times with PBS containing 0.05% Tween 20 (v/v) (PBS-Tween).

Purified gamma globulin to AV I or AV II was diluted 1:50 and 1:100 (v/v), respectively, with PBS-Tween and 200 µl was added to each well and incubated at room temperature for 2 hours. Alkaline phosphatase conjugated with goat IgG prepared against whole molecule rabbit IgG [Sigma Chemical Co., St. Louis, MO 63178], diluted 1:1000 (v/v) in PBS-Tween, was added to rinsed plates in 200 ul aliquots and incubated at 37 C for 3 hours. Plates were then washed throughly three times with PBS-Tween and 200 µl of 1 mg/ml enzyme substrate, p-nitrophenyl phosphate [Sigma Chemical Co., St. Louis, MO 63178], dissolved in substrate buffer (10% diethanolamine (v/v), pH 9.8, in distilled water with 0.02% sodium azide (w/v)). Color change after 30 to 60 min was determined spectrophotometrically at A405 nm with a microELISA Minireader [Dynatech Laboratories, Alexandria VA 22314]. Tests were considered positive for virus if A405 nm value of a sample well was greater than the mean A405 nm of the healthy control samples plus three standard deviations.

Results

Field Survey

The 1982 survey revealed widespread infection of commercial asparagus plantings in Oceana and Van Buren Counties by a single virus, isolate A, later identified as AV II (Table 2.1). This virus was present in 19 of 23 fields surveyed and most plantings were more than 50% infected. The incidence of infection with isolate A was greater for plantings 11 to 15 and 16 to 20 years old than for those 0 to 2 and 3 to 10 years of age. In the survey of asparagus seedling nurseries in Oceana County, four of five nurseries were infected with isolate A but generally at lower levels than commercial plantings (Table 2.2). No other viruses were detected in any asparagus planting during the 1982 growing season.

No additional viruses were detected in commercial asparagus plantings during the 1983 survey (data not shown). In 1984, AV I-type local lesions were detected on *C. quinoa* rub-inoculated with spears collected from two commercial asparagus plantings in Oceana County and designated isolate B (data not shown).

During the 1985 harvest season AV I was detected in five of ten newly surveyed plantings in Oceana County (Table 2.3). There was no correlation between the age of the planting and the incidence of AV I although the oldest field, planted in 1950, had the greatest percentage of plants (70%) infected by this virus. Two asparagus plantings immediately adjacent to

Table 2.1. Percent asparagus virus II (AV II) infection as determined with *Chenopodium quinoa* indicator plants for 12 asparagus fields in Oceana County and 11 fields in Van Buren County.^{z}

	Van Buren County			Oceana County			
Age of Plants (yrs)	Fields Assayed (No.)	Total Plants Assayed (No.)	Incidence for Individual Field (%)	Fields Assayed (No.)	Total Plants (No.)	Incidence for Individual Fields (%)	Mean % Infection for all Fields
0-2	3	57	60, 11, 0	3	68	0, 71, 67	34.8
3-10	3	61	15, 0, 0	3	62	60, 5, 70	25.0
11-15	3	60	50, 25, 71	3	60	75, 60, 80	60.2
16-20	2	41	55, 55	3	65	90, 70, 76	69.2

[z] Each sample was ground in 0.01 M sodium phosphate buffer, pH 6.8, with 0.1% 2-mercaptoethanol added and rub-inoculated to separate indicator plants that had first been dusted with carborundum (320 mesh) Asparagus cultivars assayed were Mary Washington and Viking KB3. Samples were kept at 4 C after collection until processed 24 to 48 hours later. Indicator plants were observed for virus symptoms 5 to 20 days after inoculation.

Table 2.2. Percent asparagus virus II infection as determined with *Chenopodium quinoa* indicator plants for five asparagus seedling nurseries in Oceana County, MI.[z]

Age of Plants (months)	Fields Assayed (No.)	Total Plants Tested (No.)	Incidence for Individual Field (%)
12	2	24	16.6, 16.6
18	2	30	0, 41.6
24	1	21	33.3

[z] Each sample was ground in 0.01 M sodium phosphate buffer, pH 6.8, with 0.1% 2-mercaptoethanol added and rub-inoculated to separate indicator plants that had first been dusted with carborundum (320 mesh). Asparagus cultivars assayed were Mary Washington and Viking. Samples were kept at 4 C after collection until processed 24 to 48 hours later. Indicator plants were observed for virus symptoms 5 to 20 days after inoculation.

Table 2.3. Incidence of asparagus virus I (AV I) in commercial asparagus in Michigan as determined with *Chenopodium quinoa* indicator plants and serologically specific electron microscopy.

Year Planted	Cultivar	Presence of asparagus virus I[y]	
		No. positives[z]/ No. tested	
Oceana County			
1950	Mary Washington	18/25	(72%)
1955	Mary Washington	0/25	(0%)
1967	Mary Washington	16/25	(64%)
1969	Mary Washington	2/25	(8%)
1970	Mary Washington	0/25	(0%)
1975	Mary Washington	3/25	(12%)
1980	Viking KB3	0/25	(0%)
1980	Viking KB3	0/25	(0%)
1981	Viking KB3	8/25	(32%)
1982	Viking KB3	0/25	(0%)
Van Buren County			
1959	Mary Washington	0/25	(0%)
1960	Mary Washington	0/25	(0%)
1965	Mary Washington	0/25	(0%)
1969	Mary Washington	0/25	(0%)
1970	Mary Washington	0/25	(0%)
1975	Mary Washington	0/25	(0%)
1975	Mary Washington	0/25	(0%)

[y] Individual asparagus spear samples were indexed on *Chenopodium quinoa* by rub-inoculation.

[z] Local necrotic lesions from *C. quinoa* leaves were checked for AV I particles using serologically specific electron microscopy.

the 35 year old field had the next greatest incidence of infection by AV I and fields at greater distances were determined to contain even fewer plants infected with this virus. Asparagus virus I was not detected in any asparagus plantings surveyed in Van Buren County during the summer of 1985.

Serology

Sap from C. quinoa plants systemically infected with isolate A and diluted 1:1 produced a single preciptin line in agar gel double diffusion tests with antiserum prepared against AV II diluted 1:3 (v/v) but did not react with other antisera tested (Figure 2.1). Sap from C. quinoa plants infected with a Washington isolate of AV II also produced a single precipitin line and no precipitin line was formed in tests with healthy sap.

Physical Properties and Host Range

The physical characteristics and host range of isolate A were the same as that of the Washington isolate of AV II. Isolate A had a dilution end-point of 10^{-3} to 10^{-4}, thermal inactivation point of 55 to 60 C and a longevity *in vitro* of 8 to 10 days. The general characteristics agree with the results obtained with the AV II isolate from Washington (17). The host range of isolate A was determined to be the same as reported for AV II in Washington (18) (Table 2.4).

Isolate B possessed physical characteristics and host range identical with those of the Washington isolate AV I (17). The isolate was determined to have a dilution end-

Figure 2.1. Ouchterlony gel double diffusion test. Center well was charged with asparagus virus II (AV II) antiserum (dilution = 1:1 or 1:2), peripheral wells were charged with asparagus virus II (Washington isolate) = A, Michigan isolate I = B, Michigan isolate II = C and D, asparagus virus I = E and sap from virus-free *Chenopodium quinoa* = F.

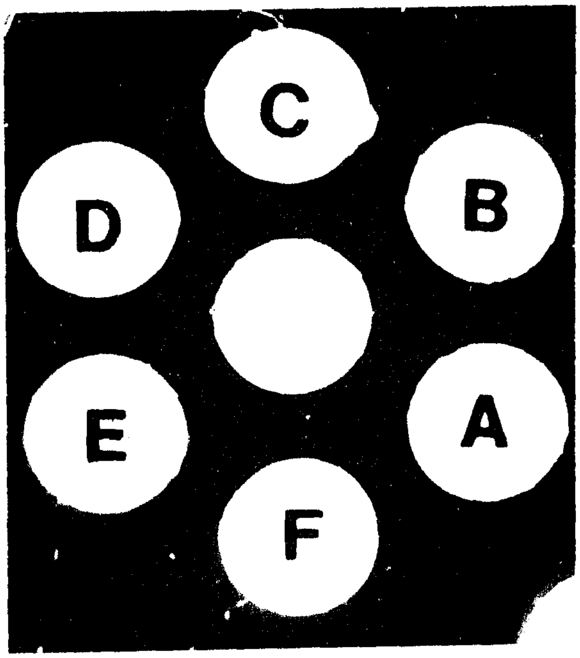

C
B
D
E
A
F

Table 2.4. Reactions of 12 plant species inoculated with a Michigan isolate of asparagus virus II (AV II).

Plant species	Symptoms
Asparagus officinalis L.	Latent systemic infection
Beta vulgaris L.	Local chlorotic ring lesions
Chenopodium amaranticolor Coste & Reye	Small local necrotic lesions
C. murale L.	Sunken local necrotic lesions No systemic infection
C. quinoa Willd.	Local chlorotic ring lesions Systemic mottle occasional slight necrosis
Cucumis sativus L.	Local chlorotic ring lesions
Cucurbita pepo L.	Systemic mottle
Gomphrena globosa L.	Necrotic ringspot lesions Mottling of lower leaves
Nicotiana tabaccum L. cv. Havana 423	Local chlorotic ring lesions
N. clevlandii A. Gray	Latent systemic infection
Phaseolus vulgaris L. cv. Bountiful	Small red local lesions
Vigna unguiculata (L.) Walp. cv. California Blackeye	Small red local lesions

point of 10^{-3} to 10^{-4}, thermal inactivation point of 55 to 60 C, and a longevity *in vitro* of 1 to 2 days. The host range of B included only members of the Chenopodiaceae, including *C. quinoa* and *C. amaranticolor* (Table 2.5).

Ultraviolet absorption profiles of purified preparations of isolate A which were sedimented in a 0-30 percent linear-log sucrose density gradient revealed two major peaks (Figure 2.2). Virus particles collected from each peaks were determined to infective when rub-inoculated onto *C. quinoa*, producing typical AV II-type symptoms 5 to 10 days after inoculation.

Serologically Specific Electron Microscopy

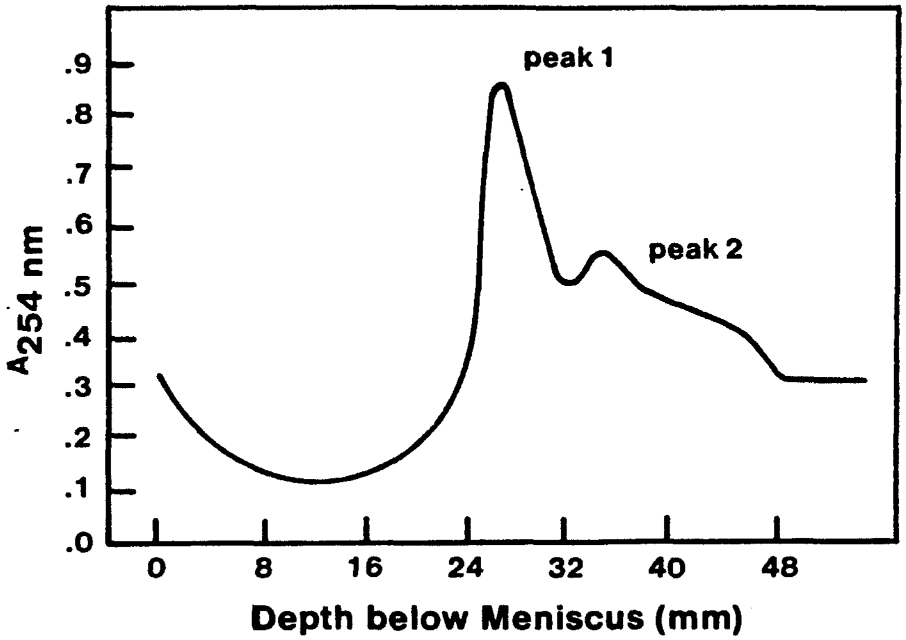
Serologically specific electron microscopy studies indicated that isolate B was serologically related to the Washington isolate of AV I. Formvar-covered, carbon-coated copper grids previously treated with a 1:10 dilution of antiserum to the Washington or Michigan isolates of AV I trapped 100 times more virus particles than did untreated grids. An average of 121 virus particles were observed in 5 min counts of grids pre-treated with antiserum prepared against the Washington isolate of AV I. An average of 10 particles were observed in 5 min counts of untreated grids. Virus particles of isolate B became decorated when they were treated with a 1:10 dilution of antiserum prepared against the Washington or Michigan isolates of AV I but TMV particles treated in the same manner were not decorated. Virus particles of isolate B treated with a 1:10 dilution of pre-

Table 2.5. Reactions of 13 plant species inoculated with a Michigan isolate of asparagus virus I (AV I).

Plant species	Symptoms
Asparagus officinalis L.	No reaction
Beta vulgaris L.	No reaction
Chenopodium amaranticolor Coste & Reyes	Local necrotic lesions
C. murale L.	Local necrotic lesions
C. quinoa Willd.	Local necrotic lesions
Cucumis sativus L.	No reaction
Cucurbita pepo L.	No reaction
Gomphrena globosa L.	No reaction
Nicotiana tabaccum L. cv. Havana 423	No reaction
N. clevlandii A. Gray	No reaction
Phaseolus vulgaris L. cv. Bountiful	No reaction
Tetragonia expansa Murr.	No reaction
Vigna unguiculata (L.) Walp. cv. California Blackeye	No reaction

[z] Indicator plants not showing virus symptoms after 14 days were back inoculated to *C. quinoa* to test for latent infection.

Figure 2.2. Ultraviolet absorption profile of a Michigan isolate of asparagus virus II (AV II) sedimented in a 0-30 percent linear-log sucrose density gradient. The direction of sedimentation is from left to right. A Beckman SW-41 rotor was used at 38,000 rpm for 90 minutes at 4 C.



peak 1
peak 2
A_{254} nm
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0
0
8
16
24
32
40
48
Depth below Meniscus (mm)

immune antiserum were not decorated (Figure 2.3).

Electron Microscopy

Isometric or quasi-isometric particles ranging in diameter from 23 to 30 nm (Figure 2.4) were observed when negatively stained purified virus preparations of isolate A were viewed on the electron microscope. Long flexous rod-shaped virus particles, 700 to 880 nm in length (Figure 2.3) were readily visible in leaf dips prepared from single necrotic local lesions of *C. quinoa* leaves inoculated with isolate B.

Figure 2.3. Results of immunosorbent electron microscopy with a A & B. Michigan isolate of asparagus virus I (AV I) or C. tobacco mosaic virus (TMV). Formvar covered copper grids were carbon coated then treated overnight at 4 C with a 1/10 dilution of antiserum prepared against the Washington isolate or Japanese isolate of AV I. A & C were decorated with the same antiserum for 1 hr at room temperature then stained with either 2% phosphotungstic acid or 2% ammonium molybdate. Scale bar = 100 nm.

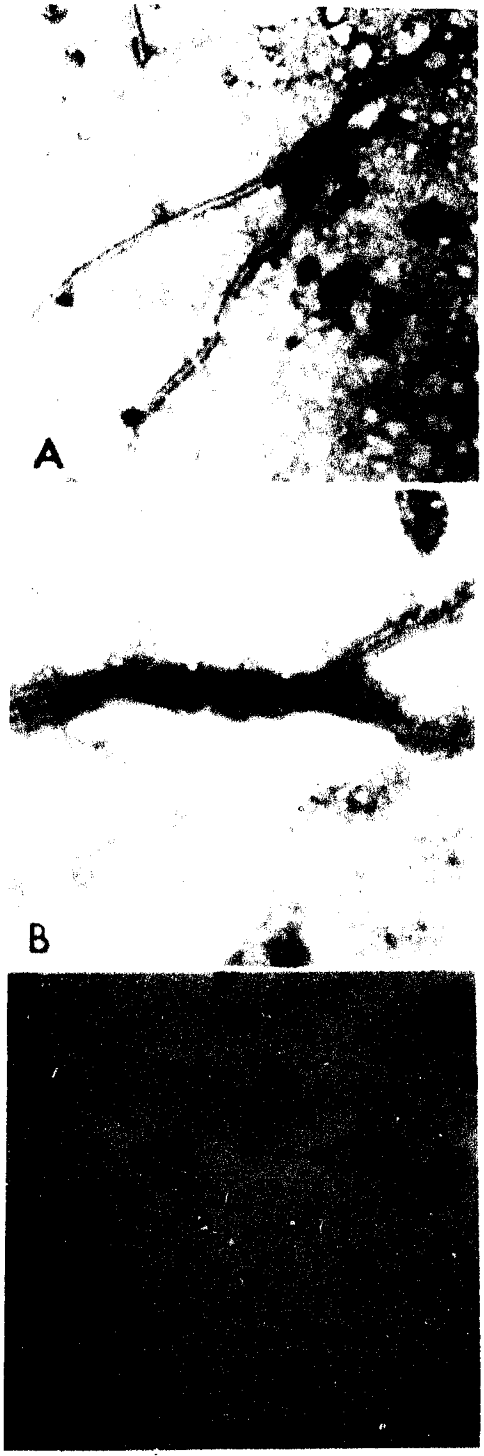
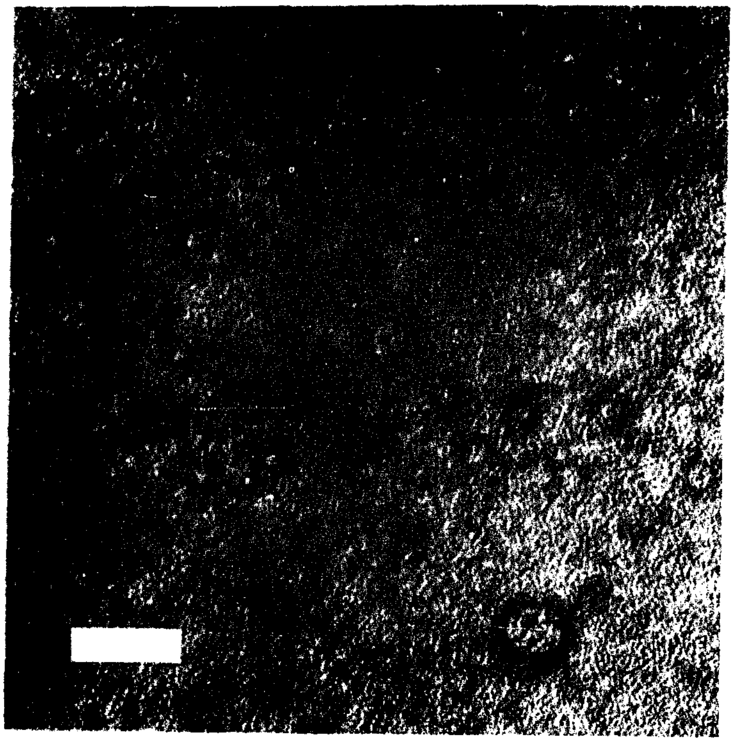

A
B

Figure 2.4. Electron micrograph of Michigan isolate of asparagus virus II (AV II). Quasi-isometric virus particles were fixed with 1% glutaralhehyde for 10 minutes then negatively stained with 2% ammonium molybdate, pH 7.0. Scale bar = 50 nm.



Discussion

Michigan isolates A and B appeared to be serologically related to AV II and AV I, respectively, described previously by Mink and Uyeda (14) in Washington. Isolate A reacted serologically only with antisera against the Washington isolate of AV II. Serological precipitin lines coalesced in agar and no spur formation was noted. Serologically specific electron microscopy indicated that isolate B is serologically related to the Washington isolate of AV I.

Asparagus virus II was determined to be widespread in almost all Michigan commercial asparagus plantings surveyed (Table 2.1). Four of five seedling nurseries surveyed were infected with AV II but generally at a lower level than older production fields (Table 2.2). Though considerable variation in the incidence of AV II within age groups exists, the incidence of AV II was greater for asparagus plantings 11 to 20 years old than for those 0 to 10 years of age and seedling nurseries generally had the lowest incidence of infection. Mean percent infection data indicate that younger fields had fewer plants infected with AV II than older fields.

Asparagus virus I was determined to be present in only a few Michigan asparagus plantings in Oceana County and its incidence varied greatly (Table 2.3). No direct correlation exists between the age of a planting and its incidence of infection with AV I. One 35 year old planting in Oceana County was determined to be 70% infected with AV I and two immediately adjacent asparagus plantings were determined to be 33% and 63% infected with AV I. Fields at even greater distances from the 35 year old planting had even lower incidences of AV I and the virus was not detected in plantings located more than one mile away from the 35 year old planting. A relationship exists between the proximity of a planting to the 35 year old field in Oceana County and its percent infection with AV I. Asparagus virus I is a member of the PVY group and is thought to be transmitted by aphids in the nonpersistent manner (6, 7, 8, 13). The data suggest that the 35 year old planting is a source from which aphids have spread the virus to nearby asparagus plantings in Oceana County.

Literature Cited

1. Brunt, A. A., and Paludan, N. 1970. The serological relationship between "asparagus stunt" and tobacco streak virus. Phytopathol. Z. 69:277-282.

2. Brunt, A. A., and Stace-Smith, R. 1976. The occurrence of the black raspberry latent strain of tobacco streak virus in wild and cultivated Rubus species in British Columbia. Acta. Hortic. 66:71-76.

3. Clark, M. F., and Adams, A. N. 1977. Characteristics of the micro-plate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-484.

4. Davis, R. F., and Garrison, S. 1985. First report of a virus present in New Jersey. Plant Dis. 66:628.

5. Derrick, K. S. 1973. Quantitative assay for plant viruses using serologically-specific electron microscopy. Virology 56:652-653.

6. Falloon, P. G. 1982. The need for asparagus breeding in New Zealand. New Zealand J. Exp. Agr. 10:101-109.

7. Falloon, P. G., Falloon, L. M., and Grogan, R. G. 1985. A survey of California asparagus for asparagus virus I (AVI), asparagus virus II (AVII) and tobacco streak virus (TSV). Plant Dis. (in press).

8. Fujisawa, I., Goto, T., Tsuchizaki, T., and Iizuka, N. 1983. Host range and some properties of asparagus virus I isolated from Asparagus officinalis in Japan. Ann. Phytopath. Soc. Japan 49:299-307.

9. Fujisawa, I., Goto, T., Tsuchizaki, T., and Iizuka, N. 1983. Some properties of asparagus virus II isolated from Asparagus officinalis in Japan. Ann. Phytopath. Soc. Japan 49:683-688.

10. Hartung, A. C., Evans, T. A., and Stephens, C. T. 1985. Occurrence of asparagus virus II in commercial asparagus fields in Michigan. Plant Dis. 69:501-504.

11. Hein, A. 1960. Uber das vorkommen einer virose an spargel. Z. Planzenkr. Pflanzenpathol. Pflanzenschutz 67:217-219.

12. Hein, A. 1963. Viroses an spargel. Mitt. Biol. Bundesanst. Land-Forstwirtsch, Berlin-Dahlem 108:70-74 (Rev. Appl. Mycol. 1964. 43:455).

13. Hein, A. 1969. Uber viruskrankheiten das spargels (Asparagus officinalis L.). Spargelvirus I. Z. Pflanzenkr. Pflanzenpathol. Pflanzenschutz 76:395-406. (Rev. Plant Pathol. 1970. 49:219).

14. Mink, G. I., and Uyeda, I. 1977. Three mechanically transmissible viruses isolated from asparagus in Washington. Plant Dis. Rep. 61:398-401.

15. Paludan, N. 1964. Virussygdomme hos Asparagus officinalis Mannedsovers. Platesygd. 407:11-16.

16. Posnette, A. F. 1969. Nematode transmitted viruses in asparagus. J. Hortic. Sci. 44:403-404.

17. Uyeda, I. 1978. Identification, characterization, and incidence of viruses isolated from asparagus. Ph.D. thesis, Washington State University. 115 pp.

18. Uyeda, I. and Mink, G. I. 1981. Properties of asparagus virus II, a new member of th Ilarvirus group. Phytopathology 71:1264-1269.

19. Voller, A., and Bidwell, D. E. 1977. Enzyme immunoassays and their potential in diagnostic virology. pages 449-457 in, Comparative Diagnosis of Viral Diseases Vol. II. E. Kurstak and C. Kurstak, eds.

20. Weissenfels, M., and Schmelzer, K. 1976. Untersuchungen ueber das schadausmass durch viren am spargel (Asparagus officinalis L.). Arch. Phytopathol. Pflanzenschutz, Berlin. 12:67-73.

21. Yang, H. 1979. Early effects of viruses on the growth and productivity of asparagus plants. HortScience 14:734-735.

CHAPTER III

TRANSMISSION AND SPREAD OF ASPARAGUS VIRUSES WITHIN MICHIGAN ASPARAGUS

Introduction

Asparagus virus II (AV II) has been detected widely within most commercial asparagus plantings in Michigan and asparagus virus I (AV I) has also been determined to be present, but not widespread.

Asparagus virus I has been classified as a member of the potyvirus (PVY) group which are transmitted in a nonpersistent manner by a wide variety of aphids (5). Members of the potyvirus group typically have flexous filamentous particles mostly 730 to 790 nm in length and seed transmission is relatively rare (12). Some members of this group are mechanically transmissible to a moderately wide range of herbaceous hosts while others have relatively restricted host ranges (12).

Asparagus virus I is widespread in asparagus plantings in Washington (19), California (8), New Jersey (4), and has been detected in a few Michigan plantings. Attempts to seed-transmit AV I within asparagus or rub-transmit the virus to healthy asparagus using sap or purified preparations of the virus have been unsuccessful (9, 21).

Asparagus virus II is a member of the ilarvirus group and its method of transmission within asparagus plantings is also unclear. Members of the ilarvirus group have isometric or quasi-isometric particles generally ranging in size from 22 to 35 nm and most are composed of two to three kinds of nucleoprotein components of different sizes (22). Many members of this group are known to be seed-transmitted and most are readily mechanically transmissible to a relatively wide range of herbaceous hosts (22).

Asparagus virus II is widespread in asparagus plantings in Washington (19), and California (8), and has been detected at low levels in New Jersey. In Michigan, 50 to 70 percent of asparagus plants are infected with AV II in most plantings 5 years or older (15). Asparagus virus II has been mechanically transmitted by some (10, 24) but not by others (21). The transmission of AV II by seed within asparagus is well documented (10, 16, 21, 22).

A member of the ilarvirus group, prunus necrotic ringspot virus (PNRSV), is pollen-borne in cherry and infects trees when they are pollinated by virus-carrying pollen (11). Virus particles have been localized on the outer walls of some pollen grains. Transmission electron microscopic examination of anthers from plants infected with bromegrass mosaic virus (BMV), southern bean mosaic virus (SBMV) or tobacco mosaic virus (TMV) showed that the exine of mature pollen was infested with virions and that homogenates of SBMV-infested pollen or dry TMV-infested pollen were infectious when assayed on appropriate hosts (13). Hamilton et al (14) demonstrated the presence of PNRSV-antigens on the exine of bee and hand-collected sweet cherry pollen using enzyme-linked immunosorbent assay (ELISA). When virus-containing pollen was washed the antigen was easily released and induced chlorotic local lesions when rub-inoculated onto Chenopodium amaranticolor and Cucumis sativa cv. Straight Eight. Scanning electron microscopic observations of pollen treated with latex-conjugated antibody specific for PNRSV revealed more latex beads bound to the surface of virus-infested than virus-free pollen.

The objective of this work was to investigate the methods of transmission for AV I and AV II and to begin to assess the relative importance of the methods of transmission in the spread of these viruses within Michigan asparagus plantings.

MATERIALS AND METHODS

Aphid Transmission of AV I

The ability of two aphid species to transmit AV I was evaluated in the greenhouse and growth chamber. Colonies of non-viruliferous green peach aphids (*Myzus persicae* Sulz.) and European asparagus aphids (*Brachycolus asparagi* Mord.). European asparagus aphids and green peach aphids were given an aquisition access period of 1 and 2 weeks, respectively, on asparagus plants infected with AV I, then transferred to 2-month old virus-free and AV II-infected asparagus seedlings. After 2 weeks all shoots were cut at ground level and plants transferred to aphid-free cages. Test plants were evaluated for the presence of AV I by rub-inoculation to *C. quinoa* and SSEM after the second new spear had emerged.

Mechanical Transmission of AV I

Spears were collected from greenhouse-grown asparagus plants known to be infected only with AV I and triturated in a 0.02 M sodium phosphate buffer, pH 7.0, containing 0.1% 2-mercaptoethanol. This homogenate was rub-inoculated onto the stems of virus-free asparagus seedlings and leaves of *C. quinoa* that were first dusted with carborundum (320 mesh). Inoculated asparagus plants were evaluated for symptom development over a 2 month period then tested for AV I using rub-inoculation to *C. quinoa*. Necrotic lesions developing on leaves of *C. quinoa* were assessed for AV I particles using SSEM.

Mechanical Transmission of AV II to Asparagus

A Michigan isolate of AV II was purified from systemically infected *C. quinoa* leaves using the method of Brunt and Stace-Smith (1) and further purified using three cycles of linear-log sucrose density gradients as previously described (15). A 1 mg/ml solution of purified AV II in 0.02 M sodium phosphate buffer, pH 7.0, was used to inoculate asparagus seedlings that had previously been screened for AV II using enzyme-linked immunosorbent assay (ELISA) following the procedures of Voller and Bidwell (23), and rub-inoculated to *C. quinoa*. Virus-free asparagus seedlings were maintained in the dark for 24 hours, then dusted with carborundum (320 mesh) and stems were rub-inoculated with purified virus or buffer using sterile cotton swabs. Plants were evaluated for symptom development over an 8 week period, after which young spears were harvested and tested for AV II using ELISA and rub-inoculation to *C. quinoa*.

Mechanical transmission from asparagus to asparagus was assessed in a similar manner. Asparagus seedlings were first screened for AV II using ELISA and rub-inoculation to *C. quinoa*. Young spears from AV II-infected and virus-free asparagus plants were triturated with a mortar and pestle at 4 C and diluted 1:10 (w/v) in 0.02 M sodium phosphate buffer, pH 7.0, containing 0.1% 2-mercaptoethanol and triturated with a mortar and pestle at 4 C. These sap preparations were used to rub-inoculate virus-free asparagus seedlings either directly on the stem, as previously described, or on the wounded tips of recently excised spears.

Pollen Transmission of AV II in Asparagus

Collection of asparagus pollen

During the winter of 1985, flowers were collected from greenhouse-grown, male asparagus seedlings previously indexed for the presence of AV II by ELISA and rub-inoculation to *C. quinoa*. Anthers were first examined under a dissection microscope and mature anthers, prior to dehiscence, were removed manually using fine forceps. The anthers were dried at room temperature for 48 hours and stored at 4 C. Pollen for individual experiments was prepared using the method of Cole et al (3). Dried anthers were swirled in a dry glass tube using a vortex tube agitator and pollen grains that adhered to the tube walls were collected. No evidence of damage was noted when suspensions of pollen were examined by light microscopy. Pollen was usually processed within 1 week of its collection.

Tests for presence of infective viruses

Pollen samples were tested for the presence of infective AV II using the methods of Hamilton et al (14). Samples were suspended in 5 ml of 0.01 M sodium phosphate buffer, pH 7.0, or in the same buffer amended with 1% polyvinyl pyrrolidone (v/v) (PVP) mol. wt. 40,000 [Sigma Chemical Co., St. Louis, MO 63178]. The tubes were shaken with a vortex tube agitator at maximum speed for 1 min. No evidence of pollen damage was observed when suspensions were observed by light microscopy. Pollen grains were sedimented at 6,000 rpm for 5 to 10 min and a 2-ml sample was withdrawn with a sterile pipette. This sample was rub-inoculated on single leaves of two C. quinoa and the plants observed for 5 to 14 days for symptom development.

Identification of AV II

The identification of AV II in pollen samples or wash solutions was carried out by ELISA using the methods of Voller and Bidwell (23) as modified by Hamilton et al (14). Pollen was divided into equal test samples of 0.05 ml dry-packed volume and suspended in 0.01 M sodium phosphate buffer as previously described. Suspensions of intact pollen grains in grinding buffer added to duplicate wells of flat bottomed polystyrene microtiter plates [Dynatech Laboratories, Alexandria, VA 22314] and incubated overnight at 4 C. Plates were washed three times with phosphate buffered saline with 0.05% Tween 20 (v/v) (PBS-Tween).

Gamma globulin specific for the Michigan isolate of AV II was purified using the procedure of Clark and Adams (2), diluted 1:50 (v/v) with PBS-Tween and 200 ul added to each well and incubated for 2 hours at room temperature. Plates were washed as before and 200 µl of alkaline phosphatase conjugated with goat IgG prepared against whole molecule rabbit IgG [Sigma Chemical Co., St. Louis, MO 63178], diluted 1:1000 (v/v) in PBS-Tween, was added. After 3 hours incubation at 37 C plates were again washed and 200 µl of 1 mg/ml enzyme substrate, p-nitrophenyl phosphate [Sigma Chemical Co., St. Louis, MO 63178], dissolved in substrate buffer (10% diethanolamine, pH 9.8, in distilled water with

0.02% sodium azide (w/v)) were added. Color change after 30 to 60 min was determined spectrophotometrically at A405 with a microELISA Minireader [Dynatech Laboratories, Alexandria, VA 22314]. A threshold value used for positive a reaction for each plate was the mean A405 nm value of the healthy control plus three standard deviations.

Pollen suspensions were incubated for 60 min at room temperature, centrifuged as previously described and a 200 µl sample of pollen wash solution was withdrawn and used as antigen in similar ELISA tests. Pollen samples were washed two more times in this same manner and resuspended in grinding buffer. No evidence of pollen damage or tapetal fragments was apparent when suspensions were examined by light microscopy. Washed pollen samples were divided in half and intact pollen used as antigen in ELISA. The remaining half of the sample was ground manually in a glass tissue homogenizer until all pollen grains were ruptured and the homogenate used as antigen as previously described.

Detection AV II antigen on the surface of asparagus pollen

Protein A-coated latex-linked antisera (PALLAS) was used to determine if AV II antigen was present on the surface of asparagus pollen using the methods of Querfurth and Paul (20) and Hamilton et al (14). A standard suspension of bacto-latex beads [0.81 µm, Difco Laboratories, Detroit, MI 48232] was first diluted with 14 volumes of 0.9% NaCL (w/v) solution then mixed 1:1 with a diluted protein A solution [Sigma Chemical Co., St. Louis, MO 63178], composed of 0.1 mg protein A dissolved in 2.0 ml 0.1 M glycine buffer, pH 8.2. This solution was incubated for 2 to 4 hours at room temperature with occasional stirring then allowed to stand overnight at 4 C. The protein A-latex bead complexes were washed by centrifuging at 6000 rpm for 30 min and resuspended in 0.1 M glycine buffer, pH 8.2, with 0.02% PVP (v/v) added. The washing step was repeated two more times and the final pellet resuspended in 2 ml 0.1 M glycine buffer, pH 8.2, with 0.05% sodium azide (w/v) [Sigma Chemical Co., St. Louis, MO 63178].

Purified gamma globulin specific for the Michigan isolate of AV II was conjugated with the protein A-latex bead complex. The globulin suspension (100 µg/ml in 0.1 M glycine buffer, pH 8.2) was mixed 1:1 with the protein A-latex bead complex, allowed to incubate for 2 to 4 hours at room temperature and washed as described previously.

Pollen samples were dissolved in 0.1 M glycine buffer, pH 8.2, mixed 1:1 with protein A-latex bead complex and allowed to incubate for 1 hour at room temperature with occasional shaking. The pollen-protein A-latex-antibody complex was centrifuged at 6000 rpm for 5 min and the supernatant was drawn off with a pipette. This wash step was repeated five times and the final pellet of pollen protein A-latex-antibody complex was resuspended in a small volume of glycine buffer. The solution was pipetted onto the surface of aluminium stubs previously coated with adhesive and allowed to settle for 10 min. Excess fluid was drained by touching the edge of the drop with a piece of filter paper and the pollen was allowed to air dry overnight at room temperature. The pollen samples were gold-coated and examined with an ISI-Mini scanning electron microscope (Figure 3.1).

To determine if protein A-latex bead-antibody complexes were bound specifically to AV II antigen, a "blocking" experiment was carried out. Pollen samples were pre-treated with a purified anti-AV II gamma globulin suspension (500 µg/ml in 0.1 M glycine buffer, pH 8.2) or buffer alone for 1 hour at room temperature with gentle agitation. Samples were then washed three times as previously described then tested for the presence of surface antigen using PALLAS.

Field indexing to determine the distribution and extent of AV I and AV II infection in an asparagus planting

A block of 100 plants in a 10 x 10 grid in a 4-year old asparagus planting in Oceana County was indexed in June of 1985 for the presence of AV I and AV II. A minimum of two spears were harvested from each plant and maintained at 4 C until indexing. Spear tips were either tested for AV II using the ELISA technique as previously described or triturated in 0.02 M sodium phosphate buffer, pH 7.0, and rub-inoculated onto *C. quinoa*. Inoculated plants were maintained in the greenhouse for 2 weeks and observed for symptom development. Single necrotic local lesions were excised triturated in a small volume of 0.01 M sodium phosphate buffer, pH 7.0, and tested for the presence of AV I using SSEM. Data from field indexing were evaluated for the non-randomness of AV I or AV II-infected plants using

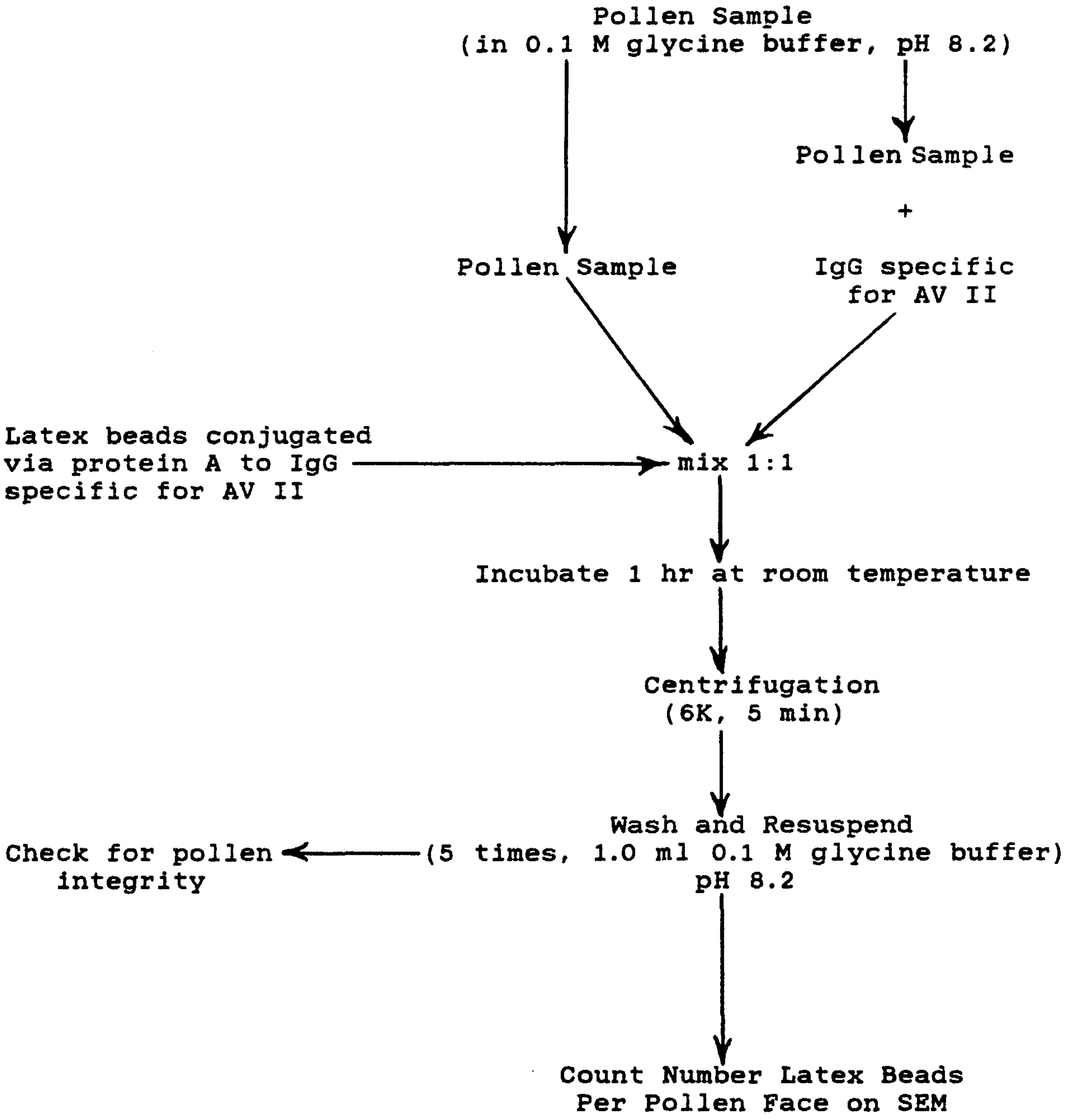


Figure 3.1. Procedure for the localization of asparagus virus II (AV II) antigen on the surface of hand-collected pollen from greenhouse grown *Asparagus officinalis* using protein A-linked latex antiserum (PALLAS).

ordinary run analysis (Madden et al, 1982). Rows of plants were combined to form an arbitrary row with a length of 100 plants. A row of plants was considered to have a non-random sequence of infected and healthy plants if $-Z_{\mu}$ was greater than 1.64 ($P = 0.05$).

Results

Aphid Transmission of AV I in Asparagus

Asparagus virus I was transmitted to asparagus by green peach aphids (*Myzus persicae*) but not by European asparagus aphids (*Brachycolus asparagi*). Twenty-one of fourty asparagus plants became infected with AV I when fed on by viruliferous green peach aphids whereas none of the 30 asparagus plants fed on by European asparagus aphids became infected with the virus.

Mechanical Transmission of AV I to Asparagus

Asparagus virus I was not successfully mechanically transmitted to asparagus plants using sap prepared from spears known to be infected only with AV I (data not shown).

Mechanical Transmission of AV II to Asparagus

Manual inoculation of healthy asparagus seedlings with the sap prepared from spears of AV II-infected asparagus plants was an effective means of transmitting the virus (Table 3.1) with 75 to 80% of the test plants becoming infected. Rub-inoculation of sap to stems or to wounded stem tips was equally effective and infected plants sometimes exhibited a mild chlorosis. However, only 1 of 30 asparagus seedlings inoculated with concentrated (1 mg/ml) purified preparations of AV II became infected.

Table 3.1. Mechanical transmission of asparagus virus II (AV II) in _Asparagus_ _officinalis_ L. using sap or concentrated, purified virus preparations.

Inoculum	Method of Inoculation	# of plants infected[Y] / # of plants tested	
Purified[Z]			
Virus from _C._ _quinoa_	Rub to stem	1/30	(3.3%)
Sap			
AV II-infected asparagus	Rub to stem	16/20	(80.0%)
	Rub to wounded stem tip	15/20	(75.0%)
Virus-free asparagus	Rub to stem	0/20	(0.0%)
	Rub to wounded stem tip	0/20	(0.0%)

[Y] Asparagus plants were indexed for AV II before and 2 months after virus inoculation by enzyme-linked immunosorbent assay and rub-inoculation to _C._ _quinoa_.

[Z] Concentrated purified AV II (1 mg/ml) in 0.01 M sodium phosphate buffer, pH 7.0.

Pollen Transmission of AV II in Asparagus

Association of AV II with asparagus pollen

Pollen samples collected from asparagus plants known to be infected with AV II were determined to contain infective virus. In a preliminary study, pollen and pollen wash solutions from both samples of AV II-infected asparagus induced chlorotic local lesions in *Chenopodium quinoa* followed by systemic mottling (Table 3.2). Single lesions from these plants were transferred sequentially, three times on *C. quinoa* and symptoms induced were identical to those of AV II. Asparagus virus II antigen was determined by ELISA to be present in pollen and pollen wash solutions of pollen samples from AV II-infected plants (Table 3.2). No viruses were detected on pollen collected from virus-free asparagus seedlings.

Asparagus virus II was determined to be an external contaminant of some asparagus pollen. In a second study, AV II antigen was again determined by ELISA to be present on pollen, in pollen wash solutions and on washed pollen collected from AV II-infected asparagus (Table 3.3). Washed and ruptured pollen from these samples did not contained detectable levels of AV II antigen. Intact pollen and pollen wash solutions from these samples induced typical AV II symptoms when rub-inoculated onto *C. quinoa*. Asparagus virus II antigen was not detected on or within pollen collected from virus-free asparagus and no symptoms were produced on *C. quinoa* leaves rub-inoculated with these samples.

Table 3.2. Detection of antigens and infectious virions of asparagus virus II (AV II) on asparagus pollen and in pollen washes by enzyme-linked immunosorbent assay (ELISA) and rub-inoculation.

		A405 nm values		
Sample	Virus Content[w]	Pollen[x]	Pollen Wash	Infectivity[y] Pollen / Wash
1	AV II	0.23[z]	0.15	+ / +
2	AV II	0.20	0.16	+ / +
3	none	0.00	0.01	- / -
4	none	0.01	0.00	- / -

[w] Plants were previously indexed for the presence or absence of AV II using rub-inoculation to *Chenopodium quinoa* leaves and ELISA.

[x] Pollen was washed in 0.01 M phosphate buffer, pH 7.0, with 1% polyvinylpyrollidone for 60 min then centrifuged at 6,000 rpm for 5-10 min.

[y] Virus infectivity was determined by rub-inoculation of each sample onto the leaves of two *Chenopodium quinoa* plants.

[z] The critical value for virus detection was the mean of the healthy control plus three standard deviations (A405 nm = 0.06).

Table 3.3. Detection of asparagus virus II (AV II) antigens on asparagus pollen, in pollen washes and within pollen by enzyme-linked immunosorbent assay (ELISA).

		A405 nm values			
Sample	Virus Content[w]	Pollen[x]	Wash	Washed Pollen	Ruptured Pollen[y]
1	AV II	0.08[z]	0.08	0.12	0.08
2	AV II	0.20	1.60	0.09	0.05
3	AV II	0.14	1.88	0.09	0.07
4	AV II	0.09	0.09	0.09	0.08
5	none	0.01	0.00	0.03	0.04
6	none	0.00	0.02	0.03	0.03
7	none	0.01	0.01	0.01	0.03
8	none	0.00	0.02	0.04	0.02

[w] Plants were previously indexed for the presence or absence of AV II using rub-inoculation to Chenopodium quinoa and by ELISA.

[x] Pollen was washed in a 0.01 M phosphate buffer, pH 7.0, with 1% polyvinylpyrollidone for 60 minutes then centrifuged at 6,000 rpm for 5-10 minutes.

[y] Pollen was ruptured using a ground glass tissue homogenizer.

[z] The critical value for virus detection were the mean of the healthy conrtrol plus three standard deviations (A405 nm = 0.06).

Localization of AV II antigen in the exine of asparagus pollen

Asparagus virus II antigen was localized in the exine of hand-collected asparagus pollen by PALLAS. Scanning electron microscopy revealed latex particles bound to the exine of pollen collected from AV II-infected asparagus treated with anti-AV II-protein A-latex conjugate, but not to the same pollen "blocked" by pre-treatment with anti-AV II IgG or pollen collected from virus-free asparagus (Figure 3.2). An average of 11.6 latex particles per pollen face were bound to pollen collected from AV II-infected asparagus and only 4.2 and 1.6 particles per face were observed on pollen collected from virus-free asparagus or "blocked" pollen, respectively (Table 3.4).

Extent and distribution of AV I and AV II within one asparagus planting

The experimental block of *Asparagus officinalis* cultivar Viking KB3 spanned 10 rows of plants (Figure 3.3) and was located 100 m from the southeast corner of the field. Of the 100 plants indexed, 30% were determined to be virus-free, 28% were infected with AV I, 36% were infected with AV II and 5% were doubly infected with both AV I and AV II. The pattern of distribution of AV I was determined by ordinary run analysis to be non-random ($-Z_{\mu} = 1.79$) whereas the distribution of AV II within that same field appeared to be random ($-Z_{\mu} = 1.23$).

Figure 3.2. Scanning electron microscopy of hand-collected asparagus pollen after exposure to latex spheres (0.81 µm diameter) conjugated with asparagus virus II (AV II) antibodies. A) Pollen from AV II-infected asparagus. B) Pollen from virus-free asparagus. C) Pollen from AV II-infected asparagus "blocked" with antiserum specific for AV II.


A
B
C

Table 3.4. Localization of asparagus virus II (AV II) antigen in the exine wall of hand-collected asparagus pollen using protein A-linked latex antiserum (PALLAS).

Virus Content of Pollen[w]	Number of latex beads/pollen face[x]
AV II	11.6 ± 3.3[y]
none	4.2 ± 1.4
AV II ("blocked"[z])	1.6 ± 1.4

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[w] Virus content of pollen was determined by enzyme-linked immunosorbent assay (ELISA) and rub-inoculation to *C. quinoa*.

[x] Samples were first gold coated then observed on an ISI mini scanning electron microscope.

[y] Values indicate the mean of a minimum of 50 pollen faces plus or minus the standard errror.

[z] Pollen determined to contain AV II antigen in exine wall by ELISA were pre-treated with purified AV II specific IgG then treated with protein-A IgG-conjugated latex beads.

N ↑

oo	oo	oo	o+	xo	o+	o+	xo	x+	oo
oo	oo	o+	o+	oo	xo	o+	xo	xo	o+
oo	o+	oo	o+	oo	o+	o+	o+	oo	xo
oo	xo	oo	x+	xo	o+	o+	oo	o+	xo
xo	o+	oo	o+	o+	oo	o+	o+	o+	o+
xo	o+	oo	o+	o+	o+	x+	x+	xo	xo
oo	o+	o+	oo	o+	xo	o+	oo	xo	xo
xo	oo	oo	o+	o+	xo	oo	xo	xo	xo
oo	o+	oo	oo	o+	xo	o+	xo	oo	oo
oo	xo	o+	xo	xo	xo	xo	oo	x+	xo
10	9	8	7	6	5	4	3	2	1

ROW NUMBERS

Figure 3.3. Distribution of asparagus virus I (AV I) and asparagus virus II (AV II) infected plants in a 4 year old asparagus planting of variety Viking KB3. oo = virus-free, xo = AV I-infected, o+ = AV II-infected and x+ = doubly infected with AV I and AV II.

Discussion

The results of these experiments demonstrated that AV II was readily transmissible from asparagus plant to asparagus plant by mechanical means and that the exine of pollen grains from some virus-infected asparagus was contaminated with infectious virions of AV II. Asparagus virus I was determined in these studies to be transmissible in asparagus by the green peach aphids (*Myzus persicae*).

Asparagus virus II has been demonstrated to spread within Michigan (15) and Washington (21, 22) asparagus plantings. It has been suggested that pollen surfaces contaminated with infectious plant viruses may serve as vehicles by which certain mechanically transmissible viruses may spread within some plant species (13). This route of virus spread would, by necessity, be more common and efficient in open-pollinated than in self-pollinated plants and could play a role in the seed transmission of some plant viruses. Asparagus is an open-pollinated plant and may become infected with AV II by mechanically means via wind-blown or bee-carried pollen infested with the virus. Honeybees have been observed routinely visiting asparagus flowers, especially in seed production blocks. Bees may mechanically inoculate plant surfaces or floral parts with AV II during their visitations. This could result in the transmission of the virus to the female plant and/or to developing seed.

In California knoves are usually used to sever asparagus spears at harvest and AV II may also be spread by the knives.

In most asparagus growing regions of the world, ferns are cut in late fall or early spring. This cultural practice may also be a means by which AV II is spread within and between asparagus plantings. Contaminated blades could mechanically transmit AV II if asparagus ferns are not allowed to completely senesce prior to cutting. Falloon et al (8) suggested that the spread of AV II within one California seed production block has been a result of cutting green fern stalks with a machete or a tractor-mounted slasher, both methods providing a means of mechanical transmission of AV II. The random distribution of AV II within our Michigan asparagus planting, as determined by ordinary run analysis, is consistent with transmission by seed, mechanical means or the random movement of pollen by wind or bees.

In asparagus in New Zealand, AV I has been reported to increase to 90 to 100 percent by the third year after planting (7) which was attributed to the possible transmission of the virus by aphids. The rate of spread of AV I in New Zealand asparagus plantings and non-random distribution of AV I within Michigan asparagus plantings is consistent with plant-to-plant transmission by aphids. Asparagus virus I was demonstrated in greenhouse studies by this author and others (17, 19) to be readily transmissible within asparagus by the green peach aphid and this is one possible method of spread for AV I within Michigan asparagus plantings. While the European asparagus aphid is widespread in most commercial growing regions of the U.S. it was not demonstrated to be a vector for the transmission of AV I in this study.

It has been observed that asparagus plants infected with AV I or AV II alone exhibit a mild reduction in growth and vigor as compared with virus-free plants (24). Asparagus infected with both AV I and AV II showed the greatest reduction in growth and often died after 2 years in the field. It has been demonstrated (Chapter 4, this manuscript) that greenhouse-grown asparagus seedlings infected with AV II more readily succumb to Fusarium crown and root rot incited by _Fusarium_ _oxysporum_ f.sp. _asparagi_ and _F_. _moniliforme_ than virus-free seedlings. Asparagus seedlings doubly infected with both AV I and AV II became more diseased when inoculated with _F_. _oxysporum_ f.sp. _asparagi_ than those seedlings infected with either AV I or AV II alone. For these reasons, it is imperative that one or both of these viruses be eradicated from asparagus plantings so that profitable yields can be maintained. Asparagus virus I is widespread in most asparagus-growing regions of the world and will probably be difficult to eradicate because of its potential transmission by aphids. Although AV II is also widespread, it would be relatively easy to eliminate from breeding and seed production blocks through meristem tissue culturing (26). Furthermore, its mechanical spread within production plantings could be minimized by discontinuing the mechanical harvest of spears and by delaying the cutting of ferns until they have fully senesced.

Literature Cited

1. Brunt, A. A., and Stace-Smith, R. 1976. The occurrence of the black raspberry latent strain of tobacco streak virus in wild and cultivated Rubus species in British Columbia. Acta. Hortic. 66:71- 76.

2. Clark, M. F., and Adams, A. N. 1977. Characteristics of the micro-plate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-484.

3. Cole, A., Mink, G. I., and Regev, S. 1982. Location of prunus necrotic ringspot virus on pollen grains from infected almond and cherry trees. Phytopathology 72:1542-1545.

4. Davis, R. F., and Garrison, S. 1985. First report of a virus present in New Jersey. Plant Dis. 66:628.

5. deBokx, J. A., and Huttinga, H. 1981. Potato virus Y. No. 242 in: Descriptions of Plant Viruses. Commonwealth Mycological Institute, Kew, Surrey, England. 4 pp.

6. Derrick, K. S. 1973. Quantitative assay for plant viruses using serologically-specific electron microscopy. Virology 56:652-653.

7. Falloon, P. G. 1982. The need for asparagus breeding in New Zealand. New Zealand J. Exp. Agr. 10:101-109.

8. Falloon, P. G., Falloon, L. M., and Grogan, R. G. 1985. A survey of California asparagus for asparagus virus I (AVI), asparagus virus II (AVII) and tobacco streak virus (TSV). Plant Dis. (in press).

9. Fujisawa, I., Goto, T., Tsuchizaki, T., and Iizuka, N. 1983. Host range and some properties of asparagus virus I isolated from Asparagus officinalis in Japan. Ann. Phytopath. Soc. Japan 49:299- 307.

10. Fujisawa, I., Goto, T., Tsuchizaki, T., and Iizuka, N. 1983. Some properties of asparagus virus II isolated from Asparagus officinalis in Japan. Ann. Phytopath. Soc. Japan 49:683-688.

11. George, J. A., and Davidson, T. R. 1963. Pollen transmission of necrotic ringspot and sour cherry yellows viruses from tree to tree. Can. J. Plant Sci. 43:276-288.

12. Gibbs, A., and Harrison, B. 1979. Plant Virology: The Principles. John Wiley and Sons, New York. 292 pp.

13. Hamilton, R. I., Leung, E., and Nichols, C. 1977. Surface contamination of pollen by plant viruses. Phytopathology 67:395-399.

14. Hamilton, R. I., Nichols, C., and Valentine, B. 1984. Survey for prunus necrotic ringspot and other viruses contaminating the exine of pollen collected by bees. Can. J. Plant Path. 6:196-199.

15. Hartung, A. C., Evans, T. A., and Stephens, C. T. 1985. Occurrence of asparagus virus II in commercial asparagus fields in Michigan. Plant Dis. 69:501-504.

16. Hein, A. 1963. Viroses am spargel. Mitt. Biol. Bundesanst. Land-Forstwirtsch, Berlin-Dahlem 108:70-74 (Rev. Appl. Mycol. 1964. 43:455).

17. Hein, A. 1969. Uber viruskrankheiten das spargels (_Asparagus_ _officinalis_ L.). Spargelvirus I. Z. Pflanzenkr. Pflanzenpathol. Pflanzenschutz 76:395-406 (Rev. Plant Pathol. 1970. 49:219).

18. Madden, L. V., Raymond Louie, J. J., and Knoke, J. K. 1982. Evaluation of tests for randomness of infected plants. Phytopathology 72:195-198.

19. Mink, G. I., and Uyeda, I. 1977. Three mechanically transmissible viruses isolated from asparagus in Washington. Plant Dis. Rep. 61:398-401.

20. Querfurth, G., and Paul, H. L. 1979. Protein A-coated latex-linked antisera (PALLAS): New reagents for a sensitive test permitting the use of antisera unsuitable for the latex test. Phytopath. Z. 94:282-285.

21. Uyeda, I. 1978. Identification, characterization, and incidence of viruses isolated from asparagus. Ph.D. thesis, Washington State University. 115 pp.

22. Uyeda, I., and Mink, G. I. 1981. Properties of asparagus virus II, a new member of the Ilarvirus group. Phytopathology 71:1264-1269.

23. Voller, A., and Bidwell, D. E. 1977. Enzyme immunoassays and their potential in diagnostic virology. Pages 449-457 in, Comparative Diagnosis of Viral Diseases Vol. II. E. Kurstak and C. Kurstak, eds.

24. Weissenfels, M., and Schmelzer, K. 1976. Untersuchungen ueber das schadaumass durch viren an spargel (_Asparagus_ _officinalis_ L.). Arch. Phytopathol. Pflanzenschutz, Berlin. 12:67-73.

25. Yang, H. 1979. Early effects of viruses on the growth and productivity of asparagus plants. HortScience 14:734-735.

26. Yang, H., and Clore, W. J. 1976. Obtaining virus-free plants of *Asparagus officinalis* L. by culturing shoot tips and apical meristems. HortScience 11:474-475.

CHAPTER IV

INTERACTION BETWEEN ASPARAGUS VIRUSES AND PATHOGENIC FUSARIUM SPECIES IN ASPARAGUS

Introduction

Declining asparagus yields in Michigan are attributed ultimately to crown and root rot incited by *Fusarium oxysporum* f.sp. *asparagi* and *Fusarium moniliforme* in stressed plants (11). It is the author's hypothesis that no single stress factor is responsible for this decline in vigor and longevity. Rather, several cultural, environmental or biological stresses on the asparagus plant may predispose it to infection by the Fusarium crown and root rotting organisms. A number of stress factors have been identified in asparagus production including tillage (17), improper soil pH (A, Putnam, unpublished data), allelopathic compounds (10), defoliation caused by rust (M, Lacy, unpublished data), virus infection (21) and the over-harvesting of spears (18).

The effects of over-harvesting on vigor and productivity of asparagus is well understood (18). Other research indicates that harvest stress may lead to a reduction in the ability of asparagus plants to wall off and lignify infection courts of pathogenic *Fusarium* species (R. Endo, personal communication). This could result in an increased incidence and severity of crown and root rot, thereby contributing to the problem of "asparagus decline".

Virus infection has been demonstrated to negatively affect the vigor and productivity of asparagus. Asparagus plantings in Germany infected with cucumber mosaic virus, asparagus virus I or asparagus virus II showed a 29 to 44% reduction in yield and a 15 to 20% decrease in number of spears and height of ferns (25). In Washington, asparagus plants infected with either asparagus virus I or asparagus virus II exhibited a mild reduction in vigor and productivity (21). Plants infected with both viruses showed severe decline and mortality in the second year in the field. Asparagus virus II has been detected widely within most commercial asparagus plantings in Michigan and asparagus virus I has also been determined to be present, but not widespread (Chapter 2, this manuscript). These viruses may constitute important biological stress factors leading to an increased incidence and severity of Fusarium crown and root rot in asparagus.

A number of studies indicated that fungal infections of plant roots were increased when plants are also infected with one or more viruses (1, 5, 19, 24). Two possible mechanisms are proposed for the increased fungal root rot induced by virus infection. Either the inherent susceptibility of root tissue is increased by virus infection or increased leakage of nutrients from roots of virus-infected plants increases the inoculum potential of the fungi in the plant rhizosphere (2).

Farley and Lockwood (7) demonstrated that pea plants became more diseased when inoculated with Fusarium solani when they were first infected with any one of four different viruses. Beute and Lockwood (3), working with the same system, determined that the increased Fusarium root rot in peas infected with bean yellow mosaic virus was not due to an increased susceptibility of root tissue but rather that virus infection increased the exudation of nutrients from roots thereby increasing the inoculum potential of the pathogen within the rhizosphere. The roots of virus-infected pea plants released more electrolytes, carbohydrates, amino acids and nucleotides than did the roots of healthy plants. They attributed this increased exudation to an increase in the permeability of cell membranes of the root.

The objective of this investigation was to evaluate the effect of infection by asparagus virus I and/or asparagus virus II on disease severity caused by the Fusarium crown and root rotting organisms in asparagus, and to investigate changes in root exudate composition and the ability of the root to lignify the area surrounding infection courts.

Materials and Methods

Plant Material

Preparation of seed

All asparagus seed was first surface disinfested of *Fusarium* with a 10,000 mg/ml benomyl solution in acetone by placing on a orbital shaker overnight. Seeds were washed free of benomyl with 100 to 200 ml of acetone and air dried. Surface disinfested asparagus seeds were pregerminated on moistened sterile filter paper in petri dishes in the dark. When radicles had emerged, they were transferred to flats containing standard commercial potting mix and maintained in a greenhouse at 21 to 24 C and a 16 hour photoperiod for 2 months.

Preparation of seedlings

Virus-free and AV II-infected asparagus seedlings were prepared using a seedlot of cultivar Mary Washington 500 (MW 500) that had been determined to be approximately 50% infected with AV II [provided by G.I. Mink]. At six weeks after germination, seedlings were indexed for AV II using the enzyme-linked immunosorbent assay (ELISA) method of Voller and Bidwell (23).

Asparagus seedlings (MW 500) either infected with AV I or doubly infected with both AV I and AV II were prepared from healthy and AV II-infected plants by aphid transmission. Green peach apids (*Myzus persicae*) were allowed to feed for 2 weeks on asparagus plants known to be infected only with AV I, then transferred to healthy or AV II-infected 6-week old asparagus plants. An equal number or non-viruliferous green peach aphids were allowed to feed on a second group of healthy or AV II-infected asparagus seedlings as a control. After 2 weeks all stems were cut at soil level and plants were transferred to aphid-free cages. Plants were indexed for the presence of AV I and AV II after the emergence of the second new shoot by rub-inoculating to C. quinoa. Plants were maintained in a greenhouse at 21 to 24 C and a 16 hour photoperiod for 5 to 14 days and observed for the development of typical AV I and AV II symptoms. Single necrotic lesions developing in 10 to 14 days were examined with serologically specific electron microscopy (SSEM) for AV I particles using the procedure of Derrick (6).

Preparation of tissue culture clones

Healthy and AV II-infected asparagus lines (cultivar Viking KB3) were prepared by culturing shoot tips and apical meristems from field grown asparagus plants known to be infected only with AV II using the methods of Yang and Clore (22). Explants of tissue were obtained from spears about 20 cm long and 2 cm in diameter. All subsequent procedures were carried out in a laminar flow hood. The surface of each spear was washed with water and then sterilized with 0.525% sodium hypochlorite solution for 10 min. The scales on the tip of each spear were removed and meristems and shoot tips were excised under a binocular dissecting microscope with a knife made from a razor blade chip attached to a wooden handle.

Two types of tissue explants were used. Apical domes less than 0.1 mm in height and free of leaf primordia were used for the production of virus-free plants and shoot tips greater than 2 mm in height with several leaf primordia for the production of AV II-infected plants. Isolated meristems and shoot tips were placed in Pyrex tubes (10 x 2.5 cm) on 10 ml of modified Murashige and Skoog's medium (MMS) (16) with 0.1 ppm naphthaleneacetic acid (NAA) and 0.1 ppm kinetin (6-furfurylamino purine). The medium was adjusted to pH 5.7 with 1 N NaOH or 1 N HCl and autoclaved at 121 C and 20 psi for 15 min. Tubes containing meristems and shoot tips were placed in a growth chamber and maintained at 25 C with a 16 hour photoperiod.

After 2 months 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 was placed into each flask and after 2 to 3 months plantlets were indexed for AV II using the procedure of Mink and Uyeda (13). A sample of the shoot tissue of each plantlet was triturated in a small volume of 0.01 M sodium phosphate buffer, pH 7.0, with 0.1% 2-mercaptethanol added. The homogenate was rubbed onto the surface of <u>Chenopodium</u> <u>quinoa</u> leaves that had previously been dusted with carborundum (320 mesh). Inoculated plants were maintained in a greenhouse at 21 to 24 C and a 16 hour photoperiod and assessed for symptom development 5 to 10 days after inoculation.

Source and Maintenance of Fungi

Isolates of *Fusarium oxysporum* (Schelcht.) emend. Snyder and Hansen f.sp. *asparagi* Cohen and *F*. *moniliforme* (Sheldon) emend. Snyder and Hansen, originally isolated from diseased asparagus, were kindly provided by M. Lacy, Michigan State University. Fungi were maintained in sterile soil at 4 C until use.

Inoculation Procedures

Preparation of colonized millet inoculum

Soil containing *F*. *oxysporum* f.sp. *asparagi* and *F*. *moniliforme* was dusted onto potato dextrose agar (PDA) [Difco Laboratories, Detroit, MI 48232] plates and grown for 14 to 21 days at 24 C. Millet colonized by *Fusarium* isolates was prepared using a modification of the method of Goth and Johnston (9). One hundred ml of glass distilled water was added to 200 g of millet in a flask and autoclaved for 1 hour at 20 psi on two consecutive days. After each autoclaving, millet was first allowed to cool and the flask was shaken vigorously until all large clumps of millet had been broken. Sterile millet was inoculated with one 4 mm diameter plug of PDA colonized by the appropriate *Fusarium* isolate and the flask was incubated at room temperature for 10 to 14 days. Each flask was shaken vigorously daily to ensure an even distribution of the fungus within the inoculum.

Inoculation of plants

Two-month old asparagus seedlings or tissue cultured asparagus clones of comparable size were challenge inoculated with 8 g of infested or uninfested millet per 1000 g of pasteurized sandy loam soil. This concentration of inoculum was previously determined to provide a moderate level of disease pressure (T. Evans, unpublished data). Plants were maintained in a greenhouse at 21 to 24 C with a 16 hour photoperiod.

Preparation of Conidial Suspensions

Conidia were removed from the agar surface with glass distilled water and gentle agitation then filtered through two layers of sterile cheescloth to remove most hyphal fragments. Conidia were washed by centrifuging at 6,000 rpm for 15 min and resuspending the pellet in 50 ml of sterile glass distilled water. This process was repeated three times and the final suspension was adjusted to the appropriate spore concentration.

Evaluation of Inoculated Plants

Disease severity of roots was evaluated 2 months after Fusarium inoculation on a scale of increasing severity from 1 to 5 where 1 = healthy plant, no visible root lesions, no rotted roots or crown discoloration; 2 = few root lesions (1-5) and/or rotted roots, no crown discoloration, no reduction in number or shoots or roots; 3 = moderate number of root lesions (6-10) and/or rotted roots, no or slight crown discoloration, shoots reduced, feeder roots reduced; 4 = many root lesions (>10) and/or rotted roots, crown discoloration, shoots reduced, feeder roots sparse; 5 = many root lesions (>10) and/or rotted roots, crown discoloration, shoots greatly reduced, feeder roots greatly reduced or absent or dead.

Influence of Virus Infection on Root Rot Severity

The effect of virus infection on subsequent infection by *F. oxysporum* f.sp. *asparagi* and *F. moniliforme* was investigated in the greenhouse. Two-month old healthy and AV II-infected asparagus seedlings or asparagus clones were challenge inoculated with *Fusarium*-infested millet as described above. The interaction between infection with AV I and/or AV II and subsequent infection by *F. oxysporum* f.sp. *asparagi* was evaluated using the procedures described above. Inoculated plants were maintained in the greenhouse at 21 to 24 C and 16 hour photoperiod and fertilized at 1 month with 100 ml of full strength Peter's 20-20-20 [W.R. Grace and Company, Fogelsvile, PA 18051]. Plants were evaluated for root rot severity 2 months after inoculation.

Influence of Root Exudates From Healthy and Virus-Infected Asparagus on Root Rot Severity

The influence of root exudates from healthy and virus-infected asparagus plants on root rot severity was investigated in the greenhouse. Five two-month old healthy or AV II-infected asparagus seedlings were grown in pasteurized sandy loam soil in 25.4 cm plastic pots and watered twice daily with 100 ml of glass distilled water for 2 months. Leachates from these pots were allowed to drain onto the soil surface of pots containing virus-free asparagus plants inoculated with either *F. oxysporum* f.sp. or *F. moniliforme*-

colonized millet. After 2 months, plants that were watered with leachates were evaluated for disease severity using the procedure described above.

Collection of Root Exudates From Healthy and Virus-Infected Asparagus

Healthy and AV-II infected clones were grown in liquid culture supported by nylon screens in staining dishes (10 x 8 x 6 cm). Four plantlets were grown in each dish with four replicates per treatment. Dishes were placed in autoclaved polypropylene bags [Bel-Art Products, Pequannock, NJ 07440] and maintained in a growth chamber at 25 C and 16 hour photoperiod. Plants were grown in full strength Hoagland's solution (4) for 6 days, washed with glass distilled water then transferred to 100 ml double distilled water for 24 hours for exudate collection. Plants were returned to fresh full strength Hoagland's solution and this pattern of exudate collection was continued for 4 weeks.

All solutions containing root exudate samples were passed through 0.20 µm filters (Nalgene Sterilization Unit, Type S) [Nalgene Company, Rochester, NY 14602] and stored at 4 C. Solutions were concentrated 10-fold in a flash evaporator at 50 C and passed through 0.45 µm Millipore filters and stored in sterile tubes at 4 C or frozen.

Chemical Analysis of Root Exudates From Healthy and Virus-Infected Asparagus

The conductivity of water culture solutions was determined with a standard conductivity bridge and the results were expressed in µmhos, the reciprocal of electrical conductivity. Total carbohydrates of root exudates were determined using a modification of Dreywood's anthrone reagent (15). The anthrone reagent was prepared by dissolving 0.4 g of anthrone in 200 ml of 9.3 M H_2SO_4. One ml of root exudate sample was mixed thoroughly with 2 ml of anthrone reagent then placed into a boiling water bath for 3 min. Samples were quickly cooled and the optical density at 620 nm measured in a Bausch & Lomb spectronic 20. A standard curve was prepared from 1 ml samples containing 10, 20, 40, 80, and 160 µg of glucose. Glucose content of root exudates was determined with an enzymatic colorimetric test (No. 115) [Sigma Chemical Co., St. Louis, MO, 63178] using the manufacturer's directions. Optical densities were read at 450 nm in a Bausch & Lomb Spectronic 20 and compared with a standard curve prepared using 1-ml samples containing 10, 20, 40, 80, and 160 µg of glucose.

Total amino acids were determined using the ninhydrin method of Moore (14). A 2-ml sample of root exudate was mixed thoroughly with 1 ml of a ninhydrin reagent solution (No. N 1632) [Sigma Chemical Co., St. Louis, MO 63178] and heated in a boiling water bath for 15 min. Samples were quickly cooled to below 30 C, vigorously shaken and optical density measure at 570 nm in a Bausch & Lomb spectronic 20. A standard curve was prepared from 2-ml samples containing 4, 8, 16 and 32 µg of glycine. Protein was determined with Folin Ciocalteu's Phenol Reagent (Micro-Protein Determination Kit) [Sigma Chemical Co., St. Louis, MO 63178] using the manufacturer's directions (12). Test samples were first diluted with a 0.85% sodium chloride solution in distilled water (w/v) so that the final protein concentration was between 150 and 1000 µg/ml. A 2.2 ml aliquot of Biuret Reagent (No. 690-1) was added to 0.2 ml of diluted test solutions and to 0.2 ml of the sodium chloride solution, mixed thoroughly and allowed to stand at room temperature for 10 min. Then, 0.1 ml of Folin Ciocalteu's Phenol Reagent (No. 690-2) was added, mixed thoroughly and allowed to stand at room temperature for 30 min. Samples were transferred to a cuvet and their optical density at 725 nm determined in a Bausch & Lomb spectronic 20. A standard curve was prepared from 0.2 ml samples containing 5, 10, 20, 40 and 80 µg of bovine serum albumin.

The Effect of Root Exudates From Healthy and Virus-Infected Asparagus on the Germination of Conidia and Germ Tube Growth

The effect of diluted root exudates on the germination of conidia and subsequent germ tube growth for F. moniliforme and F. oxysporum f.sp. asparagi was measured. Root exudates were diluted ten-fold by adding 0.1 ml sterile root exudate to a solution containing 0.1 g agar [Difco Laboratories, Detroit, MI 48232] in 9.9 ml of glass distilled water that had been previously autoclaved at 121 C and 15 psi for 20 min and cooled to 55 C in a water bath. Washed conidia were pipetted onto the surface of water agar plates amended with root exudates from virus-infected and healthy asparagus, spread uniformally with the aid of a sterile glass rod and incubated at 24 C. Germination of conidia and subsequent germ tube growth was measured hourly using a substage binocular dissecting microscope and ocular micrometer. A minimum of 25 spores were monitored on duplicate plates for each replication.

The Effect of Virus Infection on Lignin Formation Within Asparagus Roots in Response to Infection by *Fusarium* spp.

The effect of virus infection on the ability of asparagus seedling roots to lignify and wall off infection courts of *F. oxysporum* f.sp. asparagi and *F. moniliforme* was evaluated in the greenhouse. Healthy and AV II-infected two-month old asparagus seedlings were challenge inoculated with *F. oxysporum* f.sp. *asparagi* and *F. moniliforme* as previously described. Two months after inoculation plants were evaluated for disease severity as before and root tissue with distinct lesions was excised. Root lesions were separated into two categories by size: 1 mm to 5 mm in length and those 5 mm to 10 mm in length. Root tissue with lesions was hand-sectioned using a razor blade and stained with 1% phloroglucinol in 50% HCl (8) and rated for relative degree of lignification in the region surrounding the infection court. A minimum of 100 sections were evaluated on a scale of increasing lignification from 1 to 5 for each treatment.

Results

Influence of Virus Infection on Root Rot Severity

Two month old asparagus seedlings or comparable tissue culture clones infected with AV II became significantly (P=0.05) more diseased than virus-free plants when challenge inoculated with *F. oxysporum* f.sp. *asparagi* (Table 4.1). The difference in disease severity was smaller between AV II-infected and virus-free asparagus plants when challenged with *F. moniliforme* (Table 4.1). In a second experiment, asparagus seedlings infected with AV I or AV II were significantly (P=0.05) more diseased than virus-free seedlings when inoculated with *F. oxysporum* f.sp. *asparagi*-colonized millet (Table 4.2). Asparagus seedlings doubly infected with AV I and AV II became significantly (P=0.05) more diseased than seedlings infected with either AV I or AV II alone.

Influence of Leachates From Healthy and Virus-Infected Asparagus on Root Rot Severity

Root rot severity in virus-free asparagus plants watered with leachates from AV II-infected asparagus was significantly (P=0.05) greater than that of virus-free asparagus watered with leacheates from virus-free asparagus (Figure 4.1). This difference in root rot severity was greater for plants challenge inoculated with *F. oxysporum* f.sp. *asparagi* than with *F. moniliforme*.

Table 4.1. Disease severity of clones of *Asparagus officinalis* variety KB3 with and without asparagus virus II (AV II) when inoculated with *Fusarium oxysporum* f.sp. *asparagi* (FOA) or *F. moniliforme* (FM) colonized millet.

Treatment	Disease Rating[Y]	
	Virus-free	Virus-infected
FOA	1.3 ± 0.4[Z]	3.3 ± 0.4 *
FM	2.3 ± 0.3	3.0 ± 0.4 *
Control	1.0 ± 0.0	1.4 ± 0.4

[Y] Disease ratings are 1-5 with 1 = healthy plant, 2 = few root lesions and/or rotted roots, 3 = moderate number root lesions and/or rotted roots, 4 = many lesions and/or rotted roots, 5 = many root lesions and/or rotted roots or plant dead.

[Z] Values are the mean for 10 plants plus or minus the standard error.

Asterisks (* =) means for virus-infected plants differ (P=0.05) from corresponding virus-free plants according to LSD test.

Table 4.2. The effect of infection with asparagus virus I (AV I) or asparagus virus II (AV II) alone or AV I and AV II together on disease severity in seedlings of *Asparagus officinalis* when inoculated with *Fusarium oxysporum* f.sp. *asparagi* (FOA) colonized millet.

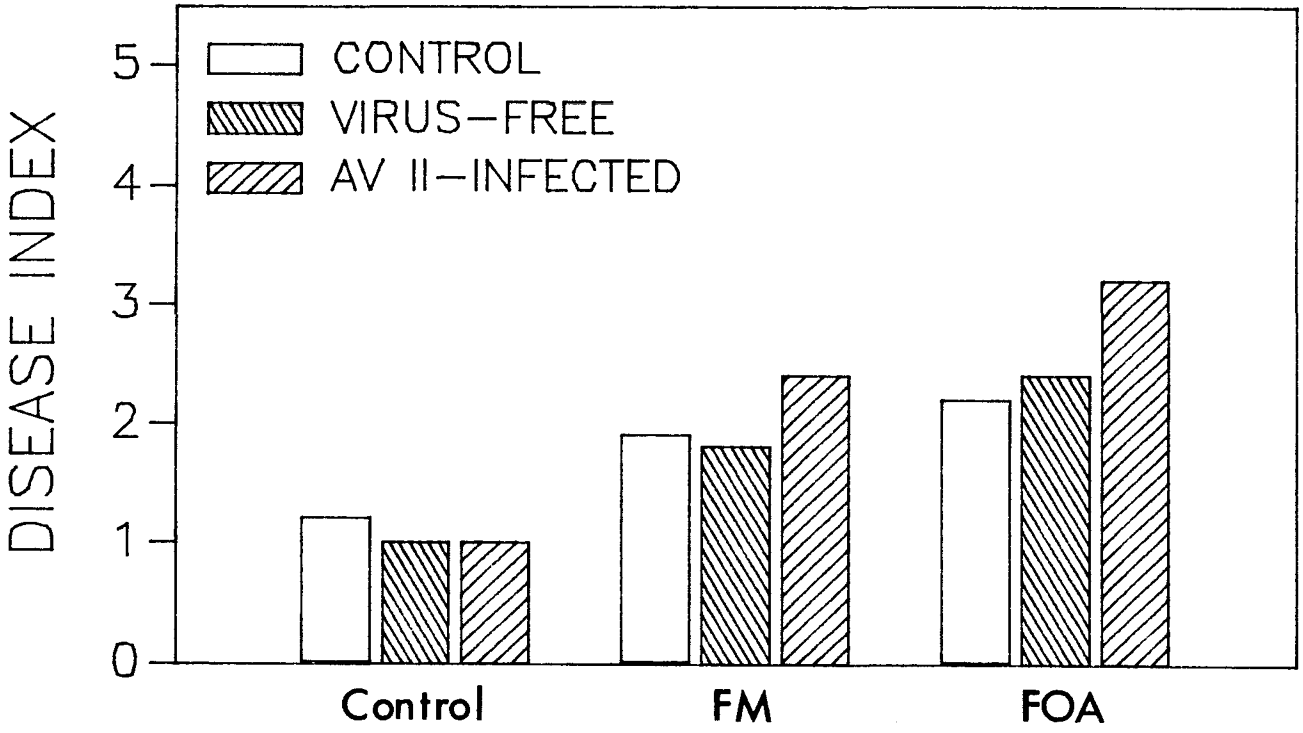
	Disease Rating[x]	
Treatment	Control[y]	FOA
Virus-free	1.1 a[z]	2.2 a
AV I	1.2 a	3.2 b
AV II	1.1 a	3.2 b
AV I & AV II	1.3 a	4.1 c

[x] Disease ratings are 1-5 with 1 = healthy plant, 2 = few root lesions and/or rotted roots, 3 = moderate number root lesions and/or rotted roots, 4 = many lesions and/or rotted roots, 5 = many root lesions and/or rotted roots or plant dead.

[y] Values are the mean for 10 plants.

[z] Within columns, numbers followed by a common letter are not significantly different (P=0.05) according to Duncan's multiple range test.

Figure 4.1. Effect of leachates from AV II-infected and virus-free asparagus on Fusarium crown and root rot. Virus-free asparagus plants inoculated with _Fusarium oxysporum_ f.sp. _asparagi_ (FOA), _F_. _moniliforme_ (FM) or sterile millet received leachings from 5 AV II-infected or healthy plants which were water with 50 ml glass distilled water twice daily over a 2 month period. Disease severity was determined on a scale of 1-5.


DISEASE INDEX
0
1
2
3
4
5
CONTROL
VIRUS–FREE
AV II–INFECTED
Control
FM
FOA

Changes in Root Exudates of Virus-Infected Asparagus Clones

Asparagus clones infected with AV II and grown in liquid culture released three times more electrolytes in a 24 hour period than did virus-free clones (Figure 4.2). In two separate experiments, AV II-infected asparagus clones grown in liquid culture released more glucose, total carbohydrates and amino acids than did virus-free asparagus clones (Figure 4.3). There were no differences in the amount of protein released by the roots of virus-infected and healthy asparagus plants. Each healthy asparagus plant released approximately 27 µg of carbohydrate (2.5 µg of this determined to be glucose) and less than 1 µg of amino acids per day. Asparagus plants infected with AV II each released about 50 ug of carbohydrate (10 µg of this determined to be glucose) and 8 µg of amino acids over the same period. There was a 100% increase in carbohydrate and a 800% increase in amino acids in the root exudates of virus-infected asparagus plants.

The Effect of Root Exudates From Healthy and Virus-Infected Asparagus on the Germination of Conidia and Germ Tube Growth

The influence of root exudates from healthy and virus infected asparagus on the germination of conidia and growth of germ tubes of _F_. _oxysporum_ f.sp. and _F_. _moniliforme_ was investigated. A significantly greater number of microconidia and macroconidia of _F_. _oxysporum_ f.sp. _asparagi_ and _F_. _moniliforme_ germinated earlier on water agar amended with root exudates from virus-infected asparagus than on water agar amended with root exudates from healthy plants or water

Figure 4.2. Increased exudation of electrolytes by AV II-infected asparagus clones in liquid culture relative to the exudation of virus-free clones. Values are the average release by 4 plantlets over 3 separate twenty-four hour periods.

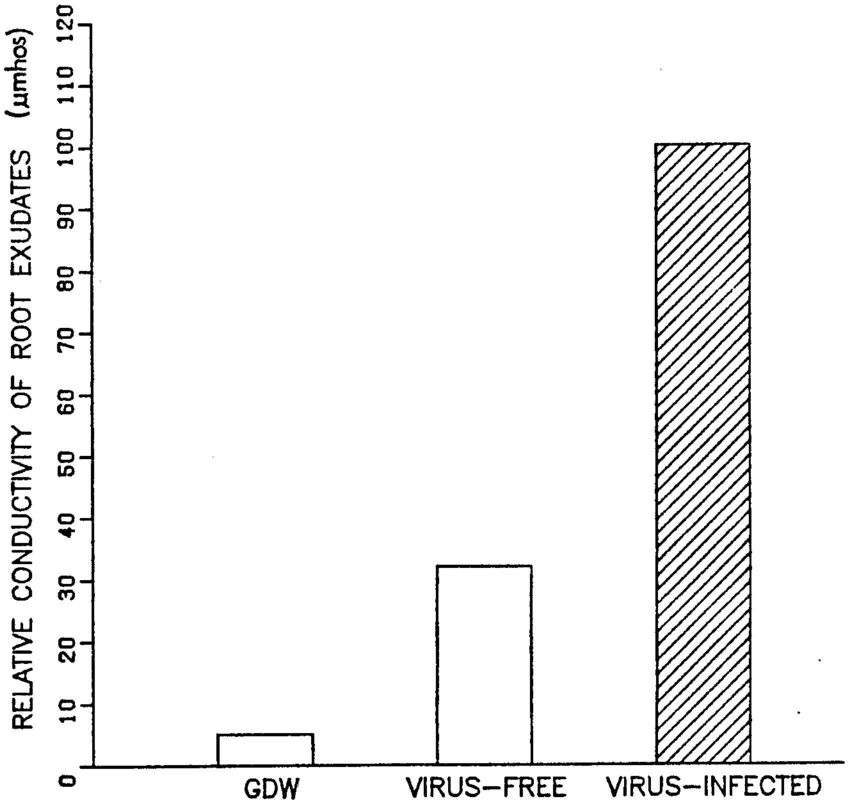
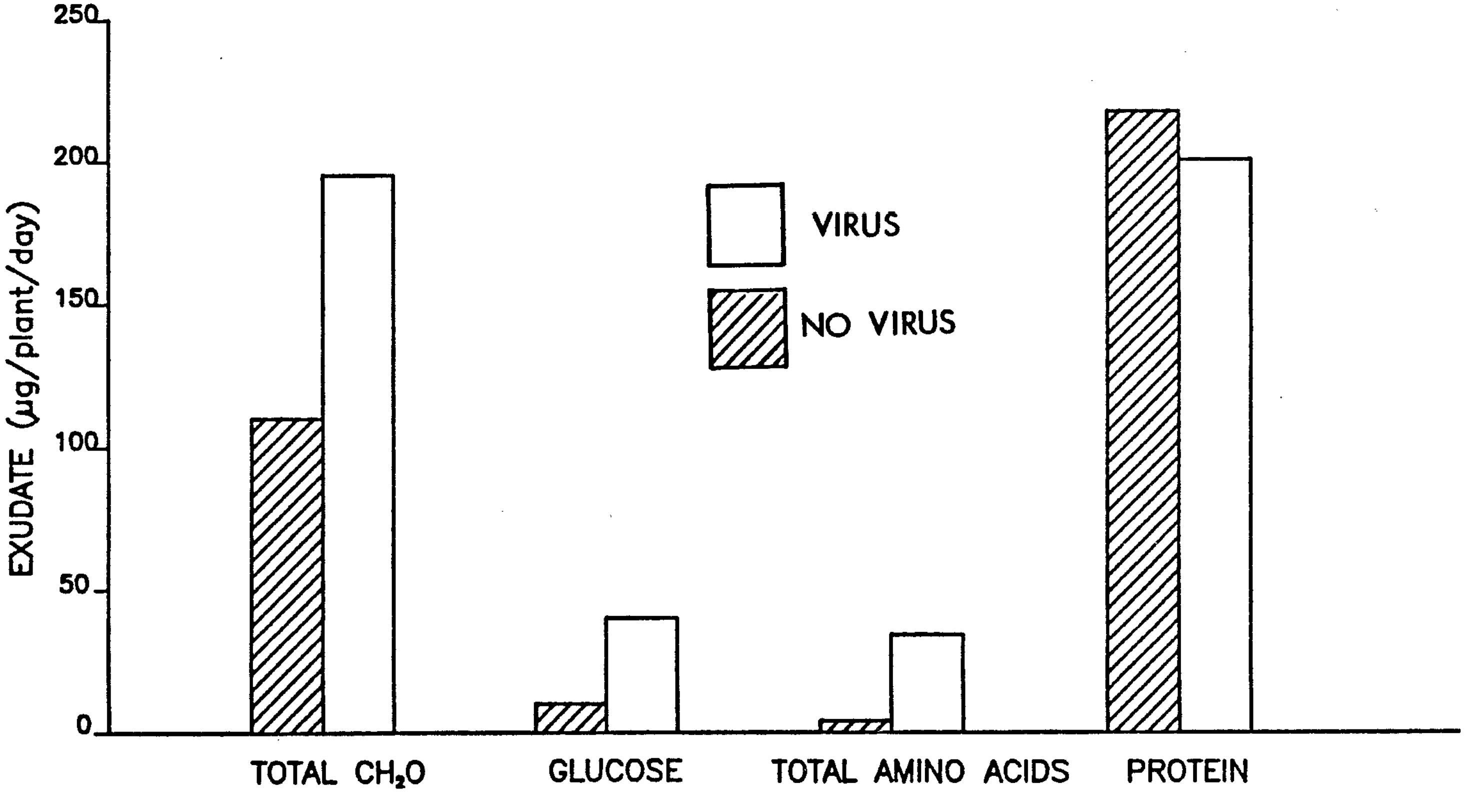

RELATIVE CONDUCTIVITY OF ROOT EXUDATES (µmhos)
0
10
20
30
40
50
60
70
80
90
100
110
120
GDW
VIRUS-FREE
VIRUS-INFECTED

Figure 4.3. Increased exudation of glucose, total carbohydrates, amino acids and proteins in AV II-infected asparagus clones grown in liquid culture relative to exudation of virus-free clones. Values are the average release by 4 plantlets over 3 separate twenty-four hour periods.

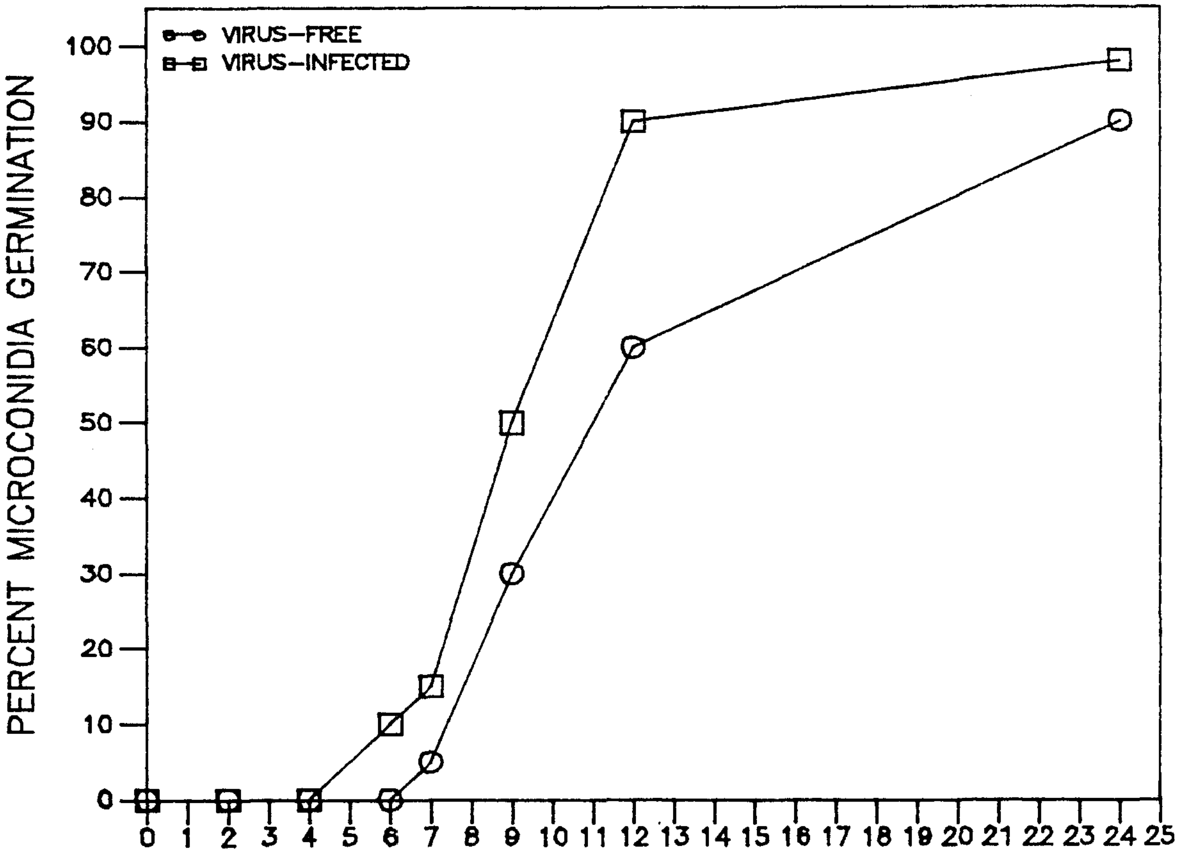

EXUDATE (µg/plant/day)
0
50
100
150
200
250
TOTAL CH₂O
GLUCOSE
TOTAL AMINO ACIDS
PROTEIN
VIRUS
NO VIRUS

agar alone (Figure 4.4). Germ tubes from microconidia or macroconida of *F. oxysporum* f.sp. *asparagi* were significantly (P=0.05) longer after 12 hours on water agar amended with root exudates from virus-infected asparagus than on water agar amended with root exudates from healthy plants or unamended water agar (Table 4.3). Root exudates from virus-free virus-infected asparagus plants had an equal influence on the growth of germ tubes from conidia of *F. moniliforme*. Germ tubes of conidia grown on unamended water agar for 12 hours were the shortest.

The Effect of Virus Infection on Lignin Formation Within Asparagus Roots in Response to Infection by Fusarium spp.

Virus infection of asparagus seedlings signifcantly (P=0.05) reduced the amount of lignin produced in the areas of the root surrounding the infection courts of *F. oxysporum* f.sp.*asparagi* and *F. moniliforme*. Virus-free asparagus plants had a mean lignification rating of 3.5 to 4.0 whereas virus-infected plants had a mean rating of only 1.3 to 1.8 (Table 4.4). There were no apparent differences in the amount of lignin produced in response to infection by *F. oxysporum* f.sp. *asparagi* or *F. moniliforme*.

Figure 4.4. The effect of root exudates from healthy and virus-infected asparagus on the germination of conidia of _Fusarium_ _moniliforme_ and _F_. _oxysporum_ f. sp. _asparagi_.


VIRUS-FREE
VIRUS-INFECTED
PERCENT MICROCONIDIA GERMINATION
0
10
20
30
40
50
60
70
80
90
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

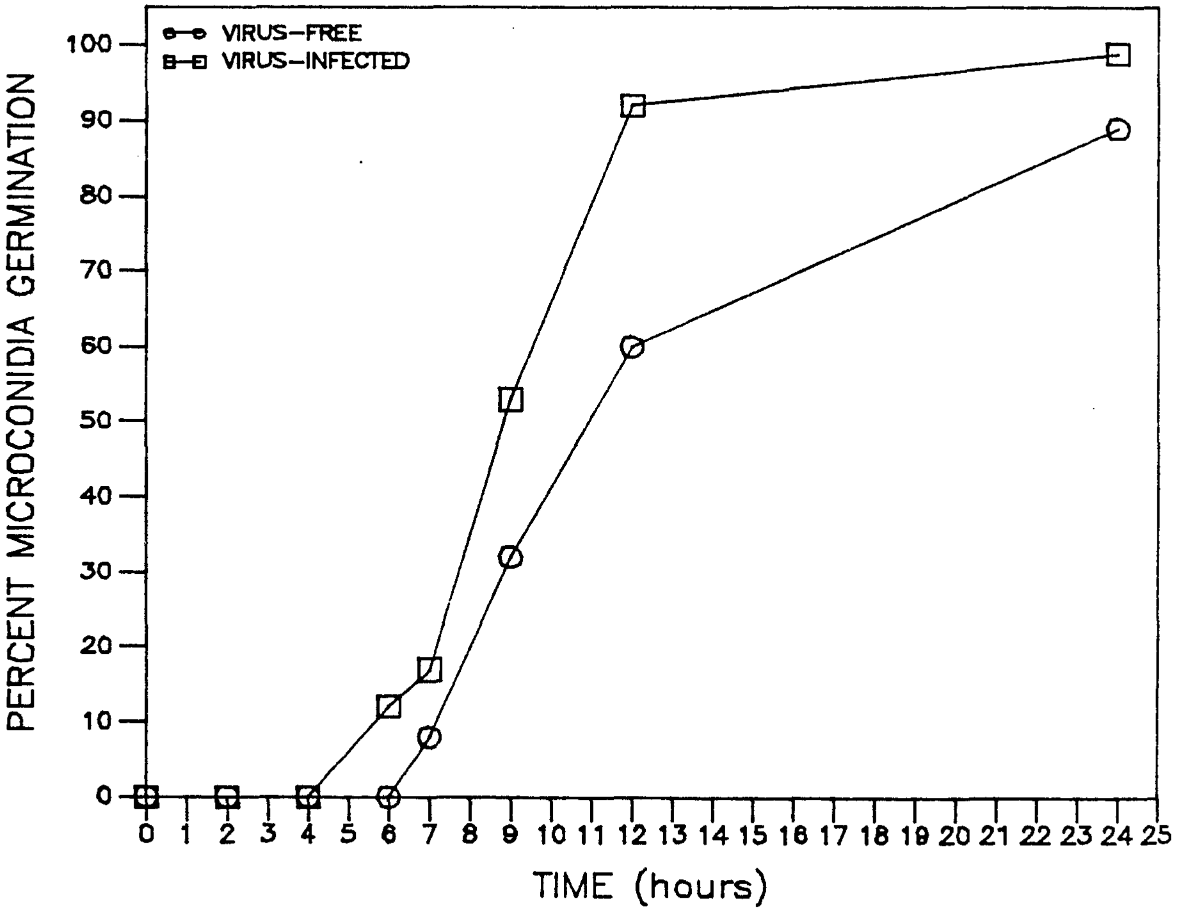

VIRUS-FREE
VIRUS-INFECTED
PERCENT MICROCONIDIA GERMINATION
0
10
20
30
40
50
60
70
80
90
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
TIME (hours)

Table 4.3. The effect of root exudates from healthy and AV II-infected asparagus clones on the growth of *Fusarium oxysporum* f.sp. *asparagi* (FOA) and *F. moniliforme* (FM) germ tubes.

	Germ Tube Length [x] (ocular micrometer units)	
Treatment	FOA	FM
Root Exudates [y]		
Virus-free	0.94 b [z]	3.50 a
AV II-infected	2.50 a	3.90 a
Water Agar Alone	1.05 b	3.50 a
PDA Alone	2.20 a	3.80 a

[x] Values are the mean germ tube lengths at 12 hours for 50 spores per treatment.

[y] Exudates from plants grown in liquid culture were diluted ten-fold in water agar.

[z] Within columns, numbers followed by a common letter are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 4.4. Effect of infection with asparagus virus II (AV II) on lignification within asparagus seedling roots in response to infection by *Fusarium oxysporum* f.sp. *asparagi* (FOA) or *F. moniliforme* (FM).

		Relative degree of lignification[y]	
	Virus Content	1 mm to 5 mm root lesions	5 mm to 10 mm root lesions
FOA	none	3.7 ± 0.8[z]	4.1 ± 0.7
	AV II	1.8 ± 0.7*	1.3 ± 0.7*
FM	none	3.6 ± 0.5	4.0 ± 0.5
	AV II	1.3 ± 0.4*	1.5 ± 0.6*

[y] Hand-sections of root lesions were first stained with 1% phloroglucinol in 50% HCl and infection courts rated on a scale of 1 to 5 for the degree of lignification with 1 = no lignification; 2 = light lignification; 3 = moderate lignification 4 = heavy lignification and 5 = very heavy lignification.

[z] Values indicate the mean of 100 sections plus or minus the standard error.

Asterisks (* =) means for AV II-infected plants differ from corresponding virus-free plants according to LSD test.

Discussion

Two-month old asparagus seedlings or comparable tissue culture clones infected with AV II became significantly more diseased than virus-free plants when inoculated with either Fusarium oxysporum f.sp. asparagi or F. moniliforme. The virus-induced increase in root rot severity observed in asparagus is due in part to an alteration in the composition of root exudates. The direct effect of root exudates on the severity of Fusarium crown and root rot was demonstrated in greenhouse experiments. Virus-free asparagus seedlings inoculated with F. oxysporum f.sp. asparagi or F. moniliforme and watered with root exudates from virus-infected plants showed increased severity of root rot compared with those plants watered with root exudates from virus-free asparagus. Electrolytes, carbohydrates and amino acids were exuded in larger amounts from the roots of asparagus plants infected with AV II than from healthy plants. Leakage of electrolytes from plant cells is indicative of increased permeablitly of cell membranes (3) and the exudates of virus-infected plants contained three times more electrolytes than those of healthy plants.

The quantity of carbohydrate exuded by roots of virus-infected asparagus plants growing in liquid culture was two times that of virus-free plants. Amino acids in the exudates of virus-infected plants was eight to ten times greater than that of virus-free plants. This large increase in the quantity of amino acids exuded by the roots of virus-infected plants may play a critical role in increasing the severity of root rot seen in these plants.

Root exudates from virus-infected asparagus plants may increase the inoculum potential in the rhizosphere of these root rot fungi thereby increasing the severity of disease in virus-infected asparagus. Soil-borne pathogens such as F. oxysporum f.sp. asparagi and F. moniliforme attack their host in a sequence of steps. These steps include germination of spores, mycelial growth, penetration of host tissue and pathogensis. Each step is influenced by the nutrient environment. Nitrogen and carbon are clearly the most important nutrients in the soil and can greatly effect the pathogenic development of fungi. Toussoun et al (20) showed that the germination of conidia of F. solani f.sp. phaseoli was favored by glucose. Glucose also stimulated saprophytic mycelial growth but delayed the penetration of the bean hypocotyls and pathogenesis. On the other hand, colonization of bean hypocotyls and pathogenesis was favored by nitrogen. Similarly, Beute and Lockwood (3) observed that disease severity in F solani-infected peas was increased by the addition of amino acids and that glucose had no affect.

Healthy asparagus roots have a propensity to ward off colonization by Fusarium prior to infection of the stele. The reduced ability of virus-infected asparagus plants to wall-off and lignify infection courts of pathogenic Fusarium contributes to the increase in disease severity observed in these plants. Histological studies by Smith and Peterson (19) have revealed that wall appositions are produced by healthy asparagus roots in response to penetration by *F. oxysporum* f.sp. *asparagi*. These apposition materials were produced in the region immediately basipetal of the root meristems and were composed of polysaccharides, acid mucopolysaccaride, callose and phenolic substances.

The mechanism by which virus-infection leads to an increase in root rot appears to be two-fold. First, virus infection leads to an increased permeability of cell membranes of the root, resulting in an increased leakage of nutrients, including carbohydrates and amino acids. Secondly, the roots of virus-infected asparagus have a reduced ability to synthesize lignin as barriers to infection by *F. oxysporum* f.sp. and *asparagi* and *F. moniliforme*.

Literature Cited

1. Bateman, D. F. 1961. Synergism between cucumber mosaic virus and Rhizoctonia in relation to rhizoctonia damping-off of cucumber. (Abstr.) Phytopathology 51:574.

2. Beute, M. K. 1970. Mechanism of increased root rot in virus-infected peas. Ph.D. Thesis. Michigan State University. 89 pp.

3. Beute, M. K., and Lockwood, J. L. 1968. Mechanism of increased root rot in virus-infected peas. Phytopathology 58:1643-1651.

4. Conger, A. 1964. A simple liquid-culture method of growing plants. Proc. Florida State Horticultural Society 77.

5. Denis, S. J., and Elliott, E. S. 1967. Decline of red clover plants infected with red clover vein mosaic virus and Fusarium species. (Abstr.) Phytopathology 57:808-809.

6. Derrick, K. S. 1973. Quantitative assay for plant viruses using serologically specific electron microscopy. Virology 56:652-653.

7. Farley J. D., and Lockwood, J. L. 1964. Increased susceptibility of root rots in virus-infected peas. Phytopathology 54:1279-1280.

8. Foster, A. S. 1942. Practicle plant anatomy. D. Van Nostrand Company, Inc. Philadelphia. 155 pp.

9. Goth, R. W., Johnston, S. A. 1979. Use of Fusarium infested desicated rye grains to evaluate wilt and crown rot reactions of mature Asparagus officinalis. (Abstr.) Phytopathology 69:535.

10. Hartung, A. C., and Stephens, C. T. 1982 Effects of allelopathic substances produced by asparagus on the incidence and severity of asparagus decline due to Fusarium crown rot. Journal of Chemical Ecology 48:224-228.

11. Lacy, M. L. 1979. Effects of chemicals on stand establishment and yields of asparagus. Plant Dis. Rep. 63:612-616.

12. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193:265-275.

13. Mink, G. I., and Uyeda, I. 1977. Three mechanically transmissible viruses isolated from asparagus in Washington. Plant Dis. Rep. 61:398-401.

14. Moore, S. 1968. Amino acid analysis: aqueous dimethyl sulfoxide as a solvent for the ninhydrin reaction. J. Biol. Chem. 243:6281-6283.

15. Morris, D. L. 1948. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science 107:254-255.

16. Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-497.

17. Putnam, A. R., and Lacy, M. L. 1977. Asparagus management with no-tillage. Michigan State University Agr. Exp. Sta. Res. Rept. #339.

18. Shelton, D. R., and Lacy, M. L. 1980. Effect of harvest duration on yield and depletion of storage carbohydrates in asparagus roots. J. Amer. Soc. Hort. Sci. 105:332-335.

19. Smith, H. C. 1962. Is barley yellow dwarf a predisposing factor in common root rot of wheat in Canada? Can. J. Plant Dis. Surv. 42:143-148.

20. Toussoun, T. A., Nash, S. M., and Snyder, W. C. 1960. The effects of nitrogen sources and glucose on the pathogenesis of _Fusarium solani_ f. _phaseoli_. Phytopathology 50:137-140.

21. Voller, A., and Bidwell, D. E. 1977. Enzyme immunoassays and their potential in diagnostic virology. Pages 449-457 _In_, Comparative Diagnosis of Viral Diseases Vol. II. E. Kurstak and C. Kurstak, eds.

22. Watson, R. D., and Guthrie, J. W. 1964. Virus-fungus interrelationships in a root rot complex in red clover. Plant Dis. Rep. 48:723-727.

23. Weissenfels, M., and Schmelzer, K. 1976. Untersuchungen ueber das schadausmass durch viren am spargel (_Asparagus officinalis_ L.). Arch. Phytopathol. Pflanzenschutz, Berlin. 12:67-73.

24. Yang, H. 1979. Early effects of viruses on the growth and productivity of asparagus plants. HortScience 14:734-735.

25. Yang, H., and Clore, W. J. 1976. Obtaining virus-free plants of _Asparagus officinalis_ L. by culturing shoot tips and apical meristems. HortScience 11:474-475.