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EFFECTS OF ALLELOPATHIC SUBSTANCES PRODUCED BY ASPARAGUS ON THE INCIDENCE AND SEVERITY OF FUSARIUM CROWN ROT

By

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ABSTRACT

EFFECTS OF ALLELOPATHIC SUBSTANCES PRODUCED BY ASPARAGUS ON THE INCIDENCE AND SEVERITY OF FUSARIUM CROWN ROT

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The effects of toxic components isolated from asparagus tissue on *Fusarium* spp. and other microorganisms and their effects on the susceptibility of asparagus to Fusarium crown rot were investigated to determine their role in Asparagus Decline. Asparagus root tissue alone and treatments in which asparagus root and rhizome tissue were combined with Fusarium inoculum significantly reduced plant growth over untreated controls and treatments with Fusarium alone. Extracts of root tissue were partitioned with solvents, eluted on a Sephadex G-25 column and separated by thin layer chromatography. Four separate components inhibitory to cress seed germination were obtained. Water extracts of root tissues were also inhibitory to growth of *Pythium ultimum* and 15 of 54 bacterial isolates, but not *Fusarium* isolates. Extracts of asparagus roots were more inhibitory to microorganisms and seed germination than extracts of fern or rhizome tissue.

DEDICATION

To my beautiful daughter, Chloe,
whose presence made this thesis possible.

To my best friend and intimate companion, John,
for his constant and loving encouragement.

To my father, who always wanted this for me.

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INTRODUCTION

Asparagus officinalis L. (asparagus) is a monocotyledonous plant in the Liliaceae first described by Linnaeus in 1735. The genus *Asparagus*, considered to be a native of Europe and Central Asia, is comprised of 300 species, some herbaceous, and some woody (30). Only *A. officinalis* var. *altilis* L. has been utilized as a commercial crop. *A. officinalis* var. *altilis* has been under cultivation for over 2,000 years in Europe and Asia where it was prized as a food by the Greeks and Romans. Pliny mentions an asparagus plant growing near Ravenna of which "three heads would weigh a pound" (18). Throughout other areas of the old world, asparagus was used mainly as a medicinal plant. Asparagus was brought to America by early colonists where it soon escaped cultivation, adapting itself to sandy fields, road sides and surviving in old garden plots and along salt marshes (13,55).

All species of asparagus are perennial and possess fleshy or tuberous roots and an underground stem (rhizome) that supports ther aerial shoots known as the ferns. The ferns contain cladophylls which are the true leaves. The green branches function as the major photosynthetic organs. The fleshy roots function as storage organs for carbohydrates which are produced during the growing season. In the spring, these reserves are used for the initiation of buds, spear and fern growth (49). The fleshy roots of the cultivated species may spread laterally 10-12 feet in the soil as they grow outward and downward from the rhizome (22). Individual asparagus plants will differ considerably in growth habit due to the dioecious nature of asparagus: plants of the same variety are not isogenic (53). Seeds are produced in a 3-celled berry that turns red at maturity. Seeds are black, comparatively large (1/8 inch diameter) and are flattened on one side (54).

Today, *A. officinalis* var. *altilis* is grown as a high cash value vegetable crop throughout the United States. In Michigan, the third largest producer of asparagus behind California and Washington, asparagus is grown on sandy soils often not suitable for other vegetable crops. Therefore, asparagus provides growers a profitable alternative crop suitable for these marginal soils. Properly maintained, asparagus plantations should remain productive for up to 20 years, producing annual yields of 3,000 pounds per acre or more. But despite increased production due to increased acreage, yields of asparagus are currently declining significantly, causing great consternation among asparagus growers. Today, asparagus fields are being removed from production after 8 to 15 years due to sparse stands and small spear size (52). In 1978, the average yield for Michigan was 1,500 pounds per acre whereas in 1981, the state average was 900 pounds per acre (1). Most fields are planted with approximately 10,000 crowns per acre, but fewer than half of the original crowns survive the first five years (Figure 1). In a 1978 field survey of asparagus fields in Michigan, the average crown population was 3,153 crowns per acre (41), representing a 70% reduction in crown survival.

This decline in both yield and number of crowns in the field is known as "Asparagus Decline", and is not confined to Michigan. Reduced yield has also led to decline in acreage planted to asparagus in other asparagus producing states. Over the past 25 years, asparagus production in New Jersey has dwindled from 30,000 acres to less than 1,000 acres (20). In California, reports acreage planted to asparagus decreased from 44,000 acres in 1974 to 28,000 acres in 1978 (52). Asparagus Decline has also been reported in the Netherlands where acreage planted to asparagus decreased from 500 ha in 1963 to 340 ha in 1970 (57).

In addition to the decreased longevity and productivity of established plantings, asparagus cannot be reestablished in fields where asparagus was previously grown. Growers have tried replanting in the past but with little success. In severe cases, only 55% of asparagus crowns survived when replanted in fields once containing asparagus (41). Hanna (1947) reported that in fields replanted immediately after plowing out an old asparagus stand, yields were never more than half the expected poundage (17). He also reported that when asparagus was direct seeded into old land, seedling mortality was practically 100% after 2-3 months.

Fusarium Root and Crown Rot of Asparagus

Asparagus Decline is frequently attributed to root and crown rot of the asparagus plant caused by *Fusarium* species. Fusaria are among the most cosmopolitan of the fungi. They are of great economic importance since they play a major role in reducing yields and quality of many important food crops of the world (37). Many of the "modern" Fusarium diseases such as corn stalk rot are caused by a complex of organisms aided and directed by a plethora of environmental factors and cultural practices (61). The Fusaria are capable of surviving in the soil almost indefinitely as chlamydospores or other resting structures. They also have the ability to infect plants by several different morphological structures, e.g. chlamydospores, macro- and microconidia. The *Fusarium* species implicated in causing root and crown rot of asparagus are *F. moniliforme* Sheldon Snyd. and Hans. and *F. oxysporum* (Schlecht.) Snyd. & Hans. f. sp. *asparagi* Cohen. *F. oxysporum* is the most frequently isolated of the *Fusarium* species in soils (36); it is very active as a saprophyte. The pathogenic *F. oxysporum* species are as specialized as the Uredineae in their specificity to host plants and play a definitive role in plant disease. *Fusarium moniliforme* is distributed throughout the world but is most common in the warmer regions. It is a major parasite on several species of the Gramineae, particularly rice. However its parasitic activity on other plants is not as well defined as other Fusaria. It is often found in association with other organisms, particularly *F. oxysporum*, acting in consort with them to produce disease (4,19,36).

Fusarium wilt and root rot of asparagus is considered to be the most common disease of asparagus in the United States (59). The above ground symptoms include a yellowing as well as stunting or wilting of the ferns (Figure 2). Eliptical reddish-brown lesions may be found at the base of the ferns near the soil

Figure 1. Asparagus plantation with long "skips" in asparagus rows. Dead plants were caused by Fusarium infection of the roots.

Figure 2. Mature asparagus fern showing above ground symptoms of Fusarium infection. Fusarium oxysporum f. sp. asparagi was isolated from the crown of this plant.

line and the vascular bundles of the crown and stalks appear discolored. The first report of the Fusarium disease on asparagus was in 1908 by Stone and Chapman in Massachusetts (50). They described a severe wilting of the young shoots and yellowing of the mature stalks of asparagus plants. An unidentified *Fusarium* species was isolated from young, wilted shoots but they conducted no pathogenicity studies. Cook, in 1923, reported that stunted, yellowed, plants showing vascular discoloration within the stems occurred in circular areas in infected stands (Figure 3) (8). He was able to complete Koch's postulates with a *Fusarium* species isolated from infected plants but could not identify the particular species responsible for the symptoms. The first comprehensive report on wilt and root rot of asparagus was written by Cohen and Heald in 1941 (7). These researchers reported the presence of wilt and root rot disease in all asparagus fields surveyed. The isolated *F. oxysporum* from vascular bundles of roots, crowns, and shoots as well as asparagus crown obtained from other areas of the country. In pathogenicity studies, they reported the "incubation period" of the disease was shorter in sandy soils than in soils with a higher amount of organic matter, and disease development was increased in experiments conducted under high temperature conditions. The isolate was not pathogenic on tomato, potato, carnations or onions, but caused a mild wilt of Alaska pea. Armstrong & Armstrong (2) later conclusively established that *F. oxysporum* f. sp. *asparagi* was very specific in its pathogenicity to asparagus. Cohen and Heald (7) felt the

Figure [illegible]. Bare circular area in an asparagus plantation attributed to Fusarium.

natural growth habit of the asparagus plant and the cultural practices employed for its maintenance were contributing factors in the pathogen's etiology. The perennial asparagus storage roots are continually replenished leaving senescing, depleated tissue in the soil that can be easily colonized by the fungus saprophytically. Moreover, since asparagus is harvested by cutting or snapping the spears from the crown, thereby producing a wound, the pathogen is able to easily infect its host. Cohen and Heald concluded that fertilization and pH adjustments of the soil would have little effect in controlling the disease. Field plots for testing fertilizers and soils of pH ranging from 6 to 8.4 showed no differences in percentage of infected plants. They felt the organism was omnipresent wherever asparagus was grown and was specific for asparagus in pathogenicity.

In 1955, K.M. Graham published an extensive report concerning infection by _Fusarium_ species on asparagus seedlings (14). He identified the pathogenic organism as _F. oxysporum_ var. _redolens_ (Wr.), and gave a detailed description of how the pathogen invades the asparagus seedling tissue. Sectioning roots from greenhouse-grown inoculated plants, he found the fungus capable of invading the meristematic region of the root tip and through the stomata of the hypocotyl and the coleoptile. Penetration was always initiated intercellularly. By examining many seedlings with many sites of infection, he concluded that most of the infections occurred in the region of the root tip. His examination of mature crowns showed that discolored vascular tissue was most frequently associated with a wound, leading him to suggest this pathogen was a "wound parasite". Endo later established that asparagus storage roots quickly "wall off" Fusarium infections and wounds which are potential infection sites. Stressed storage roots take longer to form wound periderm than non-stressed storage roots, which may give the pathogen an opportunity to cause further infection (Endo, personal communication).

Graham also became curious about the frequency of repeated isolation of F. moniliforme from seeds and dead asparagus stalks, and suspected that F. moniliforme might also be affecting seedlings. Although this fungus had been previously considered a saprophyte it caused distinct necrosis of the root apical and lateral meristems of seedlings in greenhouse tests. He did not feel this organism was responsible for the seedling blight observed in the field since symptoms on these plants were dissimilar. Yet he was skeptical of the saprophytic guise attributed to F. moniliforme and cautioned against ignoring the role this fungus may play in the asparagus ecosystem. He compared isolates of both Fusarium organisms in liquid culture and found his isolate of F. oxysporum grew more profusely on sucrose, less on basal medium plus cellulose, and had a greater ability to hydrolyse starch than F. moniliforme. As an aside, he noted F. moniliforme was incapable of producing chlamydospores at even the lowest concentrations of sugar.

Graham also examined temperature and moisture effects in greenhouse studies utilizing naturally and artificially infested soils. He placed the optimum soil temperature for the disease between 25-30°C and indicated that low and high soil moisture delayed seedling emergence, favoring pre-emergence damping off. His data from naturally and artificially Fusarium-infested soil indicated that there was a greater than 50% decrease in emergence for all levels of moisture. It was not clear whether he was using surface sterilized seeds in his experiments and this fact reduces the value of his data inasmuch as the pathogen was later shown to be seed borne (12,15,16,21).

By the 1950's, it was well accepted among growers throughout the country that asparagus could not be replanted into old fields and virgin soil must be utilized for producing seedlings. By that time, growers were running out of new land. Also, their fields were reaching peak production after 8 years then declining steadily until stands could no longer be profitably maintained. Some asparagus fields would begin to decline after only 4 or 5 years of growth (52). Since asparagus could not be harvested until the third year of growth in a plantation, maximum production of the crop was only lasting 5 to 13 years, based on previous experience, growers expected fields to remain profitable for at least 17 years. Grogan and Kimble responded to this concern and further investigated the Asparagus Decline syndrome in California (14). In the end, they concluded that the problem was the same Fusarium wilt that Cohen and Heald had described, but the strain in California was more virulent than that in Washington. They called their isolate _F. oxysporum_ f. _asparagi_. They agreed with Kimble that _F. moniliforme_ was also attributing to the problem but _F. oxysporum_ f. _asparagi_ was the primary pathogen responsible for Asparagus Decline. Although their isolate was virulent enough to quickly deplete and kill asparagus plants, the movement of the pathogen in the soil was slow. They also tested their isolate for pathogenicity to other possible hosts but found the pathogen highly specific for asparagus, readily infecting both seedlings and three-month-old crowns. They demonstrated that the pathogen could be seed-borne by washing asparagus seed with water, pouring the wash water over surface sterilized seed, planting the seed in sterile soil, and determining wilt of emerged seedlings. The Fusarium pathogen could be isolated from all seedlings that wilted. They postulated the Fusarium pathogen was spread throughout the field by cultivation when an old stand was removed. This dissemination of the pathogen was responsible for the increased seedling death death noted in the replanted land. They suggested resistant varieties were the only viable control and began the process of developing resistant clones. Lewis and Shoemaker began a screening process for resistant plants and found one line of plants that exhibited limited tolerance to Fusarium (31,32). Takatori, in California, and Ellison, in New Jersey, have also worked on breeding asparagus but to date no cultivars that are resistant to Fusarium have been developed (52). Cultivars such as UC 157 may show tolerance to Fusarium in one area of the country but not in others (Stephens, C.T., personal communication).

Although _F. moniliforme_ was a recognized pathogen of asparagus and had been reported as a seed contaminant, it was not considered important in the decline syndrome until 1979 (11,23). Johnson, Springer, and Lewis found F. moniliforme to be the dominant Fusarium species isolated from a 12 year old field (23). They conducted pathogenicity tests to compare the effects of two isolates of F. moniliforme and two isolates of F. oxysporum f. sp. asparagi on two cultivars of asparagus seedlings and crown. F. moniliforme was recovered more frequently from stem and crown lesions of plants in 12-year-old plantings whereas F. oxysporum f. sp. asparagi was isolated more frequently from discolored vascular root tissue and cortical root tissue in 2-year-old plantings. They felt F. moniliforme was more important in older plantings of asparagus and proposed the disease this pathogen caused on asparagus be named "Fusarium stem and crown rot." In passing, they mentioned their seedling transplants showed reduced vigor within 3 days of transplanting into Fusarium-infested soil. They suggested this may not be due to the Fusarium infection but to the presence of a "toxin" in the soil since plants exhibited foliar chlorosis and necrosis within a few days of transplanting. They suggested this toxin was being produced by fungal spore germination. Other researchers have studied in detail toxic compounds from Fusarium species. Many different metabolites termed "mycotoxins" have been isolated from Fusarium species in the past decade (58). However, very little evidence has ever been accumulated in support of the hypothesis that such toxins contribute to disease development of Fusarium pathogens in any way (33).

Control of the Fusarium Pathogen

Efforts to develop a chemical control for the Fusarium pathogens have not been successful. Soil fumigation and seed treatment reduce but do not eliminate decline symptoms in the field. Manning (33) reported soil fumigation and preplant crown soaks with benomyl or captafol may increase the average fresh weight of ferns when compared to non-treated plants and non-fumigated soils. However, this combination of treatments, although effective in helping establish newly planted fields does nothing to stop the demise of long term effects of Fusarium infestation in a plantation (28). More recently, soaking seed in 25,000 ppm benomyl in acetone for 24 hrs was found to eradicate Fusarium inoculum from seed (9). However, no data were given as to how eradication of Fusarium from seed may affect the incidence or severity of infestation in plantations as they continue to age. To date, there is no practical chemical control for the pathogens, nor are there any resistant cultivars available. Control is confounded by the perennial nature of asparagus and the difficulty of combating the pathogen in the soil without damaging the plant.

Allelopathy

Many components operating in the soil determine whether a plant will be attacked by a root pathogen. Although Asparagus Decline is ultimately attributed to Fusarium infection, many other factors involved in the agroecosystem contribute to asparagus decline. It is now well accepted by the scientific community that there are substances in the soil that are inhibitory to plant growth (5,40,46,51). As early as 1832, DeCandolle suggested that "soil sickness" was due to crop plant exudates (43). Since then, a large amount of scientific effort has been devoted to elucidating how root exudates and leachates from plant residues affect plant growth, interference between plant populations, and the microbial populations including plant pathogens in the agroecosystem. Schroth and Hildebrand (46) observed:

> "Plant exudates directly affect the pathogens by inducing their germination, contributing to nutritional status prior to penetration or by inhibiting their saphrophytic and pathogenic activities. The pathogens are affected indirectly by competition and antibiosis by the root microflora whose activities also are mediated by exudates".

This biochemical interaction that plants exert on their environment was termed "allelopathy" by Molisch in 1937. Allelopathy is defined as "any direct or indirect harmful affect by one plant (including microorganisms) on another through the production of chemical compounds that escape into the environment" (42). E.L. Rice further clarified this definition by stating "the effect (of allelopathy) depends on a chemical being added to the environment" (43). Allelopathy differs from competition; competition is defined as "the removal or reduction of some factor from the environment that is required by some other plant sharing the habitat (43)." Although the term allelopathy is rarely used in plant pathology literature, development of morphogenesis of pathogens, antagonism of pathogens by non-host organisms, development of disease symptoms, host-plant resistance and promotion of infection all appear to involve allelochemicals (3,42).

Allelopathic effects appear to be especially important in natural communities dominated by a single species (58). Since essentially all of the agriculture in the United States is comprised of large monocultures of plant species, it is not suprising that allelopathy may function in these agroecosystems. However, only a few researchers have examined this phenomena in any great detail in relationship to microorganisms, in particular plant pathogens. In an examination of the peach tree decline and replant problem, Chandler and Daniell (6) found peach seedlings grown in old peach soil and peach soil leachates were more susceptible to infection by Pseudomonas syringae than seedlings grown in the control soil or soil from a pecan orchard. They postulated that toxins from dead peach roots may predispose new trees to bacterial canker and thus contribute to peach decline. Patrick identified amygdalin, a cyanogenic glycoside in peach roots, as the source of toxic substances present in the soil (38). In the presence of enzymes provided by the microbial population in the soil, amygdalin is cleaved in two places and hydrogen cyanide (HCN) and benzaldehyde are produced. He demonstrated that peach trees are susceptible to damage by these compounds while other Prunus species such as apricot are less affected.

Patrick and Koch (39) reported that exposure of tobacco plants to leachates obtained from decomposing rye and timothy residues increased the susceptibility of tobacco to black root rot caused by _Thielaviopsis basicola_ (Beck. & Br.) Ferraris. Using 16 different tobacco varieties ranging from susceptible to resistant to black root rot and 6 different isolates of _T. basicola_, they showed that exposure to the leachates broke down the resistance to the pathogen in even the most highly resistant varieties. They felt the leachates were damaging the tobacco roots and,therefore, making plants more susceptible for infection and colonization by the fungus. They hypothesized toxins produced by rye or timothy accounted for the breakdown of resistance in the field since rye and timothy were often used in rotation with tobacco. They concluded these toxins may be an important "host-conditioning" factor in the disease syndrome.

Kommedahl and Ohman (26) reported that _Agropyron repens_ (L.) Beauv. (quackgrass) produces a toxin that predisposes _Medicago sativa_ L. (alfalfa) seedlings to infection by root pathogens. Their research also indicated nitrogen overcame some of the deleterious effects of the quackgrass for oats but not soybean and the quackgrass residues harbored fungi pathogenic to alfalfa (26,27). Their evidence for toxin production from quackgrass was 1) water extracts from rhizomes decreased germination in vitro of several indicator species, and 2) when indicators were grown in fields previously infested with quackgrass, a decrease in growth height occurred when compared to the same species grown on similar soil lacking quackgrass. Yields of oats and soybean were also decreased to some extent. No attempt was made to show whether there was any interaction of toxic extracts with pathogenicity of fungal species on indicator species and no statistics were performed for the data. They felt the toxic products were still a factor in disease development and their presence should not be ignored in the overall disease etiology.

Toussoun and Patrick reported toxic products of decomposing residues of rye, barley, broccoli, and broad bean greatly enhanced the pathogenesis of *F. solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk) Snyder & Hansen on beans (55). Disease enhancement, measured by lesion development on bean stems, was greatest using toxic extracts obtained during the early stages (less than one month) of decay of the residues. They postulated this enhancement was due to an additive effect of the extract and the pathogen: the extract was preconditioning the roots to fungal invasion. They noted that root rots are not necessarily caused by specific pathogens and hypothesized organisms ordinarily causing little damage might be able to "become more aggressive" if conditions were favorable for their development. Other experiments showed these toxins had a direct effect on the host cells, altering the cell permeability. They concluded the resulting increased exudation of ninhydrin-positive compounds and other substances were readily available to organisms in the infection court and were mainly responsible for predisposing the host to infection by pathogenic organisms.

Asparagus as an Allelopathic Plant

In 1970, VanBakel and Kerstens observed that soils used for asparagus production cannot be used again because of "soil sickness", but, like others before them, attributed the problem to Fusarium infestation (57). In 1972 a group of Japanese researchers (24) isolated a substance from etiolated asparagus tissue extractable by ether that inhibited lettuce, rice, rape, radish, carrot, and barnyard grass root growth in petri dish assays. Purification and identifications of this substance indicated that the compound was 1,2-dithiolane-4-carboxylic acid, colloquially called asparagusic acid. This acid inhibited root growth of the aformentioned indicator species at concentrations from 6.67×10^{-4} to 6.67×10^{-7} M.

The first report of possible allelopathic effects of asparagus was published in 1977 by Laufer and Garrison (28). Unsterilized soil amended with 4 g of asparagus root tissue delayed asparagus seed emergence for 11 days and strikingly inhibited the emergence of weed species. Crowns were less inhibitory and fern tissue not inhibitory in the same test. Water extracts of root tissue were also inhibitory to germination of asparagus seed in petri dish assays. Their data suggested asparagus might be an allelopathic plant. During the same year asparagus root growth was found to be inhibited when grown in the presence of dried, ground, asparagus root tissue in greenhouse studies (10). Shafer and Garrison followed up this piece of evidence with more comprehensive studies on seedling emergence in the presence of dried asparagus root tissue (47). Two, 4 or 6 g amounts of dried, ground asparagus tissue was allowed to "decompose" in soil for 0, 28, 50, and 90 days, then seedling emergence of lettuce, tomato, and asparagus was assayed in these soils. Possible electrical conductivity and pH confounding factors were also checked. The highest rate of dried root tissue continued to delay emergence after 90 days of decomposition. All rates delayed emergence at 0 to 28 days of decomposition. They concluded that if allelopathic substances were being produced, they were inactivated with time in the soil. Laufer and Garrison also showed water extracts from dried root tissue contained substances of MW less than 1000 that were inhibitory to lettuce and seed radicle elongation but did not affect germination (29). Putnam et al. has since established that inhibitors present in water extracts are water soluble and chloroform insoluble (41).

To date, the most comprehensive report of allelopathic properties attributed to asparagus was published by Yang (61). He used water extracts of dried plant tissue from both field grown and tissue cultured plants in germination and seedling development assays. Although germination of asparagus seed was delayed in the presence of extract, germination after 8 days was not significantly different from that of the distilled water controls. However, root length was significantly reduced when compared to the controls. This was true with stem, crown and root extract of both field grown and tissue cultured plant extracts. The root extract was most inhibitory but crown and stem extracts also affected growth to a lesser extent. Shoot length was inhibited by both crown and root extracts but not by stem extract. The root extract was most inhibitory. Substances present in the water extract were found to be heat stable.

Yang's study gave good evidence that toxic substances important in inhibition of seedling development could be extracted from asparagus plant tissues. He suggested these autotoxins may play a role in the decline problem of asparagus. He also showed that this toxic effect of the water extracts could not be diminished by adding charcoal to the preparations. He mentioned that some plant selections obtained from breeding lines in Washington were productive in old asparagus fields and suggested that varietal selection was probably the most viable way to overcome the replant problem of asparagus.

In summary, not much has been done about the replant problem in asparagus since it was first reported by Hanna in 1947. Although the problem is ultimately attributed to Fusarium infestation of asparagus fields, many other factors including root exudates of plants or allelochemicals present in the agroecosystem could be contributing to the phenomenon of Asparagus Decline. In this thesis, there are three main objectives: First, to determine if there are any interactions of the reported allelopathic substances of asparagus with _Fusarium_ spp. pathogenic to asparagus. Second, to further purify allelopathic compounds from asparagus root tissues, since these compounds may play a role in asparagus decline. And third, investigate if allelopathic compounds produce changes in the rhizosphere around the asparagus roots, consequently shifting the ecological balance of the soil and giving some competative advantage to other microorganisms in the sandy soil - specifically the asparagus pathogens, _F. oxysporum_ f. sp. _asparagi_, and _F. moniliforme_.

CHAPTER I

Asparagus Tissue and *Fusarium* spp. interaction on the Incidence and Severity of Root and Crown Rot of Asparagus

Although it is recognized that soil amendment studies are not complete proof of allelopathy, many researchers have used this method to help substantiate the role of allelopathy in plant disease development. In this section, greenhouse studies using soil amended with the different dried asparagus tissues in combination with isolates of the two Fusarium pathogens, *F. oxysporum* f. sp. *asparagi* and *F. moniliforme* were conducted a) to determine if the incidence or severity of Fusarium root and crown rot would be increased under these conditions and, b) if the phenomenon was caused by an interaction or an additive effect between plant tissue and fungal species.

MATERIALS AND METHODS

Preparation of Inoculum

Millet seeds [*Setaria italica* (L.) Beauv.] (250 g) and distilled water (100 ml) were placed in a 1 liter flask, autoclaved 1 hour, shaken to loosen the seed from sides of the flask, then left to cool overnight at room temperature. The millet seeds were reautoclaved the next day for 1 hour, allowed to cool, then inoculated with a 4 mm diameter plug of actively growing mycelium of *F. moniliforme* or *F. oxysporum* f. sp. *asparagi*. The cultures were grown at 26°C and each flask was shaken daily to facilitate mycelial spread throughout the millet seeds. The cultures were harvested after 2 wks and to cool, then inoculated with a 4 mm diameter plug of actively growing mycelium of *F. moniliforme* or *F. oxysporum* f. sp. *asparagi*. The cultures were grown at 26°C and each flask was shaken daily to facilitate mycelial spread throughout the millet seeds. The cultures were harvested after 2 wks and allowed to air dry and then stored in paper bags at 26°C until ready for use in soil amendment studies.

Preparation of Asparagus Plant Material

A commercial asparagus field in Oceana County, MI, was excavated and 'Martha Washington' asparagus plants were collected, washed, and separated into living roots and rhizomes. All dead plant material was discarded. The roots and rhizomes were oven-dried (50°C) and ground in a Wiley mill. Asparagus ferns were clipped from actively growing plants in the greenhouse and prepared in the same manner. All dried plant tissues were sterilized with propylene dioxide (58). Containers with sterilized tissue were allowed to exhaust under a hood for 24 hr to dissipate all propylene dioxide. Dried tissues were stored in plastic bags at -20°C until used.

In order to test for *Fusarium* sp. contamination, 1 g of dried tissue was spread evenly over the surface of a 9 cm petri plate containing Komada's medium. After 1 week, plates were assessed for fungal colonies growing on the media.

Interaction of Fusarium spp. and Dried Asparagus Plant Tissues with Asparagus Seedlings

The effect of combinations of the two *Fusarium* spp. and the dried root, rhizome and fern tissue was tested on asparagus seedlings. Hybrid asparagus seedlings "UC 147", 8-wk-old, were grown in steamed soil (sand:greenhouse soil, 2:1 v:v) containing the following: 1) F. moniliforme, 2) F. moniliforme and root tissue, 3) F. moniliforme and rhizome tissue, 4) F. oxysporum f. sp. asparagi, 5) F. oxysporum f. sp. asparagi and root tissue, 6) F. oxysporum f. sp. asparagi and rhizome tissue, 7) both Fusarium isolates together, 8) both isolates and root tissue, 9) both isolates and rhizome tissue, 10) root tissue, 11) rhizome tissue, and 12) a sterilized millet seed control. In a second experiment, fern tissue was used in place of root or rhizome tissue. All other treatments were the same as in the first experiment. In each experiment, infested millet seed inoculum was incorporated into soil at 8 g millet seed inoculum/4-in. pot. When both isolates were incorporated, the inoculum was applied at 4 g. Dried plant tissues were incorporated into soil at 50 g (8% of soil weight).

The inoculated seedlings were placed at random on a greenhouse bench and watered daily. The plants were fertilized twice with Peters fertilizer 20:20:20 during the time they were in the greenhouse. After 8 wk, the plants were harvested and evaluated visually for root and rhizome rot separately using a scale of 0-5. The data were subjected to analysis of variance and Duncan's Multiple Range Test.

The effect of the two Fusarium spp. in combination with different amounts of root tissue on asparagus seedling growth was tested. A 4 X 3 factorial experiment was conducted using 0, 5, 10, 20 g root tissue with either no fungus or in combination with F. moniliforme and F. oxysporum f. sp. asparagi.

were grown in steamed soil (sand:greenhouse soil, 2:1 v:v) containing the following: 1) *F. moniliforme*, 2) *F. moniliforme* and root tissue, 3) *F. moniliforme* and rhizome tissue, 4) *F. oxysporum* f. sp. *asparagi*, 5) *F. oxysporum* f. sp. *asparagi* and root tissue, 6) *F. oxysporum* f. sp. *asparagi* and rhizome tissue, 7) both *Fusarium* isolates together, 8) both isolates and root tissue, 9) both isolates and rhizome tissue, 10) root tissue, 11) rhizome tissue, and 12) a sterilized millet seed control. In a second experiment, fern tissue was used in place of root or rhizome tissue. All other treatments were the same as in the first experiment. In each experiment, infested millet seed inoculum was incorporated into soil at 8 g millet seed inoculum/4-in. pot. When both isolates were incorporated, the inoculum was applied at 4 g. Dried plant tissues were incorporated into soil at 50 g (8% of soil weight).

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The effect of the two *Fusarium* spp. in combination with different amounts of root tissue on asparagus seedling growth was tested. A 4 X 3 factorial experiment was conducted using 0, 5, 10, 20 g root tissue with either no fungus or in combination with *F. moniliforme* and *F. oxysporum* f. sp. *asparagi*.

There were 6 plants/treatment. Inoculum was applied at 8 g. Plants were grown as before, harvested after 8 wk and evaluated for root and rhizome rot, fresh weight, and dry weight. The data were subjected to an analysis of variance and Duncan's Multiple Range Test.

RESULTS

Sterilized plant material showed some residual Fusarium infestation. Eight of the 10 plates showed some fungal growth present with an average of 2.9 colonies/ plate, standard deviation of 2.45, and a range of 0-8 colonies/plate. None of these colonies were checked for pathogenicity. We considered this amount of residual colonization of the plant tissue by *Fusarium* spp. too low to contribute to infection caused by our pathogens in our experiments.

In the first experiment, dry weights of seedlings treated with root tissue alone or with root tissue and *Fusarium* spp. were significantly lower (*P*=0.05) than dry weights of controls (Table 1). Neither pathogen, rhizome tissue alone, or *Fusarium oxysporum* f. sp *asparagi* + *F. moniliforme* + rhizome tissue significantly decreased dry weights as compared to the controls. However, root rot rating of seedlings treated with *F. oxysporum* f. sp. f. sp. *asparagi* or *F. moniliforme* or asparagus plant tissues were significantlly greater than those of the controls (Figure 4 and 5). Root rot was greatest in treatments of root tissue combined with *F. moniliforme* or *F. oxysporum* f. sp. *asparagi*. Analysis of data was done using data transformed by square root.

Table 1. Dry weight and root rot rating for asparagus seedlings treated with combinations of _Fusarium_ spp. and dried asparagus tissue.

	TISSUE					
	NONE		ROOT		RHIZOME	
FUSARIUM SPP.[1]	DRY WEIGHT[2] (g)	ROOT ROT[4] RATING	DRY WEIGHT (g)	ROOT ROT RATING	DRY WEIGHT (g)	ROOT ROT RATING
None	2.85 d[3]	0.0 a	0.66 ab	3.8 e	2.42 d	1.0 b
FO	2.54 d	1.8 c	0.36 ab	4.6 f	0.95 abc	3.2 d
FM	2.07 d	2.2 c	0.24 a	4.8 f	1.41 bc	2.8 d
FO + FM	1.94 c	1.3 b	0.53 ab	3.8 e	2.03 d	3.3 de

[1] FM = _F_. _moniliforme_, FO = _F_. _oxysporum_ f. sp. _asparagi_.

[2] Dry weight = mean of 6 replications/treatment.

[3] Means without a letter in common are significantly different at P = 0.05 for Duncan's Multiple Range Test.

[4] Root rot rating scale: 0 = no root rot or rhizome discoloration,
1 = 25% root rot or few red or pink streaks in the rhizome,
2 = 50% root rot or prominent streaking in rhizome tissues,
3 = 75% root rot or death of 25% of the rhizome,
4 = greater than 75% root rot or death of 50% of the rhizome,
5 = death of the plant.

Figure 4. Root rot of asparagus caused by a combination of F. oxysporum f. sp. asparagi and asparagus dried tissue (crown residue = dried rhizome tissue, root residue = dried root tissue).

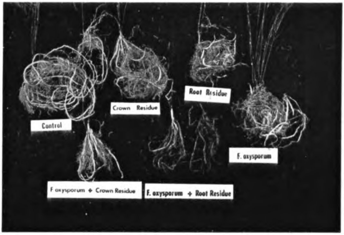
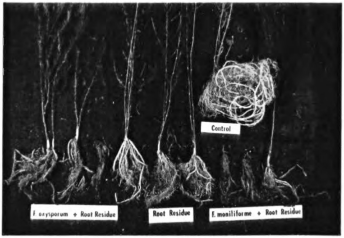


Figure 5. Root rot of asparagus caused by a combination of F. moniliforme and dried asparagus root tissue.



The AOV indicated there was no significant interaction of the *Fusarium* X tissue combination at the *P*=0.05 level (Table 2). This indicates an additive effect of the *Fusarium* spp. and root tissue.

In the second experiment, there was no significant reduction in dry weight in the treatment with fern tissue alone (Table 3). Seedling dry weight was decreased significantly by the addition of *F. oxysporum* f. sp. *asparagi* and *F. moniliforme* and even more so when the pathogens were incorporated into the soil together. However, the addition of fern tissue in combination with *F. oxysporum* f. sp. *asparagi* did not decrease dry weight to any greater extent than when *F. oxysporum* f. sp. *asparagi* was incorporated into the soil alone. When fern tissue was incorporated into the soil in combination with *F. moniliforme*, dry weight decreased more than when *F. moniliforme* alone was present.

Fern tissue alone caused no significant increase in root rot rating compared to controls (Table 3). Root rot ratings did increase when either pathogen singly or in combination was incorporated into the soil. The addition of fern tissue in combination with *F. moniliforme* caused a greater increase in root rot than when *F. moniliforme* alone or fern tissue alone was incorporated into the soil. However, there was less root rot of seedlings in those treatments where fern tissue *F. oxysporum* f. sp. *asparagi* were present than when *F. oxysporum* f. sp. *asparagi* alone was present.

In the third experiment, when asparagus seedlings were

Table 2. Analysis of variance of dry weight of 3 month old asparagus seedlings treated with *Fusarium* spp. and dried asparagus root or rhizome tissue.

Treatment[1]	Sum of Squares	Degree of Freedom[2]	Mean Square	F Statistic	Level of Significance of F Value
Fusarium spp.[3]	0.0018	1	0.0018	0.014	0.91
Root or rhizome rhizome tissue	5.237	2	2.613	20.564	0.0005
Fusarium X tissue interaction	0.336	2	0.169	1.321	0.282
Error	3.81	30	0.127		

[1] Data analysis on data transformed by square root.

[2] Treatments arranged in a 3 X 4 Factorial design with 6 replications/treatment.

[3] *Fusarium* spp. were *F. oxysporum* f. sp. *asparagi* or *F. moniliforme*.

Table 3. Dry weight and root rot rating for asparagus seedlings treated with combinations of Fusarium spp. and dried asparagus fern tissue.

Treatment	Dry Weight[3] (gm)	Root Rot[5] Rating
FM[1] + fern tissue	.03 a[4]	3.3 f
FM + FO[2] + fern tissue	.15 ab	2.3 de
FM + FO	.22 abc	4.3 g
FO + fern tissue	.42 cd	1.6 cd
FO	.51 de	2.7 ef
FM	.65 e	1.0 bc
Fern tissue	1.03 f	0.3 ab
Control	1.12 f	0.0 a

[1]FM = *F. moniliforme*.

[2]FO = *F. oxysporum* f. sp. *asparagi*.

[3]Dry weight = mean of 6 plant replications/treatment.

[4]Means without a letter in common are significantly different at *P* = 0.05 for Duncan's Multiple Range Test. Means were also analyzed using orthoganol contrasts.

[5]Root rot rating scale: 0 = no root rot or rhizome discoloration, 1 = 25% root rot or few red or pink streaks in the rhizome, 2 = 50% root rot or prominent streaking in rhizome tissue, 3 = 75% root rot or death of 25% of the rhizome, 4 = greater than 75% root rot or death of 50% of the rhizome, 5 = death of the plant.

planted into soil containing combinations of _Fusarium_ spp. and different levels of root tissue, analysis of variance of the 3 X 4 factorial experiment indicated there was a significant interaction of dried root tissue and _Fusarium_ spp. on fresh weight and dry weight of 3-month old asparagus seedlings (Tables 4 and 5).

When the 10 g or 20 g level of root tissues were incorporated into the soil in combination with _F. moniliforme_, dry weight, as well as fresh weight was decreased significantly when compared to the control or to the treatment using _F. moniliforme_ alone (Tables 6 and 7). Curiously, when _F. oxysporum_ f. sp. _asparagi_ was incorporated into the soil alone, some increase in dry weight and fresh weight were noted. However, as increasing levels of dried root tissue were incorporated into the soil with _F. oxysporum_ f. sp. _asparagi_, a progressive decrease in dry weight was observed. Root rot ratings indicated there was little disease evident in plants treated with either pathogen (Table 8). However, visual assessment of root rot increased as levels of tissue incorporated into the soil increased. Root rot rating also increased when dried tissue was incorporated into the soil in combination with either pathogen.

DISCUSSION

These experiments indicate that severity of Fusarium root and crown rot caused by _F. oxysporum_ f. sp. _asparagi_ and _F._ moniliforme is increased in the presence of dried plant tissues.

Table 4. Dry weights of 3 month old asparagus seedlings treated with varying levels of dried asparagus root tissue alone or in combination with _F. oxysporum_ f. sp. _asparagi_ or f. _moniliforme_.

Fusarium spp.[1]	Tissue Weight (g) 0	5	10	20
No Fusarium	1.27 cd[2]	1.25 bcd	1.14 bcd	0.75 a
FO	2.16 e	2.13 e	1.26 bcd	0.87 abc
FM	1.53 d	2.38 e	0.43 a	0.73 a

[1]Fusarium treatments: No Fusarium, FO=_F. oxysporum_ f. sp. _asparagi_, FM = _F. moniliforme_. No _Fusarium control_ was sterilized _millet_ seed _incorporated into_ soil.

[2]Means with out a letter in common are significantly different at P=0.05 for Duncan's Multiple Range Test.

Table 5. Fresh weight of 3 month old asparagus seedlings treated with varying levels of dried asparagus root tissue alone or in combination with *F. oxysporum* f. sp. *asparagi* or *F. moniliforme*.

Fusarium spp.[1]	Tissue Weight (g)			
	0	5	10	20
No Fusarium	4.82 e[2]	3.71 cd	3.73 cd	2.03 a
FO	6.27 g	5.97 f	4.07 de	3.13 c
FM	4.62 de	6.72 g	2.20 ab	2.63 ab

[1]Fusarium treatments: no Fusarium, FO=*F. oxysporum* f. sp. *asparagi*, FM = *F. moniliforme*. No Fusarium control was sterilized millet seed incorporated into soil.

[2]Means with out a letter in common are significantly different at *P*=0.05 for Duncan's Multiple Range Test.

Table 6. Analysis of variance of fresh weight of 3 month old asparagus seedlings treated with varying levels of dried asparagus root tissue alone or in combination with F. oxysporum f. sp. asparagi or F. moniliforme.

Treatment	Sum of squares	Degree of freedom[1]	Mean square	F statistic	Level of significance of F Value
Fusarium spp.[2]	20.3	2	10.15	6.80	0.002
Root tissue[3]	107.47	3	35.82	23.99	<0.0005
Fusarium X root tissue	34.35	6	5.73	3.83	0.003
Error	89.58	60	1.49		

[1]Experimental design was a 3 X 4 factorial with 6 replications/ treatment.

[2]Fusarium spp. treatments were; no Fusarium, F. oxysporum f. sp. asparagi, or F. moniliforme incorporated into soil at 8 gm inoculum/pot. No Fusarium control was dried, sterilized millet seed.

[3]Root tissue was incorporated into soil at 0, 5, 10 or 20 gm/pot.

Table 7. Analysis of variance of dry weight of 3 month old asparagus seedlings treated with varying levels of dried asparagus root tissue alone or in combination with _F. oxysporum_ f. sp. _asparagi_ or _F. moniliforme_.

Treatment	Sum of squares	Degree of freedom[1]	Mean square	F statistic	Level of significance of F value
Fusarium spp.[2]	3.16	2	1.58	7.93	0.001
Root tissue[3]	16.32	3	5.44	27.31	< 0.0005
Fusarium X root tissue	6.10	6	1.02	5.10	< 0.0005
Error	11.95	60	0.20		

[1]Experimental design was a 3 X 4 factorial with 6 replications/treatment.

[2]_Fusarium_ spp. treatments were; no Fusarium, _F. oxysporum_ f. sp. _asparagi_, or _F. moniliforme_ incorporated into soil at 8 gm inoculum/pot. No Fusarium control was dried, sterilized millet seed.

[3]Root tissue was incorporated into soil at 0, 5, 10 or 20 gm/pot.

Table 8. Root rot rating for asparagus seedlings treated with combinations of *Fusarium* spp. and varying levels of dried asparagus root tissue

Treatment	Root Rot[2] Rating
Control	0.0
FO[1]	0.50a[3]
FM	0.75 a
Root Tissue 5 gm	0.17 a
FM + 5 gm root tissue	0.75 a
FO + 5 gm root tissue	0.92 bc
Root tissue 10 gm	0.83 bc
Root tissue 20 gm	1.17 bc
FO + 10 gm root tissue	1.25 c
FM + 10 gm root tissue	2.08 d
FO + 20 gm root tissue	2.25 d
FM + 20 gm root tissue	2.33 d

[1]FO = *F. oxysporum* f. sp. *asparagi*; FM = *F. moniliforme*

[2]Root rot rating scale: 0 = no visible symptoms to 5 = death of the plant.

[3]Means without a letter in common are significantly different at P = 0.05 for Duncan's Multiple Range Test.

Asparagus root tissue was more toxic to asparagus seedlings than either rhizome or fern tissue. Roots grown in the presence of dried tissues were brown and distorted, and did not resemble those infected by the pathogens. Damage caused by root tissue was greater than that caused by rhizome tissue only, and fern tissue caused only a mild browning of the asparagus seedling roots. Neither rhizome nor fern tissue caused a significant decrease in dry weight, whereas root tissue alone did as compared to the control plants. These data indicated there was something present in the dried tissue that was increasing the severity of Fusarium root and crown rot on asparagus seedlings. It is hypothesized the increased damage to the root system might be the result of an additive effect of the substances released from the dried tissue and pathogen acting independently when tissue is present in the soil at high concentrations or an interactive effect between pathogen and plant tissue. Experiments using low concentrations of root tissue indicated there was an interaction of F. moniliforme or F. oxysporum f. sp. asparagi and the dried root tissue at the 10 g level. In this case, the dried tissue appeared to precondition or predispose the asparagus roots to fungal invasion. The data suggests that predisposition is the major role of these residues in root rot etiology in the asparagus agroecosystem. Pathogen colonization and establishment may be increased with the assistance of autotoxic substances released from senescing or dead tissues of this perennial plant.

Release of toxic substances from plant refuse present in an old field may be one factor in the failure to develop resistance to Fusarium root and crown rot in asparagus cultivars. Many factors influence the severity of a disease under field conditions. Patrick and Koch showed conclusively that T. basicola was equally distructive to tobacco cultivars of varying susceptibility after plants had been exposed to toxic extracts from rye or timothy tissue (39). They concluded residual tissues of these rotational crops might constitute important "host-conditioning factors" in the tobacco black root rot disease. Asparagus toxins are thought to degrade after a period of time (46). Yet, there is a build-up of asparagus tissue in soil, due to the growth habit of the plant. Therefore, toxic exudates could be released for extended periods of time. This continual stress on the roots by toxic components released from the senescing tissues may give the Fusarium pathogens an easy entrance to the plant. The variety of asparagus used in these experiments, UC 147, is known to be "tolerant" to Fusarium infection in California (C.T. Stephens, personal communication). Perhaps in Michigan the UC 147 tolerance is breaking down in the presence of the autotoxic substances present in the root tissue.

CHAPTER II

Isolation of Germination Inhibitors from Dried Asparagus Root Tissue

Previously, water extracts were found to be inhibitory to germination or would delay germination of selected indicator species as well as asparagus seed. Putnam et al., had determined that, when water extracts are extracted with chloroform, the activity remains in the water fraction (41). The objective in this section was to develop a purification scheme for separating compounds present in the water extracts that may be responsible for the allelopathic properties attributed to asparagus. Dried root tissue had been shown to be the more toxic than rhizome or fern tissue to asparagus seedlings. Therefore, dried root tissue was chosen to use in the purification procedure. Germination inhibition bioassays were employed for testing different fractions of the isolation procedure.

MATERIALS AND METHODS

Isolation of Germination Inhibitors from Dried Asparagus Root Tissue

Solvent extractions of the dried root tissues were made to determine which fractions contained active allelopathic components (Figure 6). Dried asparagus root tissue (50 *g*) was added to 500 ml of 50% methanol and ground in a Waring blender for 1 minute. The slurry was stirred overnight at 1°C, then filtered through 4 layers of cheesecloth and centrifuged at

Figure 6. Flowchart for extraction procedure of asparagus root tissue.

DRIED TISSUE (50 gm) + MeOH:H_2O (500 ml) 1:1

↓

FILTER THRU CHEESECLOTH & CENTRIFUGE

↓

ACETONE PRECIPITATION THEN CONCENTRATE

↓

SOLVENT EXTRACTION: $CHCl_3$ then Butanol

↓

CONCENTRATE-RESUSPEND IN MeOH

↓

SEPHADEX G-25 COLUMN

↓

(ELUTION SERIES)

↙ ↓ ↘

$CHCl_3$:MeOH:Acetic Acid:H_2O	MeOH:H_2O	MeOH:H_2O + 10% Acetic Acid
(19:1:4:0.6)	(1:1)	(1:1)
Fraction 1	Fraction 2	Fraction 3

↘ ↓ ↙

CONCENTRATE-RESUSPENDED IN MeOH

↓

ITLC-SA

(ISOPROPYL ALCOHOL:NH_4OH 100:7)

↓

VIEW UNDER UV LIGHT

6000 xg for 15 minutes to remove particulates. The supernatant was decanted and the methanol-water fraction was precipitated with acetone (4:1 v:v, acetone/sample) overnight at 1 °C. The precipitate was discarded and the liquid concentrated to 1/4 volume on a Buchi Rotary evaporator. This concentrate was extracted with chloroform 3 times (1:1, v:v). The chloroform was discarded and the polar fraction extracted 3 times with 2 volumes of water-saturated butanol. The butanol extracts were combined and evaporated to dryness. The extract was resuspended in 20 ml methanol, and 5 ml were removed and concentrated to 2 ml under N_2. The concentrate was applied to a Sephadex G-25 column (2 X 20 cm) prepared according to Rouser and Fleischer (44) and eluted as follows: 100 ml of chloroform:methanol:acetic acid:H_2O (19:1:4:0.6, v:v:v:v) (Fraction 1), 100 ml of methanol:H_2O (1:1 v:v) (Fraction 2) and 100 ml of 10% acetic acid in 50% methanol (Fraction 3). Each fraction was concentrated under reduced pressure, dissolved in a small volume of methanol and further separated by preparative thin-layer chromatography. Aliquots were removed and spotted on Instant Thin Layer Chromatograph-SA (TLC) polysilicic acid gel-impregnated glass fiber sheets (Gelman Sciences Inc., Ann Arbor, MI 48106) and the chromatograms developed with isopropyl alcohol:NH_4OH (58%) (100:7, v:v). The chromatograms were viewed under short and long wave UV light. The fluorescent spots were marked and Rf values were calculated. Areas of equal Rf value were removed, combined and eluted with 100 ml of methanol, concentrated to dryness with a flash evaporator, dissolved in 1 ml of methanol and bioassayed for germination inhibition on cress seed (Lepidium sativum, 'curly cress'). The whole TLC plate was bioassayed in this manner.

Extraction of Fresh Asparagus Root Tissue for Germination Inhibitors.

Asparagus crowns (UC 147) were grown in the greenhouse for 1 year, harvested, the ferns removed and crowns washed. Fresh crowns (806.5 g) were chopped up with a knife, placed in a glass container and covered with methanol. The crowns were allowed to soak in methanol for 1 wk, then the methanol was decanted, and filtered through #1 Whatman filter paper. The filtrate was concentrated to 1/2 volume under reduced pressure. This sample was treated with acetone and extracted with solvents exactly like dried root tissue (Figure 6). The Butanol, chloroform, and methanol fractions were then bioassayed on pre-germinated asparagus seed.

Bioassay for Germination Inhibition by Asparagus Root Extracts

In order to determine which components of the asparagus root tissue extract were inhibitory to plants, each extract was bioassayed for radicle elongation on pre-germinated asparagus and cress seed. After experiments had established that those fractions which inhibited asparagus germination also inhibited cress germination, cress seed were used in further bioassays. Seed of several other species were also bioassayed with the butanol fraction after solvent extraction.

Because asparagus seed exhibit wide variability in germination rates, they were pre-germinated before use in bioassay studies. Asparagus seed were surface-sterilized with 50 ml of

0.5% sodium hypochlorite and 2 drops "Tween 20" for 15 minutes, rinsed 3 times with distilled water, imbibed for 15 minutes in distilled water and placed on moistened Whatman #1 filter paper in 9 cm petri plates and sealed with Parafilm^R. The plates were placed in the dark at 26° C until radicle emergence was visible in a majority of seed. Seed with radicles just breaking through the seed coat were selected for bioassay.

Asparagus extract from each fraction was diluted in a tenfold series and 1 ml of each dilution was added to Whatman #1 filter paper in 9 cm petri dishes and allowed to air dry. Asparagus seed with radicles of equal length (10 seeds/plate) were placed on the filter paper which was then dampened with glass distilled water. The plates were sealed with Parafilm^R and placed in the dark at 26°C. Five replicate plates were prepared for a total of 50 seed per test. Appropriate solvent and glass distilled water controls were used. Growth was considered inhibited if the radicle did not elongate further.

For cress seed assays, similar dilutions were spotted on Whatman #540 filter paper, using 0.25 ml/2.1 cm disks with 4 disks/plate. Disks were allowed to air-dry, wetted with 0.25 ml glass distilled water and 10 cress seed placed on each disk. Appropriate control solvents were also spotted in a similar manner. The plates were sealed with Parafilm^R, placed on the lab bench and evaluated after 4 days. Seed were considered germinated if radicle emergence was complete. A dilution series was not performed on compounds eluted from TLC plates due to scarcity of extract material.

The butanol fraction was also bioassayed using lettuce (*Lactuca sativa*), radish (*Raphanus sativus* L.), Barnyard grass (*Echinochloa crus-galli* L. Beauv.), Tendergreen mustard (*Brassica campestris*), National pickling cucumber (*Cucumis sativus* L.), tomato (*Lycopersicon esculentum* Mill.), alfalfa (*Medicago sativum* L.), and pre-germinated asparagus seed (*Asparagus officinalis* L.). Bioassays were conducted as before on Whatman #1 filter paper using 100 or 50 seed/test. Undiluted extract was assayed in this test due to scarcity of extract material.

RESULTS

Isolation of Germination Inhibitors from Dried Asparagus Root Extract

The three fractions from the Sephadex G-25 column were separated into individual components by thin layer chromatography (TLC) (Table 9). Fraction 1 contained 5 components, fraction 2 contained 8 components, and fraction 3 contained 6 components as determined by fluorescence under UV light.

Extraction of Fresh Asparagus Root Tissue for Germination Inhibitors

In this procedure, distilled water was not added to the methanol in the first extraction step because asparagus root tissues contain such a high content of water. Solvent extraction produced three separate fractions that were bioassayed on pre-germinated asparagus seed, the chloroform, water-saturated butanol and methanol fractions. Bioassay of

Table 9. Components isolated from dried asparagus root tissue which inhibit cress seed germination.

Fraction [1]	Separate fluorescent components [2]	Number of inhibitory components	Rf of inhibitory components	Cress seed germination [3]
1	5	1	0.83	6/60
2	8	1	origin	19/40
3	6	2	0.85	22/40
			0.49	22/40

[1] Each fraction represents a single elution from Sephadex G-25 column with a different solvent system.

[2] Components separated by TLC using isopropyl alcohol:NH_4OH (100:7) as mobile phase.

[3] Number of seed that germinated out of the total number of seed tested.

each fraction showed inhibition of germinated asparagus seed in all fractions at 10^{-3} dilution factor.

Bioassay of Germination Inhibitors from Asparagus Root Extracts

To determine which components of the solvent extract from dried tissue or fresh tissue contained germination inhibitors, all fractions were assayed on cress and germinated asparagus seed. The chloroform fraction exhibited no germination inhibition and the water fraction inhibited germination only when used undiluted. However, the butanol fraction exhibited 100% germination inhibition even after a 10^{-2} dilution. Therefore the butanol fraction was chosen for further chemical isolation experiments.

All fractions eluted from the Sephadex G-25 column elution exhibited some germination inhibition of cress and germinated asparagus seed. Therefore, components of each column fraction were separated by TLC. Individual components visible under UV light were all bioassayed on cress seed (Table 9). Fraction 1 contained 1 active component of 5 components (Rf 0.83) that resulted in 90% inhibition of germination as compared to controls. The only component of 8 to inhibit germination in Fraction 2 was found at the origin (germination inhibition on cress seed of 52%). Fraction 3 contained 2 out of 6 components (Rf 0.85 and 0.49) each of which inhibited germination of cress seed by 45%.

The butanol extract inhibited germination of all eight plant species tested. Alfalfa seed were able to emerge from the seed coat but the radical did not elongate. In pre-germinated asparagus seed, no further elongation of the radical was observed in asparagus extract. Radicles of the other species were not able to emerge from their seed coat. All seeds in the control germinated 80% or more.

DISCUSSION

Asparagus seed were strongly inhibited by the separate fractions from the column elution of an extract from dried asparagus root tissue. The inhibition of asparagus seed by the fraction suggest that asparagus tissue contains compounds that are autotoxic to its growth. These data are partially in agreement with Yang who found water extract from asparagus tissues inhibitory to root growth (62). Yang, however, did not find that his extracts affected seed germination. Possibly his water extracts were more dilute than the one used here. Moreover, Yang used distilled water in his extraction procedure whereas my extraction was done with 50% methanol solvent in the first extraction step. Therefore, the extract could contain additional chemical components that are soluble in methanol.

In addition, Yang used dead, tissue-culture grown and dead, field-grown plants whereas in these experiments oven-dried living tissues were used. Chemical breakdown of inhibitory components may have taken place in the dead tissue used by Yang. Shafer and Garrison reported toxicity of decomposing root tissue diminished after plant tissue was allowed to decompose for 50 days (48). We observed that extracts kept frozen or at 4°C for periods greater than 30 days gradually decreased in toxicity to cress or asparagus seed.

As isolation of toxic components from asparagus tissue has been carried out in the laboratory as opposed to the field, interpretation as to their their possible significance in Asparagus Decline must be viewed in the light of this obvious limitation. Toxic products obtained by these methods may be produced under field conditions and may certainly affect the asparagus plantation. Whether the particular components isolated in this laboratory may be of significance in such a complex system as the soil environment, cannot be determined at this time. However, extraction from plant tissues in the laboratory is the first step in determining the presence of possible allelopathic components in asparagus. Further elucidation of the chemical nature of these components is needed. Also, it must be established that these components or their derivatives are available in the rhizosphere of the asparagus plant before the allelopathic properties of asparagus can be considered a factor in the etiology of Asparagus Decline.

Separation of different column fractions by TLC produced 4 components inhibitory in cress seed germination bioassays. This suggests that the toxicity displayed by extracts of asparagus roots is due to more than one chemical component present in the plant tissue. The means by which inhibition of seed took place was not investigated in this study. As there are several separate components in the extract, the mode of action of the components in inhibition may be different for each different component. This hypothesis is supported by results obtained from the bioassay of the butanol fraction on a wide range of plant seed. The response of the different species to the butanol extract was varied. Therefore, separate components of this fraction might be affecting different biochemical steps in the germination process.

Extracts of live plant root tissue also produced fractions inhibitory to asparagus seed radicle elongation. All three solvent extracts were inhibitory to asparagus seed at concentrations of 10^{-3}. Bioassay of dried root tissues exhibited inhibition only in the butanol and water fractions at 10^{-2} and 10^{0} concentrations respectively. Inhibitory components present in the choloroform fraction were no longer present in the dried tissue. This indicates that components in the live asparagus tissue are somehow destroyed after the tissue is dried. It was not determined as to what happens to those components in the chloroform extract when the tissue is dried, nor was it determined if dried or live tissues, or both are responsible for allelopathic properties attributed to asparagus in the soil. More research is necessary in clarifying this point.

CHAPTER III

Interaction of Asparagus Root Extracts with Microorganisms

Toxins may play a role in shifting populations of microorganisms. Compounds released from plant debris during decomposition may be harmful to some organisms but not to others present in the soil, thus causing a more favorable atmosphere for other organisms to develop. With this in mind, we examined how extracts from dried asparagus root tissue may be affecting microorganisms indigenous in sandy soils including the isolates of *F. oxysporum* f. sp. *asparagi* and *F. moniliforme*.

MATERIALS AND METHODS

Inhibition of Fungal Growth by Root Extract

Preliminary experiments were conducted with *Pythium ultimum*, *Rhizoctonia solani*, *F. oxysporum* f. sp. *asparagi*, and *F. moniliforme* to determine if soil-borne organisms are inhibited in the presence of asparagus root extract. A 4.0 mm plug of PDA containing the fungal isolate to be assayed was placed in the middle of potato-dextrose agar (PDA) plate and 2.5 g of autoclaved or nonautoclaved dried asparagus root tissue was sprinkled in a localized area on the side of the petri plate. Plates were viewed 5 days later for inhibition of fungal growth.

In another test, these same four fungal isolates were also tested for sensitivity to a water extract of dried asparagus root or rhizome tissue. The extracts were prepared by adding 20 g of the tissue to distilled water, placed in dialysis tubing

(3500 MW cutoff) and dialyzed overnight against 2300 ml distilled water. The dialyzed fraction was concentrated to 200 ml (equivalent to 1 ml/0.1 g dry wt.) under reduced pressure at 50°C, then filter sterilized. A 6 mm agar plug was removed from a PDA plate, and 0.2 ml or 0.4 ml of the dialyzed fraction from the root, rhizome or distilled water control placed in the well. A 4 mm plug of PDA containing the fungal isolate to be assayed was placed on the other side of the plate. The plates were sealed with Parafilm[R] and the cultures allowed to grow for 5 days. There were 3 replicate plates/treatment.

A 4 mm diameter plug of PDA containing an actively growing culture of *P. ultimum* was placed in 50 ml potato dextrose broth (PDB) to which was added 0, 2.5, 5.0, or 10 ml of root extract prepared as before. The cultures were incubated for 10 days on a reciprocal shaker, then harvested by filtering through pre-weighed, glass-fiber filter paper (Whatman GF/C, 7.0 cm diameter), dried and weighed. Any contaminated cultures were discarded. There were 4 replicate flasks/treatment. The data were subjected to an analysis of variance and Duncan's Multiple Range Test.

Effects of Dried Asparagus Root Tissue on Isolates of Pythium spp.

Thirteen different *Pythium* isolates were tested for sensitivity to water extracts of dried asparagus root tissue. Eleven isolates of *P. ultimum* were collected in Ohio, and one isolate each of *P. ultimum* and *P. aphanadermatum* were collected in Michigan. A water extract of dried asparagus root tissue prepared as before was sterilized with a Millipore filter (GS

0.22 μm, Millipore Corporation, Bedford, MA 01730) to remove any bacterial contamination. The extract (0.2 ml) was applied to separate PDA plates (9 cm diameter) and allowed to dry. Each plate was then inoculated with a separate Pythium isolate. Cultures were allowed to grow for 4 days at room temperature then visually assessed for hyphal inhibition. This experiment included 2 replicate plates/isolate and distilled water as a control.

Sensitivity to Bacterial Isolates Collected from Sandy Soils to Water Extract of Dried Asparagus Root Tissue

Naturally occurring species of bacteria were isolated from the soil of 3 fields suitable for asparagus production using Martin's selective medium (34). Fifty-four separate isolates were collected and maintained on complete agar. Complete agar was prepared as follows: 10 g casamino acid, 5 g yeast extract, 3 g K_2HPO_4, 1 g KH_2PO_4, 15 g agar added to 1000 ml H_2O. This mixture was autoclaved 15 minutes, allowed to cool and poured into 9 cm petri plates or slants. Soft agar overlays contained 0.15% agar. Isolates were tested for sensitivity to a water extract of dried asparagus root tissue. Separate bacteria isolates were grown on complete agar slants for 24 hr after which 1 ml of $PO_4^=$ buffer, pH 7.0, was added to the slant. The slant was vortexed 30 seconds, and 100 μl of the bacteria suspended in the phosphate buffer was used to inoculate a soft agar overlay of complete agar. The soft agar overlay was poured over complete agar plates containing the water extract of dried asparagus root tissue. The water extract was applied to the plate at 10μl/spot and allowed to dry.

RESULTS

Inhibition of Fungal Growth by Dried Asparagus Root Extracts

Preliminary experiments indicated that dried asparagus tissue had no visible effect on the growth of *Rhizoctonia solani*, *F. oxysporum* f. sp. *asparagi*, or *F. moniliforme*. However, *P. ultimum* hyphae would not grow within a 1.5 cm distance around autoclaved or non-autoclaved asparagus root tissue on PDA after 5 days (Figure 7). When 0.2 ml of a water extract of root or rhizome tissue was used on the plates, the same results were obtained; *P. ultimum* formed a distinct zone of inhibition around the area where the extract had seeped into the agar, and the three other fungi were unaffected. The water control showed no such zone of inhibition. When *P. ultimum* was grown in PDA in the presence of 2,5, 5.0, or 10 ml concentrations of asparagus root extract for 10 days, all concentrations significantly inhibited fungal growth; dry weights of fungal cultures were significantly different at *P*=0.05 by Duncan's Multiple Range Test (Table 10).

Effects of Water Extract of Dried Asparagus Root Tissue on Isolates of *Pythium* spp.

Nine of the *P. ultimum* isolates and the *P. aphanidermatum* isolate were not able to grow within 2 cm of the area where asparagus root extract was applied. In 3 of these isolates, sparse growth of the *Pythium* hyphae was observed on the area where the root extract was applied. Hyphal strands were appressed to the agar plate and did not exibit the normal fluffy appearance of the rest of the culture or the control plates.

Figure 7. <u>Pythium ultimum</u> growing in the presence of dried <u>sterilized root</u>, rhizome or fern tissue. 1) fern tissue, 2) Root tissue, 3) rhizome tissue.

TABLE 10. Weight of *Pythium ultimum* hyphae grown in PDB in the presence of various concentrations of asparagus root extract.

Extract (ml)[1]	Weight (gm)[2]
0.0	.14 b[3]
2.5	.02a
5.0	.03a
10.0	.03a

1 Amount of asparagus root extract/50 ml PDB.

2 Weight of fungal tissue after 10 days growth in PDB. Each treatment is a mean of 4 replica flasks.

3 Means without a letter in common are significant ($\underline{P}$=0.05) for Duncan's Multiple Range Test.

Sensitivity to Bacterial Isolates Collected from Sandy Soils to Water Extract of Dried Asparagus Root Tissue

Of 54 isolates tested for sensitivity to a sterilized water extract of dried asparagus root tissue, 15 isolates were unable to grow in the area of the plate that contained the 10 µl spot of the root extract. There was a distinct, clear area in the soft agar overlay over the 10 µl spot of root extract. Bacterial growth was normal in all other areas of the plate.

DISCUSSION

Dried asparagus root and rhizome tissues, and extracts of roots and rhizome tissues were inhibitory to *P. ultimum* isolates and 15 of 54 isolates of soil borne bacteria. Conversely, neither of the pathogenic *Fusarium* spp. nor *R. solani* were affected. These data suggest that toxins may play a role in shifting the soil environment to favor the *Fusarium* pathogens. Toxins produced by the plant may create an environment favorable to build-up of Fusarium inoculum thus increasing the odds of these pathogens infecting the asparagus root system. Due to the release of toxic components from senescing tissues and the effect on other microorganisms, Fusarium build-up would be favored when an old plantation is destroyed. The presence of the pathogens in the soil as plant tissue is being broken down and the formation of subsequent resting structures may prove very important in the replant problem.

Data generated in this study indicates that toxic compounds may play a role in Asparagus Decline. Direct and indirect affects on asparagus seedling root disease and the build-up of Fusarium pathogens in the soil needs further investigation.

LIST OF REFERENCES

1. Anonymous, 1977. Michigan Asparagus Survey. Michigan Crop Reporting Service.

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

3. Bell, A.A., 1977. Plant Pathology as influenced by allelopathy. In: Report of the research planning conference on the role of secondary compounds in plant interactions (allelopathy). pp. 64-99. (C.G. McwHorter, A.C. Thompson and E.W. Hauser, eds.) USDA, Agricultural Research Service, Tifton, GA.

4. Booth, C. 1971. The genus Fusarium. Commonwealth Mycological Institute, Kew, Surrey, England. 237 pp.

5. Borner, Horst. 1960. Liberation of organic substances from higher plants and their role in soil sickness problem. Bot. Rev. 26(4):393-424.

6. Chandler, W.A., J.W. Daniell. 1974. Effect of leachates from peach soil and roots on bacterial canker and growth of peach seedlings. Phytopathology 64:1281-1284.

7. Cohen, Sylvan. I and F.D. Heald, 1941. A wilt and root rot of asparagus caused by Fusarium oxysporum Schlecht. Plant Disease Reporter 25:503-509.

8. Cook, M.T. 1923. Dwarf asparagus. Phytopathology 13:284. (Abstr.).

9. Damicone, J.P. and D.R. Cooley. 1981. Benomyl in acetone eradicates Fusarium moniliforme and F. oxysporum from asparagus seed. Plant Disease 65:892-893.

10. DeFrank, J.M. 1977. Allelopathy in Asparagus. Undergraduate Report. Rutgers University. 4 pp.

11. Endo, R.M. and E.C. Burkholder. 1971. The association of Fusarium moniliforme with the crown rot complex of asparagus. Phytopathology 61:891 (Abstr.).

12. Gilbertson, R.L. and W.J. Manning. 1980. Fusarium associated with asparagus flower, fruit, and seed. Phytopathology 70:462 (Abstr.).

13. Gleason, H.A. and Arthur Cronquist. 1963. Manual of vascular plants of the northeastern United States and adjacent Canada. Van Nostrand. New York. 810 pp.

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

15. Grogan, R.G. and K.A. Kimble. 1959. The association of Fusarium wilt with the asparagus decline and replant problem in California. Phytopathology 49:122-125.

16. Grove, M.D. 1976. Fungarial disease of asparagus. Proc. Am. Phytopathol. Soc. 3:317 (Abstr.).

17. Hanna, G.C. 1947. Asparagus production in California. Calif. Agr. Ext. Serv. Circ. 91, 23 pp.

18. Henderson, Peter, 1890. Handbook of plants. Peter Henderson and Co., New York.

19. Hendrix, Jr., F.F. and L.E. Nielson. 1958. Invasion and infection of crops other than the forma suscept by _Fusarium oxysporum_ f. _batatas_ and other formae. Phytopathology 48:224-228.

20. Herner, R.C. and G. Vest. 1974. Asparagus workshop proceeding, Department of Horticulture, Michigan State University, East Lansing, MI. 79 pp.

21. Inglis, D.A. 1980. Contamination of asparagus seed by _Fusarium oxysporum_ f. sp. _asparagi_ and _F. moniliforme_. Plant Disease 64:74-76.

22. Jones, H.A. and J.T. Rosu. 1928. Truck crop plants. McGraw-Hill Book Co., Inc. New York.

23. Johnson, S.A., J.K. Springer, G.D. Lewis. 1979. _Fusarium moniliforme_ as a course of stem and crown rot of asparagus and its association with asparagus decline. Phytopathology 67:778-780.

24. Kitahara, Y., H. Yanagawa, T. Kato, and N. Takahashi. 1972. Asparagusic acid, a new plant growth inhibitor in etiolated young asparagus shoots. Plant & Cell Physiology 13:923-925.

25. Komada, H. 1975. Development of a selective medium for quqantitative isolation of _Fusarium oxysporum_ from natural soils. Rev. Plant. Plot Res. 8:114-125.

26. Kommedahl, T. and J.H. Ohman. 1960. The role of Agropyron repens in the seedling blight epidemiology of alfalfa and cereals. Minnesota Acad. Sci. Proc. 28:10-15.

27. Kommedahl, T., K.M. Old, J.H. Ohman, and E.W. Ryan. 1970. Quackgrass and Nitrogen effects on succeeding crops in the field. Weed Science 18:29-32.

28. Lacy, M.L. 1979. Effects of chemicals in stand establishment and yields of asparagus. Plant Disease Reporter 63:612-616.

29. Laufer, G.A. and S.A. Garrison. 1977. The effect of asparagus tissue on seed germination and asparagus seedling growth. Possible alleopathic interactions. Horticultural Science 12:385 (Abstr.).

30. Lawrence, G.H.M., 1982. Taxonomy of Vascular Plants. MacMillan Co. New York. 823 pp.

31. Lewis, G.D. and P.B. Shoemaker. 1964. Presence of Fusarium oxysporum f. asparagi on asparagus seed and fusarium resistance in plant introduction lines of asparagus. Phytopathology 54:128 (Abstr.).

32. Lewis, G.D. and P.B. Shoemaker. 1964. Resistance of asparagus species to Fusarium oxysporum f. asparagi. Plant Disease Reporter 48:364-365.

33. MacHardy, W.E. and C.H. Beekman. 1981. Vascular wilt Fusaria: Infection in pathogenesis, In: Fusarium Diseases. Biology and Taxonomy. P.E. Nelson, T.A. Toussoun, R.J. Cook, eds. Pennsylvania State University Press. University Park. 457 pp.

34. Manning, N.J. and P.M. Vardaro. 1977. Soil fumigation and preplant fungicide crown soaks effects in plant growth and Fusarium incidence in newly planted asparagus. Plant Disease Reporter 61:355-357.

35. Martin, 1975. Comparison of agar media for counts of viable soil bacteria. Soil Biol. Biochem. 7:401-402

36. Messiaen, C.M. and R. Cassini. 1981. Taxonomy of Fusarium in: Fusarium Diseases Biology and Taxonomy. P.E. Nelson, T.A. Toussoun, R.J. Cook, eds. Pennsylvania State University Press. University Park, PA. 457 pp.

37. Nelson, P.E., T.A. Toussoun and R.J. Cook (eds.). 1981. Fusarium Diseases, Biology and Taxonomy. 457 pp.

38. Patrick, Z.A. 1955. The peach replant problem in Ontario II. Toxic substances from microbial decomposition of products of peach root residues. Can. J. Bot 33:461-486.

39. Patrick, Z.A. and L.W. Koch. 1963. The adverse influence of phytotoxic substances from decomposing plant residues on resistance of tobacco to black root rot. Can. J. Bot. 41:747-758.

40. Putnam, A.R. and W.B. Duke. 1978. Allelopathy in agroecosystems. Ann. Rev. Phytopath. 16:431-451.

41. Putnam, A.R., K. Hassett, J.F. Fobes, and R.M. Hodupp. 1982. Evidence for allelopathy and autotoxicity in Asparagus officinalis L. (unpublished).

42. Rice, E.L. 1974. Allelopathy. Academic Press. New York. 353 pp.

43. Rice, E.L. 1979. Allelopathy - An update. The Botanical Review 45:15-109.

44. Rouser, G. and S. Fleischer. 1967. Isolation characterization and determination of polar lipids of Mitochondria. In Methods in Enzymology. Vol X. R.W. Eastbrook and M.E. Pullman, Eds. Academic Press, New York and London.

45. Schreiner, O. and H.S. Reed. 1908. The toxic action of certain organic plant constituents. Bot. Gaz. 45:73-102.

46. Schroth, M.N., and D.C. Hildebrand. 1964. Influence of plant exudates on root infecting fungi. Annual Rev. Phytopathology 2:101-132.

47. Shafer, W.E. and S.A. Garrison. 1980. Effects of decomposing asparagus root tissues on lettuce, tomato and asparagus seed emergence. (Abstr.). HortScience 15:406.

48. Shafer W.E., and S.A. Garrison. 1980. Effects of asparagus root extracts on lettuce and asparagus seed germination and growth. (Abstr.) HortScience 15:406-407.

49. Shelton, D.R. 1978. Effect of stresses on growth and yield of asparagus (Asparagus officinalis L.). MS thesis. Michigan State University.

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

51. Stowe, L.G. 1979. Allelopathy and its influence on the

distribution of plants in an Illinois old-field. J. of Ecology 67:1065-1085.

52. Takatori, F., and F. Souther. 1978. Asparagus workshop proceedings. Dept. Plant Sciences, University of California, Riverside. 156 pp.

53. Tiedjens, V.A. 1924. Some physiological aspects of Asparagus officinalis. Proc. Amer. Soc. Hort. Sci. 23:189-196.

54. Thompson, H.C. and W.C. Kelly. 1957. Vegetable Crops. 5th ed. McGraw-Hill. New York. 611 pp.

55. Toussoun, T.A., and Z.A. Patrick. 1963. Effect of phytotoxic substances from decomposing plant residue on root rot of beans. Phytopathology 53:265-170.

56. Tuite, J. 1969. Plant pathological methods. Burgess Publishing Company. Minneapolis, MN.

57. VanBakel, J.M.M., and J.J.A. Kerstens. 1970. Foot rot in asparagus caused by Fusarium oxysporum f. sp. asparagi. Neth. J. Pl. Path. 76:320-325.

58. Vesonder, R.F., and C.W. Hesseltine. 1981. Metabolites of Fusarium. 350-364. In: Fusarium Diseases, Biology and Taxonomy. P.E. Nelson, T.A. Toussoun, R.J. Cook, eds. Penn. State University Press, University Park. 457 pp.

59. Walker, John Charles. 1952. Diseases of vegetable crops. McGraw-Hill Book Company. New York.

60. Whittaker, R.H. 1975. Communities and ecosystems, 2nd ed. MacMillan Pub., Inc. New York. 358 pp.

61. Woltz, S.S., and J.P. Jones. 1981. Nutritional requirements of Fusarium oxysporum: Basis for a disease control system. In: Fusarium Disease, Biology and Taxonomy, P.E. Nelson, T.A. Toussoun, R.J. Cook, eds. Penn. State University Press. 457 pp.

62. Yang, Hsu-Jen. 1982. Autotoxicity of Asparagus officinalis L. J. Amer. Soc. Hort. Sci. 107(5):860-862.