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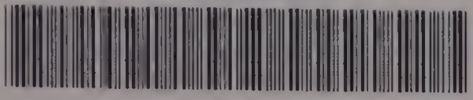
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SOURCES OF INOCULUM AND DISEASE INCREASE OF STEM, CROWN AND ROOT ROT OF ASPARAGUS CAUSED BY *FUSARIUM OXYSPORUM* AND *FUSARIUM MONILIFORME*

A Thesis Presented

By

ROBERT L. GILBERTSON

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

February 1981

DEPARTMENT OF PLANT PATHOLOGY

SOURCES OF INOCULUM AND DISEASE INCREASE
OF STEM, CROWN AND ROOT ROT OF ASPARAGUS CAUSED BY
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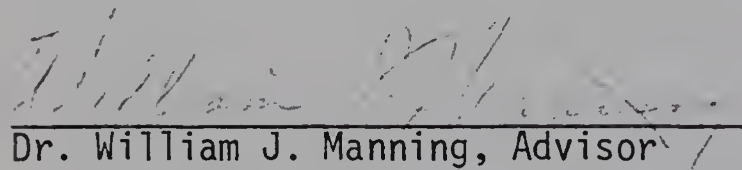

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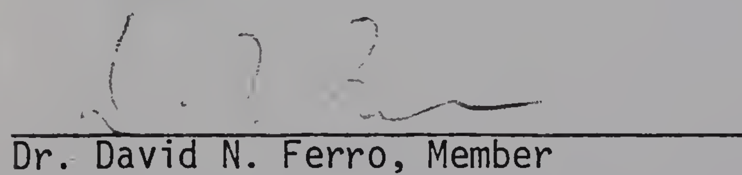

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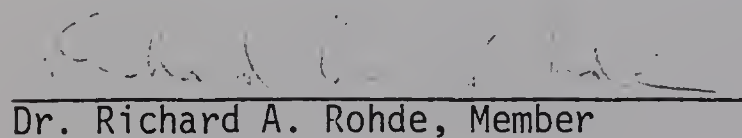

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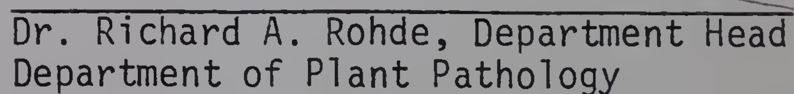

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ABSTRACT

Stem, crown, and root rot caused by *Fusarium oxysporum* and *F. moniliforme* is well established in local growers fields and is threatening the future of the asparagus industry in Massachusetts. *F. oxysporum* is soil-borne and exists in high populations in local beds. Both pathogens can be seed-borne and are disseminated with propagative stock: seeds and crowns. The pathogens attack different parts of the asparagus plant, and utilize different means of dissemination and survival. *F. oxysporum* parasitizes crowns, feeder roots and storage roots; and exists in soils as a saprophyte, as chlamydospores or in association with volunteer plants or weed hosts. *F. moniliforme* attacks aboveground plant parts such as flowers, fruits, and stems; and survives on seeds, volunteer plants, and in association with asparagus miner flies. Both pathogens are favored by the mining of asparagus miner flies and by other asparagus insect pest damage. Different control methods are necessary for management of the disease, due to the different biology of the pathogens. New beds can't be established near or in soils previously in asparagus culture, and propagative stock must be clean of *Fusarium*. Proper weed, insect control, and cultural practices must be employed for successful asparagus culture; and continued research for a resistant variety is crucial.

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INTRODUCTION

The Problem

Commercial asparagus (*Asparagus officinialis* L.) production in the United States has been steadily declining over the past twenty years. Total United States acreage has decreased over 35% since 1959 (93). The asparagus decline has been even more severe in Massachusetts. Commercial production in Massachusetts declined from 18,000 hundred weight in 1966 to 7,000 hundred weight in 1975, while acreage declined from 900 to 450 during the same period (69). This represents a decrease of over 50% in less than ten years, indicating the severity of the problem. Only the increased average price of asparagus, from $20.60 per CWT in 1966 to $41.50 in 1975 (69), has kept asparagus culture in Massachusetts profitable. Despite the high price for asparagus, many local growers are turning under asparagus fields and planting other crops such as peppers, potatoes, corn and cabbage.

Asparagus growers have been complaining of a decline in spear production and plant vigor throughout western Massachusetts, where most Massachusetts asparagus is grown. Established beds of asparagus remain productive for only 8-10 years, well below the expected 15-20 years of production. Plants yellow, wilt and die, leaving irregular skips throughout fields of plants. Beds decline to a point where the grower is no longer able to obtain profitable yields of asparagus,

despite high prices. These fields are often turned under, and planted to other crops or sold for other uses. Growers have been unable to replant asparagus in fields previously in asparagus culture. The decline of Massachusetts asparagus has occurred in all growers fields to some extent, and few fields produce to the extent they did 15-20 years ago. One grower reported that fields which once yielded 75-100 boxes of asparagus per acre now only yield 35-40 boxes. This type of reduction in production is typical of most growers and has recently threatened the entire future of the Massachusetts asparagus industry.

Massachusetts Asparagus Decline -- Why

There have been no major climatic changes in Western Massachusetts over the past 10-15 years which could explain such a dramatic decline in one particular crop plant (8). Asparagus varieties grown in Massachusetts have generally responded well to the climate of the region since people began growing asparagus in the early 1900's. Western Massachusetts farmers growing asparagus in the early and mid-1900's did follow certain different cultural practices and encountered different pest pressures than did the present day growers. I examined cultural practices and insect and disease pressure from these two different time periods to determine if differences in any of these factors influenced asparagus decline in Western Massachusetts. Table 1 summarizes the major differences in asparagus culture between the two time periods, and possible effects on present day asparagus culture

are mentioned. The information in this table was compiled from growers recommendations of 1935 and 1979, information from many local growers, and personnel observation and research.

The most significant difference is the high incidence of Fusarium stem, crown, and root rot caused by *Fusarium oxysporum* and *F. moniliforme* in present day fields. Both pathogens can be readily isolated from declining plants in growers fields throughout Western Massachusetts, and are believed to be primarily responsible for asparagus decline in Massachusetts (8,9,36,37,68). Crown and root rot caused by *F. oxysporum* has been shown responsible for asparagus decline in states such as California (1, 41,42), Washington (21,22), New Jersey (50,51) and Michigan (61); and countries including Germany (94), Taiwan (92), and Canada (38). *F. moniliforme* has also been associated with asparagus decline (29) and may play a major role in the disease in certain areas(51).

The stem, crown, and root rot disease complex may have been favored by a number of these factors. Changes in cultural practices leading to decreased organic matter in asparagus soils may cause unfavorable changes in soil structure, drainage and microflora for the plant. Harrowing of beds before, during, and after the growing season damages roots and crowns, increasing potential infections by pathogenic soil-borne fusaria. High weed populations may compete with plants for water and nutrients and harbor pathogenic fusaria. The leaving of senescing and often diseased or insect-ridden stalks in asparagus beds can provide

TABLE 1. COMPARISON OF CULTURAL PRACTICES, INSECT PESTS, AND DISEASES IN COMMERCIAL WESTERN MASSACHUSETTS BEDS, PAST AND PRESENT

FACTOR	SPECIFIC PRACTICE, INSECT PEST, OR DISEASE: PAST (1935)	PRESENT (1979)	POTENTIAL EFFECT ON ASP CULTURE (1979)
Cultural Practices	Cover crops grown prior to establishing new beds.	Not a common procedure	Decreased organic matter content of soil, loss of any potential beneficial effect on soil microflora.
	Use of both chemical fertilizers and organic matter additions (manure).	Use of chemical fertilizers only.	Lack of slow release and non-leachable nutrition.
	Weed control by cyanamid or by physical (labor) means.	Weed control by harrowing or herbicide.	Physical damage to plants, herbicide toxicity.
	Senescing stalks harrowed into field, removed, or burned.	Stalks often left in field.	No addition of nutrients to soil, potential insect and pathogen populations sustained, increased.
	Beds well maintained throughout growing season.	Beds often not maintained.	Increased weed, insect, disease pressure; plant strength not maximum, other pressures favored.
Insect	Common Asparagus Beetle major insect problem -- foliar feeder.	Common Beetle not a major problem.	Less foliar damage.
	12-spotted asparagus beetle recent introduction from Europe, not a major problem.	12-spotted beetle well established major problem, adults foliar feeders, larvae fruit feeder.	Increase of foliarand fruit damage, facilitate berry and seed infection with fungi, weaken plant substantially.

TABLE 1. Cont'd.

FACTOR	SPECIFIC PRACTICE, INSECT PEST, OR DISEASE: PAST (1935)	PRESENT (1979)	POTENTIAL EFFECT ON ASP CULTURE (1979)
Insect	Asparagus miner fly present in most fields, but not to a major pest.	Miner very common, stems damaged by larvae.	Favor stem disease, premature decline and weakening of plant.
Disease	Rust major disease factor.	Resistant varieties developed.	These varieties (i.e., Mary Wash) may be more susceptible to other diseases.
	Phoma stem blight not reported.	Phoma stem blight extremely common in some fields on dying stems.	Lead to weakening and decline of plants.
	Fusarium disease not a major problem; 5-10% fields reported afflicted.	*Fusarium* crown, stem and root rot complex in all commercial fields and the major disease problem.	Primary cause of asparagus decline in Western Massachusetts asparagus beds, (probably favored by other pressures).

potential inoculum sources for fusaria and insect pests the following growing season. The recently observed *Phoma* stem blight caused by *Phoma asparagi*, and insect pest problems may further contribute to the decline of asparagus plants.

Efforts to revive the Western Massachusetts asparagus industry depend on effective management of the stem, crown, and root rot disease complex, which is most effectively formulated based on a thorough knowledge of the asparagus plants, the fusaria involved, and other factors influencing disease expression.

Purpose

Both asparagus researchers and growers have been alarmed by the rapid decline of asparagus in Western Massachusetts over the past 10-15 years, and an effective management program for the disease complex is being sought. Much is known about the asparagus plant itself, but there is little information on the relation of the pathogens *F. oxysporum* and *F. moniliforme*, to the plant. The purpose of this study is to determine the biology of these two pathogens in respect to asparagus plants and local soils; and to determine why the *Fusarium* disease complex has recently become the most serious asparagus problem in Western Massachusetts.

The Asparagus Plant

Asparagus (*Asparagus officinialis* L.) is a member of the family Lilaceae, and is a native species of the Mediterranean region, parts

of Russia, and the British Isles (97). People have been eating asparagus for well over 2000 years (44); and it has been cultivated as a crop plant from at least the time of the Roman scholar Cato the Elder (234-149 B.C.), who described Roman asparagus culture in his volume on agriculture, De agriculture (8). The spread of asparagus through Europe was partially due to Roman troops who established asparagus beds throughout the Roman empire. Asparagus was introduced to North America and other parts of the New World by early European settlers. Asparagus is presently commercially grown throughout the world in countries such as Brazil, Taiwan, Canada, Mexico, the United States, Germany, and Great Britain; and is a highly prized vegetable crop by many peoples.

United States Production and Importance

The cultivated form of asparagus in the United States is commercially grown in states including California, New Jersey, Michigan, Washington and Massachusetts. Of the 22 major vegetable crops asparagus ranked tenth in acreage and fourteenth in value in 1973 (97). Over half of the asparagus produced in the United States between 1974-76 was processed, either canned or frozen; and the remainder was sold fresh (44). California, Washington, and Michigan accounted for close to 50% of the total processed crop, and 90% of the total fresh crop. Most of the present United States asparagus industry is concentrated in states on the West Coast and in the Great Lakes region with New Jersey the leading eastern state in asparagus production. Most Massachusetts grown asparagus is sold fresh, and a high local demand

assures local growers of high prices and a readily available market.

Plant Characteristics and Culture

Asparagus is a perennial herbaceous crop which can live over 30 years. Commercial asparagus beds have been known to have remained productive for well over 20 years. The harvested crop consists of the tender, immature, aboveground stems (spears) of the plant, which are cut when 15-25 cm. high (44). The plants must be in their third year of growth before they can be harvested, to assure normal spear size and plant longevity. Asparagus is propagated through seed, although most growers plant commercially produced 1-year-old root systems, or crowns. Some growers have begun the use of 8-10-week-old transplants for establishing new beds. The below ground part of the plant consists of a central crown or rhizome (Figure 1) from which thick fleshy storage roots arise. These fleshy roots grow laterally from the crown at a rate of 8-14" a year for 2-4 years, after which they naturally senesce and are replaced by new storage roots (26). The crown and storage roots store carbohydrates necessary for spear production in the spring. Fine absorbing roots arise from the storage roots in all directions and complete the root system of the plant, which may extend 3-5 feet from the crown. The absorbing roots take in water and nutrients necessary for plant growth. Buds arising from the crowns in the early spring develop into aerial stems. Spears cut for market are harvested every 1-5 days during a 6-10 week period beginning in late April or early May and ending in mid to late June (44). Harvesting time and pressure depends on the age of the bed and the climate in the

area. Stems which aren't cut and continue growth are called stalks, and these develop into ferns during the growing season and can reach heights of over 1 meter. Ferns develop small triangular scale like "leaves" which contain chlorophyll and photosynthesize. These needle-like "leaves" aren't true leaves, but modified stem branchlets or cladophylls (26), with the true leaves being the small vestigial scales found on stems and young shoots (Figure 1). Sucrose is the major photosynthetic product (26) and it is transported to the crown and storage roots for storage throughout the growing season, which lasts until mid to late September. The ferns senesce during the late fall and become brown and die naturally. The previous years dead ferns will often persist into the next growing season if not removed.

The asparagus plant is dioecious with separate male and female plants which produce male and female axillary flowers. Most of the flowers are imperfect and female and male flowers occur naturally in about a 1:1 ratio (26). Flowers are primarily insect pollinated and pollinated pistilate (26) flowers will give rise to fruits, which are small spherical to round berries (97). The fruits are green when immature and then turn red as they ripen later in the growing season. Ripe fruits contain 3-8 black, angular seeds which contain hemicellulose as the primary reserve carbohydrate (26). Male plants grow faster, live longer and produce more spears than female plants, which are larger in size and produce larger spears (26, 44, 97).

General Asparagus Culture

Commercial asparagus beds are started with seeds, crowns or 3-month-old transplants. Large vigorous crowns should be selected from any lots of 1-year-old crowns used, and at least 50% of the crowns are sometimes discarded to assure maximum yields (100). Good seed is difficult to obtain, but can be used to establish vigorous beds. Three-month old transplants are possibly the best method presently available. The seedlings grow vigorously in the greenhouse for 3 months, and intact root systems are planted in late spring or early summer. Resulting plants are vigorous and become large by the fall, and are ready for harvest in two seasons. Sandy soils with high amounts of organic matter are best suited to asparagus culture. Muck soils won't provide adequate aeration for proper plant growth. The soil pH must be maintained between 6-7, and hydrated lime or ground limestone can be used to adjust acidic soils to the proper pH. When establishing a new bed using crowns or transplants, the planting material should be initially planted 2-4" deep in a one foot deep furrow. The crowns should be 18" apart and transplants 6" apart. Furrows should be 3-5' apart (100). The furrows are gradually filled with soil as the plants grow during the first growing season, and furrows are filled by the end of that period. The bed is then permanently established, and harvesting can begin in one season for crowns and two for transplants. Fertilizers should be applied before and after the harvesting period, and weeds should be effectively controlled to assure a healthy, productive asparagus bed.

Asparagus Culture in Massachusetts

Asparagus has been commercially grown in Massachusetts since at least the mid to late 1800's when beds were first established in the eastern parts of the state. By 1935 asparagus acreage in Massachusetts had reached close to 2000 acres, with 2 eastern counties, Middlesex and Barnstable, accounting for nearly half the total acreage (100). Hampshire County and other western counties had much less asparagus acreage than eastern counties at this point, but low tobacco returns were causing growers to establish asparagus beds in Western Massachusetts (100). From 1935 on, eastern Massachusetts acreage began to decline due to urban development, and a severe root and crown rot disease; and western Massachusetts production began to increase. During 1940-1950 the western counties became the leading asparagus producing counties, and at some point the town of Hadley, Massachusetts in Hampshire County earned the title: "The asparagus capital of the world" due to the fine tasting fresh asparagus from that town. Presently there is little or no commercial asparagus production in eastern Massachusetts. The decline in eastern Massachusetts acreage resulted in an overall decrease in Massachusetts asparagus acreage, and in 1965 the total Massachusetts asparagus acreage was about 900 (64), most of which was in Western Massachusetts. During the last 10-15 years asparagus acreage and production has declined over 50% to around 400 total acres. Thus, western Massachusetts asparagus acreage is now declining rapidly, and this later decline in respect to the eastern part of the state can be accounted for

by the later advent of asparagus culture in western Massachusetts. The decline in both regions has been attributed to the increased incidence of the *Fusarium* stem, crown, and root rot complex in commercial asparagus beds (89, 100).

The Genus Fusarium

Fusarium species are widely distributed in soils, on plants, and on various organic substrates. They are commonly encountered by plant pathologists as parasites or saprophytes on many different plant species. Plant-parasitic fusaria cause vascular wilts, seedling blights, crown and root rots, and other diseases of crop plants, ornamentals, trees and other plants. Fusaria cause serious diseases in many crops including tomatoes, beans, potatoes, onions, sugarcane, and cotton. *Fusarium oxysporum* f. sp. *cubense*, which causes the Panama disease of bananas, almost wiped out the entire Central American banana industry until a resistant variety was found. Other fusaria cause storage rots of food materials, and some species produce mycotoxins which make stored foods unfit for human or animal consumption. Fusaria have also been associated with human and animal diseases.

Fusaria are imperfect fungi in the Class Deuteromycotina. Link established the genus *Fusarium* in 1809 for fungi with fusiform spores borne on a stroma (13). These hyphomycetous fungi produce conidiophores on aerial hyphae, and are in the Order Moniliales. Spores and conidiophores are often supported on a mass of interwoven hyphae which forms a stroma or pseudotissue termed a sporodochium. Sporodochium

formation is a characteristic of the genus *Fusarium* and the genus is thus included in the family Tuberculariaceae (fungi which form sporodochia). The major taxonomic characteristic of the genus is the asexual, multicelled, fusiform, macroconidium; which can be produced by all fusaria.

Classification

Most keys to the genus are based on macroconidia (90). Other asexual spore types including microconidia and chlamydospores may aid in the taxonomy of fusaria, depending on the species involved. Microconidia are small, usually one-celled, spherical, oval, or kidney shaped spores borne on simple or branched hyphae. Chlamydospores are thickwalled survival spores formed from single hyphae cells. These spores form in response to adverse environmental or nutritional condition. Not all fusaria form chlamydospores (i.e. *F. moniliforme*) or microconidia (i.e. most *F. roseum* cultivars). Other fusaria have Ascomycetous perfect states involving the formation of ascospores in perithecia, but these states rarely play a major role in the life cycle of the fungus (11). The genus *Fusarium* initially had over 1000 species, varieties, and forms; many of which were based on casual superficial observations (90). Possible relationships between these species were often overlooked or not considered, and many names were assigned to single species of fusaria. The need for a logical precise classification system compelled Wollenweber and Reinking (99a) to create a classification scheme for fusaria which they outlined in *Die Fusarien* in 1935(99a).

Wollenweber and Reinking reduced the genus to 143 species, varieties, and forms; which were included in 16 sections, or aggregations of related species. Though greatly decreasing the taxonomic confusion within the genus, Die Fusarien was found difficult or impractical by many plant pathologists. As knowledge of the genus increased, more practical taxonomic systems appeared, and these systems were based on Wollenweber and Reinking's original work.

During the early 1940's Snyder and Hansen proposed a system reducing all fusaria to 9 species and a number of non-taxonomic cultivars (84). Gordon ,1952, devised a system which was primarily based on Wollenweber and Reinking, but incorporated part of Snyder and Hansen's system for 2 sections (Elegans and Martiella). Booth, 1971, based his system on that of Gordon, and came up with 44 species and 7 taxonomic varieties; which were placed in 12 sections. Other systems include those of Bilay, 1971, in which there are 26 species in 9 sections; and Cassini, 1968, which is wholly based on the Snyder and Hansen system but replaces Snyder and Hansen's non-botanical, nontaxonomic cultivars with botanical, taxonomic varieties (90). The system of Snyder and Hansen, and to a lesser extent Booth were primarily used in this research.

Snyder and Hansen attempted to devise a simple, practical, and dependable system for plant pathologists and other biologists. Many of Wollenweber and Reinking's criteria for speciation involved slight differences in the length and width of conidia, spore septation, chlamydospore position and other cultural characteristics (84). Snyder

and Hansen proposed a 9 species system in which the species coincided with the sections designated by Wollenweber and Reinking. The major characteristic for species separation is the shape of macrocondia, with secondary characteristics such as presence or absence of microconidia and chlamydospores and microconidial shape also considered. The authors later added cultivars to differentiate between large groups of fusaria placed in a single species; especially the large species, F. roseum (90). Cultivars are nonbotanical, horticultural variety names which allow for modifications within the 9 species system without the addition of new species (90). Snyder and Hansen never created a key for their system, but later authors did, including Messiaen, Matuo, and Toussoun and Nelson (91). Toussoun and Nelson's pictorial guide to the fusaria was primarily used in this study.

Booth's system is primarily based on Gordon's system (38). Booth considered spore morphology to be the primary taxonomic character, along with macroconidia size, conidophore morphology, colony pigmentation and other secondary characters. Booth emphasized the morphology of the sporogenous cells as the most important character (90). Variation of the sporogenous cell has yet to be analyzed statistically, and may prove unreliable for speciation (90). Booth also differentiates some species based on perfect states, and these perithecial states are often uncommon and difficult to use for routine identification of fusaria. Booth's system is more complex than that of Snyder and Hansen, and may not account for sporogenous cell variability; but it is helpful in understanding the fusaria. Particularly useful is a summary

of diseases caused by particular fusaria in his volume The Genus Fusarium.

Plant Pathogenic Fusaria

Importance and General Characteristics

Fusarium species cause disease in many different annual and perennial crop plants, and utilize many different means of dissemination and survival. Plant pathogenic species exhibit varied host ranges and substrate preferences, and cause many different types of plant diseases (13). Certain strains of some fusaria, such as F. oxysporum and F. solani, have been shown to cause disease on specific crops; and are termed forma specialis (f. sp.). Forma specialis strains are morphologically identical, and differ only in which hosts they are able to infect (2). F. oxysporum has many forma specialis strains which cause diseases in over 100 different host plants, including tomato, beans, cucumber, potato, and chrysanthemum (13). Other strains of F. oxysporum are strictly saprophytic and cause no plant diseases (34). Some fusaria have no recognized forma specialis strains, and cause disease in many different plant species. F. moniliforme causing disease in crops such as corn, sorghum, cotton, sugar cane, and rice has not been shown to exist in distinct strains causing disease in specific host plants. A morphologically distinct cultivar of F. moniliforme, F. moniliforme var subglutinans, has been shown to exist; and causes diseases on similar crops, including sugarcane and corn (91). A cultivar of F. oxysporum, F. oxysporum var redolens has been described (91) and is believed to

cause asparagus seedling blight (90).

F. moniliforme, F. moniliforme var subglutinans and F. oxysporum are responsible for devastating plant diseases. These pathogens cause a variety of diseases ranging from root rots to wilts and yellows.

Specific Fusaria and Plant Diseases Caused

F. moniliforme

F. moniliforme is the causal agent of the bakanae disease of rice, one of the most serious rice diseases in Asia (13). The characteristic symptom of the disease is hypertrophy of young rice shoots due to increased gibberellin levels caused by the fungus (13). F. moniliforme also causes cotton seedling blight, root rot, and pink boll; and storage rots of tomato, banana, and pineapple (13). More recently, F. moniliforme has been shown to cause seedling blight and root of maize and sorghum (13), and stalk rot and kernel infection in corn (60, 68). The fungus can be readily identified in culture by the formation of microconidia in chains (91). F. moniliforme is disseminated by soil, seed, and airborne spores, and is able to overwinter in organic matter, plant debris and seed (13).

F. moniliforme var subglutinans

F. moniliforme var subglutinans has been shown to cause a number of plant diseases. F. moniliforme var subglutinans is morphologically similar to F. moniliforme except for how microconidia are borne (91). F. moniliforme var subglutinans bears microconidia on polyphialides

in falseheads while F. moniliforme bears conidia in long chains (91). F. moniliforme and F. moniliforme var subglutinans don't form chlamydospores, and may use thick-walled hyphal cells for survival (34). F. moniliforme var subglutinans has a host range which overlaps F. moniliforme, and these two fungi are easily confused, especially in diseases where both are involved. Both fungi have been shown to cause stalk rot and kernel rot of maize (13). Bolkan, Dianese, and Cupertino demonstrated F. moniliforme var subglutinans to be the cause of pineapple fruit rot and to cause infections via flowers (10, 11). Dissemination of F. moniliforme var subglutinans is by soil, seed, or air; and the fungus overwinters in plant debris, organic matter, and seeds (13).

F. oxysporum

F. oxysporum is the most economically important species of Fusarium (13). It is differentiated from F. moniliforme and F. moniliforme var subglutinans based on conidial morphology and its ability to form chlamydospores (91). These resistant spores enable F. oxysporum to survive efficiently in soils for long periods of time, unlike F. moniliforme and F. moniliforme var subglutinans which must be associated with organic matter or plant debris for soil survival (13). Pathogenic strains are frequently separated into forma specialis based on what host they are known to attack, although the demonstration of overlapping host ranges and alternate hosts for different forma specialis has raised questions about the validity of this separation (4, 45, 87, 96).

Symptoms caused by the pathogen can vary, but the most well known and commonly found symptoms are vascular discoloration and wilting. Plants of all ages can be attacked by F. oxysporum and the pathogen is frequently soil-borne (13, 34). Many of these diseases occur on annual plants such as beets, beans, spinach, onions, tomatoes, cabbage and cucumber (13). Growers are able to utilize effective management methods, such as soil fumigation, between yearly plantings of annual crops. This is not the case for the perennial host plants such as asparagus, banana, and coffee, which are constantly exposed to soil-borne populations of F. oxysporum. The perennial nature of the crop prevents the use of effective soil treatments using toxic chemicals. Diseases of perennial plants often result in prolonged declines of host plants, while in annuals a rapid vascular wilt usually kills plants in a short time period (13). Therefore, diseases of perennial plants caused by F. oxysporum must be viewed differently than disease of annual plants.

F. oxysporum f. sp. cubense which causes Panama disease of banana is a soil-borne pathogen which causes a root, rhizome, and stem rot of banana which eventually enters the plant vascular system causing wilting and death of the plant. The eventual decline and death of plants is due to a gradual increase of the pathogen population in the soil under intensive cultivation of a single crop (34). The pathogen has been shown able to invade and colonize other hosts than banana (45). A variety of weed species were shown to have F. oxysporum f. sp. cubense associated with their root systems (96). The fungus was also shown to persist in soils for well over 10 years in absence of banana host plants (34).

This was attributed to chlamydospore production and saprophytic ability. The pathogen is disseminated via soil, air, water, plant debris, and infected propagative material (13, 34). In other diseases, *F. oxysporum* is disseminated through soil, seed, drainage water, plant debris, and plant materials (13). The fungus overwinters in soil, seed, or with organic matter or plant debris in soil (13); and is a competitive saprophyte able to survive long periods in absence of host plants.

Fusaria on Asparagus

Fusarium associated with diseased asparagus was first reported in Massachusetts in 1908 by Stone and Chapman, who isolated a *Fusarium* species from young, wilted asparagus shoots. Such diseased shoots would later exhibit stem lesions and a yellowed, wilted appearance. Cook, in 1923, reported a similar disease of asparagus caused by *Fusarium* from New Jersey, and called the disease "dwarf asparagus." Plants were stunted, and vascular discoloration was evident in some plants. An unidentified *Fusarium sp.* was readily isolated from diseased crown tissue (23). Reports on plant diseases found in the United States in 1921, 1924, 1935, 1936 and 1937 mention a stem rot of asparagus caused by *Fusarium* to occur in Massachusetts, New York, New Jersey, and California (22). The reports from 1935 and 1937 attribute a yield reduction of 5 and 8% respectively to the stem rot disease. German researchers described a vascular foot rot of asparagus caused by *Fusarium culmorum* in 1929 (22). Boyd in 1930 and 1940 reported fusaria associated with diseased asparagus in Massachusetts, while Armstrong in 1930 and Kirby

in 1943 described a similar fusarial disease from South Carolina and Pennsylvania, respectively. Other later reports from across the U.S. began associating *Fusarium* species with a root, crown, and stem rot of asparagus plantings.

Cohen and Heald, in 1941 and 1946, first described the causal agent of the wilt and root rot disease of asparagus to be *Fusarium oxysporum* (Schlect) var *asparagi*. The pathogen was isolated from diseased plants exhibiting root rot and wilt in Washington. The authors believed this disease to be different from the foot rot of asparagus caused by *F. culmorum* in Germany (22). The disease appeared in the field as yellowing stalks which often exhibited a red-brown discoloration of vascular tissue. A red-brown stem rot appeared at the base of some infected stems and the pathogen often fruited on infected tissue (21). Infected storage roots showed elliptical red-brown lesions which often extended into the stele of the root and vascular tissue of the crown. Heavily diseased roots collapsed and dried out, leaving only epidermal tissue intact. Secondary invaders colonized the remaining root tissue. The pathogen was believed to infect plants via above and below ground wounds, and harvesting and harrowing of beds further increased disease severity (22). Newly planted transplants developed lesions even without wounding, indicating direct penetration of the host. The authors found many superficial discolored lesions on stems which didn't yield *F. oxysporum* and concluded that these lesions represented incipient infections. *F. oxysporum* was consistently isolated from vascular systems of diseased plants, and isolates were able to cause typical root rot and wilt symptoms

on asparagus seedlings in the greenhouse. These isolates were non-pathogenic on potato, tomato, carnation and onion. The authors concluded that the fungus was able to initiate a symptomological complex in the host and colonize roots, crowns and stems. They hypothesized that the pathogen survived as a saprophyte on senescing asparagus tissue during the winter and spring, and attacked living tissue in the summer (22). Cohen and Heald also described an asparagus seedling wilt caused by a Fusarium species in seedlings planted in a corn field, but no confirmation of the identity of the fungus was made.

Graham in 1955 reported a Fusarium seedling blight of asparagus in Ontario. Affected seedlings appeared stunted, yellowed, and wilted; and some died. Blank spaces in seedling rows indicated a high mortality rate due to the blight. Fronds, shoots, and roots of diseased seedlings showed elliptical red-brown lesions (40). Graham identified the causal agent of the blight to be F. oxysporum Schlecht. emend. Snyder and Hansen var. redolens (Wr.) Gordon, and to a lesser extent F. moniliforme. Both pathogens were restricted to cortical tissues of asparagus seedlings. F. oxysporum var. redolens effectively colonized crown, root, and shoot cortical tissues, while F. moniliforme caused root tip necrosis. F. moniliforme was isolated from seeds and stalks of asparagus plants in the field, but was rarely isolated from diseased seedlings in the field (40). F. moniliforme was also observed to form tufts or dense mats of mycelium on dead or dying asparagus stalks after periods of rain (40). Graham discounted F. moniliforme as a major causal agent of seedling blight. Soil-borne inoculum of F. oxysporum var redolens was considered

the major cause of seedling blight, and the pathogen wasn't found seed-borne by Graham (40). The pathogen was found in soil samples from fields never in asparagus culture, with the highest incidences in fields near or adjacent to established asparagus fields. Soil with known histories of asparagus culture were found to harbor *F. oxysporum* var *redolens* pathogenic to asparagus after over 10 years of culture in other crops such as potato, tomato, and raspberry. The author concluded that *F. oxysporum* var *redolens* may be indigenous to Ontario soils; and that the pathogen is favored by specific substrates, such as decaying asparagus roots, to cause seedling blight. The fungus is also able to survive saprophytically, on non-living substrates, or be associated with other non-host plants, such as alfalfa (40). *F. oxysporum* var *redolens* pathogenic to asparagus was unable to cause disease on crops such as spinach, oats, and corn; but was able to cause disease on gladiolus, another member of the Lilaceae family. Results of pathogenicity tests using inoculum of *F. oxysporum* var *redolens*, *F. moniliforme*, and both fungi together indicated an apparent specialization to substrates. Seedlings inoculated with both fungi showed a combination of symptoms including cortical decay and root tip necrosis. Seedlings inoculated with *F. moniliforme* exhibited only root tip necrosis, while those inoculated with *F. oxysporum* var *redolens* only showed cortical decay. *F. oxysporum* var *redolens* was better able to cause seedling blight (40). Graham found *F. oxysporum* var *redolens* able to utilize starch better than *F. moniliforme*, while *F. moniliforme* was able to utilize cellulose better than *F. oxysporum* var *redolens*. Not all isolates of *F. oxysporum* var *redolens* were able to cause seedling

blight, and Graham concluded that parasitic and saprophytic strains of the species exist. He also stated that further specialization within the species may result in individuals with parasitic capabilities ranging from those able to initiate disease by direct penetration to these requiring a means of entry (40).

Grogan and Kimble in 1954 and 1959 showed *F. oxysporum* f. sp. *asparagi* (Cohen and Heald) to be primarily responsible for the asparagus decline and replant problem in California. The authors described a decline of producing beds and an inability to establish new beds with crowns or seeds. Seedlings became yellowed, wilted, and died, 2-3 months after planting in soils previously in asparagus culture (42). Typical red lesions appeared on infected roots, crowns, and stems; and vascular discoloration was seen in many infected plants. Red discoloration of crown vascular tissue often originated from areas where spears had been harvested (42). Stalks would show lower stem lesions and occasional red-brown vascular discoloration. *F. oxysporum* was most frequently isolated from diseased plants, and over 75% of these isolates were pathogenic to asparagus seedlings. *F. oxysporum* isolates from asparagus were unable to cause disease on potato, tomato, or carnation. The pathogen was found to move slowly through soil and seed transmission was suspected. The authors believed this disease to be identical to the crown and root rot described by Cohen and Heald. Other *Fusarium* species were found associated with diseased plants, and isolates of *F. moniliforme* were found pathogenic to seedlings, while *F. roseum* isolates were not (42).

Grogan and Kimble also concluded that the organism causing the

cortical seedling blight in Ontario was a less virulent F. oxysporum clone. This pathogen was unable to colonize vascular elements; while more virulent clones, such as those studied by Cohen and Heald, 1946, and the authors, were able to effectively colonize vascular elements (42).

Lewis and Shoemaker, 1964, tested asparagus seed lots from New Jersey, Massachusetts, Michigan, and Ontario for the presence of F. oxysporum. Seeds were planted in fusaria free sand and fed with a nutrient solution. Plants exhumed 2.5 months later were examined for root rot. Plants from all seed lots showed a 20-100% infection with F. oxysporum f. sp. asparagi. The pathogen was isolated from root lesions.

An asparagus disease caused by F. oxysporum f. sp. asparagi was described by Van Bakel and Kerstens in 1970. The disease was distinct from the asparagus disease caused by F. culmorum. Symptoms of the disease caused by F. oxysporum included small, brown, oval shaped lesions on storage roots and lower stems (94). Plants declined due to a foot rot of stems, roots, and crowns. The authors named the disease foot rot of asparagus.

Van Bakel and Kerstens in 1974 also reported a yellowing and wilting of asparagus stalks caused by F. culmorum to be a serious concern to growers. The disease is characterized by yellow, dead stems with reddish stem lesions girdling stem bases. Such lesions would occasionally extend up the stems. Two types of infection were recognized, one where lesions at the stem base girdle and kill the stem; and a second where lesions occur higher on stem and the stem survives. Airborne dispersal of the pathogen was demonstrated (95), and the authors hypothesized that

wind plays an important role in the spread of the pathogen; though the fungus is primarily soil-borne.

Endo and Burkholder in 1971 associated F. moniliforme with crown rot of asparagus in California. 93% of infected plants yielded F. moniliforme while 22% yielded F. oxysporum. Both pathogens were isolated from red-brown lesions on crowns, stems, and storage roots (29). F. moniliforme was additionally isolated from commercial asparagus seed, volunteer seedlings, and plant debris. The authors considered F. moniliforme as a major factor in asparagus crown rot in California (29).

Both F. oxysporum and F. moniliforme were found associated with declining asparagus plants in Washington by Grove , 1976 . F. oxysporum was the prevalent species isolated. Of 192 Fusarium isolates from diseased plants collected from 23 fields, 91% were F. oxysporum, 7% were F. solani, and 2% were F. moniliforme. Of the F. oxysporum isolates 34% were highly pathogenic, 52% were moderately pathogenic, and 14% were not pathogenic. All F. moniliforme isolates were highly pathogenic while no F. solani isolates showed any pathogenicity. Pathogenic isolates of F. oxysporum were also on all seed lots sampled except for two.

Blacklow in 1976 found F. moniliforme and F. oxysporum to be responsible for asparagus decline in Western Massachusetts. F. oxysporum was the major causal agent. Both pathogens caused asparagus seedling death, and root and stem lesions on mature plants. 50% of the F. oxysporum colonies isolated from soils were found pathogenic to asparagus seedlings. F. oxysporum was also found to be seed-borne (8).

Root and crown rot caused by F. oxysporum and F. moniliforme is

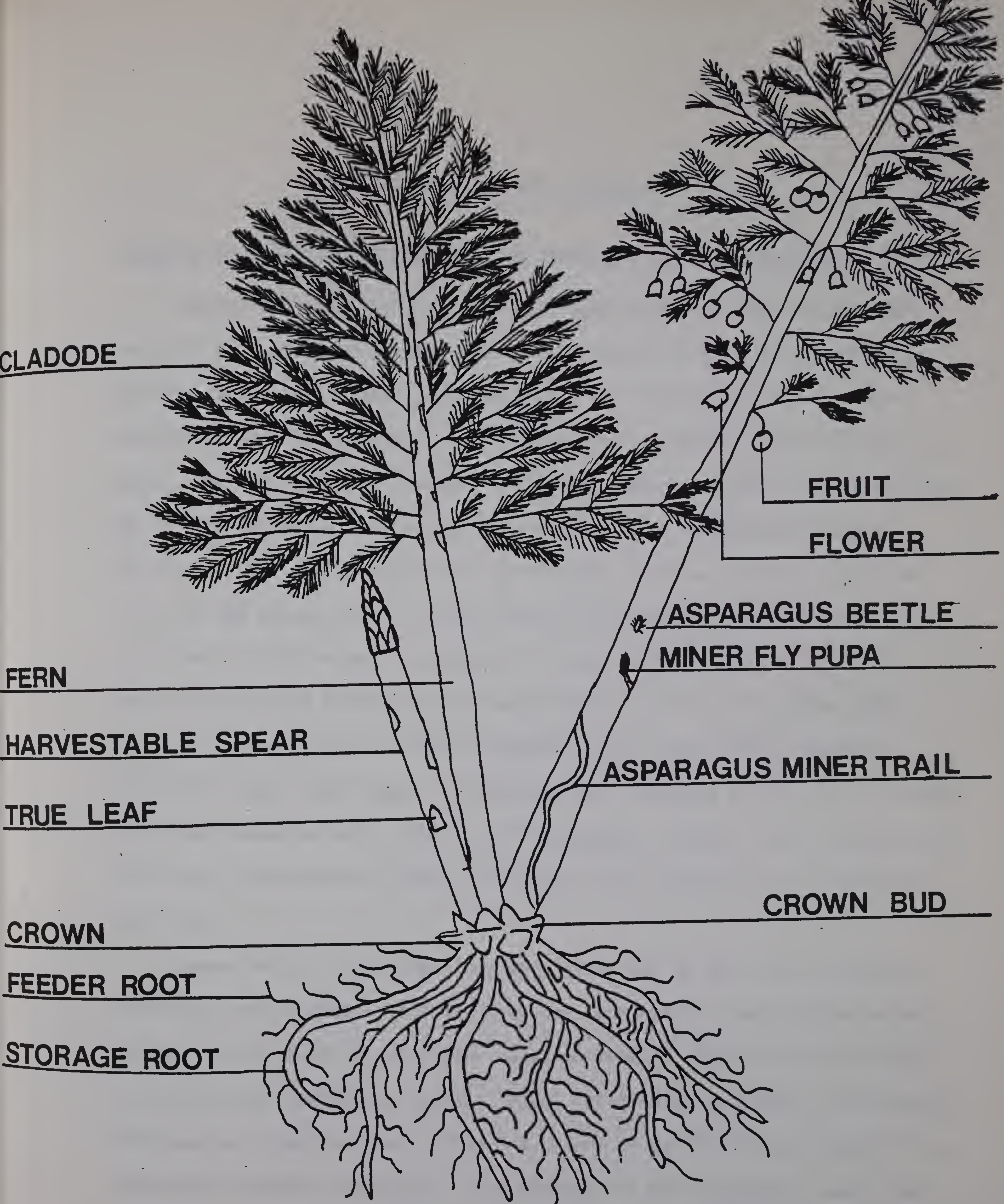
primarily responsible for asparagus decline in Michigan Lacy (1977) and New Jersey, Johnston *et al*., 1977 . Johnston (51) isolated *F. oxysporum* f. sp. *asparagi* and *F. moniliforme* from diseased plants in commercial New Jersey asparagus fields. *F. moniliforme* was most frequently isolated from stem and crown lesions of infected 12-year old plants, and less frequently from lesions on roots, crowns, and stems of 2-year-old plants. *F. oxysporum* f. sp. *asparagi* was isolated from discolored vascular root tissue and cortical root lesions, while *F. moniliforme* was primarily isolated from cortical stem and crown lesions. Based on isolations and pathogenicity tests, Johnston concluded that *F. moniliforme* causes a stem and crown rot disease separate from the wilt and root rot caused by *F. oxysporum*.

Inglis, 1978, found *F. moniliforme* and *F. oxysporum* f. sp. *asparagi* associated with asparagus seed in Washington. *F. moniliforme* was an external contaminant associated with the seed coat. Inglis hypothesized that seed infections occurred during seed extraction via infested berries and asparagus debris (48). The pathogen was isolated from asparagus berries and berry slurry. Berry inoculum was believed to be spread by wind blown sand, asparagus beetles, or by soil contact (48). *F. oxysporum* was primarily an external seed contaminant, although a small percentage was found to be internally carried.

Manning *et al*., in 1979, found *F. oxysporum* and *F. moniliforme* to cause asparagus root, crown and stem rot in commercial Massachusetts asparagus beds. Storage and feeder roots, cortical tissue of stem bases, and vascular crown tissues were invaded by both pathogens. Both

F. moniliforme and F. oxysporum were found seed-borne, and F. moniliforme was associated with asparagus flowers and fruits. Surface sterilized seed was found to yield seedlings infected with both pathogens. Volunteer plants from commercial fields were frequently infected by both pathogens. Gilbertson and Manning (36) consistently isolated F. moniliforme, F. solani, and F. tricinctum from washed asparagus flowers at various stages of development, immature and mature fruits, and from surfaces of developing seeds. F. moniliforme isolates were highly pathogenic to asparagus seedlings grown on Hoaglunds solution agar. F. moniliforme was never isolated from vascular elements of above ground plants, and air borne or insect-borne inoculum was believed to cause flower and seed infections (36). Gilbertson and Manning (37) attributed the asparagus replant problem to soil-borne F. oxysporum and a lesser extent F. moniliforme. The problem was most severe in soils previously having asparagus culture, though soils with no known history of asparagus were found to harbor pathogenic F. oxysporum isolates (37).

F. oxysporum and F. moniliforme were found to cause wilt and root rot of commercial asparagus in Taiwan. Field incidence ranged from 1.8 - 5.2%. F. oxysporum caused disease in 46% of the plants examined, F. moniliforme 17% and the rest by F. solani, Rhizoctonia solani and Pythium spp (92). Loamy sand and sand soils were found to favor disease; while the pathogen's optimum temperature was 25°C and pH was 6-7.



ASPARAGUS PLANT (EXHUMED)

Figure 1. ASPARAGUS OFFICINIALIS

GENERAL METHODOLOGY AND MATERIALS

Methods for Isolation of Fusaria from Asparagus Seed and Seedlings

Commercially and locally grown asparagus seed were examined for the presence of fusaria by plating seed on agar media or by planting seed in sterile sand. Local seeds were obtained from ripe fruits collected randomly from selected female plants. Fruits were washed in sterile distilled water, drained, and seed removed. Local and commercial seed lots were given one of the following treatments: no treatment (NT), presoaked in sterile distilled water for 24 hours (PS), pre-soaked and then surface sterilized in 10 or 25% Clorox plus 1-2 drops Tween 20 surfactant for 10 minutes (SS), or soaked in 2.5% benomyl in acetone 24 hours (25a), soaked in distilled water 24 hours and surface sterilized in 25% Clorox for 10 minutes (BA). The seeds were plated on acidified potato carrot agar (PCAL), modified Czapek-Dox agar, water agar, or PCNB agar and incubated at 24 C for 2-3 weeks. Seeds were examined every other day for Fusarium colonies, and resulting colonies were transferred to PCAL or carnation leaf agar (CLA) for identification (91).

Commercial and local seed lots were given one of the above different treatments and planted in sterile sand one inch apart. Seedlings were fertilized with a 20-20-20 fertilizer solution weekly. Seedlings were exhumed 4-6 weeks after emergence, weighed, rated for disease incidence, and assayed for fusaria. Seedlings were rated 0-3, based on the following system: 0 (no noticeable symptoms, healthy); 1 (no symptoms to mild symptoms, some crown discoloration, 1-2 storage root lesions, discolored feeder roots); 2 (moderate symptoms, distinct corwn discoloration, numerous storage root lesions,

feeder roots discolored or rotted, some wilting or yellowing evident); 3 (severe symptoms to dead, crown rotted or severely discolored, storage roots rotted or with extensive lesions, stems wilted, yellowed and often with stem lesions). 50 seedlings of each treatment group were randomly selected for Fusarium assay. Seedling crowns were excised, SS in 10% Clorox for 10 minutes and plated on PCAL. Resulting Fusarium colonies were identified directly from PCAL plates or subcultured on PCAL and later identified. Selected isolates were maintained on PCA slants and later tested for pathogenicity.

Methods for Isolation of Fusaria from Asparagus Plants

Below ground plant parts. Selected plants were exhumed, and root systems and crowns separated from above ground plant parts. Crowns and roots were washed with running tap water and visibly examined for disease symptoms. Samples were taken from crown tissues, storage roots and feeder roots. Crowns were sectioned in half and samples excised from interior crown vascular and cortical tissue. Samples were taken from storage and feeder roots exhibiting lesions or discoloration. Crown and storage root samples were surface-sterilized in a 10% Clorox solution plus 1-2 drops Tween-20. Samples in Clorox solution were agitated on a shaker. Feeder root samples were surface sterilized in a similar Clorox solution, but for 1-2 minutes. All samples were plated on acidified potato carrot agar (PCAL).

Aboveground plant parts. Female flowers were randomly collected at two

different times throughout the growing season: early season, June-July; and late season, August-September. Flowers were removed from plants with sterile forceps in three stages of development: pre-open, open, and senescent. Flowers were rinsed with running sterile distilled water for 1-2 minutes, then directly plated on PCAL. Fruits were sampled from female plants from initial stages of development. Immature (green) fruits were sectioned and either rinsed with sterile distilled water for 1-2 minutes or surface-sterilized in a 10% Clorox solution with 1-2 drops Tween 20 for 5 minutes; and plated on PCAL. Ripe (red) fruits were sampled, and surface tissue and interior pulp were separately plated on PCAL. Fruits were washed in sterile distilled water, opened with sterile forceps; and surface tissue, pulp, and developing seeds were individually plated on PCAL. Male and female asparagus stalks were randomly selected, and stem lesions excised from stalks. Lesions were sampled from either the entire length of stalks (SL), branchlets or twigs (TL), upper stalks (USL), or lower stalks (LSL). All excised stem lesions were surface sterilized in the standard 10% Clorox solution and plated on PCAL. Senescing stalks exhibiting fungal sporulation were collected from fields as the growing season progressed. Stems were sectioned; and pieces with sporodochia were microscopically examined, or scraped over plates of PCAL under a sterile hood. Resulting colonies were then mass transferred to PCAL and identified.

All plant tissue isolations were incubated at 23°C for one to two weeks. Resulting fusaria colonies were transferred to PCAL plates and/or slants, or carnation leaf agar plates (CLA) for identification. Many

colonies were then single spore isolated and cultured on CLA for further identification. Fusaria colonies were identified based on the Toussoun and Nelson pictorial guide to the Fusarium species (91).

Methods for Isolation of Fusaria from Soils

Soils with different crop histories were sampled for Fusarium spp. Soil samples were randomly taken from commercial asparagus fields, fields grown with other crop plants, and from areas not in agricultural use. Soils were assayed by soil dilution plates or by planting clean asparagus seed to bait for fusaria. Soil dilutions were prepared by adding a 1 g sample of a particular soil to a 100 ml .1% water agar blank and agitating on a shaker for 15 minutes to attain an approximate 1:100 dilution. A similar 1 g soil sample was simultaneously dried in an oven for 24 hours to determine the moisture content of the soil. Final propagule counts were adjusted based on the dry weight of the soil sample. Higher dilutions were obtained by adding 10 ml of the 1:100 dilution to sterile distilled water blanks. 10 ml of the 1:100 dilution into a 40 ml blank was used to get a 1:500 dilution, while 10 ml of the 1:100 dilution into a 90 ml blank gave a 1:1000 dilution. 1:500 and 1:1000 dilutions were found most suitable for propagule counts. One ml of the final dilution was pipetted onto each agar plate underneath a sterile hood. Media used was either PCAL or PCNB media selective for Fusarium (Papavizas, 1966). Plates were incubated at 23°C for 4-7 days and resulting colonies identified to genus by microscopic examination. 10 plates of each media were used for each soil sample, and an average

number of fusaria colonies per plate was determined. Fusarium colonies were subcultured on PCAL or CLA and identified to species.

A total propagules/gram of soil of the different fusaria identified was calculated by multiplying the average number of colonies per 10 plates by the corrected dilution factor for each soil sample. Isolates were stored on PCA slants in a refrigerator for pathogenicity tests.

Asparagus seeds were planted in different soils to bait pathogenic fusaria on seedlings. Seed lots were treated in a 2.5% benomyl in acetone solution, Damicone and Manning 1980, for 24 hours and then surface sterilized in a 10-25% Clorox solution for 15 minutes to eliminate seedborne contaminants. Seeds were planted in potted soil samples, or directly into field soils. Resulting seedlings were exhumed 6-8 weeks after planting, washed in running tap water and visibly examined for disease. The seedlings were rated for disease incidence based on a 0-5 rating system, and weighed in grams. Seedling crowns were excised, surface sterilized 10 minutes in a 10% Clorox solution plus 1-2 drops of Tween 20, and plated on PCAL. Fusaria growing from crowns were identified and a percent infection determined.

Methods for Isolation of Fusaria from Air

Airborne fusaria were isolated using Nash media selective for fusaria. Plates were exposed in asparagus fields for 15 minutes at stations on the ground and 1.5 meters in the air. All plates were incubated at 24°C for 4 days, and fusaria identified microscopically and subcultured on PCAL. Average number of colonies from 5 plates was

determined for different fields and stations. Colonies arising on plates from minute particles of organic matter were noted versus colonies arising from spores or other fungal propagules. Subcultured colonies were single spore isolated and identified on CLA.

Pathogenicity Tests Run on Fusarium Isolates

Test tube and agar plate pathogenicity tests were used to evaluate pathogenicity of isolates. Both tests involved germinating benlate - acetone treated (BA) or surface sterilized seed (SS) on agar to get disease free seedlings. In the agar plate technique, 2-week-old seedlings would be transferred with sterile forceps onto agar plates with fusaria colonies. One to two weeks after fungal contact with the seedling, pathogenicity would be determined. Pathogenicity was evaluated as low, medium, or high; with low indicating little or no effect on the seedlings, medium indicating some storage root lesions and crown discoloration or rot, and high indicating extensive crown rot, and storage root lesions. Controls were run with saprophytic fungi or no fungal colonies. In the test tube technique 2 week old seedlings were put on Hoaglunds solution agar slants and allowed to grow 2 additional weeks. Fungal isolates were placed at the base of healthy seedlings and pathogenicity was determined 2-3 weeks later. Pathogenicity was based on a 0-5 disease rating system: with 0 being no symptoms, healthy seedlings; 1, slight symptoms, some crown discoloration, storage and feeder root lesions; 3, stem lesions, crowns discolored and crown and roots exhibit some rot; 4, crown and root rot, extensive storage root

lesions and feeder root rot, seedlings yellowed, wilted; 5, death of seedling, rotted crown and roots, extensive fungal growth. Controls were run using saprophytic fungi or no fungal colonies. The pathogens were then re-isolated from diseased seedlings and re-identified.

CHAPTER I

INOCULUM SOURCES

Introduction

The first problem was how the pathogens became established in new asparagus beds, or what were primary inoculum sources of the disease. Agrios (2) defines primary inoculum as that surviving winter and causing infections the following growing season, but for the purpose of this study, primary inoculum is defined as that initiating infections in newly planted beds. Since the asparagus plant is a perennial, the pathogens are able to overwinter in the plants themselves; and by Agrios' definition, these plants are primary inoculum sources. We will consider established plants and other means of overwintering as secondary inoculum sources.

Two major primary inoculum sources were considered, the soil of the bed and the propagative material. Local growers have had new beds decline only 2-3 years after planting with both seeds and crowns, indicating soils may harbor pathogenic fusaria. Seeds and crowns have been shown to disseminate pathogenic fusaria, and may play a major role in establishing infections in asparagus beds.

Objectives

1. To assay soils in asparagus culture to determine if *F. oxysporum* and/or *F. moniliforme* are present in soils, and in what numbers.

2. To examine asparagus seed and propagative stock from local and

commercial sources for pathogenic fusaria.

3. Propose possible means of avoiding primary inoculum.

Literature Review

Soils and propagative plant parts have been shown by many researchers to harbor plant pathogenic fusaria (13). Soil populations of *Fusarium* have been quantitatively and qualitatively determined in many crop soils, including rice, cotton (64), onions (1), beans (7, 17, 72), and asparagus (8).

Nash and Snyder (72, 74) found 1000-3000 propagules per gram of the bean root rot pathogen *F. solani* f. sp. *phaseoli* existing in bean field soils. Lim (69) found rice and cotton field soils to sustain *Fusarium* populations ranging from 496-4400 propagules per gram. Both *F. oxysporum* and *F. moniliforme* were isolated from soil samples.

Both pathogens have been isolated from seeds of many crop plants including garden stock (5), cotton (24, 80), tomato (28), cowpea (53), beans (54, 73), safflower (57), bottlegourd (58, 59), corn (60, 88), sugar beets (65, 86), and other crops (13, 38, 77). Some *F. oxysporum* seed associations were internal (24, 58, 59, 80), but most were externally carried (28, 53, 54, 57, 65). *F. moniliforme* was less frequent on or in seeds, but does internally infect corn seed (60, 88). *F. moniliforme* and *F. oxysporum* have been associated with asparagus seed from Washington (48, 49), California (41, 27, 42), Ontario (40), and New Jersey (50, 63) and Massachusetts (9, 36, 68). *F. oxysporum* and *F. moniliforme* were mostly external contaminants of asparagus seed, while some *F. oxysporum* was possibly internally carried (48, 49).

Below ground propagative organs of plants such as corms, rhizomes and bulbs are often infested with soil-borne fusaria (13, 32, 34, 39). Pathogens are disseminated with infected stock (32, 34, 39) and infect growing plants. Members of the Lilaceae family including onions (1) and gladiolus (32) are known to carry pathogenic _F. oxysporum_. Bulbs of onion and corms of galdiolus grown in infested soils become rapidly infected (1, 32) and can then vector _F. oxysporum_ to other areas.

Materials and Methods

Fusarium populations in asparagus soils were determined by soil dilution plating, and isolates of _F. oxysporum_ and _F. moniliforme_ were tested for pathogenicity to asparagus seedlings. 1:500 and 1:1000 soil dilutions on PCNB media were run on soil samples from 4 asparagus fields (1,3,7,15 years old) as described in the General Methods and Materials. 25 _Fusarium_ colonies were transferred from 1:500 dilutions and identified to species, to get an indication of soil species. 1:1000 dilutions on 10 PCNB plates were run on samples from these fields to quantitatively determine _Fusarium_ populations. _Fusarium_ propagules/gram of soil were determined by multiplying the mean number of colonies per plate by the dilution factor and then correcting for soil water. The number of pathogenic propagules/gram was determined by multiplying the total number of fusaria propagules per gram by the percent pathogenic isolates. In addition to dilution plates, lots of 100 Mary Washington, Rutgers Beacon, and Viking 2K cultivar seeds were given the benlate-acetone treatment (BA) to eliminate seed-borne fusaria; and planted in

naturally infested soils to bait pathogenic fusaria. Seedlings were exhumed after 10 weeks growth and crowns surface sterilized 5 minutes in 10% Clorox and plated on PCAL. Resulting colonies were identified and tested for pathogenicity.

Seed lots from commercial and local sources were examined for seedborne fusaria by plating on agar and planting in sterile media. Local seeds were obtained by collecting ripe fruits and cleaning out viable seeds. Local and commercial seeds were surface sterilized or given no treatment, and planted in sterile sand. Resulting seedlings were exhumed after 4-6 weeks growth, rated for disease incidence, and crowns plated on PCAL. Weight and rating disease data for two experiments, one comparing the effect of surface sterilization of commercial seed, and the other comparing disease in different lots of local seeds; were statistically analyzed for differences using two way t-tests.

Commercial crowns were obtained from a local nursery (Tim Nourse, TN) and from mail order sources (Park Seed Co., PS; Raynor Bros., RBR). Crowns were washed under running tap water 5 minutes, observed for symptoms of crown and root rot, and isolations made as previously described. Isolates were identified and selected isolates tested for pathogenicity.

Results

Asparagus soil samples from all fields sustained populations of *Fusarium oxysporum*, *F. solani*, and other fusaria (Table 1). *F. oxysporum* was isolated from all soils and constituted over 50% of all colonies identified. *Fusarium* propagule counts ranged from 7586 propagules/gram of soil for the 3-year-old field, to 5111 propagules/gram of soil for the 1-year-old field (Table 2). 57-79% of *Fusarium* isolates showed pathogenicity on asparagus seedlings, and pathogenic *Fusarium* propagule counts ranged from 2913-5007 (Table 2).

Asparagus seedlings from BA-treated seeds planted in infested 3-year-old field soil were infected by fusaria after 10 weeks of growth. Seedlings showed crown discoloration, root and stem lesions,(Fig. 1), and some wilted and died. *F. oxysporum* was most frequently isolated from diseased plants, and only 4 seedlings yielded *F. moniliforme*. Of 150 crowns plated on PCAL, 62 yielded pathogenic isolates of fusaria (Table 3). Not all *F. oxysporum* crown isolates were pathogenic to asparagus (Table 11).

Commercial seed lots assayed yielded fusaria when plated on agar and planted in sterile sand. Low levels of infestation were seen by both *F. oxysporum* and *F. moniliforme* in some lots samples (Table 4). Most colonies appeared prior to seed germination, but 1 *F. moniliforme* and five *F. oxysporum* colonies appeared after germination. Three of 4 washed lots yielded *F. moniliforme*, while 2 of 4 lots yielded *F. oxysporum*. The Rutgers Beacon cultivar was most heavily contaminated

with F. moniliforme, but didn't yield F. oxysporum. The Burpee Mary Washington seed only yielded F. oxysporum. Seed infestations persisted after surface sterilization with 10% and 25% Clorox (Table 4). Eight of 400 Burpee Mary Washington seeds yielded F. oxysporum after surface sterilization with 10% Clorox, and 2 of 200 Rutgers Beacon seeds yielded F. moniliforme after surface sterilization with 25% Clorox. Surface sterilization with 95% ethanol then 25% Clorox completely eliminated seed infestation (Table 4), but inhibited seed germination. Selected isolates of both F. oxysporum and F. moniliforme from seeds showed pathogenicity to asparagus seedlings (Table 10).

Locally obtained asparagus seed also yielded fusaria (Table 5). All 4 washed seed lots yielded F. moniliforme (Figure 4), but none yielded F. oxysporum. F. tricinctum and F. solani were isolated from local seed lots (Table 5), but weren't pathogenic to asparagus seedlings (Table 11). All but 1 F. moniliforme colony appeared prior to seed germination. Surface sterilization with 10% Clorox reduced the incidence of seed-borne fusaria, while surface sterilization with 25% Clorox totally eliminated contamination (Table 5). All F. moniliforme isolates from local seed were pathogenic to asparagus seedlings (Table 10).

One-year-old seed associated with fruit debris from the Fil farm and Zigmont farm yielded pathogenic F. moniliforme (Table 6). F. tricinctum and F. solani were also isolated, but not F. oxysporum (Table 6).

Asparagus seedlings grown from commercial and local sources in sterile sand showed symptoms of fusarial infection, and yielded both F. oxysporum and F. moniliforme. Initial infections appeared as discolored crown tissue, particularly at the point of seed coat attachment. It was later observed that seedling crown tissue naturally turned red-brown at the origin of storage roots, making it more difficult to visually determine fusarial infections. Surface sterilization of commercial seed was not found to significantly decrease seedling disease ratings (Table 7), and crowns from surface sterilized and washed seeds yielded both pathogens. Seedlings from SS seed weighed significantly more than those from washed seed at the .05 level (Table 7). F. solani was also isolated from crowns from washed seed.

Seedlings from local washed seed showed symptoms of fusarial infection, and crowns yielded both F. oxysporum and F. moniliforme Seedlings from the 3-year field weighed significantly less than those from the other fields, and were rated significantly higher for disease at the .01 level (Table 8). Seedlings from the other samples didn't show significant differences in disease ratings, and only the seedlings from the 7-year field were significantly different in weight (Table 8). Many infections originated at the point of seed coat attachment, and this was a valuable way to visually rate for disease.

Mature asparagus crowns showed discolored cortical tissues, storage root lesions, and feeder root necrosis. Many storage root infections originated from points where feeder root had arisen. Both

TABLE 1

FUSARIA ISOLATED FROM ASPARAGUS FIELD SOILS, USING THE DILATION PLATE METHOD AND PCNB SELECTIVE MEDIUM[a]

Asparagus Field	Incidence of fusaria[b]				
	F. oxysporum	*F. moniliforme*	*F. solani*	*F. tricinctum*	*F. roseum*
3-year-old experimental	16	3	5	-	1
1-year-old commercial	18	2	4	-	-
7-year-old commercial	13	-	7	1	4
15-year-old commercial	14	-	4	3	4

[a]Using a 1/500 dilution factor

[b]25 colonies identified on acidified potato carrot agar

TABLE 2

FUSARIUM POPULATIONS DETERMINED FROM ASPARAGUS FIELD SOILS USING THE DILUTION PLATE METHOD AND PCNB SELECTIVE MEDIUM[a]

Asparagus Field	Mean Fusarium Colonies/Plate	Fusaria Propagules/ gram soil[b]	% Isolates Showing Pathogenicity[c]	Pathogenic Fusaria Propagules/g.
3-year-old experimental	6.6	7586	66	5007
1-year-old commercial	5.6	6021	79	4756
7-year-old commercial	4.6	5111	57	2913
15-year-old commercial	6.2	6739	60	3720

[a]Using a 1/1000 dilution factor

[b]Adjusted for soil water

[c]Agar plate pathogenicity tests

TABLE 3

PATHOGENIC FUSARIA ISOLATED FROM SEEDLINGS GROWN IN INFESTED SOIL AFTER 10 WEEKS GROWTH[a]

CULTIVAR	NUMBER OF ISOLATIONS[b]	NUMBER PATHOGENIC ISOLATES[c]
Mary Washington	50	10
Rutgers Beacon	50	29
Viking 2K	50	23
Totals	150	62

[a]Seedlings grown in naturally infested South Deerfield 3-year-old asparagus soil

[b]Crowns excised, surface sterilized, and plated on acidified potato carrot agar

[c]Agar plate pathogenicity tests

TABLE 4

FUSARIA ISOLATED FROM COMMERCIALLY OBTAINED ASPARAGUS SEED PLATED ON AGAR MEDIA[a]

CULTIVAR	SOURCES	TREATMENT	NUMBER	*F. oxysporum*	*F. moniliforme*
Mary Washington	Burpee	Wash-presoak	400	2	-
(MW)	Agway	Wash-presoak	400	-	4
Rutgers Beacon(RB)	New Jersey	Wash-presoak	400	-	7
Viking 2K	Ontario	Wash-presoak	400	1	3
		Surface sterilized			
MW	Agway	SS 10% Clorox	400	-	3
MW	Burpee	SS 10%	400	8	-
RB	New Jersey	SS 10%	400	-	2
Viking 2K	Ontario	SS 10%	400	-	1
MW	Agway	SS 25% Clorox	200	-	-
RB	New Jersey	SS 25% Clorox	200	-	2
Viking 2K	Ontario	SS 25% Clorox	200	-	-
MW	Agway	SS 95% ethanol	200	-	-
		25% Clorox	200	-	-
RB	New Jersey	SS 95% ethanol 25% Clorox	200	-	-
Viking 2K	Ontario	SS 95% ethanol 25% Clorox	200	-	-

[a]Media used: PCNB medium, acidified potato carrot agar, water agar

TABLE 5

FUSARIA ISOLATED FROM LOCALLY OBTAINED SEED PLATED ON ACIDIFIED POTATO CARROT AGAR[a]

SAMPLE	TREATMENT	NUMBER	*F. moniliforme*	*F. tricinctum*	*F. solani*
3 yr. experimental field	Washed	200	4	-	2
1 yr. commercial field	Washed	200	4	23	-
7 yr. commercial field	Washed	200	2	-	-
15 yr. commercial field	Washed	200	10	-	4
	Surface Sterilized				
1 yr. commercial field	SS 10% Clorox	100	2	5	-
7 yr. commercial field	SS 10% Clorox	100	-	-	-
15 yr. commercial field	SS 10% Clorox	100	-	-	1
1 yr. commercial field	SS 25% Clorox	100	-	-	-
7 yr. commercial field	SS 25% Clorox	100	-	-	-
15 yr. commercial field	SS 25% Clorox	100	-	-	-

[a]Ripe fruits collected August-October 1979, seeds individually removed with sterile forceps and plated on acidified potato carrot agar

TABLE 6

FUSARIA ISOLATED FROM OVERWINTERED 1-YEAR-OLD SEED IN PLANT DEBRIS FROM THE FIL FARM AND ZIGMONT FARM[a]

SOURCES	NUMBER	TREATMENT	Incidence of Fusarium species		
			F. moniliforme	F. tricinctum	F. solani
Fil Farm	200	Washed	18	14	8
Zigmont Farm	200	Washed	14	32	4

[a]Seed extracted from debris on field soil in May 1979, and plated on acidified potato carrot agar

TABLE 7

WEIGHT, DISEASE RATING, AND *FUSARIUM* INFECTIONS OF 10-WEEK-OLD SEEDLINGS GROWN FROM COMMERCIAL SEED IN STERILE SAND

SOURCE	NO. SEEDLINGS	TREATMENT	MEAN[a] WEIGHT (g)	MEAN[b] DISEASE RATING	Incidence of Fusarium species: CROWNS PLATED[c]	*F. moniliforme*	*F. oxysporum*
Mary Washington	100	Surface sterilized in 10% Clorox	.80 a	1.60a	50	7	2
Burpee	100	Washed	.71 b	1.81a	50	8	-

[a]Means followed by the same letters are not significantly different from each other at $P = 0.01$ using a paired t-test

[b]Seedlings rated on 0-3 system, with 0=clean seedling and 3 = dead

[c]Crowns excised, SS in 10% Clorox 5 min. and plated on acidified potato carrot agar

TABLE 8

WEIGHT, DISEASE RATING, AND *FUSARIUM* INFECTIONS OF 6 WEEK OLD SEEDLINGS GROWN FROM LOCALLY COLLECTED SEED IN STERILE SAND

SOURCE	NO. SEEDLINGS	TREATMENT	MEAN WEIGHT (g)[a]	MEAN DISEASE RATING[b]	NO. CROWNS PLATED[c]	Incidence of Fusarium species: *F. moniliforme*	Incidence of Fusarium species: *F. oxysporum*
3 yr. experimental field	100	Washed	.17a	1.79a	50	34	3
1 yr. commercial field	100	Washed	.27 c	1.21 b	50	20	3
7 yr. commercial field	100	Washed	.26 c	1.04 b	50	5	5
15 yr. commercial field	100	Washed	.21 b	1.12 b	50	7	4

[a]Means followed by the same letters are not significantly different from each other at P=0.01 using a paired t-test

[b]Seedlings rated on 0-3 system, with 0=clean seedling, and 3 = dead

[c]Crowns excised, SS in 10% Clorox 5 min. and plated on acidified potato carrot agar

TABLE 9

ISOLATION OF FUSARIA FROM ONE-YEAR-OLD COMMERCIALLY GROWN ASPARAGUS CROWNS[a]

SOURCE	SAMPLE	NUMBER ISOLATIONS	Incidence of Fusarium species		
			F. moniliforme	*F. oxysporum*	*F. solani*
Tim Nourse Farm					
TN	Crown	25	1	9	-
TN	Crown	25	-	8	-
TN	Crown	25	-	1	1
Raynor Bros					
RBR	Crown	25	4	4	-
Park Seed Co.					
PS	Crown	25	6	5	-
TN	Storage Root Lesion SRL	25	1	8	
TN	SRL	25	1	4	
RBR	SRL	25	-	4	
PS	SRL	25	-	1	
TN	Feeder roots	25	4	8	-
PS	Dead stems	25	3	-	-
PS	Buds	25	4	-	-
TN	Buds	25	-	6	-

[a]Samples excised from washed crowns SS in 10% Clorox 5-10 minutes and plated on acidified potato carrot agar

TABLE 10

RESULTS OF TEST TUBE PATHOGENICITY TESTS ON SOIL, SEED, AND CROWN ISOLATES[a]

ISOLATE	SOURCE	PATHOGENICITY
F. oxysporum	7 yr.-old commercial field soil	4 HIGH
F. oxysporum	1 yr.-old commercial field soil	4 HIGH
F. oxysporum	15 yr.-old commercial field soil	3 MED-HIGH
F. oxysporum	3 yr. experimental field soil	4 HIGH
F. oxysporum	Commercial seed	3 MED-HIGH
F. oxysporum	Commercial seed	5 HIGH
F. moniliforme	Commercial seed	5 HIGH
F. moniliforme	Commercial seed	4 HIGH
F. moniliforme	Local seed	4 HIGH
F. moniliforme	Local seed	5 HIGH
F. oxysporum	Crown	3 MED-HIGH
F. oxysporum	Crown	4 HIGH
F. oxysporum	Crown	4 HIGH
F. oxysporum	Crown	4 HIGH
F. moniliforme	Crown	5 HIGH
F. moniliforme	Crown	5 HIGH
F. tricinctum	Seed	0 NONE
F. solani	Crown	1 LOW
Alternaria	Crown	1 LOW
Penicillium	Crown	2 LOW-MED
Control		0 NONE
Control		0 NONE
Control		1 LOW

[a]BA acetone seed germinated on acidified potato carrot agar, then placed in slant of Hoaglund solution agar for 2 weeks prior to addition of fungal isolate

[b]Based on 0-5 rating system, 0 = clean and 5 = dead

local and mail order crowns showed disease symptoms, and yielded fusaria (Table 9). Isolations from crown tissue yielded F. oxysporum and to a lesser extent F. moniliforme, while storage root lesions yielded mostly F. oxysporum (Figure 5). Both pathogens were isolated from necrotic feeder roots and discolored bud tissue, and F. moniliforme was isolated from dead stem tissue (Table 9). Locally grown crowns were much larger and appeared less diseased. Selected isolates of F. moniliforme and F. oxysporum from crowns showed pathogenicity on asparagus seedlings (Figure 6) in test tube pathogenicity tests (Table 10).

Discussion

Asparagus soils were a major primary inoculum source for F. oxysporum, but not F. moniliforme. Soil-borne F. oxysporum attacks seedlings and crowns planted in infested soil, and causes root and crown rot. Growers have been unable to establish productive beds in soils previously in asparagus culture due to soil-borne F. oxysporum.

Not all F. oxysporum isolates were pathogenic to asparagus (Table 10), indicating that pathogenic isolates were possibly F. oxysporum f. sp. asparagi. For our purposes, we considered F. oxysporum isolates pathogenic to asparagus as simply F. oxysporum. The literature lacks a comprehensive host range study of F. oxysporum f. sp. asparagi, and initial work indicates that some F. oxysporum isolates from soils never in asparagus culture (virgin soils) are pathogenic to asparagus (37). Incidences of pathogenic F. oxysporum

from virgin soils may indicate that *F. oxysporum* is a facultative parasite able to infect asparagus and other hosts (Chapter 3), in addition to surviving saprophytically or as chlamydospores (Figure 2). Plant pathogenic *F. oxysporum* has been shown to exist in soils as saprophytes associated with organic matter (13, 17, 30) and as chlamydospores (13, 17, 30, 71, 72) and it is likely that *F. oxysporum* pathogenic to asparagus survives in soil as a parasite, a saprophyte and as chlamydospores. Thus, asparagus is not necessary for survival of *F. oxysporum* in soils. Describing a fungus with such a wide array of survival as a specialized parasite seemed illogical, and the occurrence of pathogenic strains in virgin soils also raised doubts about the specialization of the pathogen.

The wide array of survival mechanisms utilized by *F. oxysporum* makes it virtually impossible to eliminate from soils. The fungus is able to resist soil fumigation due to chlamydospore production or by association with organic matter. Volatile fumigants are unable to attack these propagules. Both fumigation and crop rotation have been ineffective in eliminating *F. oxysporum* from Western Massachusetts asparagus soils. A field out of asparagus culture for 7 years still harbored pathogenic *F. oxysporum*. Soils never in asparagus culture, but near established beds were also unsuitable for asparagus culture, indicating dissemination and/or an endemic fungus. Seedlings grown in these soils were infected by *F. oxysporum* and sometimes *F. moniliforme* and would die or decline due to crown and root rot.

Soil can be an important inoculum source for asparagus decline,

especially if beds are established in soils previously in asparagus culture. It becomes important for growers to know the history of a prospective asparagus field, and to establish new beds in virgin soils. Additional experiments were conducted with virgin soils to determine the biology and distribition of _F_. _oxysporum_, and the possibility of a soil assay technique for pathogenic fusaria (Chapter 2).

Isolates of _F_. _oxysporum_ and _F_. _moniliforme_ pathogenic to asparagus were isolated from seeds and crowns (Table 10). The occurrence of _F_. _oxysporum_ colonies after the germination of seed may indicate a low level of internal infection. Inglis (48, 49) showed the intricate association of _Fusarium_ spores and the seed coat of asparagus. _Fusarium_ spores lodged in external crevices and survived surface sterilization, as did some _F_. _oxysporum_ isolates believed internally carried (48). Surface sterilization with Clorox solutions can't be considered a 100% effective means of disinfecting asparagus seed.

The incidence of _F_. _moniliforme_ on locally obtained seeds demonstrates the importance of _F_. _moniliforme_ in Massachusetts asparagus decline. One-year-old seeds from growers fields overwintered _F_. _moniliforme_, and the pathogen may infect germinating volunteer plants (Chapter 4). The mechanism of local seed infestation by _F_. _moniliforme_ is discussed in Chapter 4.

The incidence of fusaria on seed varied with the cultivar of seed, but more importantly with the parent field. This was seen with pathogenic and saprophytic fusaria. Local seed from the15-year field yielded high amounts of saprophytic _F_. _tricinctum_; while other local

seed samples yielded no F. tricinctum, but did yield F. solani. Seed infestation reflects the major Fusarium species associated with plants from different fields. Commercial seed, particularly the Mary Washington cultivars yielded more F. oxysporum than local seed. This may reflect a higher incidence of F. oxysporum from commercial seed fields. Susceptible Mary Washington plants infected with F. oxysporum strains able to infect vascular elements, may yield seed internally infested by the pathogen. Such vascular seed infections have not been demonstrated in asparagus, but the F. oxysporum has been isolated from stem segments 60 cm above the soil line (38), and internal seed infection has been hypothesized (38, 39). F. oxysporum internally infecting seeds via vascular elements has been reported in safflower (57), and bottlegourd (58, 59).

Seedlings from commercial and local seeds grown in sterile sand yielded pathogenic and saprophytic fusaria, indicating that seed-borne fusaria can infect seedlings. Appearance of characteristic lesions on roots and stems and typical crown rot near the seed coat attachment was found as a reliable indication of fusarial infection. Assay of randomly selected crowns yielded Fusarium, and helped confirm the rating system. Seedling infection also reflected the dominant seed-borne fusaria from fields sampled. Sixty-eight percent of seedlings from 3-year field seed were infected with F. moniliforme and seedlings showed a significantly higher disease rating than other samples. F. moniliforme was a major causal agent of decline in the 3 year field (Chapter 2), and this was reflected in the experiment.

Infection of seedlings grown in sterile sand from local seed with *F. oxysporum* was not consistent with results obtained from plating on agar media. *F. oxysporum* was rarely isolated from local seeds, and contamination of seedlings by air or water-borne inoculum may have resulted in *F. oxysporum* infections. Rowe, Farley, and Coplin (80) demonstrated that *F. oxysporum* became airborne in greenhouses, and severely infected tomatoes grown in sterile media. Growth chamber experiments with asparagus seed sources may eliminate such contamination.

Growers starting new beds with heavily infested seeds will introduce pathogenic fusaria which can become loci of infections for other plants. A commercial bed established with seed obtained from a diseased local field was plowed under after 4 years due to severe decline, which was later diagnosed as stem, crown, and root rot caused by both *F. moniliforme* and *F. oxysporum*. It is likely that soil-borne and seed-borne inoculum were responsible for the decline. *F. oxysporum* was most frequently isolated from 1-year-old crowns, and infected cortical tissues and storage roots. Soil-borne *F. oxysporum* penetrated storage roots at points where feeder roots emerged, and eventually reached vascular elements of the storage root and crown. *F. moniliforme* was isolated from dead stem pieces and crown tissue, indicating an association with aboveground plant parts.

It is difficult to obtain clean crowns due to seed and soil-borne inoculum. Growers should avoid purchasing crowns with any vascular discoloration or large amounts of lesions root or crown tissue.

Only vigorous crowns with large numbers of storage roots should be used to establish new beds, and crowns should come from a reliable source. Growers must learn to recognize disease symptoms. A local grower who established a new bed with Rutgers Beacon cultivar crowns from a questionalbe source now has close to 100% infection in his 2-year-old bed, and it became evident later that many crowns showed visible symptoms of asparagus decline.

Dipping crowns in benomyl solutions was shown to decrease or eliminate fusarial infections in gladiolus (66), narcissus (39), and asparagus (67); and may prevent *Fusarium* infections. The effect of benomyl treatments in perennial asparagus appears temporary (Dr. William J. Manning, pers. comm.), and it is unknown if the technique completely eliminates crown infections. Further experiments on benomyl treated crowns may show this technique to be a management method for asparagus decline.

Conclusion

Propagative asparagus stock and soils are primary inoculum sources for stem, crown, and root rot of asparagus caused by *F. oxysporum* and and *F. moniliforme*. Asparagus seeds and crowns planted in soils previously in asparagus culture are rapidly infected by pathogenic *F. oxysporum* and to a lesser extent *F. moniliforme*. Pathogenic *F. oxysporum* is able to exist in asparagus soils for long periods of time and prevent replanting of asparagus. Asparagus seed sources carried both pathogens, and local seeds showed high rates of external contamination

with *F. moniliforme*, indicating the importance of *F. moniliforme* in asparagus decline in Massachusetts. One-year crowns carried *F. oxysporum* and *F. moniliforme*, but mostly *F. oxysporum*. Establishment of productive asparagus beds in Massachusetts can be achieved by avoiding infested soils and using clean propagative stock.

CHAPTER II

OCCURRENCE OF *FUSARIUM* SPECIES IN LOCAL SOILS

Introduction

Initial work indicated that virgin soils may harbor isolates of *F. oxysporum* pathogenic to asparagus. Graham, 1955, found pathogenic *F. oxysporum* var *redolens* in Ontario soils with no known history of asparagus culture, and hypothesized that the fungus may be endemic to Ontario soils. If *F. oxysporum* is endemic to even certain virgin soils, it is important to screen perspective asparagus soils for highly pathogenic isolates.

Objectives

1. To determine if pathogenic *F. oxysporum* isolates exist in virgin soils.
2. To assay a variety of different soil types to determine if pathogenic *F. oxysporum* isolates are present, and their respective virulence to asparagus seedlings.
3. To determine if a soil assay method utilizing asparagus seedlings could be used to evaluate perspective asparagus soils for plant vigor and disease incidence.

Materials and Methods

Soils were assayed for fusaria by planting benlate-acetone treated seed (BA) or presoaked seed (PS) in field soils or potted soil samples. Resulting seedlings were examined for symptoms of stem, crown, and root rot; and were weighed, rated for disease, and randomly selected crowns

plated on PCAL. Two experiments were designed for the study. In the first, seedlings were grown in two separate fields: Montague field (MFF), with no known history of asparagus culture; and South Deerfield (SD), known to be infested with both *F. oxysporum* and *F. moniliforme*. Treatments consisted of planting BA and PS seeds of cultivars Rutgers Beacon (RB) and Mary Washington (MW) in a random block design in both fields. 100 seedlings from each field of each treatment were sampled after 6 weeks growth, and evaluated as stated above. Amounts of disease incidence between fields, varietal and treatment differences, and potential tolerance in varieties were looked at. Soil dilution plates were run on soil samples from both fields to confirm the presence of fusaria. In the second experiment, BA seeds were planted in 8 inch pots of 10 different local sandy loam or loam soils (Table 3). Some soils had previous cultivated crop histories, including corn, asparagus, and tobacco; while others had no crop history, and included a pasture, a meadow, and an isolated woodland area. Seedlings were exhumed after 2 months growth and evaluated. 1:1000 soil dilution plates were run on each soil to determine *Fusarium* populations.

Results

Seedlings from both the Montague field and the South Deerfield field showed distinct symptoms of stem, crown, and root rot. Crowns showed reddish discoloration often originating at the point of seed coat attachment; and stems and roots had firm elliptical red lesions.

Some heavily diseased seedlings wilted, yellowed, and died; particularly from the SD field. Seedling growth was most vigorous in the SD field, and the RB cultivar was significantly more vigorous than the MW (Table 1). Seedlings from the MFF field showed significantly lower disease ratings than SD seedlings, and the RB cultivar seedlings showed significantly lower ratings than the MW cultivar (Table 1). BA seed treatments didn't significantly reduce disease ratings except for RB cultivar seeds planted in SD soil. Seedling crowns of both cultivars from the MFF field yielded more _F. oxysporum_ than those from SD (Figure 7), but SD crowns yielded more _F. moniliforme_ (Table 2). Soil dilutions confirmed the presence of both _F. oxysporum_ and _F. moniliforme_ in the SD soil, and only _F. oxysporum_ in the MFF soil. Isolates of _F. oxysporum_ and _F. moniliforme_ from SD showed medium to high pathogenicity on asparagus seedlings in test tube pathogenicity tests, while _F. oxysporum_ isolates from MFF field showed medium-low pathogenicity (Table 2).

Seedlings from all 10 soils evaluated for asparagus culture showed some symptoms of fusarial infection. Seedling growth in the tobacco and corn field soils was significantly greater than in any other soil, and seedling disease ratings were significantly lower than all other non-steamed samples (Table 5). The vegetable research and meadow soils showed good growth and significantly lower disease ratings than SD seedlings; while the SD seedlings showed good growth but high disease ratings. Woodland, pasture, and garden soil seedlings showed significantly lower growth and higher disease ratings (Table 5).

TABLE 1

WEIGHTS AND DISEASE RATINGS OF SEEDLINGS GROWN IN MONTAGUE AND SOUTH DEERFIELD SOILS[a]

SOIL	SEED VARIETY AND TREATMENT[b]	MEAN WEIGHT[c]	MEAN DISEASE RATING[d]
Montague			
	Rutgers Beacon (RB) No treatment (NT)	.63ab	.61a
	RB - benlate-acetone (BA)	.74 b	.50a
	Mary Washington (MW) NT	.37 c	1.53 c
	MW BA	.63ab	1.34 bc
South Deerfield			
	Rutgers Beacon NT	.98 d	1.41 c
	RB BA	1.17 e	1.10 b
	Mary Washington (NT)	.60a	2.86 d
	MW BA	.56a	2.92 d

[a]South Deerfield soil previously in asparagus culture and naturally infested with F. oxysporum and F. moniliforme, Montague soil had no known history of asparagus culture; 100 8-week old seedlings randomly selected.

[b]Seeds given no treatment were planted directly from the container they were received in,and benlate -acetone treated seeds were soaked in a 2.5% benomyl in acetone mixture 24 hrs., soaked in distilled water 24 hrs, surface sterilized 5 min. in 25% Clorox, and planted.

[c]Means followed by the same letters are not significantly different from each other, P=0.01 using Duncan's Multiple Range Test.

[d]Seedlings rated on 0-5 system, with 0=clean, 5=dead.

TABLE 2

PATHOGENIC FUSARIA ISOLATED FROM CROWNS OF SEEDLINGS GROWN IN MONTAGUE AND SOUTH DEERFIELD SOIL[a]

SOIL	SEED VARIETY AND TREATMENT[b]	INCIDENCE OF FUSARIA[c]		PATHOGENICITY[d]
		F. oxysporum	*F. moniliforme*	
Montague				
	Rutgers Beacon (RB) No Treatment (NT)	42	-	
	RB benalte-acetone	37	-	LOW-MEDIUM
	Mary Washington (MW) (NT)	33	-	
	MW BA	18	1	
South Deerfield				
	Rutgers Beacon NT	18	5	
	RB BA	23	6	MEDIUM-HIGH
	Mary Washington NT	12	9	
	MW BA	6	4	

[a]South Deerfield soil previously in asparagus culture and naturally infested with *F. oxysporum* and *F. moniliforme*, Montague soil had no known history of asparagus culture.

[b]Seeds planted directly from package or soaked in 2.5% benomyl in acetone, soaked in distilled water, surface sterilized in 25% Clorox and planted.

[c]100 8-week-old seedling crowns randomly selected, surface sterilized for 5 minutes in 10% Clorox and plated on acidified potato carrot agar PCAL. Plates incubated 7 days and resulting colonies identified, subcultured and kept on PCAL slants for pathogenicity testing.

[d]Pathogenicity based on ability of fungal isolate to cause stem, crown, and root rot on aseptically grown seedlings, none = no symptons high = severe-symptoms - dead.

TABLE 3

SOURCES OF SOILS EVALUATED FOR ASPARAGUS CULTURE[a]

1	Steamed Potting soil
2	Steamed South Deerfield soil[b]
3	Isolated Upland Meadow
4	Fumigated tobacco field
5	Corn field
6	Pasture
7	Garden
8	South Deerfield soil
9	Woodland
10	Vegetable Research Soil

[a]Soils collected from 5 points, corners and center of field, with disinfected spade.

[b]Soil previously in asparagus culture and infested with _F_. _oxysporum_ and _F_. _moniliforme_.

TABLE 4

CHARACTERISTICS OF SOILS EVALUATED FOR ASPARAGUS CULTURE

SOURCE	SOIL TYPE	pH	% ORGANIC MATTER	Ca	NUTRIENTS[a] K	P	Mg	NO_3	NH_4
Steamed potting	sand-loam	6.1	10.5	1600	120	100	50	30	35
Steamed SD	loam-sand	4.7	5.1	500	60	12	25	30	35
Meadow	loam	6.0	4.5	1600	250	100	125	30	35
Tobacco	sand-loam	6.5	5.7	1600	60	100	125	5	35
Corn	sand-loam	5.9	6.4	1600	60	50	50	10	35
Pasture	sand-loam	5.8	6.7	1600	250	50	50	20	35
Garden	sand-loam	6.0	2.0	900	180	100	125	5	35
SD asparagus	loam	6.2	4.5	1200	60	100	50	5	35
Woodland	sand-loam	6.1	6.8	1600	180	100	50	5	35
Vegetable research	sand-loam	5.7	8.5	1600	60	50	50	5	35

[a]Nutrients measured in ppm using the Morgan Soil Testing System.

TABLE 5

WEIGHTS AND DISEASE RATINGS OF SEEDLINGS GROWN IN SOILS EVALUATED FOR ASPARAGUS CULTURE[a]

SOIL	MEAN WEIGHT (g)[b]	MEAN DISEASE RATING[c]
Steamed potting	2.67 cd	.78a
Steamed South Deerfield	1.88 b	1.00 b
meadow	2.39 c	1.31 cde
tobacco	3.86 e	1.17 bcd
corn	3.67 e	1.39 c e
pasture	.73a	1.58 e
garden	1.79 b	1.67 f
SD asparagus	3.08 d	1.68 f
woodland	.31a	3.23 c
vegetable research	2.46 c	1.32 c e

[a]30 benlate-acetone treated seeds planted in six 8" pots of each soil and 100 12-week-old seedlings randomly selected (approximately 20 from each pot) for evaluation.

[b]Means followed by the same letters are not significantly different from each other, P= .01, using Duncan's Multiple Range Test.

[c]Seedlings rated on a 0-5 system, 0=clean, 5=dead.

TABLE 6

ISOLATION OF FUSARIUM OXYSPORUM FROM SOILS AND SEEDLING CROWNS OF SOIL EVALUATION EXPERIMENT, AND PATHOGENICITY OF SELECTED ISOLATES

SOIL	F. OXYSPORUM PROPAGULES PER GRAM OF SOIL[a]	ISOLATION OF F. OXYSPORUM FROM CROWNS[b]	PATHOGENICITY RATINGS OF SELECTED ISOLATES[c]	PATHOGENICITY
Steamed potting	128.2	14	3	MEDIUM
Steamed South Deerfield(SD)	659.3	12	1,4,4	MED-HIGH
Meadow	1071.4	38	1,2,3	LOW-MEDIUM
Tobacco	1489.4	48	2,4,2	MEDIUM
Corn	3457.5	35	1,1	LOW
Pasture	1089.3	42	3,3	MEDIUM
Garden	2298.8	37	2,3,5	MEDIUM-HIGH
SD asparagus	2738.1	36	3,4	MEDIUM-HIGH
Woodland	4321.0	24	1,1	LOW
Vegetable research	2631.6	50	1,1,3	LOW-MEDIUM

[a]Determined by running of 1/1000 soil dilutions on PCNB medium selective for Fusarium.

[b]Fifty 12-week-old seedling crowns randomly selected, surface sterilized in 10% Clorox for five minutes, and plated on acidified potato carrot agar. Plates incubated 7 days, isolates subcultured, identified and tested for pathogenicity on aseptically grown asparagus seedlings.

[c]Seedlings rated on a 0-5 system, 0=clean, 5=dead.

F. oxysporum was isolated from surface sterilized crowns from all soils, and all soils sustained *F. oxysporum* populations (Table 6). *F. moniliforme* was only isolated from SD soil and crowns, and at a low incidence. Pathogenicity of *F. oxysporum* isolates varied, and soil populations and crown infections couldn't be correlated to high disease ratings. Pathogenicity of isolates from the meadow, tobacco, and corn fields was low-medium; and was correlated with significantly lower disease ratings. No major differences in soil pH or nutrients were believed responsible for growth differences (Table 4).

Discussion

Both the South Deerfield and virgin Montague soils sustained populations of *F. oxysporum* pathogenic to asparagus, but isolates from the MFF field were less virulent. *F. oxysporum* readily colonized seedlings in the MFF field, but lower virulence of isolates resulted in less disease. More virulent *F. oxysporum* and *F. moniliforme* caused significantly more disease in SD seedlings. BA seed treatments were ineffective in preventing seedling infections in either experiment. The RB cultivar showed significantly better growth and significantly lower disease ratings than MW seedlings, though RB crowns yielded more *F. oxysporum*. This may be due to the varied pathogenicity of *F. oxysporum* isolates; and some MFF isolates were believed to be saprophytic, even though they colonized seedling crowns to some extent. The RB cultivar can be considered tolerant, while the MW cultivar was susceptible to *Fusarium* stem, crown and root rot.

Greater seedling vigor seen in the SD field was attributed to the better soil structure and nutrients of the SD field, which had been fertilized, limed, and cultivated for years. The MFF field had been in fallow for over 20 years, and wasn't prepared for agriculture like the SD field.

The occurrence of F. oxysporum in both soils was expected, but the pathogenicity of isolates from the MFF field wasn't. These results indicate that isolates from the MFF field were possibly facultative parasites able to colonize seedling crown tissue and cause disease. Garrett (34) reported saprophytic F. oxysporum strains acting as facultative parasites causing disease in bananas, beans, and other crops; in addition to surviving saprophytically or in association with other hosts. F. oxysporum in the MFF field soils may behave in a similar manner. MFF isolates weren't highly virulent, but a constant exposure to perennial asparagus may lead to increased virulence. The presence of virulent F. oxysporum and F. moniliforme isolates in the SD field was attributed to previous asparagus culture in the field.

Additional factors during the experiment could also have been involved in disease expression. There was a long hot dry spell, and water stress on plants may have resulted in higher infection rates in the MFF seedlings. The upper soil layer of the MFF field was observed to dry out faster than the SD field, and resulting water stress may have made seedlings more susceptible to less virulent F. oxysporum isolates. The occurrence of highly virulent F. oxysporum and F. moniliforme isolates in SD soil would prevent replanting asparagus, but the low

virulence of _F_. _oxysporum_ and absence of _F_. _moniliforme_ may allow establishment of a successful asparagus bed in MFF field.

The soil evaluation experiment verified the endemic occurrence of _F_. _oxysporum_ pathogenic to asparagus in some virgin soils. Virulence of isolates varied depending on the soil source, and was correlated to disease ratings and seedling vigor in most cases. Asparagus culture would be discouraged in the garden, woodland, pasture and SD soils due to low seedling vigor, high disease ratings, and virulence of _F_. _oxysporum_ isoaltes. Potentially successful asparagus could be established in the corn and tobacco field soils, and possibly in the vegetable research and meadow soils. Long term establishment of asparagus beds and the evaluation of growth and disease incidence would be valuable in determining the validity of the assay system; and to see if low virulent _F_. _oxysporum_ isolates developed increased virulence to asparagus.

Conclusion

_F_. _oxysporum_ populations exist in most cultivated and non-cultivated soils. Isolates from soils show varied pathogenicity to asparagus seedlings, and virulent isolates can prevent the establishment of a healthy asparagus bed. The efficient soil survival of _F_. _oxysporum_ allows for the fungus to remain in soils indefinitely, and it is important to evaluate a prospective asparagus soil to get an indication of disease incidence (i.e. virulent isolates) and plant growth. Soils giving favorable responses may sustain productive beds if proper asparagus culture is practiced.

CHAPTER III

LOCAL GROWERS FIELDS SURVEY

Introduction

Local asparagus beds have been declining steadily over the past 10-15 years. Isolations from diseased plants yield a number of *Fusarium* species in addition to other fungi. No survey of Western Massachusetts asparagus fields has been made to determine the extent of the *Fusarium* disease complex and the major pathogen(s) involved. It is well known that *Fusarium oxysporum* and *F. moniliforme* are the primary causal agents in most asparagus growing regions (8, 21, 29, 36, 40, 41, 42, 48, 49, 50, 51, 68); but little is known about the frequency, location in respect to the asparagus plant, and the importance of these and other *Fusarium* species in commercial growers fields. Some researchers have discounted *F. moniliforme* as a major problem (41, 42) or have not found *F. moniliforme* associated with asparagus decline (21). More recent work indicates that *F. moniliforme* is a factor in asparagus decline (29, 36, 40, 48, 49, 50, 51). Johnston (51) found *F. moniliforme* to be extensively associated with asparagus decline in New Jersey, and speculated that *F. moniliforme* and *F. oxysporum* may cause separate diseases on asparagus; with *F. moniliforme* associated with stems and crowns, and *F. oxysporum* associated with crowns and roots. Differences in the characteristics of these two fungi in respect to plant parts attacked and means of survival may partially explain the severe increase in the *fusarium* stem,

crown, and root rot in Western Massachusetts.

Objectives

1) To assay commercial asparagus fields for incidence of stem, crown, and root rot caused by _F_. _oxysporum_ and _F_. _moniliforme_.

2) To get an indication of the frequency, location, and importance of _Fusaria_ associated with diseased asparagus plants.

3) To determine any characteristics of the pathogens which favor successful infection of asparagus plants and disease buildup in asparagus beds.

Methods and Materials

Five commercial growers fields (MF, TZ-1, TZ-2, SB, AV) and one experimental field (SD) from Hampshire County were surveyed (Table 1). Visible symptoms of asparagus decline were noted and 25 mature plants were randomly exhumed from each field for examination. Crowns and root systems of sampled plants were washed under running tap water for five minutes, examined for symptoms of crown and root rot, and isolations made from crowns and roots as previously described. Female flowers were randomly selected from mature female plants. Flowers were collected twice during the growing season: early season, June-July and late season, August-September. Pre-opened, opened and senescent flowers were collected and plated as previously described. Stem lesions and immature and ripe fruits were similarly collected and plated on PCAL as previously described. Stalks were washed and lesions excised from various areas

of the stems. Late in the growing season (Sept.-Oct.) senescing stalks exhibiting sporodochia were collected. Sporodochia were microscopically examined, scraped over PCAL and resulting colonies identified. Airborne fungi were isolated from the MF and TZ-2 field as previously described. Test tube or agar plate pathogenicity tests were run on selected isolates from all experiments.

Results

All asparagus fields surveyed showed visible symptoms of asparagus decline. Yellowing ferns and skips in rows of plants indicated the presence of the asparagus disease complex. Stems pulled from all surveyed fields showed extensive red elongated lesions on white lower stem areas beneath the soil line (Figure 8). Upper stems often had 1-4 mm long red elliptical lesions which were confined to cortical and epidermal tissues. Cross sections of upper stems rarely revealed vascular discoloration. Cross sections of diseased crown tissue did reveal patterns of red-brown discoloration. Washed storage roots frequently exhibited sunken dark brown lesions (Figure 9), many of which originated from where feeder roots had arisen from storage roots. Feeder roots were often discolored or rotted.

Isolations from exhumed crowns yielded both _F. oxysporum_ and _F. moniliforme_. _F. oxysporum_ was most frequently isolated (Table 2), then _F. solani_ and _F. moniliforme_. An average of 12% of interior crown tissue samples from 4 different fields yielded _F. oxysporum_. _F. oxysporum_ was isolated from 50% of sampled storage root lesions (SRL), and

25 SRL sampled from the MF field yielded 18 colonies of F. oxysporum (Table 2). F. moniliforme was isolated from SRL at lower incidences than F. oxysporum (Table 2). Discolored feeder roots sampled from the SB field yielded 14 F. oxysporum colonies, 7 of F. solani, and none of F. moniliforme (Table 2). Selected isolates of F. oxysporum and F. moniliforme were pathogenic to asparagus seedlings, while F. solani isolates were non-pathogenic or mildly pathogenic (Table 7).

Some female asparagus flowers had small lesions or large areas of necrosis on pedicels or flower surfaces, but these lesions didn't always yield fusaria. Flowers sampled early and late in the growing season yielded a wide array of Fusarium spp. including F. moniliforme, F. tricinctum, F. solani, F. roseum cultivar equeseti, F. moniliforme var subglutinans, and F. oxysporum (Table 3). Fusaria were isolated from washed flowers at all different stages of development. F. oxysporum was rarely isolated from flowers, and only 2 flowers from the TZ-15 sample yielded F. oxysporum. F. moniliforme was commonly isolated from all sampled flowers at all stages of development (Figure 10). Frequency of isolation ranged from 1-28 colonies of F. moniliforme per 100 flower samples (Table 3), for an overall isolation of over 4% from sampled flowers. The (SD) field flowers sampled showed low rates of Fusarium contamination early in the growing season, but high rates late in the season (Table 3). This was not the case in the MF field (Table 3). F. solani, F. tricinctum and F. roseum were also isolated from flowers (Table 3). Frequency of isolation of saprophytic fusaria increased

later in the growing season, and a higher incidence of fusarial contamination was seen later in the growing season (Table 3).

Other contaminants such as Alternaria, Pennicillium, Aspergillis, and Botrytis were also isolated from washed flowers. F. moniliforme and F. oxysporum were pathogenic to asparagus seedlings, while saprophytic fusaria showed little or no pathogenicity (Table 9).

Isolations from both washed and surface sterilized immature asparagus fruits yielded F. moniliforme, F. tricinctum, F. solani, and to a lesser extent F. oxysporum (Table 6). All but 2 samples yielded F. moniliforme ranging from incidences of 1-3 colonies per 25 sampled fruits, for an overall average incidence of 7% F. moniliforme from immature fruits. Isolates grew from both the interior and exterior of fruits. F. tricinctum and F. solani were frequently isolated from immature fruits (Table 4). F. oxysporum was isolated from one fruit sample (Table 4). Only F. moniliforme and F. oxysporum isolates showed pathogenicity to asparagus seedlings (Table 9).

Ripe asparagus fruits yielded F. moniliforme, F. tricinctum, and F. solani (Table 5). Surfaces of fruits from 3 of 4 fields yielded F. moniliforme (Table 5), for an overall average isolation of over 6%. F. tricinctum was found on the surface of 18 of 25 of the TZ-1 samples, but only 1-4 colonies were isolated from other samples. Internal pulp extracted from ripe fruits yielded F. moniliforme (Figure 11) from all fields sampled (Table 5), for an overall average of almost 10%. External fruit surfaces often yielded a wide array of fungal inhabitants such as Alternaria, Penicillium, Rhizopus, etc.; but internal pulp wasn't

as infested with such saprophytic fungi. Lesser amounts of F. tricinctum and F. solani were also isolated from mature fruit pulp, but only F. moniliforme was pathogenic to asparagus seedlings.

Stem lesions yielded F. moniliforme, F. oxysporum, F. tricinctum, F. solani, and F. roseum (Table 6). Lesions ranged from 1-4 mm long and were confined to cortical tissue except for severe cases of decline. Not all lesions yielded fusaria. F. moniliforme (Figure 12) was isolated from stem lesions from all three fields sampled at incidences of 2-19 colonies per sample of 50 lesions, for an average isolation of nearly 20%. Upper stem lesions (USL) and twig lesions yielded the most F. moniliforme colonies (Table 6). F. oxysporum was infrequently isolated from USL and TL, but was isolated from 35% of lower stem lesions sampled (Table 6). F. tricinctum, F. solani, and F. roseum were also isolated from stem lesions (Table 6). Only F. moniliforme and F. oxysporum isolates were pathogenic to asparagus seedlings, while F. tricinctum, F. solani and F. roseum isolates were not pathogenic (Table 9). There was a large difference between the incidence of fusaria from stem lesions sampled early in the season versus late in the season (Table 6). The percent isolation of fusaria from stem lesions increased for F. moniliforme, F. oxysporum, F. tricinctum and F. roseum late in the growing season and decreased only for F. solani. F. oxysporum was isolated from none of the early lower stem lesions, and from 20 of 50 late season LSL (Table 6). Microscopic examinations of stem cross sections showed lesions to be confined to cortical tissues.

Dead and senescing stems were observed to have fungi sporulating

on the stem surfaces late in the growing season (Figure 13). Entire stems would appear pink or orange due to intense fungal sporulation. Sporodochia formed at the bases of infected stalks, and progressed up the stalks until the entire stem was covered. Stems with heavy sporulation were abundant late in the growing season from all fields sampled. Sporodochia with microconidia and macroconidia similar to Fusarium were observed on infected stem sections under the dissecting microscope. Further examination showed microconidia distinctly formed in chains, indicating F. moniliforme and scrapings of such sporodochia onto slides showed macroconidia resembling F. moniliforme. Sporodochia were scraped over PCAL plates yielded abundant Fusarium colonies after 4-5 days. F. moniliforme, F. solani, F. tricinctum, and F. roseum equiseti were identified from single spore isolates of subcultured colonies from stem sporodochia (Table 7). Pink sporodochia were most commonly encountered and yielded primarily F. moniliforme (Table 7). White and orange sporodochia yielded colonies of F. moniliforme, F, oxysporum, F. solani, F. tricinctum, and F. roseum equiseti. Most fusarium colonies identified from sporodochia were F. moniliforme, with lesser amounts of F. oxysporum, F. solani, F. tricinctum, F. roseum, and other fungi (Table 7). F. oxysporum and F. moniliforme isolates were pathogenic to asparagus.

Fusarium colonies developed in Nash media plates left unopened in asparagus growers fields (Table 8). Exposed plates left on the ground yielded the most fusaria colonies, 3.7 colonies per plate. Plates placed in the air yielded 11 total colonies or 1.1 colonies per plate. The

TABLE 1

LOCAL ASPARAGUS BEDS SAMPLED IN SURVEY
FOR INCIDENCE OF *FUSARIUM* CROWN, STEM, AND ROOT ROT

COMMERCIAL GROWERS FIELDS	AGE	TOWN
Thomas Zigmont (TZ)	1, 15 years	Hatfield
Mary Fil (MF)	7 years	Hadley
Stan Baj (SB)	4 years	Hadley
Tim Nourse (TN)	1 year	Hadley
Aqua Vitae Farm (AV)	2 year	Hadley
Experimental Field		
UMASS Experimental Farm	3 years	South Deerfield

TABLE 2

ISOLATION OF FUSARIA FROM MATURE ASPARAGUS PLANTS EXHUMED FROM COMMERCIAL GROWERS FIELDS[a]

FIELD	SAMPLE	NUMBER	INCIDENCE OF *FUSARIUM* SPECIES[b]		
			F. oxysporum	*F. moniliforme*	*F. solani*
MF-7 year	Crown	25	12	5	4
TZ-1 year	Crown	25	9	-	10
TZ-15 year	Crown	25	15	3	6
SB-4 year	Crown	25	7	8	3
MF-7 year	Storage root lesion (SRL)	25	10	3	1
TZ-1 year	SRL	25	10	5	2
TZ-15 year	SRL	25	7	14	-
SB-4 year	SRL	25	14	-	7

[a]Plants randomly exhumed July-August 1979.

[b]Plants washed in running tap water 5 minutes and samples excised with razor blades, surface sterilized in 10% Clorox 10 minutes, and plated on acidified potato carrot agar (PCAL). Plates incubated 1-2 weeks in incubator at 23°C, and colonies subcultured and identified on PCAL.

TABLE 3

ISOLATION OF FUSARIA FROM WASHED FEMALE ASPARAGUS FLOWERS[a]

FIELD	FLOWER STAGE	NUMBER	INCIDENCE OF FUSARIUM SPECIES[b] F. *oxysporum*	F. *moniliforme*	F. *tricinctum*	F. *solani*	F. *roseum*[c]
Early Season[d]							
TZ-1 year	pre-open	100	-	4	8	-	-
	open	100	-	1	12	-	1
	senescent	100	-	1	11	-	-
TZ-15 year	preopen	100	-	7	12	-	-
	open	100	-	4	13	-	3
	senescent	100	2	5	9	2	-
MF-7 year	pre-open	100	-	2	-	-	1
	open	100	-	-	-	6	-
	senescent	100	-	6	13	11	6
SD	pre-open	100	-	1	1	-	-
	open	-	2	1	-	-	
	senescent	100	-	4	2	-	-
Late Season							
MF-7 year	pre-open	100	-	8	-	16	-
	open	100	-	5	2	8	-
	senescent	100	-	-	-	12	-
SD-3 year	pre-open	100	-	16	30	10	-
	open	100	-	8	42	10	-
	senescent	100	-	28	40	12	-
AV-2 year	pre-open	100	-	3	12	7	10
	open	100	-	8	16	4	5
	senescent	100	-	13	42	6	2

TABLE 3

ISOLATION OF FUSARIA FROM WASHED FEMALE ASPARAGUS FLOWERS[a]

(Cont'd)

---

[a]Flowers randomly selected from female plants using forceps.

[b]Flowers individually washed in running sterile distilled water for 2 minutes and plated on PCAL. Plates incubated and isolates subcultured and identified on PCAL or carnation leaf agar (CLA).

[c]All *F. roseum* cultivars included.

[d]Early season collection June-July 1979 and late season August-September 1980.

TABLE 4

ISOLATION OF FUSARIA FROM IMMATURE (GREEN) ASPARAGUS FRUIT[a]

FIELD	TREATMENT	NUMBER	INCIDENCE OF FUSARIUM SPECIES[b]			
			F. oxysporum	*F. moniliforme*	*F. solani*	*F. tricinctum*
SD-3 year	washed	25	-	-	-	2
	Surface sterilized	25	-	3	-	1
MF-7 year	washed	25	-	3	2	12
	SS	25	-	2	2	5
TZ-1 year	washed	25	-	3	-	4
	SS	25	-	1	4	-
TZ-15 year	washed	25	1	2	-	5
	SS	25	-	-	-	-

[a]Fruits randomly selected from female plants, June 1979-July 1979 with forceps.

[b]Fruits washed in sterile distilled water 5 minutes or surface sterilized in 5% Clorox 5 minutes, then fruits excised in half and pieces plated on acidified potato carrot agar (PCAL). Plates incubated, and isolates subcultured and identified on PCAL.

TABLE 5

ISOLATION OF FUSARIA FROM MATURE OR RIPE (RED) FRUITS[a]

			INCIDENCE OF FUSARIUM SPECIES			
FIELD	SAMPLE[b]	NUMBER	*F. oxysporum*	*F. moniliforme*	*F. tricinctum*	*F. solani*
TZ-1 year	Surface	25	-	1	18	-
TZ-15 year	Surface	25	-	2	1	-
MF-7 year	Surface	25	-	-	-	1
SD-3 year	Surface	25	-	4	-	2
TZ-1 year	Pulp	25	-	4	2	-
TZ-15 year	Pulp	25	-	2	2	-
MF-7 year	Pulp	25	-	4	4	-
SD-3 year	Pulp	25	-	2	-	3

[a]Fruits randomly sampled from female plants September-October 1979 with forceps.

[b]Fruits surface sterilized in 5% Clorox 1 minute, then surface tissue excised with razor blades and plated on PCAL; and internal pulp removed with sterile forceps and plated on PCAL.

TABLE 6

ISOLATION OF FUSARIA FROM EXCISED ASPARAGUS STEM LESIONS[a]

FIELD	SOURCE	NUMBER	INCIDENCE OF FUSARIUM SPECIES[b]				
			F. oxysporum	*F. moniliforme*	*F. tricinctum*	*F. solani*	*F. roseum*
SB-4 year	Stem Lesion (SL)	50	6	19	1	1	2
MF-7 year	Upper Stem Lesion (USL)	50	1	17	5	3	-
	Twig Lesion (TL)	50	-	9	1	-	-
	Lower Stem Lesion(LSL)	50	15	10	8	3	-
	Early Season[c]						
SD-3 year	SL	50	-	4	3	16	-
	Late Season						
SD-3 year	USL	50	-	7	5	6	3
	LSL	50	20	2	3	2	-

[a]Red-brown Fusarium lesions excised from selected stem July-September 1979.

[b]Tissue pieces surface sterilized in 10% Clorox for 5 minutes, then plated on PCAL.

[c]Early season June-July, late season August-September.

TABLE 7

ISOLATION OF FUSARIA FROM SPORODOCHIA ON ASPARAGUS STEM TISSUE[a]

FIELD	SOURCE	SPORODOCHIAL COLOR	NUMBER	INCIDENCE OF FUSARIUM SPECIES				
				F. oxysporum	*F. moniliforme*	*F. solani*	*F. tricinctum*	*F. roseum*
MF-7 year	Upper Stem	Pink	5	-	5	-	-	-
MF-7 year	Lower Stem	Pink-White	5	1	1	2	1	-
TZ-1 year	Lower Stem	White	5	1	2	1	1	-
TZ-1 year	Lower Stem	Orange	5	-	-	1	2	-
TZ-15 year	Lower Stem	Orange	5	-	2	1	-	-
TZ-15 year	Upper Stem	Pink	5	1	3	-	-	1
AV-2 year	Upper Stem	Pink	5	-	4	-	-	1
AV-2 year	Upper Stem	Pink	5	-	5	-	-	-
AV-2 year	Upper Stem	White	5	2	-	-	-	3

[a]Sporodochial color and source were visually determined, and sporodochia were scraped over PCAL with sterile scalpel under a sterile hood.

[b]Plates were incubated 4-7 days in a growth chamber at 23°C. Resulting colonies were subcultured on PCAL, incubated 7-14 days and identified on CLA.

TABLE 8

ISOLATION OF AIRBORNE FUSARIA FROM LOCAL COMMERCIAL ASPARAGUS BEDS[a]

FIELD	STATION	*FUSARIUM* SPECIES IDENTIFIED	COLONIES ASSOCIATED W/ ORGANIC MATTERS	INCIDENCE OF FUSARIUM SPECIES[b]				
				F. oxysporum	*F. moniliforme*	*F. tricinctum*	*F. solani*	*F. roseum*
MF-7 year	Ground	21	6	1	2	7	5	6
MF-7 year	3 feet	7	-	-	1	3	-	3
TZ-1 year	Ground	16	8	2	4	4	3	3
TZ- 1 year	3 feet	4	-	-	1	2	-	-
TOTAL		48	14	3	7	16	8	12

[a]Fusaria isolated from 4 plates of Nash media, selective for *Fusarium*, exposed in the field for 15 minutes, at each station.

[b]*Fusarium* colonies visually determined on plates after 4 days, subcultured on PCAL, and single spore isolated on CLA.

TABLE 9

TEST TUBE PATHOGENICITY TESTS ON SELECTED ISOLATES[a]

ISOLATE	FIELD	SOURCE	PATHOGENICITY[b]
F. moniliforme	SD	flower	4
F. moniliforme	SD	flower	4
F. solani	SD	flower	2
F. tricinctum	SD	stem lesion	0
F. moniliforme	SB	stem lesion	5
F. moniliforme	SB	crown	4
F. moniliforme	SB	stem lesion	5
F. oxysporum	SB	crown	4
F. moniliforme	SB	seed	4
F. oxysporum	TZ	crown	3
F. moniliforme	TZ	crown	5
F. oxysporum	MF	crown	4
F. moniliforme	SD	seed	4
F. moniliforme	SD	seed	5
F. moniliforme	SD	crown	4
F. moniliforme	MF	flower	5
F. moniliforme	MF	air	5
F. moniliforme	TZ	air	4
F. oxysporum	TZ	air	2
F. moniliforme	SD	berry	4
F. oxysporum	SD	berry	3
F. solani	TZ	crown	1
F. tricinctum	MF	stem	2
F. tricinctum	MF	flower	0
Control	-	-	0
Control	-	-	1
Control	-	-	0

[a]Clean seedlings grown on Hoagland's solution agar slants 2 weeks, fungal isolate added, visual rating taken 2-4 weeks later.

[b]0-5 rating system, 0=clean 5=dead

two most frequently encountered *Fusarium* species were *F. tricinctum* and *F. roseum* var *equiseti*, which constituted 34 of 48 colonies identified (Table 8) and were isolated from all sampling stations. *F. moniliforme* was also found at all sampling stations and constituted 8 of 48 colonies identified. *F. oxysporum* was isolated from the ground stations of both fields and constituted 3 of the 48 identified colonies. *F. solani* was also found only on ground station plates and constituted 8 of 48 identified colonies (Table 8). *F. solani* and *F. roseum* colonies often originated from small airborne organic matter particles, while *F. tricinctum*, *F. oxysporum* and *F. moniliforme* did not (Table 8).

Pathogenicity tests on selected isolates showed *F. moniliforme* and *F. oxysporum* isolates from diseased asparagus plants to be highly pathogenic to asparagus (Figure 14). *F. solani* and *F. roseum* showed some pathogenicity but were rarely very virulent. *F. tricinctum* was always avirulent (Table 9).

Discussion

The survey showed the *Fusarium* stem, crown, and root rot complex to be well established in all fields sampled. Diseased plants showed yellowed fronds early in the season; and reddish lesions on stems, crowns, and roots yielded *F. moniliforme* and *F. oxysporum*. The white, blanched lower stems of plants showed numerous red-brown elliptical lesions of varied size, indicating a heavy degree of infection in established beds. Not all lesions yielded *Fusarium*, and some were believed to be unsuccessful infections. This agreed with the findings of

Cohen and Heald (21, 22), who described small lesions on lower asparagus stems to be incipient infections by F. oxysporum. High numbers of lesions on stems indicated high populations of parasitic fusaria in soils. As the growing season progressed, the number of lesions per stem increased, and smaller lesions coalesced into larger lesions. Larger lesions led to stem and crown rot of mature plants, and F. oxysporum and F. moniliforme were consistently isolated from larger lesions. Observations showed stem lesions to be initially limited to cortical tissues and stem sections didn't show vascular discoloration until the plant was near death. Crown cortical tissues were also initially parasitized and vascular tissue was colonized later in the disease. Infections would begin in cortical tissue and gradually progress into crown vascular tissue. Storage root lesions often involved vascular tissue and may provide an entry route for pathogens to reach crown vascular tissue. Classic symptoms of decline such as wilting and yellowing of fronds appeared after invasion of crown vascular elements. Vascular tissue from diseased stems rarely yielded the pathogens except from lower stems near infected crowns. Entry of vascular elements appeared to be a slow process and led to a more rapid decline of plants. When the crown rots, water and nutrients are no longer transported, and the plant dies leaving an empty space in the bed. The more rapid decline seen as F. oxysporum and F. moniliforme enter crown vascular elements may not allow sufficient time for invasion of the upper vascular system, as in vascular wilts of other crop plants. In most F. oxysporum diseases the fungus enters the vascular system, and F. oxysporum f. sp. conglutinans

develops in cabbage xylem tissues exclusively. _F_. _oxysporum_ attacking Massachusetts asparagus maybe a less effective pathogen than other strains of _F_. _oxysporum_ causing vascular wilts in asparagus and other crops. Cohen and Heald (21) and Grogan and Kimble (42) reported _F_. _oxysporum_ to cause a distinct vascular wilt of asparagus. The pathogen enters crown, root, and stem vascular elements; unlike _F_. _oxysporum_ in Massachusetts asparagus. _F_. _oxysporum_ isolates from Massachusetts may be more similar to the cortical pathogen _F_. _oxysporum_ var _redolens_, described by Graham (40) causing seedling blight of asparagus in Ontario. Attempts to key out _F_. _oxysporum_ isolates as _F_. _oxysporum_ or _F_. _oxysporum_ var _redolens_ were inconclusive due to macroconidial variation of isolates, and it can't be said if all _F_. _oxysporum_ strains attacking Massachusetts asparagus are _F_. _oxysporum_ var _redolens_ or _F_. _oxysporum_. I believe both fungi are involved in the decline. Lower virulence of Massachusetts _F_. _oxysporum_ strains may account for the inability of _F_. _oxysporum_ to invade vascular elements early in infection, though differences in the asparagus vascular system may also make it more difficult for parasitism by _F_. _oxysporum_. A similar situation is seen in tomato, where _F_. _oxysporum_ f. sp. _lycopersici_ causes a vascular wilt and _F_. _oxysporum_ causes a cortical stem rot (84a).

_F_. _oxysporum_ parasitizing Massachusetts asparagus was associated with below ground plant parts including lower stems, crowns, storage and feeder roots (Table 2); and was rarely isolated from aboveground plant parts. In all fields _F_. _oxysporum_ was limited to soils and lower plant parts, and its presence in soils was correlated with high

disease incidences in mature beds. This is consistent with the fact that most parasitic and saprophytic strains of F. oxysporum exist in soils (13, 30, 37, 64, 74).

Saprophytic fungi were isolated crowns, storage root lesions, and soils, such as F. solani. The role of such saprophytes is uncertain, but these fungi may invade tissues previously attacked by parasitic fusaria. Saprophytic fusaria are able to colonize lesions initiated by parasitic fungi and plants weakened by unfavorable conditions (13, 75).

F. moniliforme was most often isolated from aboveground plant parts such as flowers, fruits, and upper stems. It was also isolated from storage root lesions and crowns. F. moniliforme parasitizing plants has been associated with seeds, stalks, flower parts, roots, and crowns (10, 11, 13, 29, 36, 38, 48, 49, 50, 51, 60, 88). F. moniliforme was shown to parasitize banana flowers (13), and F. moniliforme var subglutinans to attack pineapple flowers (10, 11). Some F. moniliforme var subglutinans flower infections were able to infect developing pineapple fruits, and cause a serious fruit rot.

F. moniliforme and F. moniliforme var subglutinans were isolated from asparagus flowers, but for the purpose of the survey the two fungi are considered as F. moniliforme (Table 3). Asparagus flower lesions didn't always yield fusaria, and such lesions may be unsuccessful infections. F. moniliforme may survive on flowers by growing on and colonizing flower surfaces; or by remaining on flowers as dormant propagules until a proper substrate is available, such as a developing

fruit. *F. moniliforme* and *F. moniliforme* var *subglutinans* can infect flowers via airborne, water-borne, or insect-borne inoculum; but not via vascular elements. Vascular system isolations from female plants yielding infected flowers never yielded *F. moniliforme*, and colonies growing from flowers appeared randomly on external and internal surfaces. Surface sterilization with a 1% Clorox solution was able to completely eliminate flower infections, indicating a superficial association.

The stage of flower development didn't affect *Fusarium* incidence, indicating a random infestation (Table 3). The high incidence of saprophytic fusaria such as *F. tricinctum*, *F. solani*, and *F. roseum* cultivars, points to a non-specific infestation based on random contact with airborne, water-borne, or insect-borne spores. Differences in *Fusarium* populations on flowers from different fields may indicate dominant species in asparagus beds (Table 3). The greater number of flower infections observed later in the season was correlated with increased amounts of inoculum from a greater number of infected plants later in the season. Sporodochia on diseased plants appeared later in the growing season on stems, and allowed for increased amounts of inoculum to contact flowers (36). Greater insect-fusaria interactions later in the season could also create additional flower infections and/or multiply initial infections.

Fusaria associated with flowers were also isolated from developing fruits. The fungi contact pollinated flowers, survive on flower

surfaces, and infect developing fruits. Spores contacting fruits during fruit development can create additional external infections; but surface sterilization of fruits with Clorox solutions didn't completely eliminate fusarial contamination, indicating an internal association (Table 4). Fungi associated with fruit, such as *F. moniliforme*, can become associated with the seed and be disseminated with seeds or berry debris. Both pathogenic *F. moniliforme* and saprophytic *F. solani* and *F. tricinctum* were associated with fruits, showing a random association independent of pathogenicity to asparagus (Table 4, 5). *F. oxysporum* was isolated from only a single fruit, and this was consistent with the soil-borne habit of this fungus.

Ripened fruits continued to yield fusaria from internal and external tissues (Table 5). Internal infections may represent early contamination of flowers, while external infections may originate from *Fusarium* spores contacting the outside of developing fruits. Insects associated with fruits such as asparagus beetles also contributed to fruit infections, particularly by *F. moniliforme*. Seed extracted from fruits yielded *F. moniliforme* (Figure 3) from external seed coat surfaces, indicating that the pathogen is able to become associated with seeds inside ripe fruits. The surfaces of asparagus flowers and fruits yielded a wide array of fungi including the genera *Alternaria*, *Botrytis*, *Pennicillium*, *Cladosporium*, *Gloeosporium*, *Epicoccum* and *Aspergillus*; indicating a large and changing planosphere population. Fungi such as *Alternaria* and *Penicillium* were found both on external and internal fruit tissues demonstrating their ability to survive and

grow on asparagus fruits. Botrytis and Rhizopus were frequently encountered on external fruit and flower surfaces, but rarely from internal fruit surfaces. These fungi maybe transient members of the asparagus planosphere unable to colonize internal fruit tissues. Other studies of planosphere fungi on plants have shown these fungi to be commonly encountered on plant surfaces. Both F. moniliforme and F. oxysporum were associated with stem lesions (Table 6). Stem lesions were initiated by airborne or water-borne spores contacting stem surfaces, or by spores associated with insects in contact with stems. Damage to stems by asparagus miner flies, asparagus beetles, or European corn borers (Ostrinia nubilalis), may allow for infection of cortical or vascular tissues by F. oxysporum and F. moniliforme; but stem injury didn't appear to be necessary for smaller lesions. Many of these smaller lesions didn't yield fusaria and were unsuccessful infections. Sporodochia would form from active lesions under proper environmental conditions such as moist, humid, cool weather; creating potential sources of inoculum. Isolation of F. moniliforme and other saprophytic fungi from lesions such as F. tricinctum, was consistent with their association with aboveground plant parts. F. oxysporum was only associated with lower stem lesions.

Greater incidences of fusaria from stem lesions sampled later in the season showed the gradual increase in Fusarium populations throughout the growing season. Dead and senescing stems at the end of the growing season would be most heavily colonized by fusaria, particularly F. moniliforme. F. moniliforme effectively survived on

dead or dying stalks, and sporulated heavily on the surfaces of such stalks. Spores formed on sporodochia could be disseminated by air, wind, rain, or insects into soils or onto neighboring plants. This creates numerous possibilities for new infections on uninfected plants or previously infected plants. Sporulation can cause considerable infections in existing beds in a relatively short period of time under the proper conditions (36).

The ability of Fusarium spp. spores to become airborne has been demonstrated for many species (46, 47). F. oxysporum f. sp. lycopersici attacking tomatoes in the greenhouse (50) and in the field (13) has been shown to be disseminated via airborne inoculum. F. moniliforme and F. moniliforme subglutinans spores have been shown to be airborne in diseases of corn and sugar cane respectively (13, 60). F. moniliforme was isolated from the air in asparagus beds on the ground and 3 feet in the air, a height approximating that of asparagus flowers. Airborne spores are able to randomly contact aboveground asparagus plant parts, including flowers, and infect plants or survive saprophytically in the asparagus planosphere. Airborne dissemination is an effective and long range means of dissemination and is favored by cultivation, harvesting, and wind movement in fields. The ability of the pathogens to become airborne may partly explain the buildup of disease.

F. moniliforme overwinters with senesced stalks, plant debris, asparagus seed, and in association with asparagus miner fly pupae (Chapter V). Every new growing season, the F. moniliforme population

slowly increases by the various means of dissemination previously described. The initial starting population is increased after every growing season. This has resulted in large _F. moniliforme_ populations in Western Massachusetts, making this pathogen a major factor in Massachusetts asparagus decline.

Conclusion

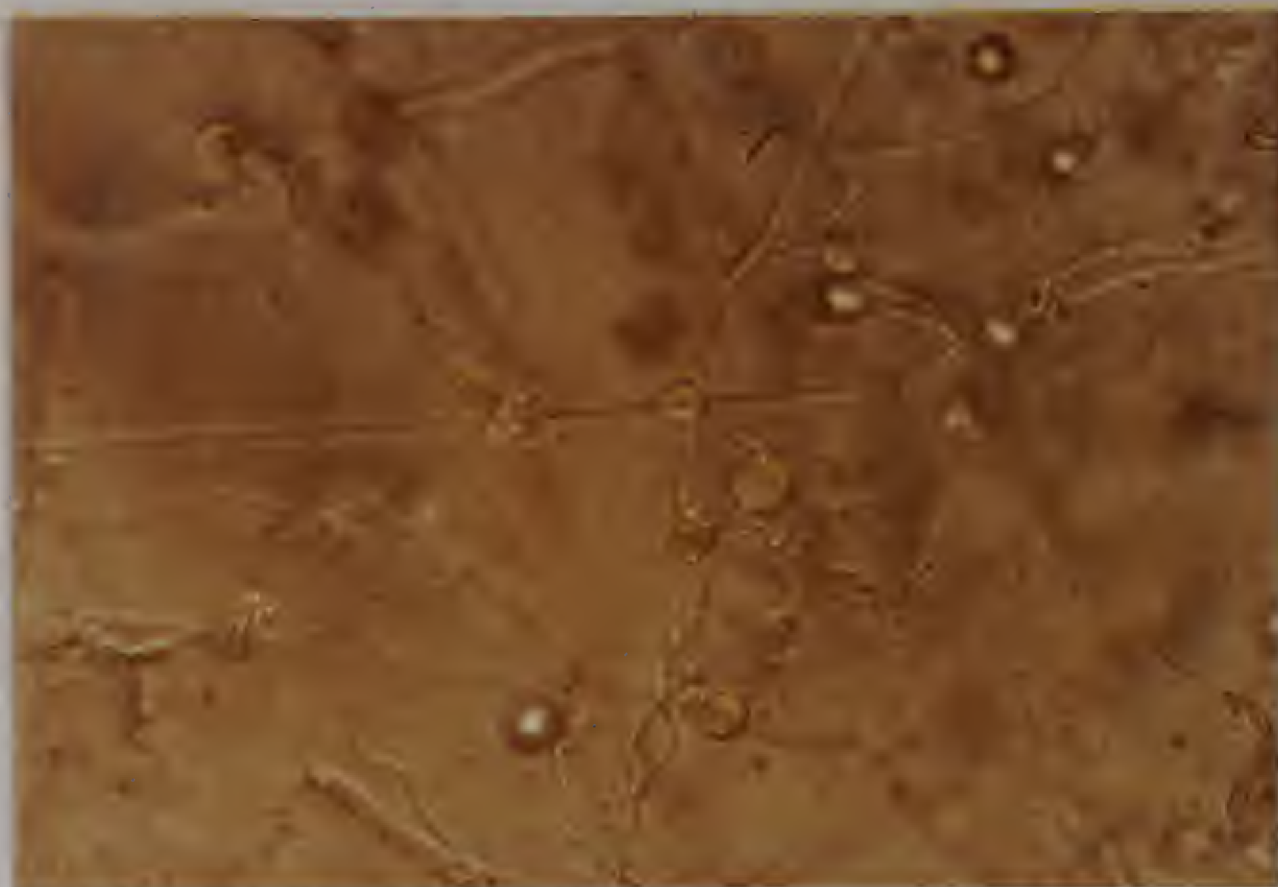
The survey of local growers fields showed _Fusarium_ stem, crown, and root rot to be the most serious problem facing asparagus growers. _Fusarium_ populations were high in all fields sampled, and almost every plant sampled was infected with _F. oxysporum_ or _F. moniliforme_. _F. oxysporum_ was found in soils and associated with below groound plant parts, while _F. moniliforme_ was found mainly on aboveground plant parts. _F. oxysporum_ populations increase in soil, using saprophytic and parasitic means of survival (Chapter I). _F. moniliforme_ can enter fields with seeds, crowns, via the air, or with insects. Populations increase in asparagus beds each successive season. _F. moniliforme_ colonies senescing asparagus stalks and sporulate on the aboveground surface of stalks. Inoculum from sporodochia infects other plants by being washed into soils and colonizing roots, crowns, and lower stems; or by being blown or carried to stems and flowers by air or insects. Flower infections result in infested seed, and _F. moniliforme_ can be disseminated with seed. The final result in a yearly build-up of _F. moniliforme_. in asparagus beds which cause considerable disease.

F. oxysporum and _F. moniliforme_ greatly differ in their biology

and life cycles, but both fungi cause severe disease in asparagus beds. The pathogens parasitize different parts of the plant and avoid competing for the same substrate. It was difficult to separate symptoms caused by *F. moniliforme* or *F. oxysporum* into separate diseases because they both cause the same symptoms on seedlings, crowns, and roots. No field was detected to be more infected with *F. moniliforme* or *F. oxysporum*. The different survival and dissemination means of these pathogens necessitates using different methods to manage the disease complex in Western Massachusetts properly.

Figure 2. Formation of chlamydospores in culture by _Fusarium oxysporum_ (400x).

Figure 3. Crown and stem rot symptoms on a seedling infected by _Fusarium_.



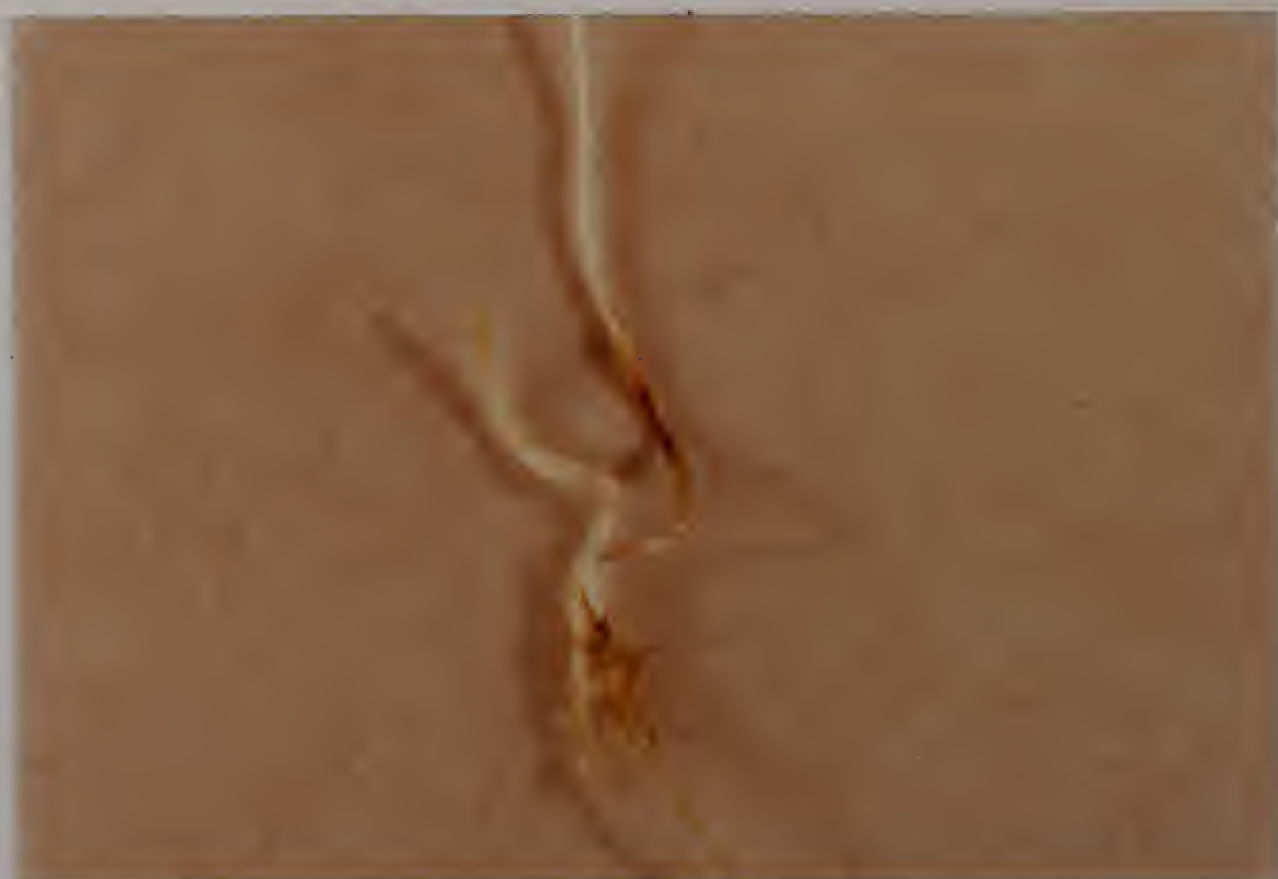
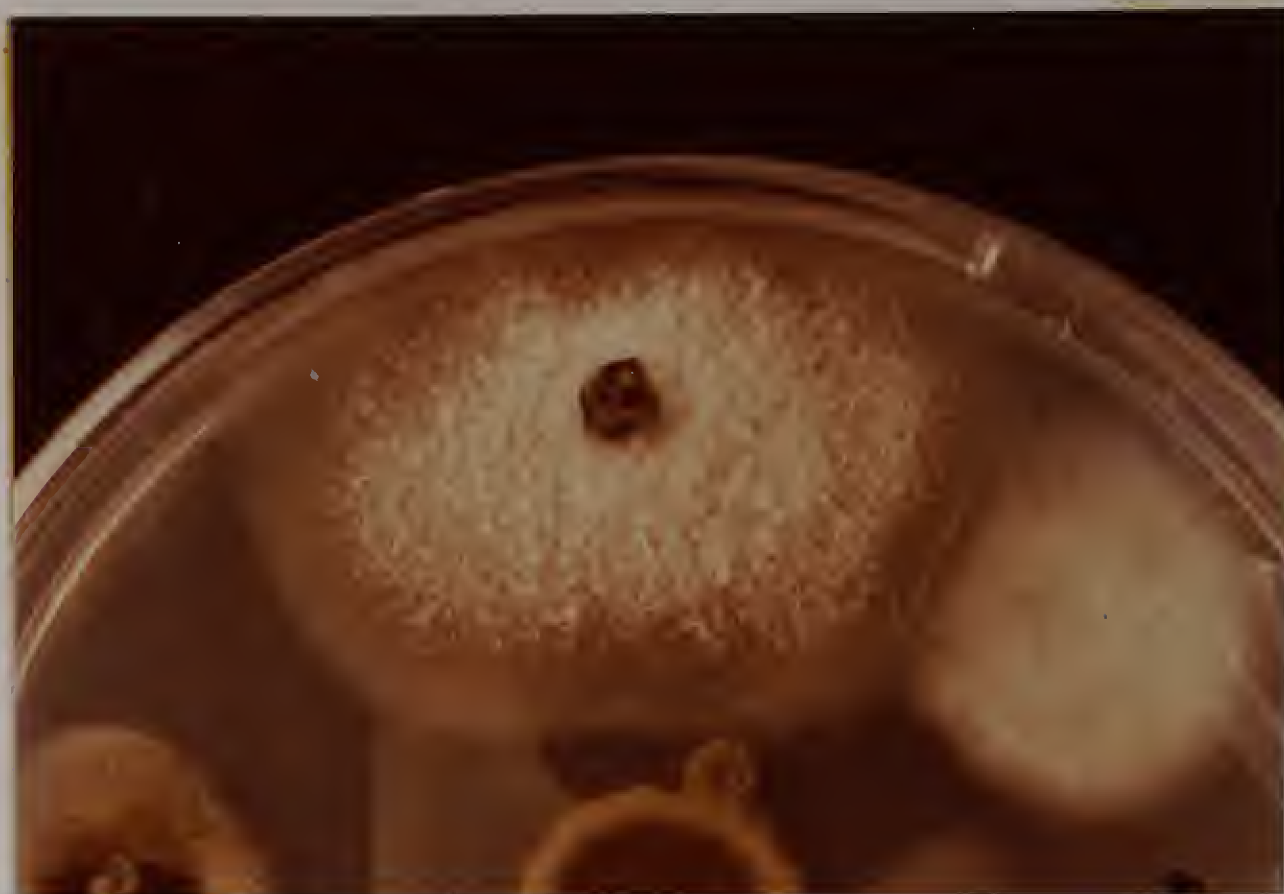


Figure 4. Colony of *F. moniliforme* isolated from a locally obtained asparagus seed plated on acidified potato carrot agar (PCAL).

Figure 5. Colony of *F. oxysporum* isolated from a storage root lesion on the roots of a locally grown 1-year-old crown.



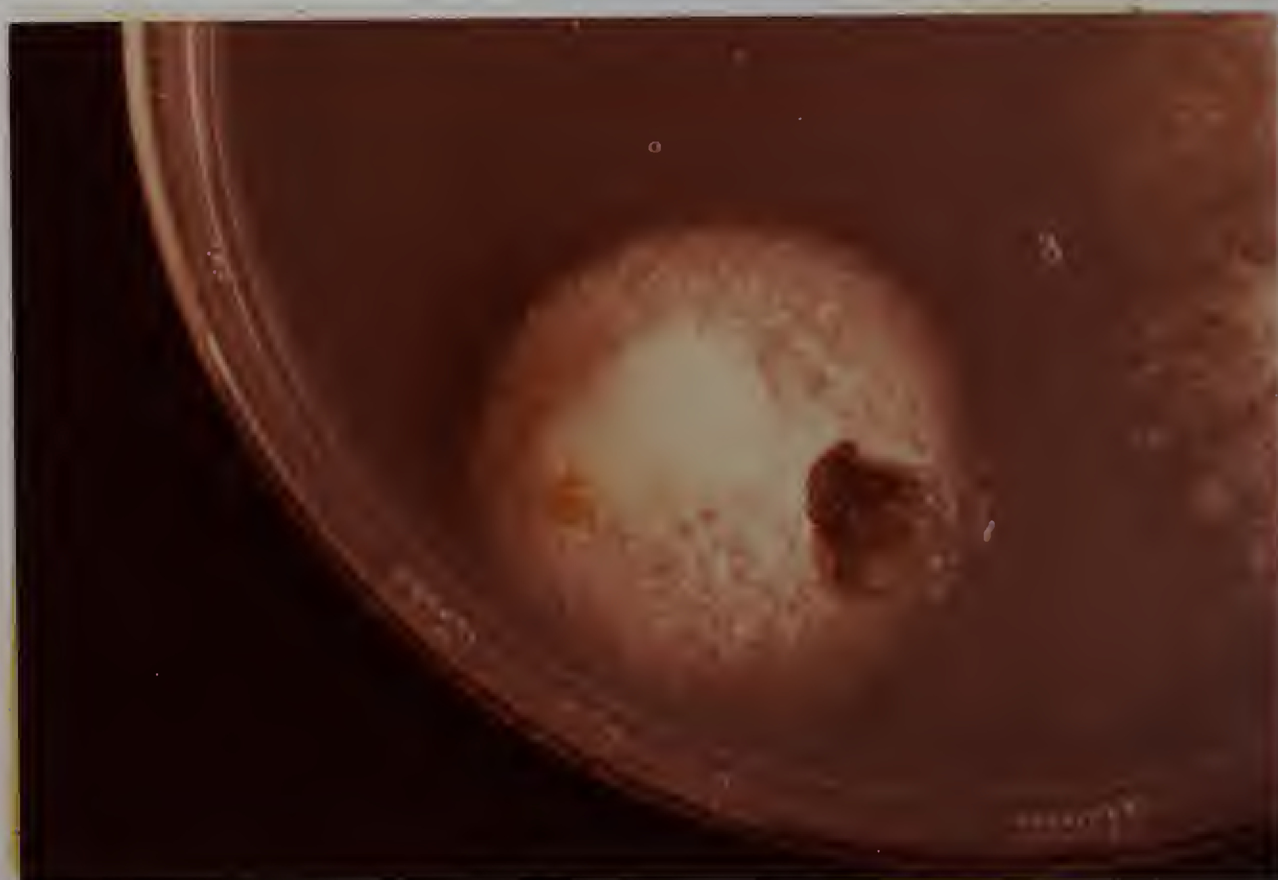


Figure 6. Pathogenicity of F. moniliforme from local seed (far right) and F. oxysporum from crown tissue (second from right) on roots of aseptically grown seedlings, compared to controls with no fungal isolates (left).

Figure 7. Colony of F. oxysporum isolated from a surface sterilized asparagus seedling crown grown in Montague field soil.

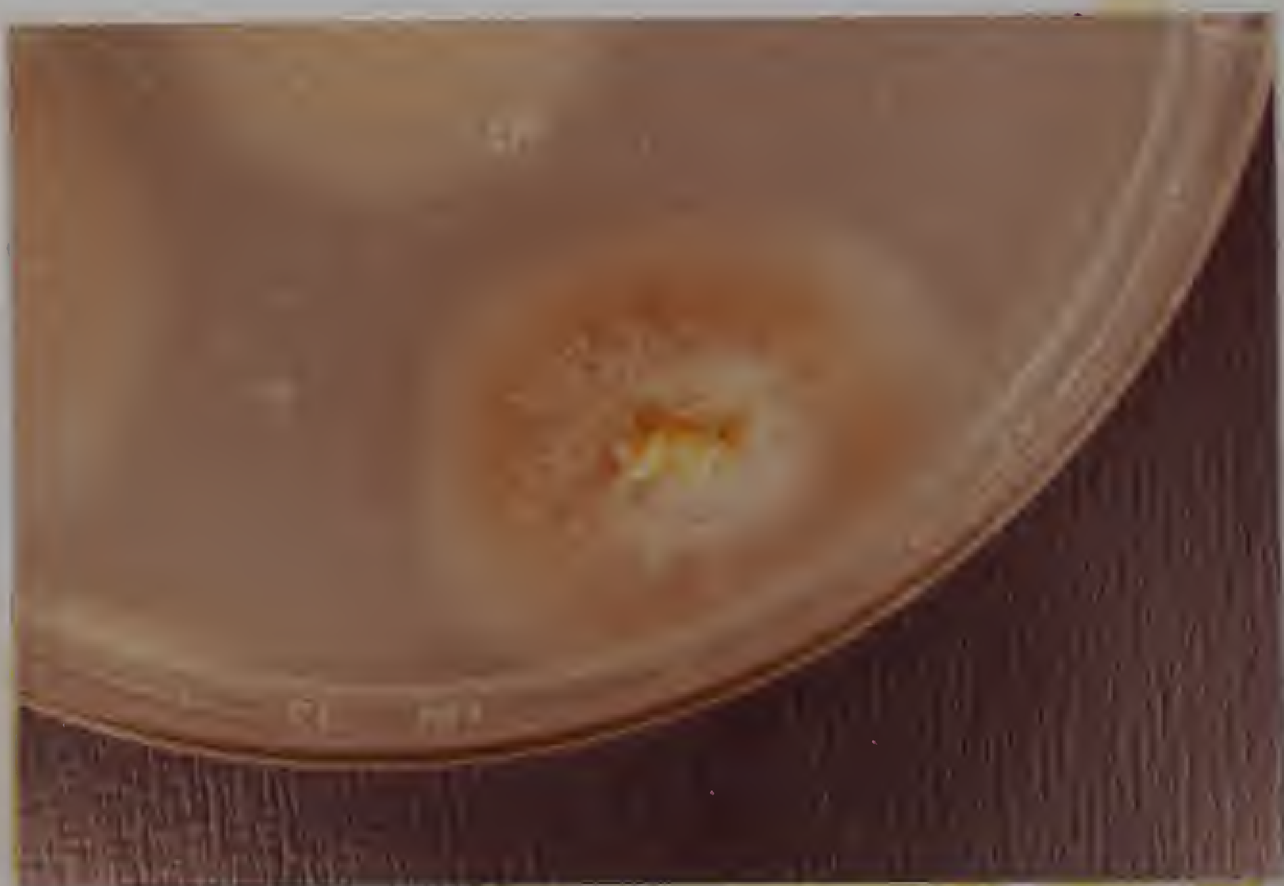


Figure 8. Underground stem of an asparagus fern showing typical red-brown elliptical lesions caused by soil-borne *Fusarium*.

Figure 9. Storage root lesions on roots of infected asparagus plant (note many lesions originating at points of feeder root growth).

Figure 10. Colony of *F. oxysporum* (top left) and *F. moniliforme* (bottom left) isolated from washed asparagus flowers on acidified potato carrot agar (PCAL).

Figure 11. Colony of *F. moniliforme* isolated from a ripe asparagus fruit on acidified potato carrot agar.

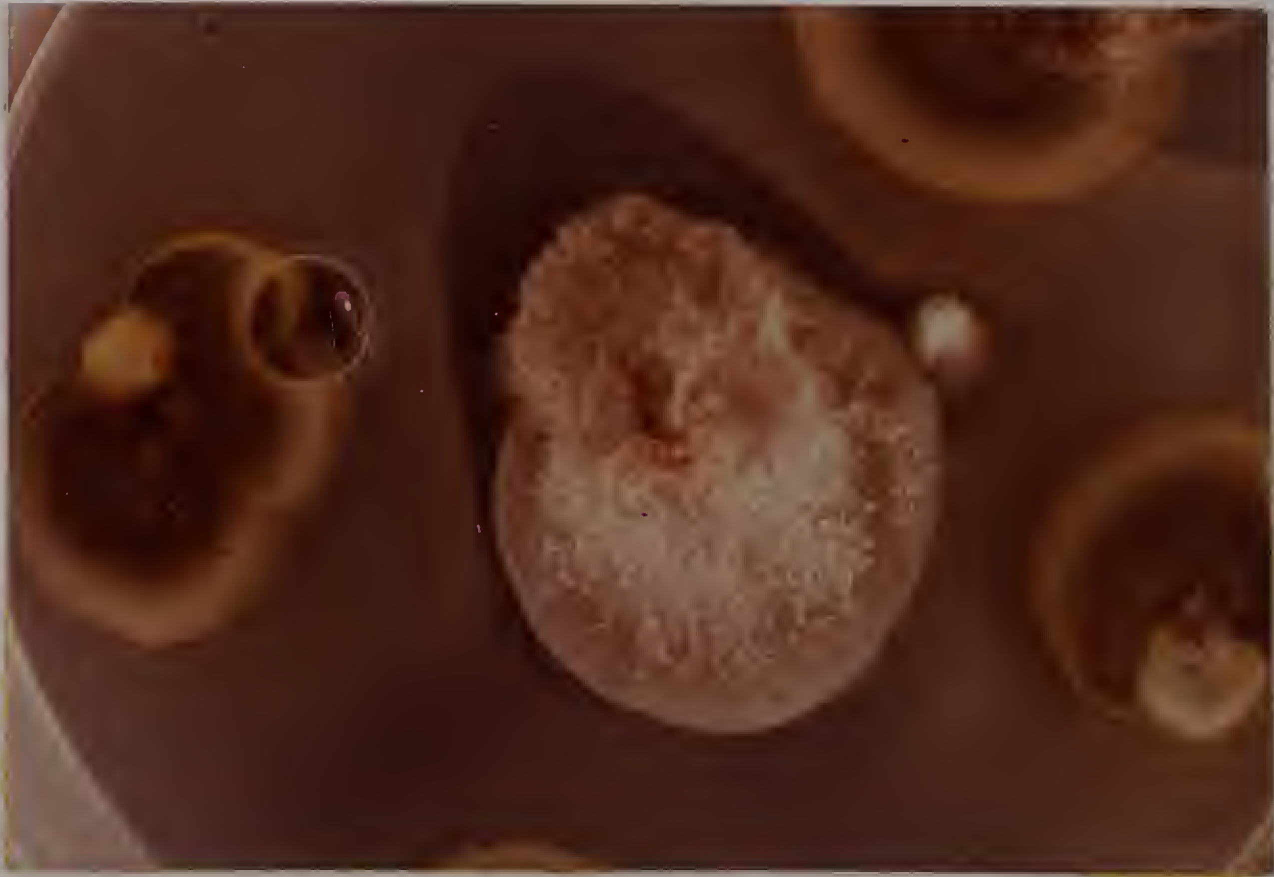


Figure 12. Colony of *F. moniliforme* isolated from a surface sterilized stem lesion section on acidified potato carrot agar.

Figure 13. Sporulation of *F. moniliforme* on a senescing asparagus stalk.

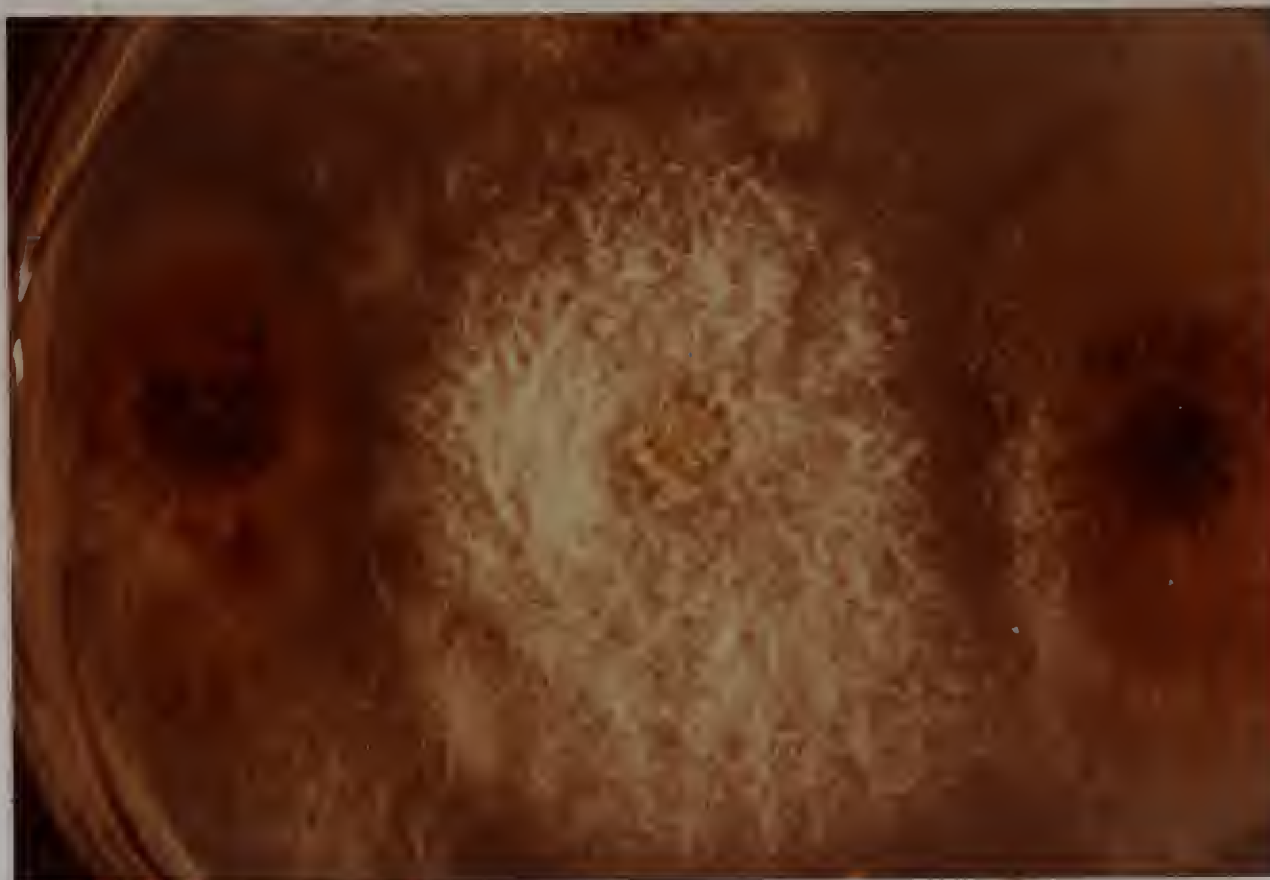


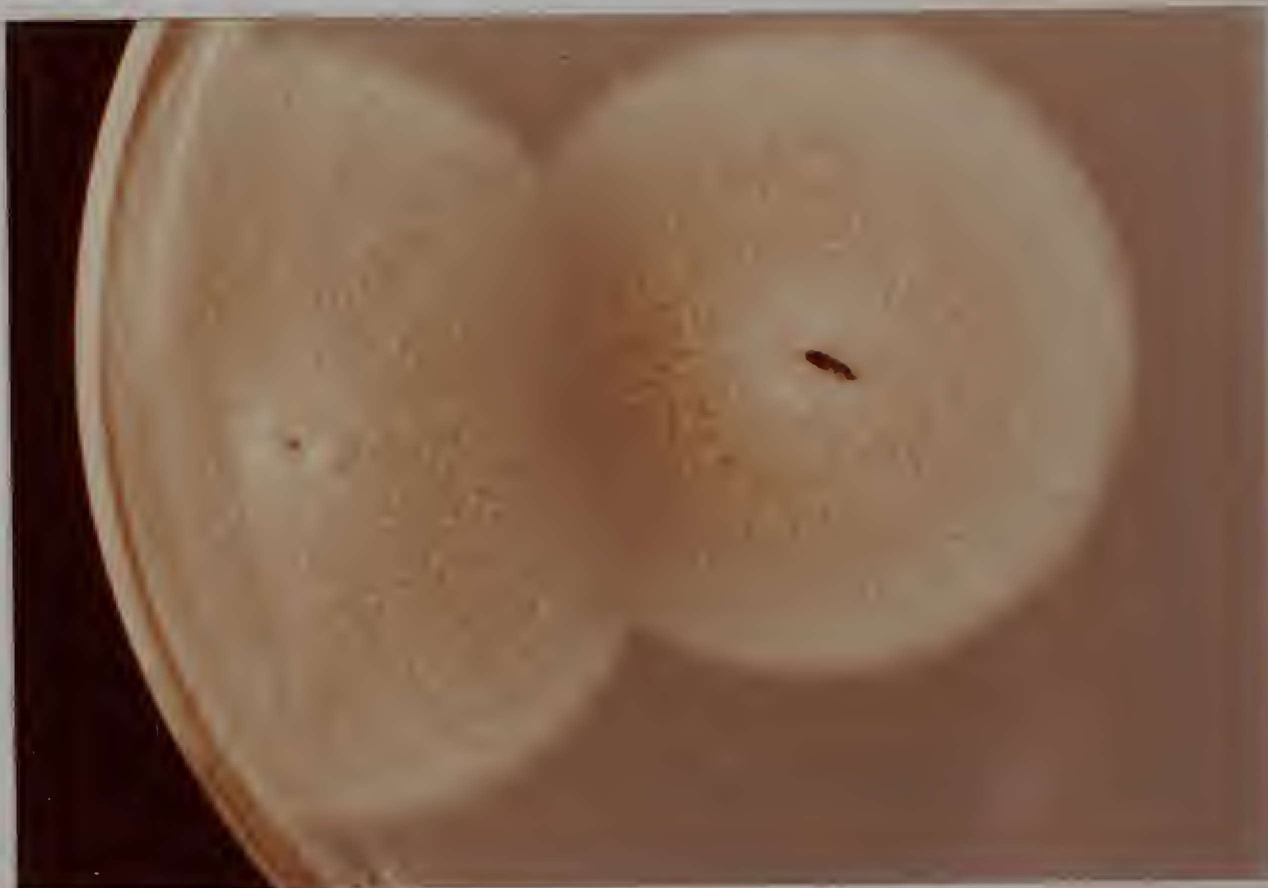
Figure 14. Pathogenicity of _F_. _moniliforme_ isolates for the air (left) and from an asparagus miner puparium (center), compared to control with no isolate (right).

Figure 15. Adult asparagus miner on asparagus inflorescence.

Figure 16. Stem mines by the asparagus miner on asparagus stalk showing fungal sporulation and discoloration.

Figure 17. Early season stem mines caused by feeding larvae of the asparagus miner.

Figure 18. Colonies of *F. moniliforme* isolated from a pupae (left) and an adult (right).



CHAPTER IV

ROLE OF VOLUNTEER AND WEED HOSTS

Introduction

Western Massachusetts asparagus fields often contain high populations of volunteer plants and weeds. Volunteer plants start as fruits containing viable seeds drop from female plants to the ground. Fruit tissue decomposes in the soil, and seeds germinate the following growing season. Clumps of one-year-old volunteer seedlings reveal where fruits had fallen the previous year. Some first year volunteers survive through the growing season and become second year plants, though these were less common than one year plants. High populations of volunteer plants were seen in two of three fields surveyed, and initial observations indicated that one and two year volunteers showed typical symptoms of *Fusarium* stem, crown, and root rot.

Though most local growers use pre-emergence herbicides for weed control, large populations of various weed species were observed in beds after harvesting. Weed species such as pigweed (*Amaranthus retroflexus*), lambsquarter (*Chenopodium spp.*), purslane (*Portulaca oleracea*), common chickweed (*Stellaria media*) horsetail or bottlebrush (*Equisetum arvense*), common milkweed (*Asclepias syriaca*), field bindweed or small morning glory (*Convolvulus arvensis*), large crab grass (*Digitaria sanguinalis*), quackgrass (*Agropyron repens*) smartweed (*Polygonum pensylvanicum*), and others were observed in commercial beds. In some beds, weeds outgrew mature asparagus ferns and dominated entire

areas of the bed. One grower's field was so weed-infested that the ferns were almost invisible. Many weeds went to seed and created additional generations for that growing season. Observations of weeds showed occasional crown discoloration or root lesions possibly caused by fusaria and weeds may serve as carriers of pathogenic fusaria.

Objectives

1. To examine volunteer plants and selected weed species for symptoms of Fusarium stem, crown, and root rot; and to attempt to isolate the pathogens from these plants.

2. To determine if pathogenic Fusarium oxysporum or F. moniliforme exist on symptomless weed hosts.

3. To determine if a wide array of weed species are associated with Fusarium species, and the possible role weeds and volunteers play in asparagus decline.

Literature Review

Volunteer plants in crop fields are inoculum sources for various plant plant pathogens, including soil-borne fungi. Volunteers maintain Fusarium in tobacco fields, Verticillum in cotton fields, and Phytophthora in potato fields (44). Fusaria pathogenic to asparagus was associated with volunteer asparagus plants by Graham (40) in Ontario; and Manning, et. al. (68) in Massachusetts.

Weeds harbor many plant pathogens, including viruses, bacteria, nematodes, and fungi (56). Infected weeds may show symptoms or be

symptomless carriers, and act as inoculum sources for plant pathogens.

Boosalis and Scharen (12) showed pigweed (Amaranthus retroflexus) susceptible to fifteen races of Rhizoctonia solani in Nebraska. The fungus caused lesions on roots and stems, and isolates of R. solani from lesions caused similar symptoms on potato plants. Residues of pigweed plants served as an important inoculum source for R. solani in potato fields. Oshima, et al. (8) found lambsquarter (Chenopodium album) and purslane (Portulaca oleracea) to be symptomless carriers of R. solani in Colorado potato fields.

Evans (30) showed a wide variety of weeds to be symptomless carriers of Verticillium dahliae pathogenic to cotton. Weeds are believed to perpetuate cotton wilt by V. dahliae in Australia, and to allow for a build-up of soil-borne inoculum (30).

Pathogenic F. oxysporum strains have been shown to infect non-host crop plants and weed species. Armstrong and Armstrong (4) and Hendrix and Neilson (45) demonstrated F. oxysporum forma specialis to readily cross infect non-host crop plants without causing disease symptoms. The causal agent of Panama disease of bananas, F. oxysporum f. sp. cubense, was isolated from related weed species in Central American banana plantations (13, 34, 97). F. oxysporum f. sp. lycopersici was associated with weeds in Israel by Katan (52). The author isolated F. oxysporum from many weeds, but only four species: pigweed (Amaranthus retroflexus), mallow (Malva silvestris), crabgrass (Digitaria sanguinatis), and mountain rice (Oryzopsis milliaceae), yielded isolates pathogenic to tomatoes. These weeds were symptomless carriers, and weed tissue contained less propagules/gram of F. oxysporum f. sp. lycopersici than

infected tomato plant tissue (52). The importance of weed hosts in disease incidence wasn't determined but weed infections were believed to be a common occurrence.

Methods and Materials

Volunteers and weeds were collected from local growers fields and washed under running tap water for 5 minutes. Weed crowns, roots, and stems were washed in sterile distilled water or surface sterilized in a 2% Clorox solution for 30 seconds to 5 minutes depending on the plant part, and plated on PCAL. Weeds were identified at least to genus, and in some cases to species. Three common weeds found in growers fields; lambsquarter, purslane, and pigweed, were grown in boxes containing soil naturally infested with *F. oxysporum* and *F. moniliforme*. Soil dilutions confirmed the presence of both pathogens. Weeds were sampled from boxes after 4 weeks growth, and washed under running tap water for 5 minutes. Plants were observed for disease symptoms such as crown discoloration, stem and root lesions, and yellowing or wilting of aboveground plant parts. Plants were sectioned into above and below ground parts for plating on PCAL. Crowns, roots, and stems of 1 year volunteer plants were surface sterilized 1 minute in 5% Clorox and plated on PCAL, while 2 year volunteer tissues were surface sterilized 5 minutes in 10% Clorox and similarly plated. *Fusarium* isolates from weeds and volunteers were subcultured and identified as previously described, and agar plate and/or test tube pathogenicity tests were run on selected isolates.

Results

One and two-year-old volunteer plants sampled from the 4, 7, and 15-year-old fields showed visible symptoms of stem, crown and root rot. Diseased plants had reddish-brown crown discoloration, storage root lesions, and stem lesions. Heavily diseased 1 year volunteers frequently yellowed, wilted, and died; and crown and root systems were rotted and necrotic. Infections often originated at the area of seed coat attachment, and sunken red lesions later developed on roots, stems, and crowns. Lesions extended up stems of heavily infected plants, and girdled and killed plants. Initial infections appeared confined to cortical tissues, but rapidly progressed throughout the whole plant. Fewer 2-year-old volunteers were observed due to a high mortality rate in 1 year plants, but 2 year volunteers also showed disease symptoms.

Fusarium oxysporum and *F. moniliforme* were isolated from crowns, stems, storage roots, and feeder roots of 1 and 2 year volunteers (Tables 1, 2). Twenty-two of 50 crowns and SRL from 1 year volunteers from the 7 year field yielded *F. oxysporum* while 14 of 25 crowns and and 25 of 40 SRL from the 4 year field yielded *F. moniliforme*. *F. moniliforme* was most frequently isolated from stem lesions, and *F. oxysporum* was mostly isolated from diseased feeder roots (Tables 1,2). Two year volunteers were found only in the 7 and 15 year fields, and yielded both pathogens (Table 2). Thirty-five crowns from 2 year volunteers from the 7 year field yielded 8 colonies of *F. oxysporum* and of *F. moniliforme*, while 40 crowns from the 15 year field yielded 8 colonies of *F. oxysporum* and 14 of *F. moniliforme*. Storage and feeder

roots yielded both pathogens, while stem lesions primarily yielded *F. moniliforme* (Table 2). Selected isolates of both pathogens showed pathogenicity to asparagus seedlings (Table 3). *F. moniliforme* consistently killed seedlings, while *F. oxysporum* isolates showed varied pathogenicity.

Weeds from growers fields and soil boxes rarely exhibited disease symptoms. Dandelions, chickweed, and grass species showed some root necrosis and dying of lower leaves, but symptoms weren't attributed to *Fusarium*. Isolations from symptomless weeds yielded *F. oxysporum* (Tables 4, 5). The incidence of isolation was low for most species, but many weeds yielded *F. oxysporum* after surface sterilization. Dandelion (*Taraxacum officiale*), lambsquarter (*Chenopodium* spp.), chickweed (*Stellaria media*), crabgrass (*Digitaria sanguinalis*) and quackgrass (*Agropyron repens*) most frequently yielded *F. oxysporum*. Surface sterilized or washed root samples from dandelion, lambsquarter, crabgrass, quackgrass, and chickweed yielded *F. oxysporum* (Table 4).

Isolates showed varied pathogenicity to asparagus seedlings ranging from high virulence to no virulence. Agar plate pathogenicity tests of quackgrass, crabgrass, chickweed, and shepherd's purse isolates showed high pathogenicity on seedlings; while oxalis, purslane, and some crabgrass and quackgrass isolates showed medium to low virulence (Table 6). Isolates with ratings of 3 were highly virulent and caused crown rot and death, while isolates with ratings of 2 showed medium virulence and caused storage root lesions and crown discoloration, but didn't kill seedlings. Agar plate tests were valuable for

TABLE 1

FUSARIA ISOLATED FROM 1-YEAR-OLD VOLUNTEER PLANTS FROM LOCAL COMMERCIAL GROWERS FIELDS[a]

FIELD	SOURCE	NUMBER	INCIDENCE OF FUSARIA[b]				
			F. oxysporum	*F. moniliforme*	*F. solani*	*F. tricinctum*	*F. roseum*
4 year	Crown	25	3	14	-	-	0
	Storage Root Lesion (SRL)	40	9	19	-	-	3
	Feeder Root Lesion (FRL)	25	14	0	-	-	4
	Stem Lesion	25	3	9	-	-	1
1 year	Crown	20	4	5	1	2	-
	SRL	40	25	3	-	-	8
	FRL	20	15	2	-	-	-
7 year	Crown	50	22	3	15	-	-
	SRL	25	16	4	4	-	-

[a]Plants randomly collected July 1979, plants were exhumed using a trowel and individually stored.

[b]Plants were washed 5 minutes in running tap water, sectioned with razor blades, and samples surface sterilized in Clorox solution and plated on acidified potato carrot agar (PCAL). Plates were incubated 7 days and colonies identified or subcultured to PCAL or carnation leaf agar and identified.

TABLE 2

FUSARIA ISOLATED FROM 2-YEAR-OLD VOLUNTEER PLANTS FROM LOCAL COMMERCIAL GROWERS FIELDS[a]

FIELD	SOURCE	NUMBER	INCIDENCE OF FUSARIA[b]				
			F. oxysporum	*F. moniliforme*	*F. solani*	*F. tricinctum*	*F. roseum*
1 year	Crown	40	8	14	6	4	3
	Storage Root Lesion (SRL)	30	10	8	4	-	0
	Stem Lesion	25	1	11	6	-	4
7 year	Crown	35	8	7	6	2	3
	SRL	30	11	8	7	-	1
	Stem Lesion	25	0	9	12	-	4
	Feeder Root Lesion	20	6	10	4	1	0

[a]Plants randomly collected July-August 1979, plants were exhumed with a trowel and individually stored.

[b]Plants washed in running tap water 5 minutes, sectioned, and samples plated on PCAL. Isolates identified as in Table 1.

TABLE 3

RESULTS OF TEST TUBE PATHOGENICITY TESTS ON VOLUNTEER PLANT ISOLATES[a]

ISOLATE	SOURCE	PATHOGENICITY[b]
Fusarium oxysporum	1 year volunteer (crown)	4
F. oxysporum	1 year volunteer (storage root lesion,SRL)	3
F. oxysporum	2 year volunteer (SRL)	3
F. oxysporum	2 year volunteer (crown)	5
F. moniliforme	2 year volunteer (stem lesion)	5
F. moniliforme	1 year volunteer (crown	5
F. moniliforme	1 year volunteer (SRL)	5

[a]Benomyl-acetone treated seed germinated on acidified potato carrot agar, then seedling placed on slant of Hoaglund's solution agar for 2 weeks, and fungal isolates added and pathogenicity determined 3-4 weeks later.

[b]Based on 0-5 rating system, 0=clean and 5=dead.

TABLE 4

FUSARIA ISOLATED FROM WEEDS FROM LOCAL COMMERCIAL GROWERS FIELDS[a]

SCIENTIFIC NAME	COMMON NAME	SOURCE	TREATMENT	NUMBER	INCIDENCE OF FUSARIA[b]	
					F. oxysporum	*F. solani*
Taraxacum officinale	Dandelion	root	Surface sterilization (SS) in Clorox 5 min.	5	-	-
Chenopodium spp.	Lambsquarter	root, stem	SS 2 min.	10	4	2
		root, stem	SS 5 min.	10	1	-
		feeder root	SS 2 min.	10	2	-
Portulaca oleracea	Purslane	root	SS 2 min.	5	-	-
		root	SS 1 min.	5	-	-
		crown	SS 1 min.	5	1	-
Agropyron repens	Quackgrass	root/stem	SS 2 min.	5	3	1
		fine roots	SS 1 min.	10	3	-
Capsella bursa-pastoris	Shepherd's Purse	root/stem	SS 2 min.	10	-	-
		fine root	SS 1 min.	5	1	-
Iva axillaris	Poverty Weed	root, stem	SS 2 min.	10	1	-
		root, stem	SS 2 min.	10	1	-
Digitaria sanguinalis	Crabgrass	root	wash in sterile distilled water	4	-	-
		root	SS 2 min.	4	1	-
		crown,root	SS 3 min.	4	1	-
		crown	SS 5 min.	4	2	-
		crown,leaves	wash	4	2	-

TABLE 4 (Cont'd)

FUSARIA ISOLATED FROM WEEDS FROM LOCAL COMMERCIAL GROWERS FIELDS[a]

SCIENTIFIC NAME	COMMON NAME	SOURCE	TREATMENT	NUMBER	INCIDENCE OF FUSARIA[b]	
					F. oxysporum	*F. solani*
Stellaria media	Chickweed	crown	SS 5 min.	4	-	-
		root	wash	4	-	-
		leaves	wash	4	1	1
Oxalis spp.	Oxalis	crown	wash	4	1	-
		root	wash	4	-	-
		leaves	wash	4	-	-
Polysonum pensylvanicum	Smartweed	root	SS 2 min.	5	-	-
		crown	SS 5 min.	5	-	-
		seed	SS 2 min.	10	-	-
		flower	wash	10	-	-

[a]Weeds randomly collected from fields June 1979-October 1979, with trowel.

[b]Plants washed under running tap water 5 minutes, sectioned with razor blades, and samples plated on acidified potato carrot agar (PCAL). Plates were incubated 7-14 days, and colonies identified or subcultured on PCAL and identified.

TABLE 5

FUSARIA ISOLATED FROM WEEDS[a] GROWN IN NATURALLY INFESTED SOIL IN SOIL BOXES[b]

WEED SPECIES	NUMBER	*F. oxysporum*	*F. moniliforme*	*F. solani*	MEAN PATHOGENICITY OF ISOLATES[c]	PATHOGENICITY DESCRIPTION
Lambsquarter (*Chenopodium spp.*)	10	3	-	-	2.6	MEDIUM-HIGH
Pigweed (*Amaranthus retroflexus*)	10	5	-	1	4.0	HIGH
Purslane (*Portulaca oleracea*)	10	4	-	-	1.5	LOW

[a]Weeds sampled 4 weeks after germination, washed under running tap water 5 minutes, sectioned, and crowns surface sterilized in 2% Clorox for 5 minutes or plated on acidified potato carrot agar. Plates incubated 7 days and isolates subcultured on PCAL for identification and pathogenicity testing.

[b]Soil from South Deerfield 3 year experimental asparagus field, and occurrence of pathogenic fusaria confirmed by dilution plating.

[c]Based on 0-5 rating system, 0=clean and 5=dead.

TABLE 6

RESULTS OF AGAR PLATE PATHOGENICITY TESTS OF WEED ISOLATES[a]

ISOLATE	SOURCE	PATHOGENICITY[b]
Fusarium oxysporum	grass spp. (root)	1 LOW
F. oxysporum	grass spp. (crown)	3 HIGH
F. oxysporum	grass spp. (crown)	3 HIGH
F. oxysporum	grass spp. (crown)	2 MEDIUM
F. oxysporum	grass spp. (crown, leaves)	2 MEDIUM
F. oxysporum	chickweed (crown)	3 HIGH
F. oxysporum	chickweed (root)	2 MEDIUM
F. oxysporum	chickweed (crown)	3 HIGH
F. oxysporum	chickweed (root)	3 HIGH
F. oxysporum	oxalis (crown)	2 MEDIUM
F. oxysporum	purslane (root)	2 MEDIUM
F. oxysporum	purslane (root)	2 MEDIUM
F. oxysporum	shepherd's purse (root)	3 HIGH
F. solani	chickweed (root)	1 LOW
Penicillium	chickweed (stem)	2 MEDIUM
Penicillium	oxalis (crown)	1 LOW
Alternaria	grass spp. (crown)	0 LOW
Trichoderma	grass spp. (crown)	1 LOW
Control	-	1 LOW
Control	-	0 LOW

[a]Isolates grown on acidified potato carrot agar for 5 days, then benlate-acetone treated seedling placed next to isolate. Pathogenicity determined 5-10 days later.

[b]Based on 0-3 rating system, 0=clean and 3=dead.

TABLE 7

RESULTS OF TEST TUBE PATHOGENICITY TEST OF WEED ISOLATES[a]

ISOLATE	SOURCE	PATHOGENICITY[b]
Fusarium oxysporum	pigweed (field)	2 LOW
F. oxysporum	pigweed (soil box)	3 MEDIUM
F. oxysporum	pigweed (soil box)	4 HIGH
F. oxysporum	pigweed (soil box)	5 HIGH
F. oxysporum	*Chenopodium* (soil box)	5 HIGH
F. oxysporum	*Chenopodium* (soil box)	2 LOW
F. oxysporum	*Chenopodium* (soil box)	1 LOW
F. oxysporum	*Chenopodium* (field)	1 LOW
F. oxysporum	purslane (soil box)	2 LOW
F. oxysporum	purslane (soil box)	2 LOW-MED.
F. oxysporum	purslane (soil box)	2 LOW
F. oxysporum	purslane (soil box)	0 LOW
F. oxysporum	dandelion (field)	3 MED-HIGH
F. oxysporum	dandelion (field)	2 LOW
F. oxysporum	dandelion (field)	3 MEDIUM
F. solani	dandelion (field)	1 LOW
F. oxysporum	smartweed (field)	5 HIGH
F. roseum	smartweed (field)	3 MED-HIGH

[a]Benomyl-acetone treated seed germinated on PCAL, then seedling plated on slant of Hoagland's solution agar for 2 weeks and fungal isolate added,and pathogenicity determined 3-4 weeks later.

[b]Based on 0-5 rating system, 0=clean and 5=dead.

fast evaluation of highly virulent isolates, but gave little indication of less virulent isolates due to the development of confusing symptoms on control seedlings with no fungi. Test tube pathogenicity tests on isolates from dandelions showed low to medium virulence, while smartweed isolates showed medium to high virulence (Table 7). Isolates from pigweed and *Chenopodium* from asparagus fields showed low virulence (Table 7). Controls using other fungi or no fungal isolates showed slight crown discoloration and root tip necrosis, but controls never showed typical crown rot symptoms.

Crown and root samples of weeds grown in boxes with infested soil yielded *F. oxysporum* but not *F. moniliforme* (Table 5). The fungus was isolated from 3 of 10 lambsquarter samples, 5 of 10 pigweed samples, and 4 of 10 purslane samples. Test tube pathogenicity tests on *F. oxysporum* isolates from all sampled weeds showed varied virulence (Tables 5, 7). Purslane isolates showed low virulence, lambsquarter isolates medium virulence and pigweed isolates high virulence.

Discussion

Volunteer plants were heavily infected by *F. oxysporum* and *F. moniliforme*. One year volunteers were parasitized by both pathogens in commercial asparagus beds, and showed severe symptoms of stem, crown, and root rot.

Volunteer plant infections occur when soil-borne inoculum randomly contacts germinating seeds, and infects seedlings via seed coat attachments. Infections progress to roots and stems, and plants become

heavily parasitized by pathogenic fusaria. As infected volunteers die, fungal mycelium and spores are reintroduced into the soil. The fungus can survive in the soil, infect other plants, or remain as dormant propagules such as chlamydospores. F. oxysporum infects volunteers in this manner, and can increase soil inoculum via volunteers.

F. moniliforme, which is a poor soil inhabitant, colonizes volunteers in a different manner. F. moniliforme infections originate from inoculum on seeds or from fruit debris. As infected fruits drop to the ground, F. moniliforme colonizes decomposing fruit tissues and becomes associated with seeds. The pathogen overwinters with seed and/or fruit debris (Chapter 1), and attacks germinating volunteers the following year.

The high incidence of volunteer plant infections shows that a substantial build-up of both pathogens may occur via volunteers. Many local growers fields have high volunteer populations, and harrowing under of volunteers introduces inoculum and additional asparagus tissue for colonization by soil-borne fusaria. This provides a means of increasing soil-borne inoculum for both pathogens, and maybe one of the few ways F. moniliforme is introduced into soils.

An efficient means of eliminating volunteer plants would be disposal of senescing stalks at the end of the growing season. This eliminates ripened fruits, insect pupae (Chapter V), and aboveground Fusarium inoculum (Chapter III). Burning or removing stalks would decrease inoculum of both pathogens, and burning would reintroduce nutrients to the soil. Growers recommendations of 1935 recommended

burning stalks late in the growing season, but few growers presently follow this practice. Burning of stalks would be valuable in newly established beds if practiced annually, and would be part of an effective integrated pest management program.

Pathogenic isolates of F. oxysporum, but not F. moniliforme were isolated from roots of various weed species. The fungus didn't cause noticeable symptoms on weeds, but was sometimes pathogenic to asparagus seedlings. F. oxysporum was able to survive surface sterilization of crown and root tissues, indicating that the fungus can invade tissues without causing symptoms. This phenomenon is an example of the theory that parasitism and pathogenicity are not necessarily related (52). Armstrong and Armstrong (4) demonstrated a similar phenomenon when non-host plants were inoculated with F. oxysporum forma specialis pathogenic to other crop plants. The fungus could be isolated from inoculated non-host plants showing no symptoms of disease. The relation between the parasite and symptomless carrier is a commensel one, possibly due to the inability of the fungus to produce toxins or enzymes in non-hosts (52). This relationship allows for living substrates to be available to a parasite over long periods of time, especially in the absence of host plants. Weeds in asparagus fields provide living substrates for F. oxysporum and contribute to the build-up and longevity of the pathogen in the field. The large number of weeds species colonized by F. oxysporum indicates a wide host range for the fungus, and is consistent with the parasitic and saprophytic abilities of the organism (13).

Heavy infestations of commercial asparagus beds by weeds favors interactions with soil-borne F. oxysporum. The fungus invades weed tissues and may exist in the weed plant rhizosphere. Root interactions between parasitized weeds and mature asparagus plants may initiate pathogenic infections on asparagus. This is a more rapid means of soil dissemination that fungal movement through soil, which has been demonstrated to be quite slow (40). Survival on weeds allows for F. oxysporum to exist in soils without asparagus culture; and combined with its saprophytic ability and chlamydospore production, would allow F. oxysporum to survive well over 10 years in absence of asparagus culture. F. moniliforme wasn't associated with weeds, which is consistent with the aboveground occurrence of the fungus and its lesser ability to colonize roots and root rhizospheres.

The evolution of F. oxysporum and symptomless carriers was discussed by Katan (52), and may explain the asparagus Fusarium-weed interaction. Primitive forms of F. oxysporum were thought to have evolved parasitic abilities allowing for invasion of belowground plant parts. Further evolution of these forms led to the pathogenic associations seen with some crops, especially perennial crops like asparagus; but pathogenic relations were not evolved toward most plants, resulting in symptomless carriers.

Possibly even more improtant than fungus-weed interactions is the pressure exerted on asparagus plants by weeds. Weeds compete for sunlight, nutrients, water, and growing space. High weed populations can stress mature plants and make them more susceptible to plant

pathogens (56). Although most growers treat beds with pre-emergence herbicides such as Karmex (diuron), Princep (simizine), and Sencor (metribuzin); weeds grow back into beds after the cutting season and compete with asparagus plants. Growers should continue weed control with post-emergence sprays of an appropriate herbicide to eliminate weed pressure on asparagus plants and inoculum sources for _F_. _oxysporum_. Effective weed control was a practice stressed in the 1935 growers recommendations that is no longer followed. Proper weed control would be an important part of an integrated control program for asparagus in Massachusetts.

CHAPTER V

ASPARAGUS STEM MINER

Introduction

The efficient dissemination and survival of *Fusarium oxysporum* and *F. moniliforme* has favored a gradual and severe increase of stem, crown, and root rot in Massachusetts asparagus fields. The perennial nature of the asparagus plant further facilitates disease build-up. The pathogens are disseminated with seed, soil, plant debris, and through the air (36, 38). Asparagus plant parts, including flowers, stems, and fruits have been shown to yield parasitic fusaria and play a role in the disease build-up (36). *Fusarium species* have also been isolated from stem mines of the asparagus miner fly, *Ophiomyia simplex* (Loew), on local asparagus (Figure 15). High populations of the fly were noted during the 1979 growing season, and a possible association between the disease complex and the asparagus miner fly was investigated.

Objectives

1. To determine if large asparagus miner fly populations exist in Massachusetts asparagus beds.
2. To determine if miner fly mines and/or life stages are associated with *Fusarium oxysporum* and/or *F. moniliforme*.
3. To determine if the asparagus miner fly plays a role in the build-up of the *Fusarium* disease problem.

Literature Review

Insects as Vectors of Fusarium moniliforme

Fusarium moniliforme, causing disease in sugar cane and figs, has been shown to be insect-disseminated (13, 62). *F. moniliforme* causes endosepsis in figs, a firm dry rot of the fig fruit. The disease is a problem in California fig orchards (62), and is principally vectored by the fig wasp (*Blastophaga psenes* L.). Wasps overwinter in galls on fig florets which are frequently parasitized by *F. moniliforme*. The adult wasps become contaminated as they emerge from galls, and disseminate the fungus as they feed and reproduce on developing fig fruits.

Sugarcane stalk rot caused by *F. moniliforme* is disseminated by the sugarcane stalk borer (*Diatrae saccharalis* F.). The adult, larval and pupal stages of the insect are all involved in dissemination of the pathogen (13).

Asparagus Miner Fly - History and Distribution

The asparagus miner fly is in the family Agromyzidae (Order: Diptera), and was first described by Loew in 1861 from Pennsylvania, New Jersey, and New York (31). The fly was first reported in Europe from Hungary in 1896 (85). Chittendon collected the small black metallic flies from Maryland asparagus fields in 1897, and suggested that the flies lived at the expense of the plant (19). Sirrine reported similar flies from Long Island asparagus fields in 1896, and suggested that the pest be called the 'asparagus miner' (82). Shortly after its initial

description, the fly was reported from all over the United States; including Massachusetts, Connecticut, New York, Pennsylvania, New Jersey, and Tennessee (19, 32). It was first reported in California in 1905. High populations were reported from Massachusetts and Connecticut in 1907 and 1908, and extensive damage to asparagus stalks was reported from Concord, Massachusetts in 1908 (19). Later researchers including Fink (31) in New York, Barnes (7) in Great Britain and the Netherlands, and Eichmann (27) in Washington; studied the biology and life cycle of the fly. Spencer (85) recently determined the distribution of the fly to include the entire Eastern United States from New York to Tennessee; in addition to California, Ontario, Quebec, France, and Germany. Spencer believed the fly to be present wherever asparagus is grown. Fink described the fly as a native species to the United States; however, Spencer reported the fly to have been introduced to the U.S. with asparagus by early settlers (31). Spencer placed the fly in an isolated taxonomic position in the family Ophiomyia, and assumed that this differentiation had to have taken place over a long period of restricted association with its only host, asparagus.

Life Cycle and Description of Life Stages

The life cycle is initiated as adult flies emerge from overwintering pupae in asparagus stalks, and lay eggs at the base of newly emerging asparagus stalks. Eggs are laid in the early spring on volunteer asparagus plants or in seedling beds, but not on stalks in commercial fields (19). The female fly deposits eggs beneath the epidermis of

asparagus stalks near or just below the soil surface. Eggs are whitish in color and are about .5 mm x .1 mm (32). The egg stage lasts 12-21 days (7, 31) and eggs hatch into white maggots which later turn cream-white color. Larvae reach up to 5 mm in length, and Fink and Eichmann reported 3 larval instars. Larvae feed beneath epidermal tissue on parenchyma cells , and create mines as they move up and down stalks feeding. Larvae feed for 2-3 weeks and pupate.

Pupae are initially light-brown, and later turn dark brown. Pupae measure about 3.5 mm x 1 mm (85), and have a pair of hooks on each end allowing firm attachment to stalks (31). There is frequently more than 1 pupa per stalk, and Fink (31) found 3-12 pupae on each of 80 stalks sampled from a heavily infested field. The pupal stage was shown to last 17-21 days by Fink and 22-25 days by Eichmann and Barnes.

Small black metallic flies about 2-3 mm long emerge from the pupae and represent the second generation of adult flies. The adult fly has a prominent head and eyes, with a broad frons almost twice the width of the eye (85). Wings are clear and range from 2.2 mm for males and 3 mm for females. Sirrine, 1900, observed adult flies resting on flowers and branchlets of asparagus, especially on plants attacked by the asparagus beetle. Second generation adults mate and deposit second generation eggs. These eggs give rise to second generation larvae and pupae. Second generation pupae serve as the overwintering stage in the life cycle, and undergo a period of diapause. Adult flies emerge from pupae the following spring (31). Sirrine and Fink noted 2 generations of flies in New York, while Eichmann perceived 3 generations in Washington.

Economic Importance

Damage to asparagus is primarily due to mining by larvae (19, 25, 31). Sirrine, in 1900, first described the larvae working beneath the epidermis of the plant and devouring the green portion of the plant between the epidermis and 'fiber or wood' of the plant. He described how 5 or 6 larvae girdled a stalk, but was unable to detect noticeable injury on plants in established cutting beds (82). Damage was observed on seedlings and newly set beds, and Sirrine described injury as yellowing and premature death of young plants. He concluded that plants in established beds were weakened by fly damage later in the growing season (82). Severe injury was reported from the Philadelphia area in 1901 (19), and several asparagus beds were reportedly destroyed by the miner. Chittendon reported heavy infestations in Concord, Massachusetts in 1907; with every field visited infested. The fly was as common as the asparagus beetle, and severe injury due to the miner fly was noted in 1908 (19). Chittendon believed the miner fly to be a potential pest of considerable economic importance.

Fink (31) found heavy infestations in New York asparagus fields in 1913. Tissues between the outer epidermis and 'wood' or vascular bundles were eaten by larvae; and as a result epidermal tissues became dry, yellowish and shriveled up, eventually causing stalks to wilt and die. Asparagus miner trails were associated with yellowed and dying asparagus stalks.

Drake and Harris, in 1932, considered asparagus miner injury to be severe in Iowa asparagus beds; but noted that miner injury was

unnoticed by growers or was attributed to other causes. The authors attributed premature wilting and yellowing of stalks and foliage to damage by the larvae of the fly (25).

Young, in 1935, reported the asparagus miner occurring in Massachusetts asparagus beds. He reported injury to rarely be serious, though its importance was believed to be increasing yearly. Occasionally mines were observed girdling stalks, and causing yellowing and death of stalks. Two generations were detected, and pulling and burning of stalks in the late fall or early spring was recommended as a control measure (100).

Eichmann studied the miner fly in Washington in 1943. He found larval feeding confined to lower cortical tissues of stalks. The vascular system was untouched, and the author concluded that cortical damage had no effect on the vital functions of the plant (27). Heavily mined stalks often remained green, while unmined stalks could turn yellow prematurely (27). Premature yellowing and wilting of stalks was attributed to infections by _Fusarium oxysporum_. Cohen and Heald (21) first described a wilt and root rot caused by _F. oxysporum_ in Washington, and discounted miner fly injury as a cause of yellowing stalks. Cohen (22) later hypothesized that miner injury might serve as an entry for the fungus. Eichmann discounted this possibility because the puncture made by the ovipositor is not in contact with the soil, and the mining of the larvae does not break to epidermis. Eichmann reported that though dead epidermal tissue may slough off, stem mines calloused over (27). Normal harvesting and cultivation practices were thought to favor fungal

pathogens more than mining. Eichmann concluded that the miner caused no serious damage to asparagus, and that the insect should not be considered an insect pest in Washington.

Van Bakel and Kersten, in 1970, described a foot rot of asparagus caused by *F. oxysporum* f. sp. *asparagi* in Germany. The authors speculated that the asparagus miner fly was involved with the disease in Germany (94). Van Bakel and Bethe (96) hypothesized that the miner fly favored infection by *F. oxysporum* in the Netherlands. Tests indicated that stems with miner injury yielded more *F. oxysporum* isolates than stems without injury (96).

Spencer, in his volume on the family Agromyzidae, reviewed previous work on the fly. He stated that it would be of interest to confirm Eichmann's findings that *O. simplex* is not really a pest of asparagus by more detailed studies of this insect in other asparagus growing areas.

Methods and Materials

Asparagus miner populations in local asparagus fields were determined at the end of the 1979 growing season by counting numbers of pupae in mature asparagus stalks. Stalks were randomly sampled from 4 different growers fields, and emerged and live pupae counted per stalk. The number of mines per stalk was determined and all larvae noted. Mines were observed microscopically under a dissecting scope with epidermal tissue present and removed. Exposed mines were scraped over plates of acidified potato agar (PCAL) under a hood. Fungal sporodochia on outer epidermal and internal cortical tissues were scraped over PCAL. Sporodochial color and source were noted. Plates were incubated 4 days at 24°C, then examined. Fusaria colonies were microscopically determined from 10 plates of mine scrapings and 10 plates of sporodochial scrapings, then subcultured on PCAL. Some colonies were single spore isolated and identified on carnation leaf agar (CLA). Tissue samples were excised from stem mines on 200 sampled stalks, surface sterilized 5 minutes in the standard 10% Clorox solution, and plated on PCAL. Plates were incubated at 24°C for 7-14 days. Resulting _Fusarium_ colonies were identified directly from plates, or mass transferred and single spore identified on CLA.

Cross sections of mines were made with razor blades to determine the extent of mining and any accompanying discoloration. Samples of vascular tissue near mines were excised from stalks, surface sterilized 1 or 10 minutes in 10% Clorox, and plated on PCAL. Plates were incubated at 24°C for 7-14 days, as were all of the following isolations

unless otherwise noted. Fusaria colonies were identified as previously described.

75 pupae were directly extracted from mines, washed in sterile distilled water, and plated on PCAL. A similar treatment was given to 10 larvae removed from mines. Three lots of 60 pupae were given one of the following surface sterilization treatments: standard 10% Clorox solution for 10 minutes, standard 25% Clorox solution 10 minutes or 95% ethanol for 5 minutes then 25% Clorox for 15 minutes; to determine spore viability on pupae and to find a method to disinfect the surface of pupae. 75 live pupae were extracted, given the ethanol-Clorox treatment, and incubated on PCAL plates for four days to assure no surface contamination. Pupae were then crushed with sterile forceps to expose puparia to agar, and to determine if puparia were contaminated. A group of 150 pupae were individually stored in a cold room 4 months, separated into groups of 50, and given no treatment, washed in sterile distilled water, or given the ethanol-Clorox treatment and crushed. The pupae were all plated on PCAL.

250 live pupae were extracted from mines, given the ethanol-Clorox treatment, and placed in vials of sand with or without moistened vermiculite. Emerging adult flies and parasites were noted, and adults were cooled in a freezer and plated on PCAL under a hood. Fusarium colonies were determined as before.

Representative Fusarium colonies from stem mines, larvae, pupae, and adults were kept on PCA slants. Test tube pathogenicity tests were run on isolates.

Results

Sampled stalks from all 4 fields showed large numbers of pupae and mines. Stalks averaged 2.88 pupae and 1.88 mines (Table 1). As many as 15 pupae were counted on individual stalks. Of all pupae extracted 54% (584/1068) had emerged.

Mines extended from below the soil line to crowns, to well aboveground. Individual mines extended as high as 1 meter on stems, though most mines and pupae occurred within the lower 10 cm of stalks. Few larvae were observed on stalks sampled late in the growing season. Examination of mines showed overlying epidermal tissue had died and turned brown. The tissue often cracked and detached from the entire lower stem areas where mines girdled stalks. Fungi were observed sporulating on dead epidermal tissue, and sporulation often extended up stalks on this tissue. Sporodochial scrapings yielded *Fusarium moniliforme*, *F. tricinctum*, and *F. solani* (Table 3). Examination of mines with epidermal tissue removed revealed large amounts of frass (plant fragments mixed with excrement) from larval feeding and cortical discoloration in older mines. Mines observed early in the season would initially appear pale green (Figure 16), and later show red-brown discoloration (Figure 17). Mycelium and sporodochia were observed in mines, but not on surrounding healthy tissues. Scrapings of mines yielded an array of fungi in the genera *Fusarium*, *Cladosporium*, *Penicillium*, *Epicoccum*, *Alternaria*, *Trichoderma*, and others. *Fusarium moniliforme* and *F. oxysporum* were identified, in addition to other

Fusarium (Table 2). *F. moniliforme* was most frequently isolated.

Excised mine tissue yielded 152 colonies of *F. moniliforme*, 20 of *F. oxysporum* and other saprophytic *Fusarium* (Table 4). Stem mine sections showed mines to extend 1-2 mm into cortical tissue and discoloration to be confined to cortical tissue. Vascular tissue near mines failed to yield fusaria.

Washed pupae yielded *F. moniliforme* (Figure 18), *F. oxysporum*, and saprophytic fusaria (Table 4). Surface sterilized pupae continued to yield fusaria. Pupae surface sterilized in 10% Clorox yielded 20 colonies of *F. moniliforme* and 8 of *F. oxysporum*, while pupae surface sterilized in 25% Clorox yielded 6 colonies of *F. moniliforme* and 9 of *F. oxysporum* (Table 4). Pupae given the ethanol-Clorox treatment were completely surface disinfested (Table 4); however, pupae given the ethanol-Clorox treatment then crushed, still yielded *Fusarium*. Puparia were infested with *F. moniliforme*, *F. oxysporum* and *F. solani* (Table 4). Stored pupae continued to yield Fusaria. Untreated and washed pupae yielded *F. moniliforme* and *F. oxysporum* even after 4 months cold storage (Table 5). Puparia also yielded both pathogens at lower rates (Table 5).

Twenty-six adults were reared from pupae. Two colonies of *F. moniliforme* were isolated from adults (Figure 18), in addition to other fungi like *Alternaria* and *Penicillium*.

Pathogenicity tests run on fusaria from mines, larvae, pupae, puparia, and adults showed *F. moniliforme* and *F. oxysporum* to be highly pathogenic (Figure 14), while *F. tricinctum* and *F. solani* were not

(Table 6). *F. moniliforme* isolates consistently showed high pathogenicity, while not all *F. oxysporum* isolates were as highly pathogenic.

TABLE 1

LATE SEASON ASPARAGUS MINER FLY (*OPHIOMYIA SIMPLEX LOEW*)

POPULATION COUNT[a]

TOTAL STEMS	TOTAL MINES	MINES PER STALK	TOTAL PUPAE	PUPAE PER STALK	# PUPAE EMERGED[b]	% EMERGED
375	706	1.88	1068	2.88	584	54

[a]Stems from 3 local growers fields were sampled during September-November 1979, and pupae were extracted and mines counted.

[b]Pupae were visually determined as empty or live, and sometimes crushed for confirmation of visual determination.

TABLE 2

ISOLATION OF FUSARIA FROM SPORODOCHIA ASSOCIATED WITH STEM MINES[a]

PLATE	SPORODOCHIAL COLOR	SOURCE	FUSARIA ISOLATED AND IDENTIFIED[b]
1	pink	epidermal	*F. moniliforme*
2	pink-white	epidermal	*F. moniliforme*
3	orange-white	epidermal	*F. tricinctum*, *F. solani*
4	orange-white	cortical	*F. moniliforme*, *F. tricinctum*
5	green-white	cortical	None
6	white	cortical	*F. moniliforme*, *F. oxysporum*, *F. solani*
7	orange	epidermal	*F. tricinctum*
8	orange	epidermal	*F. tricinctum*, *F. solani*
9	pink	epidermal	*F. moniliforme*
10	green	epidermal	None

[a]Sporodochial color and source was visually determined, and sporodochia were scraped over acidified potato carrot agar (PCAL) with a sterile scalpel under a sterile hood.

[b]Plates were incubated 4-7 days in a growth chamber at 23°C. Resulting colonies were subcultured on PCAL, incubated 7-14 days, and identified on carnation leaf agar (CLA).

TABLE 3

ISOLATION OF FUSARIA FROM SCRAPINGS OF EXPOSED STEM MINES[a]

PLATE	FUSARIA ISOLATED AND IDENTIFIED
1	*Fusarium moniliforme*, *F. tricinctum*
2	*F. oxysporum*, *F. tricinctum*
3	None
4	*F. moniliforme*, *F. tricinctum*, *F. solani*
5	None
6	None
7	*F. moniliforme*
8	*F. moniliforme*, *F. oxysporum*, *F. solani*
9	None
10	None

[a]Overlying epidermal tissue excised with sterile forceps, and mines scraped over acidified potato carrot agar (PCAL) with sterile scalpel in a sterile hood. Scrapings included cortical tissues, fungal propagules, and frass.

[b]Resulting colonies were subcultured on PCAL after 4 days growth in an incubator at 23°C, and single spore identified on CLA.

TABLE 4

ISOLATION OF FUSARIA FROM EXCISED ASPARAGUS MINER FLY (*OPHIOMYIA SIMPLEX* LOEW) MINE TISSUE AND MINER FLY LIFE STAGES[a]

SAMPLE	NUMBER	TREATMENT	NUMBER ISOLATED			
			F. moniliforme	*F. oxysporum*	*F. tricinctum*	*F. solani*
Mine tissue	200	Surface sterilized (SS) with 10% Clorox 5 min.	152	20	12	2
Larvae	10	Washed in sterile distilled water	3	1	3	-
Pupae	75	Washed in sterile distilled water	14	14	20	6
Pupae	60	SS with 10% Clorox	20	13	5	-
Pupae	60	SS with 25% Clorox	6	9	-	-
Pupae	60	SS with 95% ethanol 5 min, then 25% Clorox 15 min.	-	-	-	-
Puparia	75	SS with ethanol-Clorox, then crushed	6	2	-	2

[a]Samples individually collected and plated on PCAL.

[b]Resulting colonies were subcultured on PCAL and identified on CLA.

TABLE 5

ISOLATION OF FUSARIA FROM STORED PUPAE AND PUPARIA[a]

SAMPLE	NUMBER	TREATMENT	NUMBER ISOLATED[b]			
			F. moniliforme	*F. oxysporum*	*F. tricinctum*	*F. solani*
Pupae	50	None	11	11	4	-
Pupae	50	Washed in sterile distilled water	4	7	3	3
Puparia	50	Surface sterilized with 95% ethanol 5 min. then 25% Clorox for 15 min, plated on agar then crushed.	4	2	-	1

[a]Pupae were collected from randomly selected stalks in October 1979, individually stored in tissue culture plates in a cold chamber at 10-15°C for 4 months, then plated on PCAL.

[b]Resulting colonies were subcultured on PCAL and identified on CLA.

TABLE 6

RESULTS OF PATHOGENICITY TESTS ON FUNGAL ISOLATES FROM ASPARAGUS MINER MINES, LARVAE, PUPAE, PUPARIA, AND ADULTS[a]

GENUS-SPECIES	SOURCE	PATHOGENICITY[b]	
F. moniliforme	mine	3	High
F. moniliforme	mine	4	High
F. moniliforme	washed pupae	5	High
F. moniliforme	washed pupae	3	Moderately high
F. moniliforme	puparia	5	High
F. moniliforme	puparia	3	Moderately high
F. moniliforme	adult	5	High
F. moniliforme	adult	5	High
F. oxysporum	mine	4	High
F. oxysporum	mine	3	Moderately high
F. oxysporum	larvae	3	Moderately high
F. oxysporum	pupae	4	High
F. oxysporum	pupae	1	None
F. oxysporum	puparia	5	High
F. solani	mine	2	Low
F. tricinctum	pupae	1	None
Penicillium	mine	2	Low
Penicillium	pupae	1	None
Alternaria	pupae	1	None
Control	-	0	None
Control	-	0	None
Control	-	1	None

[a]BA seed germinated on PCAL, then placed in slant of Hoagland's solution agar for 2 weeks prior to addition of fungal isolate.

[b]Based on 0-5 rating system, 0=clear and 5=dead.

Discussion

The results indicate that substantial populations of asparagus miner flies exist in commercial fields. By the end of the 1979 growing season, almost all mature stalks had mines and pupae.

Larvae mined extensively up and down stalks feeding beneath the epidermis, and causing browning and death of overlying epidermal tissue. Dead epidermal tissue would crack or peel away, allowing *F. moniliforme*, *F. oxysporum* and saprophytic fusaria to colonize epidermal and cortical tissues. On heavily infested stalks, mines coalesced on lower stems; resulting in the death and complete detachment of the lower epidermis. Epidermal tissues would rapidly slough off under conditions of high moisture and humidity often seen in dense clumps of asparagus stalks. Physical damage done by mining was not correlated with visible symptoms on stalks, but colonization of stalks by fungi was favored by mining. Mines didn't reach the vascular elements of the plant, and didn't severely impede the basic life processes of the plant.

Fungal sporulation was observed on dead epidermal tissue over mines, and on cortical tissue of mines. Dead epidermal tissue was most effectively colonized by a pink sporulating fungus, identified as *F. moniliforme*. *F. moniliforme* was consistently identified from scrapings of pink sporodochia, and utilized dead epidermal tissue as a growth substrate. Graham described *F. moniliforme* colonizing dead and dying asparagus stalks in Ontario, and showed the fungus to effectively utilize cellulose substrates (40). Gilbertson and Manning, in 1979,

showed _F_. _moniliforme_ to be the major sporulating fungus on above-ground asparagus stalks late in the growing season. Sporodochia extended well aboveground, often giving stalks a pinkish color (36). Fungi also grew and sporulated on frass in mines. Saprophytic and parasitic fusaria colonized frass , but especially _F_. _moniliforme_. It is likely that cellulose in frass was used by _F_. _moniliforme_ as a growth substrate.

Examination of mines indicated that mycelial growth originated from lower stem areas and progressed up stalks often using mines as a growth substrate. Colonization of lower stems by _Fusarium_ species was favored by the proximity of soil and water-borne inoculum, the mining of stalks, and the humid, moist, microclimate provided by close bunches of stems. Heavy sporulation would appear on lower stems late in the season, and extend well aboveground on mines. Domination of upper stem mines by _F_. _moniliforme_ was consistent with the theory that _F_. _moniliforme_ primarily colonizes aboveground stems, and _F_. _oxysporum_ and _F_. _moniliforme_ attack lower stems. The ability of _F_. _moniliforme_ to sporulate on aboveground plant parts, particularly dead tissue, allows for efficient production of airborne and waterborne inoculum, Gilbertson and Manning 1979 .

The red-brown discoloration outlining mines later in the season (Figure 17) was associated with fungal growth and sporulation in and on mines. Stem lesions caused by _F_. _monilforme_ and _F_. _oxysporum_ on asparagus stalks are outlined by a distinct red-brown discoloration of cortical tissues (68), which is a diagnostic characteristic of fusarial

disease on asparagus. *F. moniliforme* and *F. oxysporum* were consistently isolated from discolored mine tissue (Table 4), and discoloration was indicative of fungal growth up stalks. Discoloration was most pronounced on lower stems, but later extended well aboveground with mines. The rate of isolation of *F. moniliforme* from discolored mines exceeded rates of isolation of the fungus from typical *Fusarium* stem lesions on stalks (Chapter III, Table 4), showing mines to be a favored substrate as the growing season progressed.

Isolation of *F. moniliforme* and *F. oxysporum* from larvae and pupae showed an external contamination by fungal mycelium and/or spores of these life stages. Surface sterilization eliminated fungal contamination from soft-bodied larvae, but not from sclerotized pupae. Pupae surface sterilized in 10% Clorox showed higher rates of contamination than washed pupae (Table 4). Fungal propagules become closely associated with pupae, and resist oxidation by Clorox.

The rough surface of the pupae, as observed under the dissecting microscope, may provide crevices and cavities where spores avoid surface sterilization. Spores or mycelium associated with external surfaces of pupae may lodge in these cavities during sclerotization and secondary segmentation of the pupa. Inglis (48) showed cavities in the asparagus seed coat where *Fusarium* spores lodged and resisted surface sterilization. Electron microscopy of asparagus seed coats showed cavities and spores lodged within them.

Surface sterilization using 95% ethanol and 25% Clorox allowed for isolations from puparia, and puparia were contaminated by F. moniliforme and F. oxysporum even after a 4 month cold storage. This indicates that the fungi exist inside pupae, and for long periods of time. It can't be said where in the puparia this association is, but the association probably initiated during larval feeding and movement on stalks.

The association of fusaria with pupae is not a brief superficial one, but a more prolonged intricate one. Contamination of puparia from 4 month stored pupae evidenced this, and indicated that both fungi can overwinter with pupae in senescing stalks. The fungus can possibly resume growth the following season, or contaminate emerging adults. Local growers leave senescing stalks in the field, and allow considerable inoculum to remain in the field for the next season.

Contamination of adults reared in the laboratory further showed the overwintering of Fusarium with pupae. F. moniliforme isolates from reared adults retained their pathogenicity (Table 6), and could have created new infections if vectored to an asparagus host. The extent and importance of pupal overwintering and adult contamination is unknown; but maybe a factor in disease and inoculum build-up, particularly by F. moniliforme.

Eichmann's conclusion that the miner fly is not a pest of Washington asparagus may be valid, and I didn't find larval mining correlated to yellowing dying stems. I did find larval mining to facilitate the build-upof Fusarium stem rot in Massachusetts asparagus, which is a major part of the Fusarium disease complex. Though heavily mined stalks

did remain green, I never observed a rapid callousing of mines able to prevent fungal infection, as described by Eichmann (27). Epidermal tissue sloughed off, and exposed cortical tissue to fungal colonization. This was most evident near and below the soil line where inoculum of F. oxysporum was heavy. Unmined stems rarely showed the loss of epidermal tissue and the heavy sporulation as seen on mined stems Eichmann's conclusions were likely made before the Fusarium disease complex became a major problem, and can't be applied to areas where the disease complex is well established. It would be of interest to recheck Eichmann's conclusions in Washington, where the disease has recently become more important (43, 48, 49).

The high incidence of saprophytic fusaria associated with mines and fly life stages indicates the randomness of the association of the fly with pathogenic species. The relationship is not specific, and the insect is not the major means of dissemination for the pathogens; though the activity of the fly does increase disease severity.

The results of pathogenicity tests on F. oxysporum and F. moniliforme isolates from stem mines, larvae, pupae, puparia, and adults were similar to results from isolates from diseased plants. All F. moniliforme isolates showed some pathogenicity, while not all F. oxysporum isolates did. Controls using no fungal isolate, or saprophytic fungi showed little or no pathogenicity.

A control program utilizing timed insecticide sprays combined with removal of senescing stalks in the fall, could control miner fly damage and slow the spread of the Fusarium disease complex. Control of the fly

should be considered in an integrated pest management program for asparagus.

Conclusion

There is a relationship between the fungal pathogens *F. oxysporum* and *F. moniliforme*, and the asparagus miner fly (*Ophiomyia simplex*). The relationship is in part due to the increase in *Fusarium* stem, crown, and root rot in Massachusetts; and heavy infestations of asparagus by *Fusarium* and the miner fly favors the relationship. As larvae feed on infected lower stems, they contact *Fusarium* mycelium and spores associated with stem lesions. Fungal propagules are carried externally and internally as larvae mine up and down stems. Propagules infect dying epidermal tissue and exposed cortical tissue, as evidenced by fungal sporulation and the red-brown discoloration surrounding older mines. Sporulation, particularly by the stem rot pathogen *F. moniliforme*, occurs on dead or dying epidermal tissue, exposed cortical tissue, and on frass. Heavy mining girdles lower stems, causing detachment and death of epidermal tissue. *F. oxysporum* and *F. moniliforme* colonize these exposed stems, facilitating stem rot. The fungi become associated with the insect life stages; and overwinter with pupae and puparia, sometimes contaminating adults. The fly favors a more rapid dissemination of the *Fusarium* disease complex, and should be considered in a disease management program.

Future Work

1. Microscopic examination of pupae and larvae for _Fusarium_ spores.

2. Sectioning of mines to demonstrate tissues affected.

3. Further population studies on eggs, larvae, first generation pupae to determine when control practices should be implemented.

4. Further isolations from larvae and adults.

5. Demonstration of spores on pupae using the scanning electron microscope.

6. Trapping of adults in commercial beds and plating on agar media.

7. Transmission experiments with adults and fungal isolates.

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Future Directions

1. Continued work on the role of *Fusarium moniliforme* var *subglutinans* and *F. oxysporum* var *redolens* in asparagus stem, crown and root rot.

2. Develop a spot-plate seedling assay technique for evaluation of potential asparagus bed soils.

3. Publish extension materials on the importance of virgin land, use of clean propagative stock, and cultural practices in the management of stem, crown, and root rot. Also publish more information on the disease.

4. Continue planting asparagus in virgin soils and looking for *Fusarium* infections; and continue planting asparagus in soils having various crop histories such as corn or tobacco, over long periods of time.

5. Use of baiting studies using crop residues or organic matter for determining preference of soil-borne *F. oxysporum*, and for determining the presence of soil-borne fusaria and the effect of cover crops on *Fusarium*.

6. Research into the ability of *F. oxysporum* isolates from virgin soils to be pathogenic to asparagus, and the frequency of such isolates.

7. Determine if isolates of *F. oxysporum* showing low virulence can develop into highly virulent isolates with prolonged contact with asparagus.

8. Determine the role of saprophytic rhizosphere and phyloplane fusaria, such as *F. solani*, *F. tricinctum* and *F. roseum* in the disease complex.

9. Research into the role of water stress and nutrient availability on *Fusarium* infection and disease development.

10. Further work on the relation of asparagus beetles and *Fusarium*.