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Biological management of Fusarium crown rot of asparagus seedlings with saprophytic microorganisms.

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BIOLOGICAL MANAGEMENT OF FUSARIUM CROWN ROT OF ASPARAGUS SEEDLINGS WITH SAPROPHYTIC MICROORGANISMS

A Thesis Presented

By

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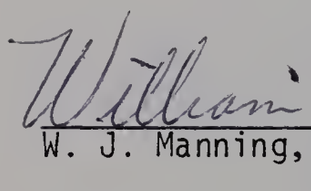

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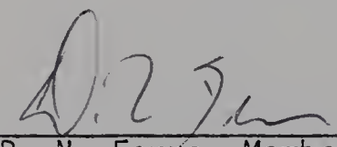

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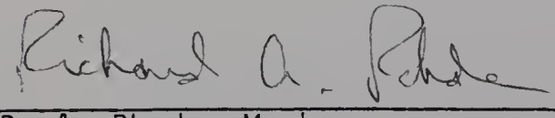

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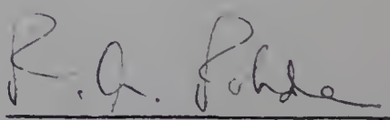

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ABSTRACT

Conidia of Trichoderma harzianum, Fusarum oxysporum (saprophytic), and an Aspergillus sp. were suspended in 4% methyl cellulose and applied to seed (pelleted) and transplants (at 4-6 x 10^6 spores/ml) of asparagus (Asparagus officinalis L.) cultivars Mary Washington (MW) and Rutgers Beacon (RB). Treated, non-treated, and fungicide-treated seedlings from a field naturally infested with both F. oxysporum and F. moniliforme and from soil from that field in flats in the greenhouse were compared. T. harzianum and Aspergillus sp. seed treatments significantly reduced crown infections in the greenhouse, but gave variable results in the field. RB seedlings were more vigorous and gave better results. A 0.12% captan soak, prior to innoculation with T. harzianum, gave better results than with either treatment alone. Dipping root systems of RB and MW transplants in conidial suspensions of F. oxysporum (saprophytic), prior to planting, resulted in larger and more vigorous plants.

CHAPTER I

INTRODUCTION

Asparagus (Asparagus officinalis L.) is a perennial monocot in the lily family. The plant forms an underground stem or rhizome, commonly known as a crown. The crown consists of a cluster of persistent buds from which shoots and a root system emerge during the growing season. Two distinct morphological types of roots develop: thick and fleshy "storage roots," and fine "feeder roots." The former are a store of starch reserves while the latter serve to absorb water and nutrients from the soil [1]. Root systems extend several feet through the soil in all directions.

Emerging buds or spears are harvested as a spring vegetable in Massachusetts from late April to early June (approximately a six-week cutting period). Remaining shoots that emerge are allowed to develop into branched shoots called ferns or fronds. The needle-like photosynthetic structures formed on shoots are not true leaves but cladophylls. True leaves are reduced, small triangular scales first visible on the spears and later on the stem and are found mainly at the axials of branches [1]. A sufficient number of ferns must be allowed to mature to maintain plant vigor and to insure survival.

Asparagus is a dioecious species with both male and female plants. Male plants have staminate flowers and generally produce more spears than females [1]. The diameter of these spears, however, is usually less than that of female plants. Occasional perfect flowers are found,

but they occur rarely and only on males. Female plants have pistillate flowers and normally produce fewer but larger spears [1]. Once pollinated, female flowers develop into red berries which contain four to six hard, black seeds when mature in the fall. The species is cross pollinated and the resulting seed is often genetically variable [1]. For this reason, it is difficult to breed lines that are true to type. The most common variety grown in the area is "Mary Washington" (MW) which was bred for resistance to asparagus rust [1]. This variety has been shown to be susceptible to fusarium crown rot [40]. Recently a selection of that variety called "Rutgers Beacon" (RB) was released which has increased vigor and tolerance to crown rot.

One-year-old dormant crowns are usually transplanted into a permanent bed. Two full seasons of growth are required before harvesting of spears begins in the third year. In the past, asparagus beds often remained productive for up to twenty years. Recently plantings have declined prematurely to unproductive yields in as little as five years [38,40]. It has also been difficult to establish new plantings on land previously cropped to asparagus as seedlings die and yields are not substantial [40,56]. The syndrome known as "asparagus decline and replant problem" has been extensively reviewed in the literature. All reports have concluded that species of the soil borne fungus Fusarium are the causal agents involved [4,6,10,20,21,26,28,40].

Fusarium crown rot was first reported in Massachusetts in 1908 [4]. Premature yellowing and dying stalks were noted in isolated areas and an unknown species of Fusarium was associated with the diseased plants. Cohen [20,21] reported a wilt and root rot of asparagus caused

by Fusarium oxysporum Schlecht. Reddish-brown lesions were found on stems, crowns, and roots of affected plants and seedling damping-off was common in contaminated soils. The problem was found in New York, New Jersey, North Carolina, South Carolina, Pennsylvania, Illinois, Massachusetts, Missouri, California, and Washington [21].

Graham [28] described a similar disease in Ontario in 1955. The article dealt with the "seedling blight" phase of the disease and revealed that it had occurred in epidemic proportions in previous years. Fusarium oxysporum and F. moniliforme Sheldon were isolated from diseased plants and both were considered pathogenic. Graham also determined that the pathogens penetrated the host via the root tips and hypocotyl stomata and felt that the fusaria were confined to the cortex of the root and stem. Grogan and Kimble [30] investigated a fusarium "wilt" of asparagus in California and found F. oxysporum to be a vascular system pathogen. Van Bakel and Kerstens [6] described a "foot rot" in the Netherlands in 1970 caused by F. oxysporum f. sp. asparagi and confirmed that reddish-brown lesions on the stems, crowns, and roots were typical symptoms of the syndrome.

Fusarium moniliforme was further implicated in the "crown rot complex" of established asparagus beds in California [26]. Blacklow and Manning [10] also found the two species to be involved in Western Massachusetts in 1976. Johnson et al. [39,40] went further and concluded that the crown rot complex involved a "wilt and root rot" caused by F. oxysporum f. sp. asparagi, and "stem and crown rot" caused by F. moniliforme. They defended their two disease theory by the fact that they found F. oxysporum more prevalent in young plantings (one to two

years) and F. moniliforme most important in older "declining" beds (twelve years). Isolates of F. moniliforme were also found to be more virulent to asparagus seedlings than pathogenic isolates of F. oxysporum.

Fusarium is a genus of soil-borne fungi in the Deuteromycotina (Fungi Imperfecti) that is common in most agricultural soils [65,77]. Perfect states including Nectria, Calonectria and Gibberella spp. have been found but are not normally produced in culture [77]. F. oxysporum and F. moniliforme are morphologically similar but can be distinguished using Snyder and Hansen's [77] classification scheme for the genus. Both species produce two types of asexual conidia (micro-conidia and macroconidia). Only F. oxysporum produces thick walled, resistant, chlamydospores. Other classifications of the genus have been compiled and often divide the group into over one hundred species. Snyder and Hansen's [77] scheme is the most widely used and divides the group into nine species with some having variations termed "cultivars."

Both species of Fusarium are now considered to be present in all asparagus growing regions of North America [38,40]. Land infested with the pathogens remains contaminated for at least ten years [28]. The fungi are also seed-borne on asparagus [27,37,38,54,66]. Conidia of the pathogens are found mainly as surface contaminants, although a small percentage of the seeds may be internally infested [37,38]. Inglis [37,38] found that the seeds were contaminated during threshing and extraction processes used commercially. Some seed remained contaminated after surface sterilization but no histological evidence was

found demonstrating internal infestation during an electron microscopic survey.

No effective management strategies are currently available against the disease. No resistant varieties are available for Massachusetts conditions. Chemical control has met with limited success [51,55,85], with soil fumigation and fungicide crown dips increasing only early yields. No attempts at biological control of the disease have been reported. Successful biological management of other soilborne diseases have shown that this approach is a feasible area of research. For these reasons, a study was undertaken to look at the potential for biological management of this disease.

Objectives of Study

1. Isolate and obtain microorganisms antagonistic to F. oxysporum and F. moniliforme, the causes of crown rot of asparagus.
2. Screen isolates in the lab, using the paired culture and axenic seedling culture techniques, to select potentially successful microbes for further study.
3. Introduce selected microorganisms into the rhizosphere of asparagus via seed and transplant innoculations.
4. Evaluate treatments in the field, using old land previously cropped to asparagus, and new land not previously cropped to asparagus using the asparagus cultivar Mary Washington.
5. Evaluate treatments in the greenhouse using asparagus cultivars Mary Washington and Rutgers Beacon planted in naturally infested field soil.

CHAPTER II

REVIEW OF LITERATURE

Biological control of soil-borne diseases of plants has received considerable attention in the past thirty to forty years. The use of resistant varieties has been the most effective management strategy for these diseases, but are not always available or suited to a particular climate. For this reason, attempts have been made to utilize "antagonistic" microorganisms which could protect the host from pathogenic organisms. Direct biological control can be achieved by the introduction of microorganisms to the host rhizosphere via seed or seedling inoculations or to the soil [5,44,63]. Indirect biological control [8,25] involves the stimulation of naturally occurring antagonists with soil amendments. Research into the direct approach was the main focus of this study.

There are three basic mechanisms of microbial protection of plant roots [8]. They are competition for space (substrate) and/or nutrients, production of antibiotics or plant growth regulators [12,73], and parasitism of the pathogen (hyperparasitism) [7,11,43,86]. One or more of these mechanisms may be in operation at any one time [8,44]. In an ideal situation, the antagonistic microbes become established in the host rhizosphere over a long period [44].

Fungi [3,45], bacteria [13,36,52], and actinomycetes [18] have been the most commonly used biological agents against fungal pathogens. These organisms have been obtained from soil dilution plates, plant

material, organic debris, and many other sources [15,47,64,80]. Once isolated, screening procedures are used to find potentially successful microbes for further testing [47]. The most commonly used method is pairing the potential antagonist with the pathogen in culture [18,19, 64,83]. The cultures are examined for zones of inhibition against the pathogen or hyperparasitism. Using this technique, Trichoderma was found to be hyperparasitic on Fusarium [19], Micromonospora antagonistic toward Fusarium oxysporum [74] and Bacillus subtilis produced an antibiotic against F. moniliforme [60]. Another screening procedure involves axenic culture of seedlings followed by dual inoculations of the antagonist and pathogen [23]. These two systems are artificial and may be substrate dependent however, and positive results does not guarantee good performance in the greenhouse or field [8,49,86].

Bacteria [73,76] and fungi [24,41] have been applied to transplants and propagating stock of crops to introduce microbes into the host rhizosphere. Aldrich and Baker [2] dipped carnation cuttings into suspensions of Bacillus subtilis to protect against Fusarium roseum f. sp. dianthi. Michael and Nelson [62] applied a Psuedomonas sp. to carnation cuttings to inhibit F. roseum "Culmorum." Verticillium wilt of strawberry was controlled in the field by inoculating transplants with several bacteria and fungi such as Pennicillium, Trichoderma, and Bacillus spp. [41]

Seed inoculations have also been used to introduce bacterial [11,43,73] and fungal [46,80,87,88] antagonists into plant rhizospheres. The microbes can colonize the emerging radical, become established, and protect the developing root systems from fungal pathogens

[44]. Chang and Kommedahl [16,17] coated corn kernels with Bacillus subtilis and Chaetomium globosum to control seedling blight in the field caused by F. roseum and F. moniliforme. Kommedahl et al. [47, 48,49] coated pea, lettuce, watermelon, cabbage, radish, and bean seeds with microbes to increase plant stands and control root rots in the field caused by Fusarium and Rhizoctonia spp. Tveit and Wood [78, 79] achieved biocontrol of seedling blight of oats caused by F. nivale and F. roseum by coating grain with psuedothecia of Chaetomium spp. Wilt of tomato, incited by F. oxysporum f. sp. lycopersici, has also been reduced with isolates of Chaetomium [82] and Cephalosporium [70].

The fungal mycoparasite [83] Trichoderma has often been used as a biological control agent against soil-borne diseases. The fungus is common in some soils and is easily isolated. Chi [19] noted it as a hyper parasite of Fusarium, and Wiendling [83] reported that it attacked other pathogens such as Pythium, Rhizoctonia, Phytophthora, Sclerotium, and Rhizopus. Trichoderma harzianum has been used in the field to control seedling blight of corn [48], and Sclerotium rolfsii on tomato, peanut, and lupine [84]. The fungus has also increased plant stands of carnation, strawberry, and tomato [34] in the field. In the greenhouse using artificially infested soil, T. harzianum reduces Pythium infection of beet [55], Phytophthora cinnamomi on pine seedlings [42], and Rhizoctonia solani on eggplant, tomato, and bean [31]. The fungus also produces antibiotics against other fungi in culture [19,31].

Another approach to biological management of root diseases has been the use of saprophytic or mildly pathogenic species, form species,

or races of the pathogen to protect the host against virulent forms [57]. Successful biocontrol using this method has been termed "cross protection" [23,72], "induced resistance" [57], and "immunization" [57]. Much work done in this area involves fusarium wilt diseases, but other diseases such as virus infections [57], Verticillium wilt of cotton [72], and black shank of tobacco [59] have been managed using this approach. This technique involves a pre-inoculation with the saprophyte (mild parasite), a period of incubation, and finally exposure to the pathogenic (virulent) form [57]. The saprophyte colonizes the cortex and/or vascular system without causing severe symptoms and is able to ward off colonization by the pathogen [57].

Numerous attempts at cross protection have been made and successes have been reported in the field and greenhouse. McClure [58] and Bega [9] protected sweet potato from wilt caused by Fusarium oxysporum with pre-inoculations with F. solani. F. solani causes a mild foot rot of the sprouts, but when it precedes the pathogen, wilt can be reduced by 90-100 percent. Graham [28] tried mixed inoculations of asparagus with both F. oxysporum and F. moniliforme which resulted in less seedling blight than when either isolate was used alone. It was postulated that the reduction in disease was due to competition between the two pathogens at the infection site. Buxton and Perry [14, 69] reduced pea wilt caused by F. oxysporum by prior or mild inoculation with F. solani in the greenhouse and field. They revealed that F. solani was a more aggressive colonizer of the root cortex which resulted in a hypersensitive reaction in the host tissue that prevented entrance by the wilt fungus.

Davis [23] ran cross protection studies with fusarium wilts of tomato, carnation, flax, and cabbage. Protection was achieved in axenic culture with pre-inoculation of the hosts with non-pathogenic f. sp. of F. oxysporum. Partial control resulted in the greenhouse with pre-inoculations of four different non-pathogenic isolates of the wilt fungus (from four different crops) [24]. Langton [53] protected tomato cuttings from F. oxysporum f. sp. lycopersici with mixed or pre-inoculations of a pea wilt isolate of the same species. The successful control was due to the colonization of the roots by the non-pathogen which resulted in "mechanical exclusion" or "localized resistance" [53]. Meyer and Maraite [61] achieved the same results with fusarium wilt of muskmelon. Their experiments were run with biotin deficient protrophic strains of F. oxysporum to aid in the recovery of the antagonists. Using this technique it was found that the host could be infected with multiple strains of the wilt pathogen and protection occurred when the milder strain was present in a higher proportion than the virulent strain. Results from these studies indicate that cross-protection in fusarium diseases is possible.

Biological control can also be integrated with chemical treatments to achieve better results [35]. The antagonist used should be resistant to the fungicide so that its performance is not hindered. Henis et al. [35] used PCNB and the fungus Trichoderma harzianum to control Rhizoctonia solani induced damping-off of table beet seedlings. Kommedahl (1979 unpublished report) also used T. harzianum in combination with captan to achieve superior control of seedling blight of

corn. The above combinations gave better results than when either component was tested alone.

The availability of pathogen-free seed and seedlings is an important prerequisite to attempts at biological control. This can pose a problem when seed is infected with the pathogen involved. Chemical [32,68] and hot water [7] treatments can be effective procedures for obtaining pathogen-free seed. Infusion of fungicides into seed via organic solvents such as acetone can effectively control deep-seated infestations not affected by surface treatments [32,68]. Benomyl solutions have been shown to reduce Fusarium sp. on asparagus seed [38].

CHAPTER III

METHODS AND MATERIALS

Obtaining Antagonistic Microorganisms

Antagonistic microbes were obtained via isolations from soils and plant materials [15,47,64,80] and from other investigators. Selections of fungi, bacteria, and actinomycetes were made at random and cultures were saved for later screening procedures. Culture medium used for all isolations was potato carrot agar (PCA) which was prepared in the following manner.

1. Boil 20 g potato and 20 g carrots in 1 liter of distilled water.
2. Pour extract through cheesecloth and bring volume to 1 liter with distilled water.
3. Add 20 g agar, melt down, and autoclave (pH = 5.5).
4. Acidified PCA (PCA-L) was obtained by adding 10 drops of 50% lactic acid per 250 ml. PCA after autoclaving (46°C) (pH = 4.5).

One gram soil samples were used to make dilutions, ranging from 1/500 to $1/10^7$, using sterile .1% water agar as the diluent. One ml. of the various dilutions was evenly distributed over PCA and PCA-L plates. The plates were incubated for 4-7 days at 24°C. Microorganisms were transferred to PCA and were stored on PCA slants at 4°C.

Isolations were made from asparagus and bean (Phaseolus vulgaris L.) tissues. Internally borne bacteria and fungi were isolated following surface sterilization of the tissue pieces (roots, crowns, lower stems) in 10% chlorine bleach (Clorox^R) for 10 minutes. The tissue pieces were plated on PCA and PCA-L plates and incubated for 7-10 days

at 24°C. Surface microflora were isolated from tissue that was successively washed in sterile water blanks on the shaker. After the tenth washing, the root, crown, or lower stem segments were rolled across PCA and PCA-L plates and incubated as described.

Isolates were also obtained from other investigators that had made claims of success with bio-control agents. Cultures of Trichoderma harzianum, T. viride, and Streptomyses gresius were received from the American Type Culture Collection (ATCC). Three isolates of T. harzianum (T-1, T-5, H-54) were also received from Dr. H. D. Wells (University of Georgia).

Screening Microorganisms in the Lab

All antagonistic microorganisms were placed in paired culture [23,29,47] with both F. oxysporum and F. moniliforme. A 5 mm plug of each pathogen was placed in the center of 3 plates (6 total) per test antagonist containing potato destrose agar (Difco^{R} PDA). Four equidistant 3 cm streaks of the test antagonists were made around the pathogen and the plates were incubated at 24°C for 2 weeks [47]. The plates were then examined for zones of inhibition against the pathogens (antibiotic production) or hyperparasitism (extensive overgrowth) of the pathogens by the test microbes [60,74,80]. Positive results using this test may indicate an isolate has potential as a biocontrol agent and warrants further study [47].

Another technique used to screen microbes in the lab is axenic seedling culture [23,24] done in a growth chamber. Seedlings were

grown on Hoaglands solution agar slants (25 mm test tubes) which contained the following ingredients:

1000 ml	distilled water
15 g	agar
1 ml	M-KH_2PO_4
5 ml	M-KNO_3
5 ml	M-$CA(NO_3)_2$
2 ml	M-$MgSO_4$
1 ml	Trace elements (H_3BO_3, $MNCl_2 \cdot 4H_2O$, $2NSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, and $H_2MoO_4 \cdot H_2O$)
1 ml	.5% chelated iron

Asparagus seed (MW) was pregerminated on 2% water agar and clean seedlings were aseptically transferred to the slants and were established in the growth chamber (27°C day, 24°C night, 14 hr. day length) for 72 hours.

Selected antagonists were inoculated at the base of the crowns and the plants were grown for two weeks. This was done to check for pathogenicity of the potential antagonists on asparagus and to determine the candidate's ability to colonize the root system of developing seedlings. Inoculated plants were compared to an uninoculated control to determine pathogenicity. Isolations were made from non-surface-sterilized root and crown segments to check for colonization ability. Isolates that were non-pathogenic (no tissue discoloration) and that were able to colonize the rhizoplane of the seedlings were saved for further study. Six plants per test antagonist were evaluated.

Cross-inoculations [23,24] were made to determine whether the antagonists could protect asparagus seedlings from subsequent exposure to the pathogen. Six plants were inoculated first with the test microorganisms and twenty-four hours later with a pathogenic isolate of F. oxysporum. Cross-inoculated plants were compared to

non-inoculated and pathogen inoculated (F. oxysporum) plants to determine the capability of the microbes to reduce or eliminate infection and/or symptom development by the pathogen.

Introduction of Selected Microorganisms Into the Asparagus Rhizosphere

Methods for eradicating seed borne Fusarium sp. infestations were compared to select a successful treatment that could be used throughout the study. Treatments examined were hot water soaks (50°, 55°, and 60°C for 15 min.) [7], 10% chlorine bleach (10 minutes) [38,40], 2.5% benomyl in acetone (24 hrs.) [32,68], 2% benomyl in water (24 hrs.) [38], acetone only (24 hrs.) and no treatment (control). 500 MW asparagus seeds (presoaked for 24 hours in sterile distilled water prior to planting) were evaluated per treatment. Seeds were assayed for infestation by plating seeds on PCA-L plates and incubation for 18 days at 24°C. Evaluation involved germination, and the number of seeds per treatment giving rise to F. oxysporum, F. moniliforme, or F. roseum [77] colonies. Treatments were statistically analyzed using Duncan's multiple range test at the $P = 0.05$ level of significance.

Antagonists selected for greenhouse and/or field were grown on culture media in the lab [46,84]. Fungal isolates were grown on either PDA (Difco^R) [46], or V-8 juice agar (Locke 1979 unpublished report) for 10 days at 24°C. V-8 juice agar contains the following ingredients:

20 g	agar
3 g	$CaCO_3$
200 ml	distilled water
800 ml	V-8 juice

Bacterial isolates were grown on Czapex solution agar (Difco^R) for 3 days at 27°C. All microbes were harvested by scraping them from the culture dish with a rubber spatula. The fungal spores or bacterial cells were suspended in a 4% methyl cellulose solution (Locke 1979) which acts as an inert sticker to adhere the microbes to roots or seeds. For seed treatments, slurries or pastes of the antagonists were prepared. Transplants were inoculated with fungal spore suspensions containing 4-6 x 10^6 spores/ml calculated using a stage hemacytometer. Bacterial suspensions contained 10^{10} cells/ml. determined by a dilution endpoint series.

Transplants were grown using Fusarium-free (2.5% benomyl in acetone treated) MW asparagus seed. The potting mix used contained one part peat, one part soil, and one part sand steam pasteurized for forty minutes. Seeds were planted in cell-pack^R flats containing sixty-four plants per flat. The seedlings were grown for six weeks in the greenhouse. Prior to treatment, the root systems were washed free of soil under running tap water.

The antagonists were introduced to the asparagus rhizosphere via seed pelleting and transplant dips. Fusarium-free seed was coated with a slurry of the microbes and dried prior to planting. Transplants were treated by dipping the crown, lower stem, and root system of the seedlings into the suspensions and were incubated in polytehylene bags for twenty-four hours at room temperature to establish the antagonists. The tops were misted periodically to prevent severe wilting.

Field Tests

Seed and transplant treatments were evaluated in an old field previously cropped to asparagus (South Deerfield farm, pH. 6.8) and in a new field not previously cropped to asparagus (Montague farm, pH. 6.8). Seed treatments consisted of three replications of one hundred seeds per treatment, repeated twice in each of the old and new fields. Transplant treatments involved three replications of thirty plants per treatment also repeated twice in each field. The plots were laid out in a randomized block design and were maintained for eight weeks. The biological treatments were compared to an untreated control and to a fungicide treatment. Thiram (Arasan 75^{R}) was used as a seed dressing and a .1% benonmyl dip was used for treating transplants.

The evaluation of the treatments included the criteria of fresh weight, plant stand, and a disease rating for visible symptoms using a 0-5 scale (0=clean, no infection; 1=crown discoloration, 2=crown and root or lower stem discoloration; 3=crown, root, and lower stem discoloration; 4=severe crown rot; 5=dead plant). Seed treatments were further evaluated for crown infection by assaying excised crowns (surface-sterilized for 10 minutes in 10% chlorine bleach) on PCA-L plates. The plates were incubated for 14 days at 24°C and crowns associated with colonies of F. oxysporum and F. moniliforme [77] were considered infected. The data obtained from these tests was statistically analyzed using Duncan's multiple range test for significance at the $P = 0.05$ level.

Greenhouse Tests

Isolates that performed well in the field and other new isolates were tested as seed and/or transplant treatments in naturally infected field soil (South Deerfield farm, pH 6.8) in the greenhouse. Seed treatments consisted of three replications of forty seeds per treatment planted in 6" x 8" flats completely randomized on the greenhouse benches. The experiments were repeated twice, once with cultivar MW and once with RB. Untreated plants were compared to biological treatments, a chemical treatment (.12% captan in water soak for 24 hours), and integrated fungicide/biological treatments (Kommedahl 1979 [35]) using the same criteria for evaluation after eight weeks as described in the field tests.

Transplant treatments consisted of RB transplants grown on 2% water agar for 18 days at 24°C used for dip inoculations. The treated transplants were incubated for six hours prior to planting in naturally infected field soil. Three replications of forty plants per treatment were planted in 6" x 8" flats. Plants were grown for eight weeks and evaluated in the manner described above.

CHAPTER IV

RESULTS

Isolation and Selection of Antagonistic Microorganisms

Sixty fungal, forty bacterial and seven actinomycete isolates were used. Fungal isolates included Trichoderma, Chaetomium, Penicillium, Gliocladium, and Fusarium (saprophytic) spp. Bacteria and actinomycetes were not identified. After screening, in paired culture against both pathogenic fusaria, several isolates were saved for further testing in axenic seeling culture and in field and/or greenhouse trials. These isolates and their origins are given in Table 1.

The majority of antagonistic microorganisms tested in paired culture were ineffective and completely overgrown by both species of pathogenic fusaria. Two isolates of gram negative (rod-shpaed) bacterial, an Aspergillus sp. with blue conidia, a Penicillium sp., and Trichoderma viride (ATCC no. 34650) did, however, produce consistent zones of inhibition against both pathogens, suggesting antibiotic production. Periodic checks were made on each to determine if they retained their ability to produce inhibitory substances after storage for several months on PCA slants at 4°C, and after several transfers. Isolates of Trichoderma harzianum grew well on culture media and usually overgrew the fusaria tested in paired culture, limiting the pathogens' growth. Close association of the T. harzianum mycelium and the mucelium of the pathogens was observed microscopically showing hyperparasitism. Saprophytic fusaria isolated from bean root surfaces were single spored

TABLE 1

ANTAGONISTIC MICROORGANISMS SELECTED FOR TESTING IN THE GREENHOUSE AND/OR FIELD AND THEIR ORIGIN

Isolate	Source
1. *Trichoderma harzianum*[a]	ATCC No. 24274
2. *T. harzianum* (B)	bean root surface
3. *T. harzianum* (T-1)	H. D. Wells, GA.
4. *T. harzianum* (T-5)	H. D. Wells, GA.
5. *T. harzianum* (H-54)	H. D. Wells, GA
6. *T. viride*[a]	ATCC No. 34650
7. *Aspergillus* sp.	asparagus seed
8. *Penicillium* sp.	asparagus seed
9. Bacterium (A) (gram negative rod)	asparagus flower
10. Bacterium (B) (gram negative rod)	soil dilution plate
11. *Fusarium oxysporum* (B)	bean root surface
12. *F. oxysporum* (11)	bean root surface
13. *F. oxysporum* (6W)	bean root surface
14. *F. solani* (1W)	bean root surface

[a]American-Type Culture Collection

and saved for other screening procedures even though no apparent antagonism occurred when paired with the pathogenic forms. The rationale was that the saprophytic fusarias' effectiveness as biocontrol agents would be due to other mechanisms not revealed in culture [33,50,61,67]. All actinomycetes tested were overgrown by the pathogens. All microorganisms tested reacted in the same manner to both species of pathogenic fusaria.

All isolates of saprophytic fusaria and those antagonists that performed well in paired culture were further screened in axenic culture with asparagus seedlings. Three weeks after inoculating seedlings

with paired culture selected antagonists, comparisons were made with non-inoculated and pathogen-inoculated seedlings. Pathogenic fusaria caused severe crown and root discoloration (and seedling death) while all selected antagonists were avirulent (no visual symptoms). Saprophytic fusaria isolated from bean root surfaces, on the other hand, causes a wide range of responses following inoculation of seedlings. Isolates of F. oxysporum caused mild symptoms (slight crown discoloration), moderate symptoms (crown and root discoloration), and in some cases severe crown rot. Several isolates of F. solani were also tested and caused no visible symptoms. All microbes tested could be reisolated from root, crown, and lower stem surfaces suggesting that they were capable of colonizing the rhizosphere.

The potential biocontrol agents were screened by cross inoculations with the pathogens on aseptically grown seedlings. Selected antagonists and saprophytic fusaria that caused mild or no symptoms were inoculated on seedlings and followed twenty-four hours later with a challenge inoculation with a pathogenic isolate of F. oxysporum. Using this technique Aspergillus, Trichoderma, and F. solani completely inhibited symptom development. The two bacterial isolates, Penicillium, and selected (mildly pathogenic) isolates of F. oxysporum resulted in only mild crown discoloration, preventing moderate to severe crown rot caused by the pathogen alone. Isolates that passed these screening tests (Table 1) were selected for field and/or greenhouse testing.

Introduction of Microbes into Asparagus Rhizosphere

A seed pre-treatment was developed that completely eliminated Fusarium spp. from Mary Washington seed lots. The particular lot tested in Table 2 was infested with 7-10% fusaria following a pre-soak in sterile distilled water for 24 hours. Chlorine bleach (10%) reduced levels of infestation to .8% F. oxysporum and 1.2% F. moniliforme significantly as did acetone (24 hours) and .2% benomyl in water (24 hours). The 2.5% benomyl in acetone (24 hours) treatment completely eliminated both pathogens as well as F. roseum. None of the treatments reduced germination compared to the control and the chlorine treatment, which is the standard treatment for asparagus seed used by growers and researchers. Hot water treatments were ineffective in eliminating infestations. Germination was severely inhibited at all temperatures used.

The benomyl in acetone pre-treatment was essential for obtaining Fusarium free seed, seedlings for axenic culture, and transplants for field and greenhouse tests. The pre-treatment was used as a standard procedure throughout the study. Freshly treated seed was plated on PCA plates seeded with conidia of F. oxysporum or F. moniliforme (five plates each, twelve seeds/plate) to bio-assay for benomyl residues that might inhibit biological seed treatments. No zones of inhibition of spore germination were observed around any seeds indicating fungicide residues on the seeds were minimal. Filter paper discs infiltrated with .2% benomyl in water solution gave distinct zones of inhibition

TABLE 2

CHEMICAL SEED TREATMENTS EVALUATED FOR ERADICATION OF FUSARIA FROM MARY WASHINGTON ASPARAGUS SEED. VALUES ARE MEAN NUMBER OF COLONIES/100 SEEDS OF F.O. (*FUSARIUM OXYSPORUM*), F.M. (*F. MONILIFORME*), F.R. (*F. ROSEUM*) F. TOTAL (TOTAL FUSARIA), AND MEAN GERMINATION AFTER 21 DAYS ON PCA-L

Treatment[a]	F.O.	F.M.	F.R.	F. Total	Germination
1. Control	7.2 b	9.8 b	12.6 c	29.6 c	70.0 ab
2. 10% Clorox[R] (10 min.)	0.8 a	1.2 a	1.4 ab	3.4 b	70.6 ab
3. Acetone (24 hrs.)	1.4 a	0.6 a	1.6 b	3.6 b	65.4 a
4. 25% benomyl in acetone (24 hrs.)	0.0 a	0.0 a	0.0 a	0.0 a	74.8 b
5. .2% benomyl in water (24 hrs.)	0.4 a	0.4 a	0.0 a	0.8 a	67.2 a

[a]5 replicates, 100 seeds; seeds were soaked in sterile distilled water after treatment for 24 hours.

Means in each column followed by the same letter are not significantly different at the P = 0.05 level, using Duncan's multiple range test..

against both fusaria suggesting that the organisms are sensitive to low levels of the fungicide if present.

Field Tests

Seed treatments were conducted and evaluated in an old asparagus field, pH 6.8 at South Deerfield, and in a new field, pH 6 at Montague farm. Seed germination was low (30-40%) in both fields with all treatments. Results from two trials are listed in Table 3 and 4 for the old field. No increases in germination or fresh weight were evident in either trial. T. harzianium (B) and Aspergillus sp. did significantly decrease disease symptoms and crown infection in both trials. Other treatments were not effective including the thiram seed dressing. Similar results were obtained in the new field with no differences in germination occurring in either trial. T. harzianum (ATCC) and Aspergillus sp. decreased crown infections and disease symptoms in both tests. Results were similar in both fields as disease symptom ratings and crown infection rates were equal. F. oxysporum and thiram were also evidently not effective seed treatments as results were often worse than the untreated controls.

Transplant treatments evaluated in the old field (Tables 7 and 8) and the new field (Tables 9 and 10) were similar, with the exception that plants in the old field were generally smaller in all treatments. Treatments with F. oxysporum conidia increased fresh weight in both fields significantly and reduced disease symptoms in the new field. A .1% benomyl dip increased fresh weight and decreased disease symptoms in the new field but was not effective in the old. Results with the

TABLE 3

SEED TREATMENTS (1ST TRIAL, JUNE 1979) EVALUATED FOR GERMINATION, FRESH WEIGHT, DISEASE SYMPTOMS,AND CROWN INFECTION IN AN OLD FIELD (S. DEERFIELD) FOR 8 WEEKS, USING CULTIVAR MARY WASHINGTON

Treatment[a]	Mean Germination	Mean Fresh Weight (s)	Mean Disease Rating[b]	Percent Crown Infection[c]
1. Control	38.0 bc	.65 abc	1.20 bcd	70 cd
2. Bacterium (A)	48.7 c	.61 abc	.74 a	53 bc
3. Trichoderma harzianum (B)	27.0 a	.46 a	1.20 bcd	28 a
4. T. harzianum[d]	39.7 bc	.61 abc	.70 a	40 ab
5. T. viride[d]	18.0 a	1.00 d	1.51 e	62 c
6. Penicillium	39.3 bc	1.01 d	1.30 cde	62 c
7. Aspergillus	32.0 b	.55 ab	.98 abc	26 a
8. Fusarium oxysporium (B)	31.7 ab	1.00 d	1.30 cde	88 d
9. Thiram	34.0 bc	.57 ab	2.00 f	59 bc

[a] 3 replicates, 100 seeds each.

[b] Crown, stem, and root rot index 0-5 (0=no symptoms, 5=dead)

[c] 100 crowns plated on PCA-L; % infected with F. oxysporum or F. moniliforme.

[d] American Type Culture Collection

Means in each column followed by the same letter are not significantly different at the P = 0.05 level using Duncans' multiple range test.

TABLE 4

SEED TREATMENTS (2ND TRIAL, JUNE 1979) EVALUATED FOR GERMINATION, FRESH WEIGHT, DISEASE SYMPTOMS,AND CROWN INFECTION IN AN OLD FIELD (S. DEERFIELD) FOR 8 WEEKS, USING CULTIVAR MARY WASHINGTON

	Treatment[a]	Mean Germination	Mean Fresh Weight (g)	Mean Disease Rating[b]	Percent Crown Infection[c]
1.	Control	36.0 bc	.71 abcd	2.00 b	46 b
2.	Bacterium (A)	48.7 c	.77 bcd	.91 ab	60 bc
3.	Trichoderma harzianum (B)	38.3 bc	.78 bcd	1.20 bcd	22 a
4.	T. harzianum[d]	38.3 bc	.78 bcd	.91 ab	44 b
5.	T. viride[d]	24.7 a	.84 bcd	1.50 de	56 bc
6.	Penicillium	39.3 bc	.82 bcd	1.33 de	56 bc
7.	Aspergillus	40.0 bc	.76 abcd	.97 abc	22 a
8.	Fusarium oxysporum (B)	39.7 bc	.91 cd	1.30 cde	84 d
9.	Thiram	24.7 a	.62 abc	2.00 f	68 cd

[a] 3 replicates, 100 seeds each.

[b] Crown, stem, and root rot index 0-5 (0=no symptoms, 5=dead)

[c] 100 crowns plated on PCA-L, % infected with F. oxysporum or F. moniliforme.

[d] American Type Culture Collection

Means in each column followed by the same letter are not significantly different at the P = 0.05 level using Duncan's multiple range test.

TABLE 5

SEED TREATMENTS (1ST TRIAL, JUNE 1979) EVALUATED FOR GERMINATION, FRESH WEIGHT, DISEASE SYMPTOMS,AND CROWN INFECTION IN A NEW FIELD (MONTAGUE) FOR 8 WEEKS, USING CULTIVAR MARY WASHINGTON

	Treatment[a]	Mean Germination	Mean Fresh Weight (g)	Mean Disease Rating[b]	Percent Crown Infection[c]
1.	Control	33.7 a	.58 ab	1.37 bcd	64 cd
2.	Bacterium (A)	40.0 ab	.71 ab	.87 ab	70 cd
3.	Trichoderma harzianum (B)	43.0 a	.78 ab	.86 ab	54 bc
4.	T. harzianum[d]	41.6 ab	.61 ab	.80 a	38 ab
5.	T. viride[d]	36.3 a	.50 a	1.35 cd	72 cd
6.	Penicillium	32.3 a	.54 a	1.25 bcd	64 cd
7.	Aspergillus	46.0 ab	.63 a	.80 a	34 a
8.	Fusarium oxysporum (B)	32.3 a	.50 a	1.17 abcd	76 d
9.	Thiram	31.7 a	1.14 c	1.15 abcd	69 cd

[a] 3 replications, 100 seeds each.

[b] Crown, stem, and root rot index 0-5 (0=no symptoms, 5=dead)

[c] 100 crowns plated on PCA-L; % infected with F. oxysporum or F. moniliforme.

[d] American Type Culture Collection

Means in each column followed by the same letter are not significantly different at the P=0.05 level using Duncan's multiple range test.

TABLE 6

SEED TREATMENTS (2ND TRIAL, JULY 1979) EVALUATED FOR GERMINATION, FRESH WEIGHT, DISEASE SYMPTOMS, AND CROWN INFECTION IN A NEW FIELD (MONTAGUE) FOR 8 WEEKS, USING CULTIVAR MARY WASHINGTON

	Treatment[a]	Mean Germination	Mean Fresh Weight (g)	Mean Disease Rating[b]	Percent Crown Infection[c]
1.	Control	35.0 a	.63 ab	1.42 d	94 e
2.	Bacterium (A)	42.7 a	.70 ab	.74 abc	60 c
3.	*Trichoderma harzianum* (B)	43.0 a	.78 ab	.95 abc	47 bc
4.	*T. harzianum*[d]	44.7 ab	.64 ab	.83 ab	25 a
5.	*T. viride*[d]	37.0 a	.50	1.26 bcd	65 cd
6.	*Penicillium*	33.0 a	.50 a	1.25 bcd	60 c
7.	*Aspergillus*	45.3 a	.66 ab	.97 abc	30 ab
8.	*Fusarium oxysporum* (B)	38.0 a	.58 ab	1.22 abcd	90 e
9.	Thiram	25.3 a	.88 bc	1.48 d	82 de

[a] 3 replications, 100 seeds each

[b] Crown, stem, and root rot index 0-5 (0=no symptoms, 5=dead)

[c] 100 crowns plated on PCA-L; % infected with *F. oxysporum* or *F. moniliforme*.

[d] American Type Culture Collection

Means in each column followed by the same letter are not significantly different at the P=0.05 level by Duncan's multiple range test.

TABLE 7

TRANSPLANT TREATMENTS (1ST TRIAL, JUNE 1979) EVALUATED FOR PLANT STAND, FRESH WEIGHT, AND DISEASE SYMPTOMS IN AN OLD FIELD (S. DEERFIELD) FOR 8 WEEKS, USING CULTIVAR MARY WASHINGTON

	Treatment[a]	Mean Plant Stand	Mean Fresh Weight (g)	Mean Disease Rating[b]
1.	Control	22.3 bcde	9.6 ab	2.4 ab
2.	Bacterium (A)	17.3 abc	5.9 a	2.8 bc
3.	*Trichoderma harzianum* (B)	24.6 def	8.5 abc	2.7 b
4.	*T. harzianum*[c]	20.3 abcd	8.1 abc	2.3 ab
5.	*Penicillium*	15.0 a	8.0 abc	2.4 ab
6.	*Aspergillus*	23.0 bcdef	7.1 ab	2.7 b
7.	*Fusarium oxysporum* (B)	27.3 ef	15.2 de	2.2 ab
8.	.1% benomyl	29.0 f	10.5 abc	2.7 b

[a] 3 replications, 30 plants (6 week-old transplants) each.

[b] Crown, stem, and root rot index 0-5 (0=no symptoms, 5=dead).

[c] American Type Culture Collection.

Means in each column followed by the same letter are not significantly different at the P=0.05 level using Duncan's multiple range test.

TABLE 8

TRANSPLANT TREATMENTS (2ND TRIAL, JULY 1979) EVALUATED FOR PLANT STAND, FRESH WEIGHT, AND DISEASE SYMPTOMS IN AN OLD FIELD (S. DEERFIELD) FOR 8 WEEKS, USING CULTIVAR MARY WASHINGTON

	Treatment[a]	Mean Plant Stand	Mean Fresh Weight (g)	Mean Disease Rating[b]
1.	Control	24.6 def	10.3 abc	3.4 c
2.	Bacterium (A)	27.6 ef	12.7 cd	2.4 ab
3.	Trichoderma harzianum (B)	25.3 def	10.1 abc	2.6 b
4.	T. harzianum[c]	29.0 f	6.6 ab	2.9 bc
5.	Penicillium	16.3 ab	6.4 ab	2.3 ab
6.	Aspergillus	22.0 bcd	6.5 ab	2.4 ab
7.	Fusarium oxysporum (B)	28.0 ef	18.0 e	2.0 a
8.	.1% benomyl	29.3 f	11.4 bcd	2.7 b

[a] 3 replications, 30 plants (6 week old transplants) each.

[b] Crown, stem, and root rot index 0-5 (0=no symptoms, 5=dead)

[c] American Type Culture Collection

Means in each column followed by the same letter are not significantly different at the P = 0.05 level using Duncan's multiple range test.

TABLE 9

TRANSPLANT TREATMENTS (1ST TRIAL, JUNE 1979) EVALUATED FOR PLANT STAND, FRESH WEIGHT, AND DISEASE SYMPTOMS IN A NEW FIELD (MONTAGUE) FOR 8 WEEKS, USING CULTIVAR MARY WASHINGTON

	Treatment[a]	Mean Plant Stand	Mean Fresh Weight (g)	Mean Disease Rating[b]
1.	Control	26.3 cde	18.2 abc	2.4 fg
2.	Bacterium (A)	29.3 e	14.5 ab	2.6 g
3.	Trichoderma harzianum (B)	21.0 ab	14.1 ab	1.9 bcdef
4.	T. harzianum[c]	23.3 abc	10.4 a	2.4 fg
5.	Penicillium	23.3 abc	17.6 abc	2.1 efg
6.	Aspergillus	25.7 bcde	13.4 ab	1.9 cdef
7.	Fusarium oxysporum	30.0 e	32.3 de	1.1 a
8.	.1% benomyl	29.7 e	55.0 f	1.1 a

[a] 3 replications, 30 plants (6 week old transplants) each.

[b] Crown, stem, and root rot index 0-5 (0=no symptoms, 5=dead)

[c] American Type Culture Collection

Means in each column followed by the same letter are not significantly different at the P=0.05 level using Duncan's multiple range test.

TABLE 10

TRANSPLANT TREATMENTS (2ND TRIAL, JULY 1979) EVALUATED FOR PLANT STAND, FRESH WEIGHT, AND DISEASE SYMPTOMS IN A NEW FIELD (MONTAGUE) FOR 8 WEEKS, USING CULTIVAR MARY WASHINGTON

	Treatment[a]	Mean Plant Stand	Mean Fresh Weight (g)	Mean Disease Rating[b]
1.	Control	25.7 bcde	16.5 ab	2.5 fg
2.	Bacterium (A)	27.0 cde	25.9 bcde	1.5 abcde
3.	Trichoderma harzianum (B)	24.3 bcd	15.2 ab	2.1 defg
4.	T. harzianum[c]	28.7 de	25.5 bcde	1.4 abcd
5.	Penicillium	19.3 a	19.5 abcd	2.6 g
6.	Aspergillus	26.7 cde	15.0 ab	2.1 defg
7.	Fusarium oxysporum (B)	29.3 e	31.4 cde	1.2 ab
8.	.1% benomyl	29.3 e	35.5 e	1.4 abc

[a] 3 replications, 30 plants (6 week old transplants) each.

[b] Crown, stem, and root rot index 0-5 (0=no symptoms, 5=dead).

[c] American Type Culture Collection

Means in each column followed by the same letter are not significantly different at the P=0.05 level by Duncan's multiple range test.

saprophytic fusaria were as good as the fungicide in the new field, and better in the old. Other treatments were variable and generally not effective.

Greenhouse Tests

Seed treatments were generally more effective in the greenhouse in naturally infested field soil (S. Deerfield pH 6.8) than in the field. Germination was improved under controlled conditions with both cultivars. The microbial treatments also gave uniform results under these conditions when evaluated. The use of steamed soil gave complete disease control. The captan fungicide treatment gave better results than thiram and enabled the integration of biological and chemical treatments. Results for cultivars MW and RB are given in Tables 11 and 12 respectively.

Treatments with cultivar RB gave uniform results and larger and more vigorous plants than MW. Germination levels with RB were also higher and none of the treatments differed significantly. T. harzianum isolates T-1, T-5, and H-54 significantly increased fresh weight and decreased crown infections and disease symptoms. Integration of captan with these three isolates resulted in higher fresh weight with T-5 + captan and H-54 + captan than with T-5 or H-54 used alone. T. harzianum treatments were equally as effective as captan. Aspergillus sp., T. harzianum (B), and Bacterium A previously tested in the field decreased crown rot symptoms and crown infection levels. Bacterium B increased fresh weight and lowered crown rot with this cultivar. The

TABLE 11

SEED TREATMENTS (NOVEMBER 1979) EVALUATED FOR GERMINATION, FRESH WEIGHT, DISEASE SYMPTOMS, AND CROWN INFECTION IN THE GREENHOUSE USING NATURALLY INFESTED FIELD SOIL AND CULTIVAR MARY WASHINGTON, FOR 8 WEEKS

Treatment[a]	Mean Germination	Mean Fresh Weight (g)	Mean Disease Rating[b]	Percent Crown Infection
1. Control	32.0 def	.20 ab	.91 g	44 ef
2. Bacterium (A)	26.3 abc	.16 a	.05 a	25 bcd
3. Trichoderma harzianum (B)	29.9 bcde	.21 b	.29 b	24 bcd
4. T. harzianum (H-54)	23.7 a	.23 bc	.77 fg	54 f
5. T. harzianum (T-1)	32.0 def	.24 bcd	.77 fg	40 def
6. T. harzianum (T-5)	30.0 cdef	.23 bc	.62 def	20 bc
7. H-54 + .12% captan	29.7 bcde	.34 f	.76 fg	28 bcde
8. T-1 + .12% captan	32.7 ef	.34 f	.68 ef	24 bcd
9. T-5 + .12% captan	35.3 f	.28 de	.73 efg	20 bc
10. .12% captan	31.0 cdef	.20 ab	.48 cd	16 abc
11. Aspergillus	29.4 bcde	.26 cd	.43 bc	40 def
12. Bacterium (B)	24.7 abcd	.31 ef	.57 cde	32 cde
13. Steamed soil	34.7 ef	.39 g	.11 a	0 a

[a] 3 replications, 40 seeds each

[b] Crown, stem, and root rot index 0-5 (0=no symptoms, 5=dead)

[c] 50 crowns plated on PCA-L; % infected with F. oxysporum and F. moniliforme.

Means in each column followed by the same letter are not significantly different at the P=0.05 level using Duncan's multiple range test.

TABLE 12

SEED TREATMENTS (NOVEMBER 1979) EVALUATED FOR GERMINATION, FRESH WEIGHT, DISEASE SYMPTOMS, AND CROWN INFECTION IN THE GREENHOUSE USING NATURALLY INFESTED FIELD SOIL AND CULTIVAR RUTGERS BEACON, FOR 8 WEEKS

Treatment[a]	Mean Germination	Mean Fresh Weight (g)	Mean Disease Rating[b]	Percent Crown Infection
1. Control	34.3 ab	.30 ab	1.50 i	62 e
2. Trichoderma harzianum (B)	32.6 a	.31 ab	.16 bc	10 ab
3. T. harzianum (H-54)	36.0 ab	.40 def	.98 gh	44 d
4. T. harzianum (T-1)	35.7 ab	.37 de	.82 ef	26 bcd
5. T. harzianum (T-5)	37.0 b	.36 cde	.97 gh	22 bc
6. H-54 + .12% captan	35.0 ab	.48 g	.93 fgh	24 bc
7. T-1 + .12% captan	34.7 ab	.43 fg	.90 fg	22 bc
8. T-5 + .12% captan	35.0 ab	.45 fg	.77 e	20 b
9. .12% captan	34.3 ab	.42 ef	1.00 h	22 bc
10. Aspergillus	35.7 ab	.29 ab	.26 c	16 ab
11. Bacterium (A)	36.6 a	.28 a	.06 ab	40 cd
12. Bacterium (B)	37.7 ab	.39 def	.64 d	40 cd
13. Steamed soil	35.7 ab	.41 def	.00 a	0 a

[a] 3 replications, 40 seeds each

[b] Crown, stem, and root rot index 0-5 (0=no symptoms, 5=dead)

[c] 50 crowns plated on PCA-L, % infected with F. oxysporum and F. moniliforme.

Means in each column followed by the same letter are not significantly different at the P = 0.05 level using Duncan's multiple range test.

best treatment was steamed soil, as no crown rot was evident and large, vigorous plants were obtained.

MW seed treatment results were better in the greenhouse than in the field, but were not as uniformly successful as RB treatments. Plants were significantly larger with *T. harzianum* plus captan than with either alone. All treatments lowered crown rot symptoms and reduced crown infection levels with the exception of *T. harzianum* (H-54, T-1) and *Aspergillus* sp.

Transplant treatments with saprophytic fusaria were evaluated in the greenhouse using cultivar RB. No fungicide test was included because the young, tender, water agar-germinated transplants were damaged by the .1% benomyl solution. Results for RB transplants are given in Table 13. Plant stands for all treatments were not significantly different. *F. oxysporum* (B), previously tested in the field significantly increased fresh weight and lowered disease symptoms. *F. oxysporum* (6w) and (11) and *F. solani* (1w) reduced disease symptom ratings but did not significantly affect fresh weight. The effectiveness of *F. oxysporum* (B) in increasing plant vigor was again clearly demonstrated.

TABLE 13

TRANSPLANT TREATMENTS (DECEMBER 1979) EVALUATED FOR PLANT STAND, FRESH WEIGHT, AND DISEASE SYMPTOMS IN THE GREENHOUSE USING NATURALLY INFESTED FIELD SOIL AND CULTIVAR RUTGERS BEACON, FOR 8 WEEKS

	Treatment[a]	Mean Plant Stand	Mean Fresh Weight (g)	Mean Disease Rating[b]
1.	Control	40.0 a	.99 a	2.20 b
2.	Fusarium oxysporum (B)	40.0 a	1.50 b	.91 a
3.	F. oxysporum (6w)	39.7 a	1.21 a	1.27 a
4.	F. oxysporum (11)	40.0 a	1.20 a	1.10 a
5.	F. solani (1w)	39.7 a	1.19 a	1.18 a

[a] 3 replications, 40 plants each.

[b] Crown, stem, and root rot index 0-5 (0=no symptoms, 5=dead)

Means in each column followed by the same letter are not significantly different at the P=0.05 level using Duncan's multiple range test.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Numerous attempts at biological management of soil-borne diseases have resulted in very little practical application. Results are variable and often non-spectacular. In this study, treatment of transplants with saprophytic fusaria did result in growth responses, but limited success with other treatments was only evident after plant excavation and lab assays. The fact that the plant is a slow growing perennial and the disease involved is a slow developing "decline," may have hindered more pronounced responses to the microbial treatments.

A very effective seed pre-treatment for eradicating seed borne fusaria form asparagus seed was developed during this study. Early in this study, extreme difficulty in growing Fusarium-free seedling was encountered due to the seed-borne nature of the disease. Growing transplants in steam-pasteurized soil resulted in a high incidence of crown rot (70-80%) due to the introduction of the pathogen on the seed. The pathogens are apparently able to rapidly colonize steam-pasteurized soil in the absence of other microbial competitors [85]. The 2.5% benomyl in acetone treatment eliminated the seed-borne fusaria and made it possible to grow disease-free plants needed in this work.

The seed treatment trials in the field were disappointing due to the low germination levels in all treatments (30-45%) which was well below the 70-80% germination expected with asparagus seed. A dry period in June and early July followed plantings in both fields and may

have caused the drastic germination reduction. Spot irrigation by hand was done to keep the drier areas moist, but no controlled irrigation system was available. These low moisture levels may have also hindered some transplant treatments. Dryness may have inhibited the ability of the microbes to colonize the rhizosphere also.

Seed treatments under controlled conditions in the greenhouse were more promising and germination often exceeded expected levels of 70% for both cultivars. The most effective treatments were with Trichoderma harzianum isolates alone and integrated with captan. Other microbes such as Aspergillus and the bacterial isolates were also promising in the greenhouse. An interesting observation made with these seed treatments was the fact that 70-90% of the T. harzianum (greenhouse) and 75% Aspergillus sp. (greenhouse and field) were recovered (reisolated) from crowns when assayed for infection on PCA-L. These crowns had been surface sterilized suggesting that the antagonists had invaded at least the crown tissues with some treatments. These observations were not included in the evaluations of the treatments, but may indicate antagonism is occurring in the crown itself. None of the other antagonists tested were recovered from the crowns but may have been present in the rhizosphere which was not examined.

The best transplant treatment in both field and greenhouse was the saprophytic F. oxysporum (B) isolate which increased fresh weights in all situations and even exceeded the benomyl treatment in one field. This isolate was superior to others tried and remained effective even after successive transfers (single spores) and storage on PCA plants. The fusaria are known to readily mutate when kept in culture over long

periods [77] and loss of pathogenicity often occurs. The storage in culture did not reduce the saprophytic ability of this particular isolate. The ineffectiveness of other microbes such as T. harzianum and Aspergillus as transplant treatments was worth noting as these treatments were good seed treatments. F. oxysporum (B) was not effective as a seed treatment but was with transplants. The method of application of a particular microbe influences the results obtained.

The mode of action of certain microbial treatments at increasing fresh weight and reducing disease symptoms and crown infection was not investigated during this study, but substantial research in this area allows some interest and speculations. The ability of the fusaria to "cross protect" hosts against other fusaria is based on the differential pathogenicity of isolates on certain plants. Fusaria causing wilt diseases (and some root rots) have been thought to be reasonably host-specific as far as symptom development is concerned [67]. Davis [22] showed that fusaria pathogenic on one species, will often invade the tissues of non susceptible hosts without causing severe symptoms. The "cross infection" of these fusaria was found to occur almost without exception [22]. Davis also noted that non-susceptible hosts often physiologically reacted to the presence of an uncongenial Fusarium by increased production of gums, tylosis, and vascular browning in the absence of external symptoms.

Taylor and Parkinson [75] showed that F. oxysporum is a normal colonizer of the rhizoplane of bean roots in the absence of disease. Throughout most of the plant's life cycle, F. oxysporum was found to be the major fungal component associated with "healthy" roots. The

fungus could often be found internally in the epidermis and cortex of such symptomless roots. Further evidence for the ability of fusaria to invade and colonize non-susceptible hosts was shown by Hendrix and Nielsen [3]. The sweet potato wilt fungus, F. oxysporum f. sp. batatas was found to invade seven other crops tested, causing no symptoms in cowpea, watermelon, and corn; and causing reddish-brown discoloration in soybean, cabbage, cotton, and snapbean. No severe symptoms (wilt) resulted in any of the seven crops. The above evidence suggests that colonization of uncongenial hosts by fusaria "saprophytic" on that crop is widespread and normally found with most plants [33,57].

Matta [57] reviewed the subject of invasion of uncongenial hosts by pathogens and non-pathogens (saprophytes) alike. Once inside the tissues of a non-susceptible host, a microbe can: (1) grow and cause variable symptoms, (2) grow and cause no symptoms, and (3) enter and be inhibited by the host or another microbe present. The latter two responses could account for biological management of a pathogen via two different mechanisms. If a microbe enters a host and is inhibited by a hypersensitive reaction from the host, the chemical (phenolic compounds) and/or mechanical (gums, tylosis, cell wall thickenings) barriers could protect the host from virulent forms [57]. This process is termed "induced" or "localized" resistance and could be a possible mechanism of the saprophytic Fusariam in reducing crown rot symptoms and increasing plant vigor.

If a microbe enters a host, grows (colonizes the substrate), and causes no symptoms; competition could occur and account for decreased infection by a pathogen at the infection site [50,55,57]. This could

result in the ability of such antagonists as T. harzianum, Aspergillus, and F. oxysporum in reducing disease symptoms and crown infections. The fact that T. harzianum and Aspergillus were found internally in many of the treated crowns, in the absence of severe symptoms, supports this theory. The rhizosphere and rhizoplane are extremely competitive microbial substrates [8] due to the fact that microbial growth is stimulated in this region. "Niche replacement" of an infection site by a saprophyte instead of a pathogen, could prevent successful pathogen colonization of such sites.

The significance of this and other related work in the area of biological management of soil-borne diseases is not yet clear. The economic applications of biological treatments against root diseases have not been realized. The fact that other control strategies have been more effective initially, may mislead researchers and growers into thinking immediate results might occur. Fungicide applications and the use of resistant varieties usually give such immediate results when effective and are preferred to the complex treatment procedures and often nonspectacular effects of microbial antagonists. The fact that asparagus breeding is considered difficult [1] and the plant's perennial habit, may make this disease particularly suited to biological management strategies. The methods used in this study and/or other avenues of biological management warrant further study.

Other important aspects of this and other work need further investigation. The long-term effects of the establishment of antagonistic microbes in the rhizosphere of a perennial field crop have yet to be looked into. Most work with economic crops and microbial

antagonists has involved mainly annual grains and vegetables. Over time, some of the treatments used in this study may have more dramatic effects than were initially evident on young seedlings. None of the treatments in this study were looked at longer than eight weeks due to time limitations and extensive preliminary work needed. Observations (unquantified) made during this study included the repeated isolation of saprophytic isolates of T. harzianium, F. oxysporum, and F. solani from vigorous surviving plants in both old (fifteen years) and new (seedlings) plantings of asparagus in the Connecticut River valley. High levels of these saprophytes may be accounting for natural biological control occurring in fields heavily infested with crown rot pathogens. Determining the extent of this phenomenon in local plantings could shed more light on the potential for biological management of this disease.

Other questions concerning the disease and biological management were uncovered. The ability of a single isolate of Fusarium oxysporum to cause a wide range of responses on many crops leads one to question the term "pathogenic isolate." The genetics, physiology, and host parasite relationship of fusarium to asparagus needs to be further investigated. Also the fact that these pathogens are usually studied only in association with their f. sp. host does not attempt to clarify the pathogenicity of fusarium to other crops and the related ecological implications. Finally, other antagonistic microorganisms, other approaches to biological (indirect) control, and soil conditions could be examined and integrated into an effective management strategy for this disease.

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APPENDIX

Fig. 1. Macroconidia and microconidia of Fusarium oxysporum pathogenic to asparagus (400X)

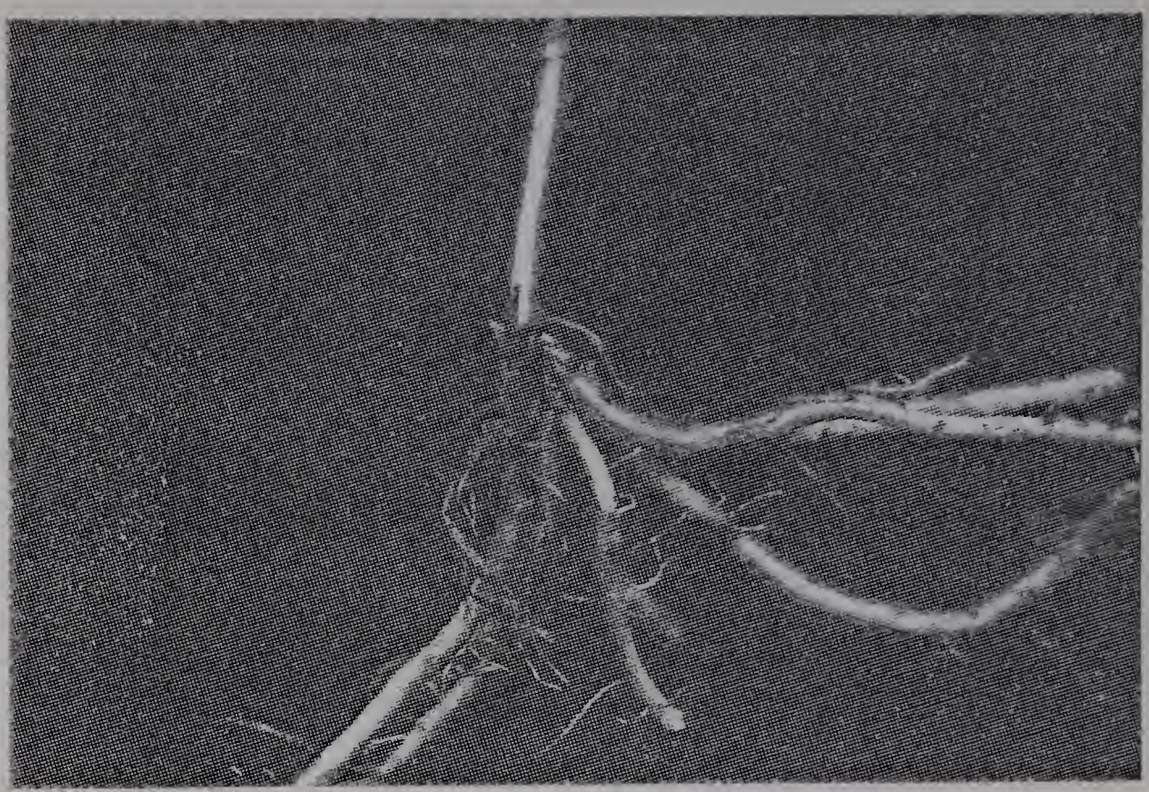
Fig. 2. Macroconidia and micronidia of Fusarium moniliforme pathogenic to asparagus (400X)

Fig. 3. 1 year-old Mary Washington crown showing crown, storage root, and feeder root rot caused by Fusarium oxysporum and F. moniliforme.

Fig. 4. Symptomless 2 month-old Mary Washington seedlings grown from 2.5% benomyl in acetone treated seed in steam pasteurized in the greenhouse.

Fig. 3. 1 year-old Mary Washington crown showing crown, storage root, and feeder root rot caused by Fusarium oxysporum and F. moniliforme.

Fig. 4. Symptomless 2 month-old Mary Washington seedlings grown from 2.5% benomyl in acetone treated seed in steam pasteurized in the greenhouse.



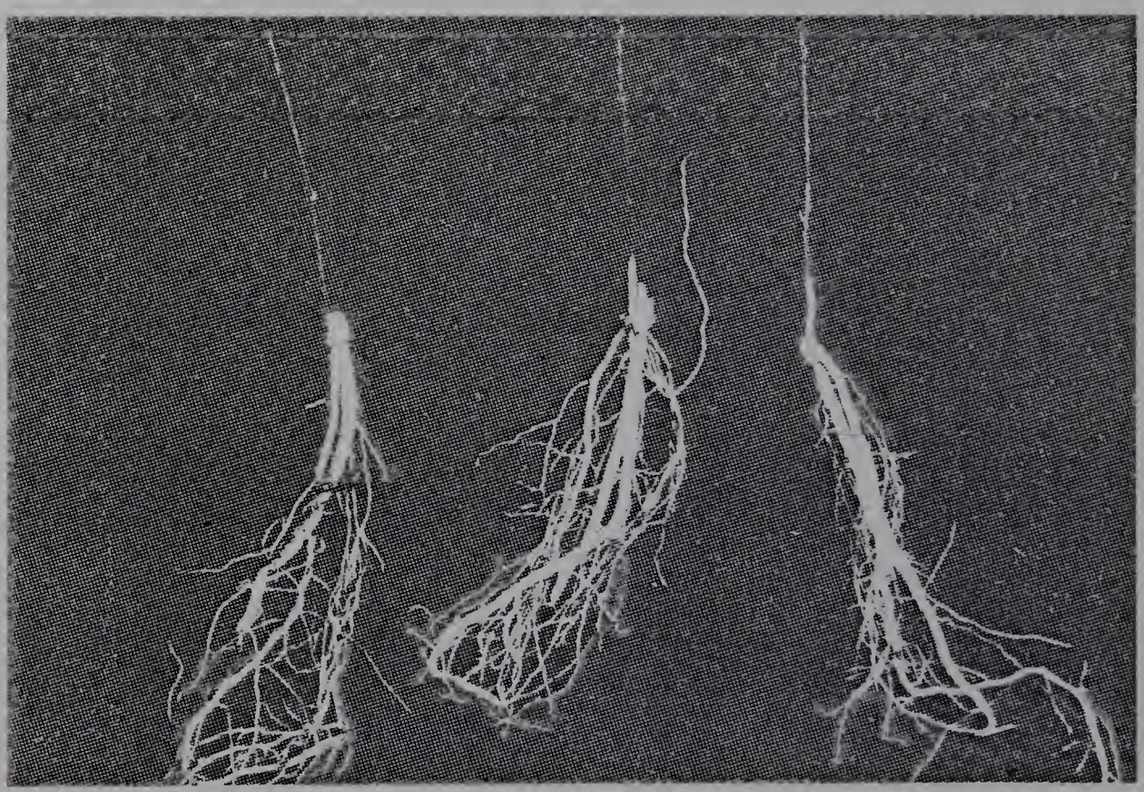
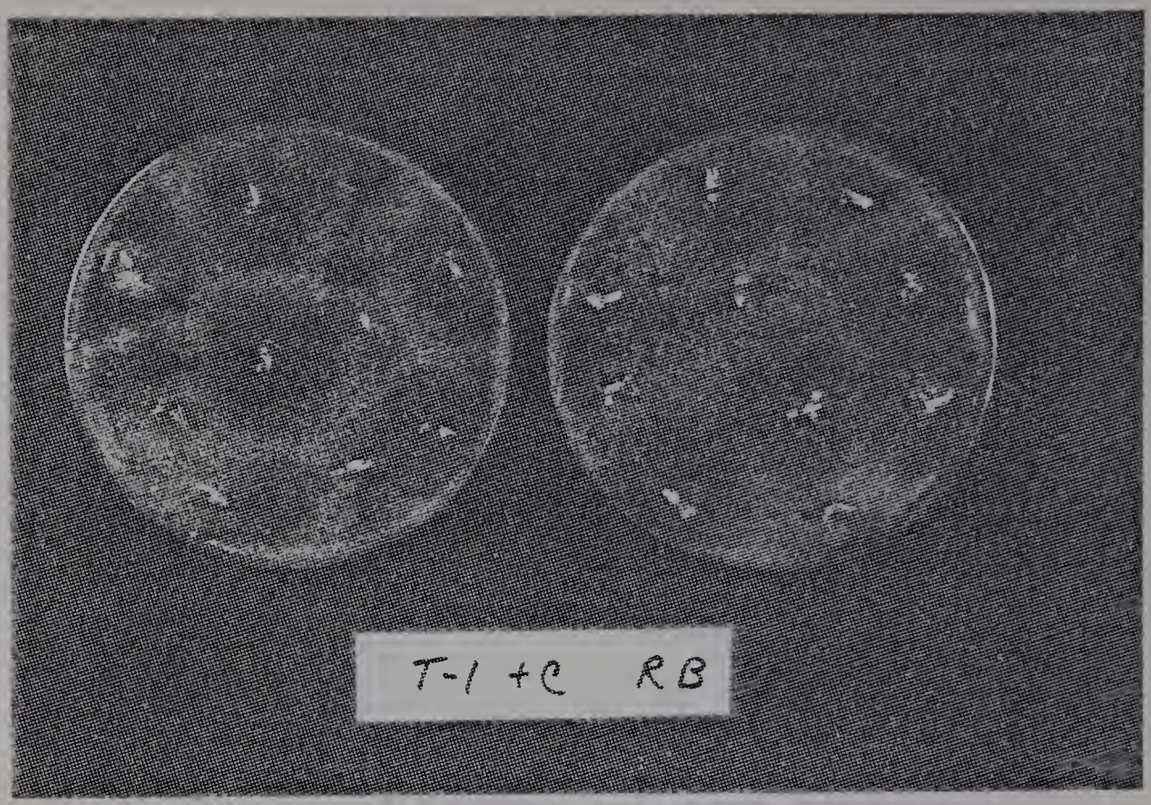


Fig. 5. Recovery of Trichoderma harzianum (T-1) from 8 week-old surface-sterilized crowns grown from T-1 + .12% captan treated Rutgers Beacon seed in naturally infested field soil in the greenhouse.

Fig. 6. Recovery of Aspergillus sp. from 8-week old surface-sterilized crowns grown from Aspergillus treated Mary Washington seed in naturally infested field soil in the greenhouse.


T-1 +C RB

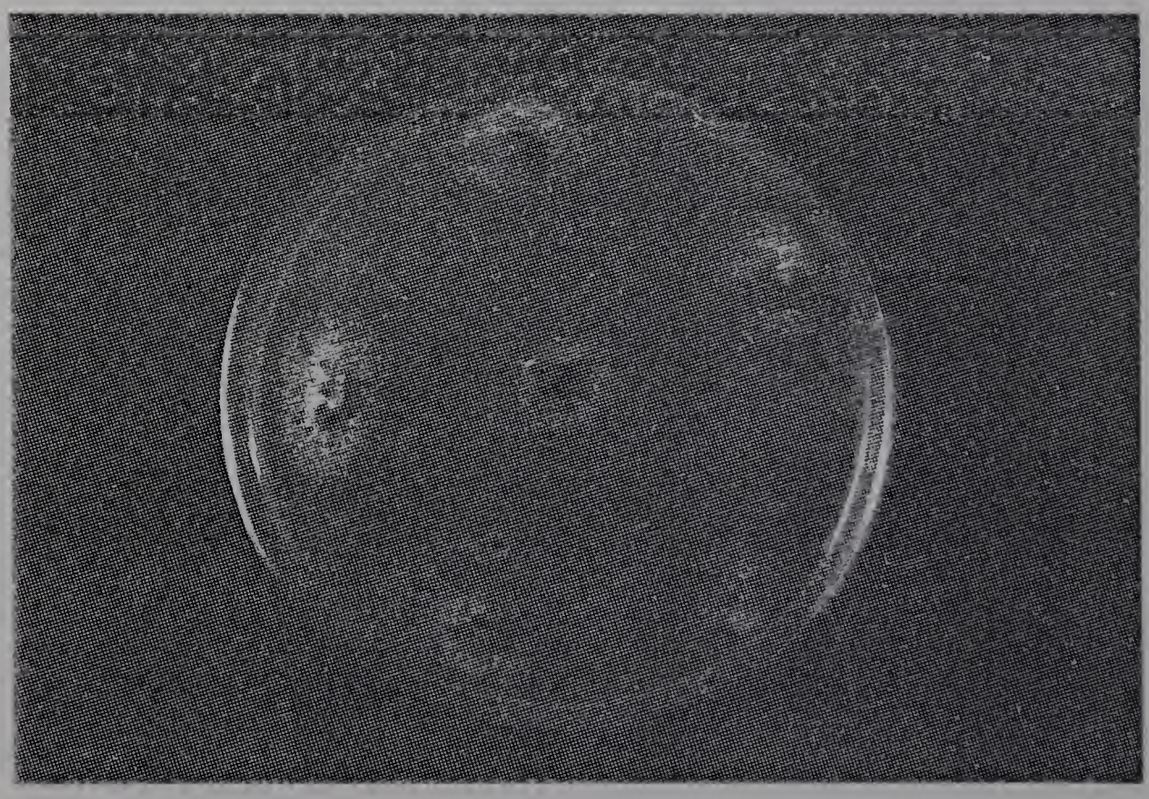


Fig. 7. 8 week-old surface-sterilized crowns grown from Rutgers Beacon seed in steam-pasteurized soil in the greenhouse.

Fig. 8. Recovery of Fusarium sp. from 8 week-old surface-sterilized crowns grown from untreated Rutgers Beacon seed in naturally infested field soil in the greenhouse.


STEAMED (BA)

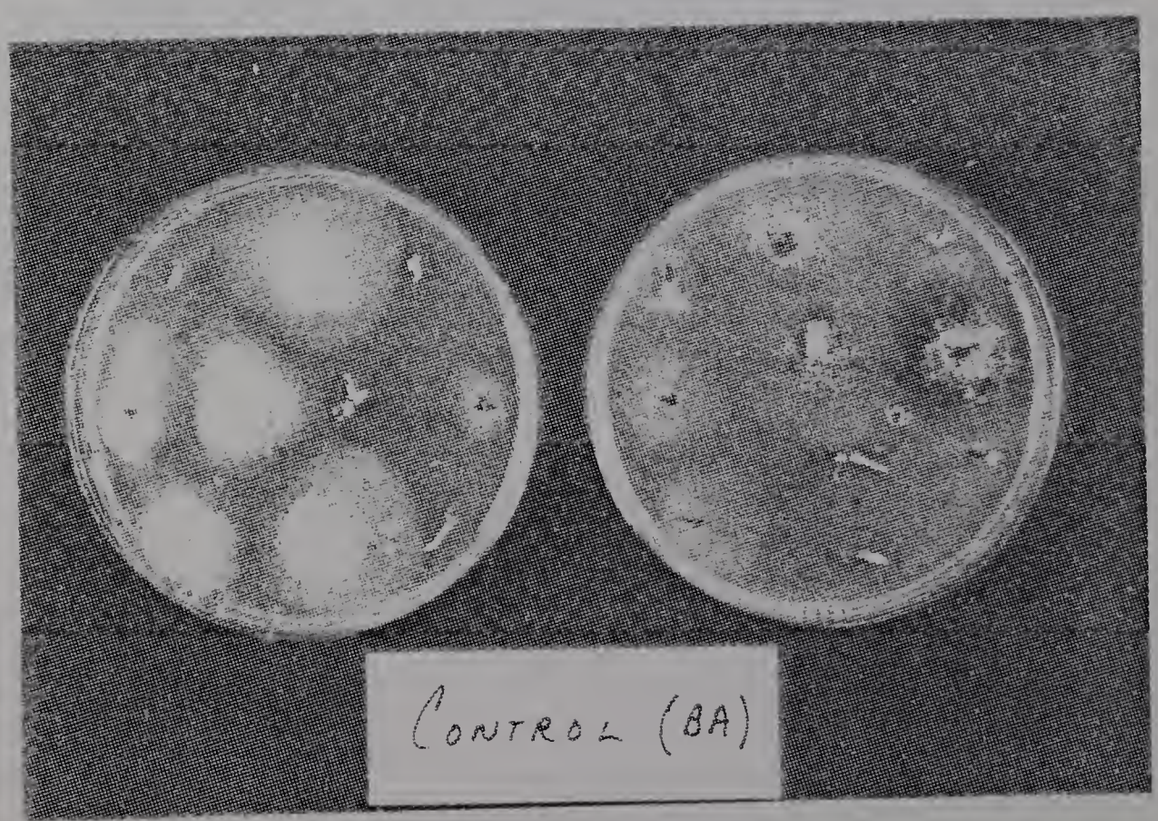

CONTROL (BA)

Fig. 9. Rutgers Beacon seedlings grown in naturally infested field soil in the greenhouse for 8 weeks from transplants; untreated (left), and treated with $4\text{-}6 \times 10^{-6}$ conidia/ml. of Fusarium oxysporum (B) (saprophytic) (right).


CONTROL
F.O. (B.)