DISSERTATION

BIOCONTROL OF FUSARIUM CROWN AND ROOT ROT OF FRESH MARKET TOMATO WITH *TRICHODERMA HARZIANUM* STRAINS UNDER GREENHOUSE CONDITIONS

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY NUSRET OZBAY ENTITLED

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ABSTRACT OF DISSERTATION

BIOCONTROL OF FUSARIUM CROWN AND ROOT ROT OF FRESH MARKET TOMATO WITH *TRICHODERMA HARZIANUM* STRAINS UNDER GREENHOUSE CONDITIONS

Greenhouse tomato growers in the United States have few products available for chemical control of plant pathogens. Biological control of soilborne plant pathogens by antagonistic microorganisms is a potential alternative to the use of chemical pesticides during greenhouse production. Biological control experiments were conducted to test the effects of commercial and noncommercial strains of *Trichoderma harzianum* against *Fusarium oxysporum f. sp. radicis-lycopersici* on tomato plants grown in two different hydroponic media, coir and rockwool. This study also investigated effects of strains on growth of tomato seedlings under greenhouse conditions. *Trichoderma harzianum* is a fungus that attacks a range of economically important phytopathogenic fungi. Tomato (*Lycopersicon esculentum* Mill., cultivar Caruso) plants were inoculated with *T. harzianum* strains (PlantShield^{TM}, T22 and T95) prior to challenge with the pathogen. They were applied to growing media prior to sowing and to roots at transplanting at two inocula densities, 10^6 or 10^7 conidia/ml. The results of this study demonstrated that *T. harzianum* strains, especially applied at transplanting, decreased Fusarium crown and root rot incidence 79% for coir and 73% for rockwool, decreased disease severity 45% for coir and 48% for rockwool, and increased fruit yield 37% for coir and 25% for rockwool on tomato for control. The results also demonstrated that *Trichoderma harzianum* strains improved tomato seedling growth.

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TABLE OF CONTENTS

List of Tables

List of Figures

CHAPTER I

GENERAL INTRODUCTION

Tomato is one of the most important greenhouse vegetable crops in the United States. In the U.S.A., the total acreage in greenhouse tomato production increased by 40 percent between 1996 and 1999. In 1999 estimates of US greenhouse vegetable acreage was 325 hectares, with tomatoes accounting for 304 of those hectares (Snyder, 1995). The leading states in greenhouse vegetable production are California, Florida, Colorado, Arizona, Ohio, Texas, and Pennsylvania, each with more than one million square feet in production (CEA, 1997). During the past decade Colorado has been the number one state in the rate of increase of the tomato greenhouse industry with cash receipts increasing about seven fold from $3.8 million in 1993 to $29 million in 1996; these currently exceed $60 million in annual value (Hudson and Fretwell, 1998). More than 48 hectares were cultivated in Colorado in 1997 and presently almost half of the total US greenhouse tomato production occurs in the state (Naegely, 1997).

Plant diseases, especially root diseases, cause significant losses in agricultural and horticultural crops every year. These losses can result in reduced food supplies, poorer-quality agricultural products, economic hardship for growers and processors, and, ultimately, higher prices. For example, soil-borne fungal pathogens cause an annual crop loss of at least four billion dollars in the United State alone (Papavizas 1985; Jewell, 1987; Monte, 2001). Typically, control of these diseases is achieved by repeated applications of pesticides (Maloy, 1993). The need to protect high value crops from damage caused by soilborne pathogens frequently leads growers to excessive application

of chemicals. There has been significant increase over the past several years in the amount of chemicals used in controlling plant diseases. In 1995, for example, 15,300 tons of fungicides are used for vegetable crops production in the U.S.A. (Barnard et al., 1997). However, pesticides have created new problems such as: pesticide-resistant pathogens; affecting non-target organisms; contaminating underground water; and posing a danger to humans from toxic residues. Because of environmental issues and legislative regulation, developing alternative control measures to chemicals has become a priority of many scientists (Lumsden and Lewis, 1989).

Diseases caused by *Fusarium* spp. are important limiting factors in the production of tomato. Several types of diseases associated with these pathogenic fungi have been identified, including Fusarium wilt, foot rot and crown and root rot disease. One of the most damaging soilborne pathogens of tomato is *Fusarium oxysporum* f. sp. *radicis-lycopersici,* which causes Fusarium crown and root rot (FCRR). Commercial yields have been reported to be reduced by 15-65 percent in the field grown tomatoes (Sonada, 1976; Anonymous, 1999). Several control procedures have been attempted for managing FCRR in the greenhouse and field. Possibilities to manage fusarium wilt, rot e.g. by using fungicides or resistant cultivars, are limited. The use of Fusarium-resistant tomato cultivars can provide some degree of control of this disease, but the occurrence and development of new pathogenic races is a continuing problem, and currently there are no commercially acceptable cultivars with adequate resistance to *F. oxysporum* f. sp. *radicis-lycopersici* (Jarvis, 1988; Jones et al., 1991). Although fungicides such as benomyl or captafol have been demonstrated to be effective, captafol is no longer labeled for usage, and there is an imminent possibility of fungicide resistance.

Fumigation with methyl bromide (MBr) + chloropicrin formulations have been the most commonly used pre-plant practice for control of FCRR. Application of methyl bromide + chloropicrin significantly reduces the incidence and severity of the disease (McGovern et al., 1996). However, disease incidence is still very high. Even with the use of methyl bromide as a pre-plant fumigant, epidemics of fusarium crown and root have occurred in commercial production fields (Chellemi, 1997).

Tomatoes represent the largest single-crop use of MBr in the United States, accounting for 25% of the total MBr use for soil fumigation, or over 5,000 t/year (UNEP, 1994). MBr is a powerful soil fumigant providing effective control of a wide range of soil-borne pathogens and pests, including fungi, bacteria, nematodes, insects, mites, weeds and parasitic plants. It is also relatively economical and convenient to use. Despite these major advantages, the use of MBr has been associated with major problems, including the depletion of the ozone layer (EPA, 1997). Because of this, MBr production and use will be phased out on a worldwide scale, by 2005 in the U.S. and other developed countries and by 2015 in the developing countries (Rowlands, 1993). Therefore, there is an urgent need to define and implement alternative solutions for managing soilborne pathogens (Noiling and Becker, 1994). For these and other reasons, researchers in academic institutions and private companies have increased their efforts to develop non-chemical approaches to plant disease control. The search for alternative strategies also has been stimulated by public concerns about the adverse effects of soil pesticides (Weller et al., 2002). Biological control is such an alternative.

The antagonistic interactions between microorganisms and plant pathogens can be utilized for biological control (Lynch, 1990). Biological control agents have the potential

to fill the gap created by the withdrawal of the broad-spectrum fungicides. Microorganisms with ability to suppress disease causing fungi and insect-pests are potentially important alternatives to chemical pesticides and have been reported by many researchers. Biological control methods using naturally occurring bacteria, fungi or viruses have in the past received limited approval from users, as their ability to protect plants has often been inferior to results obtained by chemical means. However, biological control of plant diseases, especially soilborne plant pathogens, has been the subject of much research in the last two decades. In the last few years research interest in biological control of plant diseases has became extremely active and productive. A variety of soil microorganisms have demonstrated activity in the control of various soilborne plant pathogens, including *Fusarium.*

Biocontrol with antagonistic microbes such as the fungus *Trichoderma* is one area of research. *Trichoderma* spp. are well documented as effective biological control agents of plant diseases caused by both soilborne fungi (Sivan et al., 1984; Chet, 1987; Jin et al., 1991; Coley-Smith et al., 1991) and leaf- and fruit-infecting plant pathogenic fungi, including *Botrytis cinerea* (Tronsmo, 1991; Gullino, 1992; Elad et al., 1993). *Trichoderma* spp. are often very fast growing and rapidly colonize substrates, thus excluding pathogens such as *Fusarium* spp. Several of these fungi are also parasitic to other fungi including plant pathogens (Papavizas 1985). *Trichoderma harzianum* Rifai is an efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. *T. harzianum* alone or in combination with other *Trichoderma* species can be used in biological control of several plant diseases

(Papavizas 1985; Chet 1987; Samuels 1996). An additional advantage for *T. harzianum* is that it increases growth in various plants.

Chang et al. (1986) noted that steamed or raw soil inoculated with *T. harzianum* hastened flowering of periwinkle, increased the number of blooms per plant on chrysanthemum and increased the height and weight of other plants. Windham et al. (1986) also reported increased rate of emergence of tomato and tobacco seedlings in autoclaved soil treated with *Trichoderma spp.* and the rate of seed germination was increased in vitro, and they concluded that *Trichoderma spp.* produced a growth-regulating factor. It has also been shown to be effective in controlling FCRR of tomato under greenhouse and field conditions (Chet and Henis, 1985; Sivan et al., 1987; Hartman and Fletcher, 1991; Datnoff et al., 1995).

The effectiveness of the *Trichoderma* was evaluated in terms of the reduction of disease incidence in comparison to the control plots that received only methyl bromide + chloropicrin treatments. Over all tests, *Trichoderma* reduced crown rot, with a calculated mean reduction in disease severity of 42% and a calculated reduction of disease incidence of 39%, compared to the control (Nemec et al., 1996). Under field conditions, the combination of *T. harzianum* with soil solarization or with a reduced dose of methyl bromide resulted in significant disease control of FCRR of tomato induced by FORL (Sivan and Chet, 1993). Datnoff et al. (1995) conducted field experiments in Florida to evaluate commercial formulation of two fungi, *T. harzianum* and *Glomus itraradices*, for control of FCRR of tomato. Compared to controls, significant reduction in disease incidence was obtained with treatment of biocontrol agents.

Under greenhouse conditions, the incidence of crown rot of tomato was reduced by up to 80% 75 days after sowing when *T. harzianum* T35 was applied as either seed coating or a wheat bran-peat preparation (Chet, 1990). *Paenibacillus macerans* and *T. harzianum* were evaluated for promoting plant growth and suppressing Fusarium crown and root rot (FCRR) under fumigated and non-fumigated field conditions. *Trichoderma harzianum* and *Paenibacillus macerans* significantly reduced severity of Fusarium crown and root rot. No differences were observed between the biologicals and an untreated control in methyl bromide-treated plots. These results support the use of microorganisms for growth promotion and biocontrol (Datnoff and Pernezny, 2001). *Trichoderma* has been used for *Rhizoctonia* control in greenhouse grown carnations in Colorado. Currently, no strains labeled for use on tomatoes.

The main objective of this study was to examine existing *T. harzianum* strains, including commercial formulations, with known activity against soilborne fungal pathogens for their efficacy in controlling Fusarium crown and root rot of tomato. This study also investigated effects of strains on growth of tomato seedlings under greenhouse conditions.

CHAPTER II

LITERATURE REVIEW

Biological Control

Biological control methods have been used to control pathogens for many years. Microorganisms have been added to the soil, added to furrows, drilled into the ground with seeds, applied to roots, sprayed onto foliage, and used as seed treatments (Taylor et al., 1990; Tjamos et al. 1992). There are many definitions for biological control. In the simplest terms, biological control involves suppressing pest organisms with other organisms. The basic idea involves a strategy for reducing disease incidence or severity by direct or indirect manipulation of microorganisms (Maloy, 1993). Baker and Cook (1974) defined biological control as "the reduction of inoculum density or disease-producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host or antagonists, or mass introduction of one or more antagonists."

The concept of biological control today embodies not only introduction of antagonists into cropping systems, but also manipulation of the environment designed to favor resident beneficial organisms via crop rotation , residue management, and a wide range of other cultural practices. For example, crop rotation may help control soil-borne and residue inhabiting pathogens. Insects, mites, plant diseases and vertebrates all may be targets of biological control.

A beneficial organism used to control a pathogen is referred to as a biological control agent or, often, as an antagonist, because it antagonizes or interferes with target

pathogen organism. Fungi and bacteria are the chief biological control agents that have been studied for the control of plant pathogens, particularly soil-borne fungi. In addition, viruses, amoebae, nematodes, and arthropods have been mentioned as possible biocontrol agents (Whipps and McQuilken, 1993). Some biocontrol agents have been genetically modified to enhance their biocontrol capabilities or desirable characteristics. Biological control agents must be able to compete and persist in the environment in which, they operate and be able to colonize and proliferate on existing and newly formed plant parts at times well after application.

Biological control can occur naturally and is an effective factor leading to ecological balance between pathogens and their antagonists. Chemicals extracted from plants or microbes and used for pest control are not biological controls. Biological control is a population-level process in which one species population lowers the numbers of another species by mechanisms such as predation, parasitism, pathogenicity, or competition (Van Drieshe and Bellows, 1996).

Different facets of biological control of plant pathogens are grouped under three broad headings (Mukerji and Garg, 1988; Axelrood, 1991).

1. The reduction of pathogen population through use of antagonistic microorganisms that destroy the pathogen inoculum and reduce the vigor or aggressiveness of the inoculum.
2. The protection of plant surface with microorganisms established in wounds, on leaves, or in the rhizosphere, where they serve as a biological barrier through their competitive, antibiotic, or parasitic action inhibitory to the pathogens.

3. The establishment of nonpathogenic microorganisms or agents within the plant or infected area to stimulate greater resistance of the plant to pathogen or to occupy the infected site and starve the pathogen or displace it in the lesion.

Although this strategy (biological control) has existed for a long time, a marked increase in research in this area has occurred recently.

Biological control of plant pathogens is now an established sub-discipline in the science of plant pathology. Although its beginnings can be traced back over 70 years, it was not until the early 1960s that theory and practice came together in the proceedings of one of the first biocontrol meetings (Baker and Snyder, 1965). Over the past 20 years, interest in biological control has increased fuelled by public concerns over the use of chemicals in the environment in general, and the need to find alternatives to the use of chemicals for disease control (Whipps, 2001). Presently, there are over 80 products for biocontrol of pathogens worldwide (Whipps and Davies, 2000), a significant improvement over the past ten years. Some of the microbial taxa that have been successfully commercialized and are currently marketed as Environmental Protection Agency (EPA)-registered biopesticides in the United States include bacteria belonging to the genera *Agrobacterium, Bacillus*, *Pseudomonas*, and *Streptomyces* and fungi belonging to the genera *Ampelomyces, Candida, Coniothyrium,* and *Trichoderma*.

Among the species and isolates that have been examined as biocontrol agents, *Trichoderma* species clearly dominate the market, perhaps reflecting their ease of growth and wide host range (Whipps and Lumsden, 2001). Of the biological control agents patented by early 1999, 84% were bacteria and 16% were fungi (FIS, 2000). At the end of 2001, there were approximately 195 registered biopesticide active ingredients and 780

products (USEPA, 2002). In biological control, the most common pathogen targets by are *Pythium* species, *Fusarium* species and *Rhizoctonia solani* reflecting their worldwide importance and perhaps their relative ease of control under protected cropping systems, although numerous other pathogens have been examine (Whipps, 2001).

Successful application of biological controls requires more knowledge-intensive management (BCWG, 1998). To this end, extension personnel cropadvising and growers must also become more fully aware of the costs and benefits that biopesticides/biofungicices can provide. They must understand when and where biological control of plant pathogens can be profitable within integrated pest management systems (Jacobsen, 1997).

Biological control offers an environmentally friendly approach to the management of plant disease and can be incorporated into cultural and physical controls and limited chemical usage for an effective integrated pest management (IPM) system. Biological control can be a major component in the development of more sustainable agriculture systems.

Some Advantages of Biological Control

Biological control is considered sustainable and safe. They are relatively free from problems of pest resistance and are extremely safe to human health. Biopesticides often are effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides (USEPA, 2002). In general, cultural practices are less likely to be in conflict with natural enemies than pesticides. In contrast to chemical control, biological control lessens long-term damage to the environment by persistent chemicals.

It may be a more economical alternative to some insecticide and fungicide applications. Unlike most fungicides, biological control is often very specific for a particular pathogen, thus other beneficial microorganisms or people can go completely unaffected or undisturbed by their use. There is less impact on the environment and water quality (Orr et al., 1997). Fungicides are typically used as protectants against foliar diseases. Pathogens in the soil are very difficult to control with fungicides, because the active ingredient must be delivered through the soil to contact the inoculum and because of losses from the soil by leaching and microbial breakdown. Biological control is cost-effective for specialized applications and where no chemical controls exist. When used as a component of Integrated Pest Management programs, biopesticides can greatly decrease the use of conventional pesticides, while crop yields remain high (Whipps and Lumsden, 2001; USEPA, 2002). Biological control would seem to be ideally suited for controlling root diseases, especially for soil-less systems in closed structures (Paulitz, 1997).

Some Disadvantages of Biological Control

Biological control also has some drawbacks. Biological control takes more intensive management and planning. It can take more time; require more record keeping, more patience, and sometimes more education and training. Successful use of biological control requires a through understanding of the biology of both the pathogen and antagonist (Orr et al., 1997). Biological control agents act slowly. Growers are accustomed to using conventional chemicals, where they may see rapid kill of target organisms especially insect and weeds within hours of application. Many pesticides for control of plant disease have a broad spectrum of activity, are applicable in a variety of

crops and settings, may act either prophylactically, therapeutically or both. Biological controls, in contrast, often have narrow ranges of activity and may work in only a few crops or soil types. Though often an advantage, this can be a disadvantage (Andrews, 1992).

Biological control on aerial plant surfaces is much less well developed than in the soil rhizosphere environment (Mukerji and Garg, 1998). It is possible that a pathogenic fungus may have an inhibitory or stimulatory effect on a biological control agent. For example, Burton and Coley-Smith (1985) demonstrated that extracts from sclerotia of several *Rhizoctonia* species inhibited growth, in culture, of various soil bacteria and fungi, including isolates of *Gliocladium virens* and *Trichoderma viride*. Thus, ecological studies should consider the potential influence of pathogen activities on soil antagonists as well as opposite effects. Having been developed and tested in controlled environments in the laboratory or greenhouse, most biological control agents do not perform well when subjected to the uncontrolled environment of the field (Strashnow et al., 1985; Lewis et al., 1990; Tronsmo, 1996). Biological control is inconsistent and often provides low levels of control. It may lack persistence to give long-tem control. It is expensive and more difficult to use than chemicals. Biological control is not practical for large-acreage agronomic crops and may not be compatible with accepted practices. It is subject to environmental influences (Whipps and Lumsden, 2001).

Main Approaches to Biological control

There are three ways to practice biological control, which are importing, conserving and augmenting and natural enemies. The first, known as a classical biological control, involves introducing exotic natural enemies to control pests. The

second, conservation, means modifying any environmental factors that adverse to biological control. The third, called augmentation focuses on improving the effectiveness of natural enemies (Johnson and Wilson, 1995). Organisms for biological control of plant disease can be used in various ways, but most attention has been given to their conservation and augmentation rather than the importation and addition of new species as is often done for insect and weed control (Bellows, 1999).

Conservation is applicable in situations where microorganisms important in limiting-disease causing organisms already occur, primarily in the soil and plant residues but in some cases also on leaf surfaces. They may be conserved by avoiding practices that negatively affect them (such as soil treatments with fungicides). Conserving of existing flora may be important in limiting the extent of a number of leaf diseases (Campbell, 1989). The elimination of the saprotrophic flora by the fungicide removes their natural suppressing influence on the disease organisms, resulting in greater potential disease incidence. Careful use of selective fungicides will be crucial to conserving the important antagonistic flora and permitting their beneficial action (Bellows, 1999).

Biological control of plant pathogens through augmentation is based on mass culturing antagonistic species and adding them to cropping system. This is considered augmentation because the organisms used are usually present in the system, but lower numbers or in locations different from those desired. However, many of these organisms occur naturally in the environment and the additional benefit contributed by releases may be marginal (Mahr and Ridgway, 1993). Biological control by adding large amounts of *Trichoderma harzianum* with its food base to soil is exemplified by the work of Wells et al. (1972). These researchers were among the first the report the larges-scale use of

Trichoderma preparations on solid media (ground annual ryegrass seed) for field control of *Sclerotium rolfsii* on tomato transplants. Another method of augmentation is breeding better biological control agents, which can attack or find their target more effectively. The augmentation method relies upon continual human management and does not provide a permanent solution unlike the importation or conservation approaches may (Landis and Orr, 1995).

Mechanisms of Action of Biological Control Agents

The development of successful strategies for the use of biological control agents to protect plants from pathogens require a detailed knowledge of the mechanisms employed by the biological control agents. Understanding the mechanism of biological control of plant diseases through the interactions between biological control agent and pathogen may allow us to manipulate the soil environment to create conditions conducive for successful biological control or to improve biological control strategies (Fravel, 1988; Handelsman and Parke, 1989).

The mechanism of biological control have been discussed and reviewed in several papers and books (Cook and baker, 1983; Adams, 1990; Lam and Gaffney, 1993; Tronsmo, 1996; Howell, 2003). Several mechanisms, operating alone or in concert, are known to be involved in antagonistic interactions. The mode of action of biologically active organism is complex and extremely varied; bacteria and fungi can suppress pathogens by competition, antibiosis, mycoparasitism, and induced resistance are the major mechanisms (Cook and Baker, 1983; Campbell, 1989; Handelsman, and Stabb, 1996). Each of these modes of actions has advantages and disadvantages that affect performance. Each mode of action will be discussed in detail later. The key disadvantage

is that any single mode of action gives activity against a very narrow spectrum of pathogens (Mukhopadhyay, 1994). Harman and Nelson (1994) compared the mechanism of biological action of three bacteria (strains of *Enterobacter, Pseudomonasand, and Serratia*) and two fungi (from the genera *Trichoderma* and *Gliocladium).* They concluded, from the scientific information available, that the biological control effect of the bacteria resulted from (i) the production of antibiotics and toxicant; (ii) the production of cell wall degrading enzymes; (iii) competition through inactivation of molecules in seed exudates necessary for germination of fungi and fungal propagules and, in a few examples, from siderophore competition for iron; and (iv) adherence of bacterial cells to hyphae affecting their viability. Mechanism of action of the fungi included: (i) the production of antibiotics and toxicants; and (ii) mycoparasitism and the production of cell wall degrading enzymes.

None of the mechanisms are necessarily mutually exclusive and frequently several modes of action are exhibited by a single biological control agent. For some biological control agents, different mechanisms or combinations of mechanisms may be involved in the suppression of different plant diseases.

Competition

Competition is a process that is considered to be an indirect interaction whereby pathogens are excluded by depletion of a food base or by physical occupation of a site; however, it is difficult to study (Tronsmo, 1996). Competition occurs when two or more organisms require the same nutrient and use by one reduces the amount available to the other (Baker and Cook 1974; Campbell 1989). Microbes compete in the soil and on plant surfaces for a limited number of resources including carbon compounds, nitrogen, soluble

iron and space (Scher et al., 1984). According to Baker (1968), carbon, iron, nitrogen and vitamins are all-important in this respect because they determine the growth and infection of soilborne plant pathogen in competition with other organisms. Plant roots growing in soils are a major source of carbon and energy to microorganisms in the form of root exudates, cells detached from old parts of the root, or the root itself after plant death (Cook and Baker, 1983). Pathogenic and nonpathogenic microorganisms typically occupy the same environmental niches (Axelrood, 1991). Whichever becomes established first usually is able to resist colonization by other organisms. For example, if a biological control agent is first to colonize the rhizosphere, pathogens may be excluded if all colonization niches are occupied. From the standpoint of biological control, the goal is to manipulate the growing environment so nonpathogens are favored over pathogens in competition for limiting factors (Campbell, 1989). Manipulation of soil water content by draining or irrigation may affect the balance between competing microorganisms. Liming affects plant growth directly, but changes in soil pH may also influence the competition between microbes. Changing the soil environment in favor of antagonists to plant pathogens can be considered biological control (Sundheim, 2002).

In 1926, Sanford was one of the first to recognize competition between saprophytic and pathogenic organisms for nutrients at the site of initial infection as a form of biological control of disease. He observed that potato scab caused by S*treptomyces scabies* could be reduced by adding organic matter (grass clippings) to soil (Van Driesche and Bellows, 1996). Competition is believed to be one of the mechanisms by which fungal biocontrol agents affect plant pathogens (Sivan and Chet, 1989). Soil treatments with *T. harzianum* spores suppressed infestations of *Fusarium oxysporum* f.

sp. *vasinfectum* and *F. oxysporum* f. sp. *melonis* mainly by competitive interaction for nutrients (Sivan and Chet 1989). Sutton and Peng (1993) evaluated *Gliocladium roseum* and determined that suppression of *Botrytis cinerea* by this antagonist was probably a result of competition. Alabouvette (1990) compared root colonization, chlamydospore germination, and population dynamics of *Fusarium* in conducive and suppressive soils, and concluded that disease suppression was due to nutrient competition between pathogenic and nonpathogenic *Fusarium* spp. in the rhizosphere. Schneider (1984), on the other hand, proposed that the nonpathogenic *Fusarium* spp. compete with the pathogen for infection sites at the root surface. He isolated nonpathogenic strains of *F. oxysporum* from suppressive soils in California, and demonstrated that their addition to soil infested with *F. oxysporum* f. sp. *apii* limited the severity of Fusarium wilt of celery.

Competition for iron may play a role in the biocontrol interactions. Systems such as siderophores, involved in the acquisition of iron under iron-limited conditions, may play a role in microbial interactions (Kloepper et al., 1980). Under iron-limiting conditions, bacteria produce a range of iron chelating compounds or siderophores which have a very high affinity for ferric iron. These bacterial iron chelators are thought to sequester the limited supply of iron available in the rhizosphere making it unavailable to pathogenic fungi, thereby restricting their growth (O'Sullivan and O'Gara, 1992; Loper and Henkels, 1999). Iron competition in pseudomonads has been intensively studied and the role of the siderophore produced by many *Pseudomonas* species has been clearly demonstrated in the control of *Pythium* and *Fusarium* species. Studies on suppression of Fusarium wilt of carnation and radish, caused by *Fusarium oxysporum* f.sp. *dianthi* and *F. oxysporum* f.sp. *raphani*, respectively, established competition for iron as the

mechanism of disease reduction by *Pseudomonades putida* strain WCS358 (Bakker et al., 1993; Duijff et al., 1994). Lemanceau et al. (1992, 1993) described increased suppression of fusarium wilt of carnation by combining *P. putida* WCS358 with non-pathogenic *Fusarium oxysporum* Fo47. The enhanced disease suppression by this combination is due to siderophore-mediated competition for iron by WCS358, which makes the pathogenic *F. oxysporum* strain more sensitive to competition for glucose by the non-pathogenic strain Fo47. Biological control agents that use competition to suppress disease generally need to be applied in high densities before the pathogen is present.

Antibiosis

Antibiosis is defined as inhibition of the growth of one microorganism by another as a result of diffusion of an antibiotic metabolite (Parke, 1996). Several biological control agents produce highly antimicrobial metabolites. Biological control agents that use antagonism may not necessarily have to be present in high numbers, but need to exert their antibiotic effect before infection occurs. Once the pathogen has gained entry into the root, the antibiotic may have no effect. Antibiotics are produced by a wide variety of microorganisms, particularly those in the soil (Alexander 1971; Griffin, 1972). In many biological control systems that have been studied, one or more antibiotics have been shown to play a role in disease suppression. Antibiotic-producing microorganisms often were considered first in searching for biological control agents since the selection of microorganisms with potential to produce antibiotics can be realized with little effort by screening on agar or liquid culture (Kohl and Fokkema, 1998).

Antibiosis may be involved and may play an important role in plant disease suppression by certain bacteria and fungi. The production of antibiotics has been

demonstrated to be a widespread mechanism exerted by microorganisms to control a wide variety of phytopathogens. Among the bacteria, antibiotic agrosin84 produced by *Agrobacterium radiobacter* strain K84 is one of the best described examples of biological control used to control crown gall caused by virulent *A. tumefaciens* strains (Mukerji and Garg, 1988; Agrios, 1997). *Bacillus subtilis* strains show broad suppression of various plant pathogens by producing an antibiotic iturin A (Leyns et al., 1990; Bochow, 1991).

Treatment with *Bacillus cereus* UW85 cultures fully suppressed *Pythium torulosum*.-induced damping-off on tobacco seedlings and produced antibiotics (zwittermicin A or kanosamine) that inhibited development of oomycetes in culture (Shang, et al., 1999). *Streptomyces hyroscopicus* var. *geldanus* produces the antibiotic geldanamycin, which has been directly purified from soil and demonstrated to effectively suppress root rot of pea caused by *Rhizoctonia solani* in the field (Rotrock and Gottlieb, 1984). *Streptomyces* sp. Di-944, a rhizobacterium from tomato, suppressed Rhizoctonia damping-off and Fusarium root rot in plug transplants when applied to seeds or added to potting medium. Antibiosis was suspected as a key mechanism of biological control. The mean incidence of damping-off in tomato seedlings exposed to the formulated *Streptomyces* biological control agents was significantly lower than the mean incidence of damping-off on tomato plug transplants grown in the presence of the pathogen alone. Seed-coating with the powder formulation of *Streptomyces* Di-944 significantly reduced the incidence of damping-off in 10-day-old tomato transplants by almost 90% under growth chamber conditions (Sabaratnam and Traquair, 2002).

A number of highly effective disease-suppressive agents are found among the fluorescent pseudomonads, making this group of bacteria the most widely studied group

of antibiotic producers in the rhizosphere (Handelsman, and Stabb, 1996). Among fluorescent *Pseudomonas*, the production of 2,4-diacetylphloroglucinol (DAPG), Plt (pyoluteorin), Prn (pyrrolnitrin) and different derivatives of phenazine has been described (Thomashow and Weller, 1996). The first antibiotics clearly implicated in biological control by fluorescent pseudomonads were the phenazine derivatives that contribute to disease suppression by *Pseudomonas fluorescens* strain 2-79 and *P. aureofaciens* strain 30-84, which control take-all of wheat (Weller and Cook, 1983; Brisbane and Rovira, 1988). De la Fuente *et al.* (2000) reported that the native strain UP148 of *Pseudomonas fluorescens*, isolated from legume rhizosphere, is able to produce antibiotics against *Pythium ultimum in vitro* studies.

The role of antibiotic production by antagonistic fungi has been less studied than with bacteria. One reason may be that these substances have merely been identified, and their roles have not yet been elucidated clearly by a molecular approach (Lo, 1996). However, the role of antibiosis in biological control of fungi has been considered. Antibiotic production by fungi exhibiting biological control activity has most commonly been reported for isolates of *Trichoderma/Gliocladium* (Howell, 1998). *Gliocladium virens*, which controls Rhizoctonia fruit rot and southern blight of tomato, produces gliovirin which plays a role in biological control (Lewis et al., 1990; Ristaino et al., 1991). Peptaibols are produced by *Trichoderma harzianum,* which controls various soilborne plant pathogenic fungi (Schirmbock et al., 1994). Gliotoxin production by *Trichoderma* is also thought to be responsible for cytoplasmic leakage from *Rhizoctoni solani* observed directly on membranes in potting mix (Harris and Lumsden, 1997).

In general, antibiotics cause a reduction or cessation of growth or sporulation of pathogens or reduce spore germination (Jackson, 1970). Unfortunately, some potential pathogens are less affected by antibiotics than others. For example, *Fusarium spp.* are little affected by many antibiotics produced in the soil, compared to *Pythium spp.*, which are usually quite sensitive to antibiotics produced by a wide array of fungi and bacteria (Campbell, 1989). Many biological control agents are specifically selected for their ability to produce antibiotics when introduced into a cropping system (Baker, 1991). Their efficacy against certain target pathogens depends on pathogen responses to their antibiotics as well as soil factors that may influence amounts of antibiotics produced. In some cases, introduced antagonists may themselves be antagonized and made ineffective by the production of antibiotics from other microorganisms, including pathogens (Barnett, 1964; Campbell, 1989; Adams, 1990).

Mycoparasitism

Mycoparasitism is a process by which biocontrol fungi may attack pathogenic fungi and extract nutrients from the pathogen (Chet, 1987; Harman and Nelson, 1994). This mode of action is probably less frequent than other mechanisms of suppressing disease. For this mechanism to be effective in controlling disease, the biological control agents must be present in the rhizosphere at the same time or before the pathogen appears. The biological control agent parasitizes mycelia, propagules (conidia, oospores chlamydospores), or overwintering structures (sclerotia, oospores, chlamydospores) of other fungi. Mycoparasitism occurs on a wide range of fungi and some of them have been proposed to play an important role in disease control (Adams, 1990; Maloy, 1993). Mycoparasitism depends on close contact between antagonist and host, on the secretion

of enzymes, and on the active growth of the hyperparasite into host (Kohl and Fokkema, 1998). The process involved in mycoparasitism may consist of sensing the host, followed by directed growth, contact, recognition, attachment, penetration, and exit. Although not all these features occur in every fungal-fungal interaction, the key factor is nutrient transfer from host to mycoparasite (Whipps, 2001). There are two kinds of mycoparasites: biotrophic mycoparasites that have a persistant contact with or occupation of living cells, whereas necrotrophic mycoparasites kill host cells, often in advance of contact and penetration (Dennis and Webster, 1971a, 1971b).

The most common example of mycoparasitism is that of Trichoderma spp. which attack a great variety of phytopathogenic fungi responsible for the most important diseases suffered by crops of major economic importance worldwide (Papavizas, 1985). These fungi either penetrate resting structures such as sclerotia and chlamydoapores or parasitze growing hyphae of pathogens (Papavizas 1985). The parasite extends hyphal branches toward the target host, coils around and attaches to it with appressorium-like bodies, and punctures its mycelium (Chet et al., 1981; Goldman et al., 1994). Digestion of host cell walls is accomplished by a battery of excreted enzymes, including proteases, chitinases, and glucanases (Di Pietro et al., 1992, 1993; Lorito et al., 1993). This whole process can be described as a four-step process (Chet, 1987).

1. Chemotropic growth: The first stage of mycoparasitism is chemotropic growth. The biocontrol fungus starts to branch in an atypical way and these branches grow towards the pathogenic fungus that produces chemical stimuli. This stimulus directs the growth of the parasite (Tronsmo, 1996). This was detected in *Trichoderma* as early as 1981 (Chet et al, 1981).

2. Recognition: There is a specific interaction between biological control agents and pathogens. Antagonists are rather specific and attack only a few pathogens. Lectins of pathogens and carbohydrates receptors on the surface of biocontrol fungus may be involved in this specific interaction (Barak et al., 1985; Inbar and Chet, 1992). Dennis and Webster (1971b) observed that *Trichoderma spp.* did not show coiling around plastic threads, but they did coil around certain fungi. This indicates that a specific recognition is necessary before a mycoparasitic reaction can take place.
3. Attachment: Mycoparasites can usually either coil around host hyphae or grow alongside it (Chet, 1987).
4. Degradation of the pathogen wall: Biological control fungi produce cell wall-degrading enzymes to attack the target fungus (Cherif and Benhamou, 1990). Several mycoparasites have been shown to secrete host-wall-degrading enzymes such as β-1,3-glucanes, chitinase and cellulase in liquid culture, the synthesis being induced by the presence of host fungi or host cell wall components (Elad et al., 1982; Lorito et al., 1993).

Here are some biological control examples by mycoparasitism: The fungus, *Trichoderma virens (Gliocladium virens),* is reported to be a mycoparasite of the cotton seedling pathogen, *Rhizoctonia solani* Kühn (Weindling, 1932). Application of *Trichoderma lignorum* as a wheat-bran preparation, conidial suspension, or seed coating greatly decreased the number of infested seeds by *Rhizoctonia solani* as well as damping-off percentages and hence controlling the fungal disease (Aziz et al., 1997). Scanning electron microscopy and fluorescence microscopy showed that both *T. harzianum* and *T. hamatum* were mycoparasites of both *Sclerotium rolfsii* and *Rhizoctonia solani*. The

antagonist attached to the pathogen and secreted glucanase and chitinase enzymes that eroded the cell wall (Elad et al., 1983). The fungus *Coniothyrium minitans* is parasitic on sclerotia and has been developed into a commercial product used for control of the white mould fungus *Sclerotinia sclerotiorum,* a common pathogen on a large number of vegetables and ornamentals. There are also examples of one species being parasitic on other species within the same genus. Strains of *Fusarium oxysporum* are used in control of diseases caused by *Fusarium* spp. (Sundheim, 2002).

Induced resistance

Induced resistance is a form of disease control that uses the natural defense responses of the plant, which may include production of phytoalexins, additional lignification of cells, hypersensitive reaction, and pathogen related proteins and hydoxyproline-rich glycoproteins to defend the plant against pathogenic infections (Horsfall and Crowling, 1980; Kuc and Strobel, 1992; Van Drieshe and Bellows, 1996). This resistance is caused by reducing, restricting, or blocking the pathogen's ability to produce disease in the host plant. Induced resistance can be triggered by certain chemicals, nonpathogens, avirulent forms of pathogens, incompatible races of pathogens, or by virulent pathogens under circumstances where infection is stalled owing to environmental conditions. Generally, induced resistance is systemic, because the defensive capacity is increased not only in the primary infected plant parts, but also in non-infected, spatially separated tissues (Ryals et al., 1996; Sticher et al., 1997). In this regard, induced resistance differs from cross-protection. In cross-protection, following inoculation with avirulent strains of pathogens or other microorganisms, both inducing microorganisms and challenge pathogens occur on or within the protected tissue.

Induced resistance has been demonstrated in many plant species and various presentations extended the range investigated by showing induction by fungi, bacteria, microbial elicitors and chemicals. Although induced resistance has been attracting attention recently (Hammerschmidt and Kuc, 1995; Ryals et al., 1996), the first systematic enquiry into induced resistance was made by Ross (1961a, 1961b). He observed that the inducible resistance response to tobacco mosaic virus (TMV) in *N* gene-containing, hypersensitively reacting tobacco was not confined to the immediate vicinity of the resulting local necrotic lesions, but extended to other plant parts. Enhanced resistance induced by nonpathogenic *Fusarium* spp. also was demonstrated in protection against pathogenic *Fusarium* spp. Induced resistance is accomplished by the inoculation of a plant with an inducer agent (nonpathogenic isolate) prior to, or concomitant with, a second (challenge) inoculation with a pathogen.

Working with Fusarium wilts of tomato, cabbage, flax, carnation and watermelon, Davis (1967) observed that different formae speciales of *F. oxysporum* were more effective in inducing resistance to a given host's pathogenic formae specialis than were other root pathogens *(Verticillium alboatrum* and *Rhizoctonia solani)* or nonpathogens *(Penicillium notatum* and *Neurospora crassa).* Komada (1990) demonstrated that sweet potato plants were protected against Fusarium wilt, caused by *F. oxysporum* f. sp. *batatas*, in naturally infested soil by prior inoculation with nonpathogenic *F. oxysporum* isolates, which were often found in the vessels of healthy sweet potato plants and natural soils. Biles and Martyn (1989) and Martyn et al. (1991) found that nonpathogenic races of *F. oxysporum* f.sp. *niveum* (races 0 and 1) were better inducers of resistance on watermelon than the related forma specialis *F. oxysporum* f. sp. *cucumerinum.* Gessler

and Kuc (1982) reported that several formae speciales of *F. oxysporum* induced resistance in cucumber to *F. oxysporum* f. sp. *cucumerinum* in flask culture, and that a 3-day interval between induction and challenge was necessary for adequate protection. Kroon et al. (1991) used the experimental design of split root system to observe the induced resistance in tomato plants against *F. oxysporum* f. sp. *lycopersici.* Because *F. oxysporum* f. sp. *dianthi* reduced disease symptoms caused by *F. oxysporum* f. sp. *lycopersici* without any direct interactions with this pathogen, it was concluded that *F. oxysporum* f. sp. *dianthi* was able to induce resistance against *F. oxysporum* f. sp. *lycopersici* in tomato plants. Simultaneous inoculation of tomato with microconidia of avirulent *F. oxysporum* f.sp. lycopersici race 1 and *F. oxysporum* f.sp *melonis* provided significant protection against the virulent race (Huertas-Gonzalez et al., 1999).

Fuchs et al. (1997 and 1999) demonstrated that strain Fo47 of nonpathogenic *F. oxysporum* protected tomato plants against fusarium wilt disease, inducing resistance in tomato by increasing chitinase, β-1,3-glucanase and β-1,4-gluosidase activity in plants. Benhamou et al. (2002) reported that one of the mechanisms in biocontrol activity of non pathogenic F. oxysporum strain Fo47 in controlling *P. ultimim* was induced resistance. Positive correlations between Fo47 treatment and induced resistance to infection by *P. ultimum* in cucumber were confirmed by (i) the reduction of pathogen viability; (ii) the elaboration of newly formed barriers, a phenomenon which was not seen in Fo47-free plants, where the pathogen proliferated in all root tissues within a few days; and (iii) the occlusion of intercellular spaces with a dense material likely enriched in phenolics. Larkin and Farvel (1998) identified several isolates of nonpathogenic *Fusarium* spp. *(F. oxysporum* and and *F. solani*) that effectively controlled Fusarium wilt of tomato,

watermelon, and muskmelon in greenhouse tests. The mechanism of action for selected isolates was shown to involve induced resistance.

A low-molecular weight protein, termed oligandrin, was purified to homogeneity from the culture filtrate of the mycoparasitic fungus *Pythium oligandrum*. When applied to decapitated plants, this protein displayed the ability to induce plant defense reactions that contributed to restrict stem cell invasion by the pathogenic fungus *Phytophthora parasitica* (Picard et al., 2000).

Induced resistance by bacteria has also been demonstrated. Nejad and Johnson (2000) reported that endophytic bacteria isolates, originated from wild and cultivated oilseed rape, not only significantly improved seed germination, seedling length, and plant growth of oilseed rape and tomato, they significantly reduced disease symptoms caused by the vascular wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici*. Salicylic acid produced by *Pseudomonas aeruginosa* induced resistance to *Botrytis cinerea* in bean (De Meyer and Hofte, 1997). Audenaert et al. (2000) reported that *P. aeruginosa* 7NSK2 induced resistance in tomato to the necrotrophic pathogen *B. cinerea.* Isolates of *Pseudomonas* spp. have been demonstrated to induce resistance to root rot of cucumbers caused by *Pythium aphanideramtu*m. In these experiments, the roots of cucumbers were split into two containers; the pathogen was applied to one side and the bacteria to the other. Reductions in disease were observed even though the pathogen and inducing bacterium was spatially separated (Paulitz et al., 2000).

Plant growth-promoting rhizobacteria (PGPR) strains INR7 *(Bacillus pumilus),* GB03 *(Bacillus subtilis),* and ME1 *(Curtobacterium flaccumfaciens)* were tested singly and in combinations for biological control to protect cucumber against anthracnose,

angular leaf spot, and cucurbit wilt diseases. The efficacy of induced systemic resistance activity was determined against introduced cucumber pathogens (Raupach and Kloepper, 1998). The biocontrol bacterium, *Bacillus thruringiensis*, has also been reported to induce resistance to leaf rust on coffee plants (Roveratti et al., 1989).

It appears that all of the mechanisms of antagonism that have been discussed; competition, antibiosis, mycoparasitism and induced resistance, play important roles in the biological control of plant pathogens. More knowledge is needed to understand the complex modes of action of the antagonistic strains and to apply them in the best conditions to achieve optimal biological control of plant diseases. Mechanisms of specific biocontrol agents for controlling tomato diseases summarized in **Table 2.1**.

Table 2.1. Biocontrol agents that have been studied for controlling tomato diseases

Biocontrol agent	Isolate / Strain	Disease/Pathogen	Mechanism	Application	References
Agrobacterium radiobacter	K84	Crown gall (*A. Tumefaciens*)	antibiosis	inoculation on stems	Kerr and Htay, 1974; Cooksey and Moore, 1980
Aspergillus awamori		*Fusarium oxysoprum* f. sp. *Lycopersici (FOL)*		Inoculation to roots	Khan and Khan, 2002
Aspergillus niger		Fusarium wilt		Inoculation to roots	Khan and Khan, 2002
Aspergillus ochracus		Fusarium crown and root rot (FCRR)	mycoparasitism	Inoculation to soil	Marois et al. 1981
Aurebasidium pullulans	Y47,Y48	Early blight (*Alternaria solani*)	mycoparasitism	Inoculation to leave	Brame and Flood, 1983
Bacillus pumilus	SE 34	FCRR	Induced Resistance	Inoculation to seeds	Benhamou et al., 1998
Bacillus subtilis	FZB C, FZB G, FZB 13, and FZB 44	*Phytophthora nicotianae,* FCRR	Antibiosis		Dolej and Bochow, 1996; Krebs et al.1998; Grosch et al., 1999
Bacillus subtilis	RB14	Damping-off (*Rhizoctonia solani, Pythium spp., Phytophthora spp.*)	antibiosis, competition	Inoculation to seed and soil	Asaka et al., 1996; Weller, 1988; Yehia, et al., 1981
Bacillus subtilis	Quantum 4000 HB®, Kodiak®	FCRR	antibiosis, competition	Inoculation to growing medium	Newhook, 1957

Biocontrol agent	Isolate / Strain	Disease/Pathogen	Mechanism	Application	References
Bacillus subtilis	T99	Corky root rot disease (*Pyrenochaeta lycopersici)*	antibiosis, competition	Inoculation to soil	Bochow, 1991
Bacillus subtilis		Fusarium wilt		Inoculation to roots	Khan and Khan, 2002
Beauveria bassiana		Rhizoctonia damping-off	Mycoparasitism	alginate prills (mycelia)	Seth et al., 2001
Burkholderia cepacia	Bc-F	*Rhizoctonia Solani, Sclerotium rolfsii, Pythium ultimum, Phytophthora capsici, Fusarium oxysoprum sp.*		Seed coating Root drenching	Mao et al., 1998
Chitosan		FCRR	Induced Resistance	Inoculation to growing media	Benhamou et al., 1998
Cladosporium cladosporioides	658b, 677, 712b	Grey mold (*Botrytis cinerea*)	competition	Foliar spray	Newhook, 1957; Eden et al.,1996
Cyanobacteria *(Seaweed extracts)*		Damping-off	antibiosis, competition	Inoculum to seed and leaves	Kulik, 1995
Endophytic Bacteria	PA, PF	Fusarium wilt	Antibiosis	Soil Drench / seed inoculation	Nejad and Johnson, 2000
Flavobacterium spp.	8506 CMI	Bacterial canker (*C. Michiganense pv.* Michi.)	antibiosis, acid production	Inoculation to leaves	Tsiantos, and Stevens, 1987

Biocontrol agent	Isolate / Strain	Disease/Pathogen	Mechanism	Application	References
Fusarium oxysporium f. sp. dianthi		Fusarium wilt	Induced resistance	Inoculation to roots and soil	Kroon, et al., 1991; Van Driesche and , Bellows, 1996; Wymore, 1978
Gliocladium catenulatum		Grey mold (*Botrytis cinerea*)	competition, mycoparasitism	Inoculation to whole plant	Elad et al., 1994
Gliocladium virens	SoilGard ®	FCRR, Stem canker caused by *B. cinerea*	Competition	Inoculation to soil, Foliar spray	Utkhede et al., 2001; Datnoff and Pernezny, 1998
Gliocladium virens	GI-21	Rhizoctonia fruit rot, *Rhizoctonia solani*), Southern blight (*Sclerotium rolfsii*)	competition, mycoparasitism	Inoculation to soil	Lewis et al., 1990; Ristaino, et al., 1991
Gliocladium virens	GI-3	*Rhizoctonia Solani, Sclerotium rolfsii, Pythium ultimum, Phytophthora capsici,* Fusarium wilt		Seed coating, Root drenching	Mao et al., 1998
Glomus intraradices	UT133	FCRR	competition, mycoparasitism	Inoculation to growing medium	Datnoff et al., 1995; Nemec et al., 1996
Glomus intraradices		FCRR		Inoculation to soil	Caron et al., 1985, 1986
H-S Mixture		Bacterial wilt (*Pseudomonas solanacearum)*	Reduction of population	Inoculation to soil	Lin et al., 1990

Biocontrol agent	Isolate / Strain	Disease/Pathogen	Mechanism	Application	References
Non-pathogenic *Fusarium oxysporum*	Fo47	Fusarium wilt, FCRR	Competition, Induced resistance	Inoculation to roots	Alabouvette and Couteadier,1992; Fuchs and Defago, 1991; Fuchs et al., 1997
Non-pathogenic *Fusarium oxysporum sp.*		Fusarium wilt	induced resistance	Immersing roots, Inoculation to soi	Tamietti and Matta, 1991
Non-pathogenic *Fusarium oxysporum*	26B, 43A, 43AN1-2	FCRR	Competition, Induced resistance	Inoculation to growing medium	Hartman, and Fletcher, 1991
Non-pathogenic *Fusarium oxysoprum* f. sp. *lycopersici*	218	Fusarium wilt	Induced Resistance	Inoculation to roots	Huertas-González et al., 1999
Non-pathogenic *Fusarium oxysoprum* f. sp. *lycopersici*	Fo47	Fusarium wilt	Induced Resistance	Inoculation to growing media	Fuchs et al. 1999; Cotxarrera et al., 2002
Non-pathogenic Fusarium oxysporum sp.	Fo47, CS-20	Fusarium Wilt	Induced Resistance	drench	Larkin and Fravel, 1998
Nonpathogenic *P. solanacearum*	U-10A	Fusarium wilt	mycoparasitism	Inoculation to roots	Toyoda et al., 1988
Nonpathogenic *Pseudomonas solanacearum*		Pseudomonas solanacearum	Competion Induced Resistance	Inoculation to growing media	Frey et al., 1994

Biocontrol agent	Isolate / Strain	Disease/Pathogen	Mechanism	Application	References
Oligandrin (*Pythium oligandrum)*		FCRR, *Phytophthora parasitica*	Induced Resistance	Inoculation to plants	Benhamou et al., 2001; Picard et al., 2000
Penicillium digitatum		Fusarium wilt		Inoculation to roots	Khan and Khan, 2002
Penicillium Funiculosum Thom		FCRR	mycoparasitism	Inoculation to soil	Marois et al., 1981; Marois and Mitchell, 1981
Penicillium oxalicum		Fusarium wilt	Competition, Induced resistance	Inoculation to soil	De Cal et al., 1997b
Penicillium purpurogenum	828	Fusarium wilt,	Mycoparasitism	Inoculation to growing medium	Larena and Melgaerjo, 1996
Pseudomonas aeruginosa	B8503 CMI	Bacterial canker	Antibiosis	Inoculation to leaves	Tsiantos and Stevens, 1987
Pseudomonas fluorescens	P5, RKO	Damping-off (*Pythium spp.*)	antibiosis, competition	Inoculation to seeds	Zhang et al., 1990
P. fluorescens	M29, M40	Bacterial wilt	antibiosis, competition	Inoculation to seedlings	Kim and Misaghi, 1996
P. fluorescens	208, 381	Bacterial speck	antibiosis	Spray to plant	Colin and Chafik, 1986
P. fluorescens	5.014, 5-2/4	*Pythium ultimum*	Competition	Inoculation to seed	Hultberg et al., 2000

Biocontrol agent	Isolate / Strain	Disease/Pathogen	Mechanism	Application	References
P. fluorescens		Fusarium wilt		Inoculation to roots	Khan and Khan, 2002
P. fluorescens	63-28	FCRR	Induced Resistance	Inoculation to seeds	M'Piga et al., 1997
P. fluorescens	CHA0, 679-2	FCRR, Fusarium wilt	Antibiosis, competition, induced resistance	Inoculation to growing medium	Duffy and Defago, 1997; Fuchs and Defago, 1991
Pseudomonas spp. Avirulent *P. solanacearum*	C7, CMI8172	Fusarium wilt, Bacterial wilt	antibiosis, competition	Inoculation to soil and root	Frey et al., 1994; Lemanceau and Alabouvette, 1991 and 1993
Rhodosporidium diobovatum	S33	Stem canker caused by *B. cinerea*	Antibiosis	Foliar spray	Utkhede et al., 2001
Streptomyces corchorusi		Fusarium wilt	Induced Resistance	Inoculation to soil	Abd E-Raheem et al., 1996
Streptomyces graminofaciens	N6	Corky root rot	antibiosis	Inoculation to soil	Bochow, 1989
Streptomyces griseoviridis	Mycostop™	Fusarium crown and root rot, Grey mold	antibiosis, competition	Inoculation to growing media, Seed coating	Nemec et al., 1996; Lamboy, 1997; Lahdenperä, 2000
Streptomyces mutabilis		Pseudomonas solanacearum	Induced Resistance	Inoculation to soil	Abd E-Raheem et al., 1996

Biocontrol agent	Isolate / Strain	Disease/Pathogen	Mechanism	Application	References
Streptomyces sp.	Di-944	damping-off, FCRR	Antibiosis	Seed coating	Sabaratnam and Traquair, 1996; Sabaratnam, et al., 2001; Sabaratnam and Traquair, 2002
Streptomyces spp.	K61, Mycostop	Damping-off (*Fusarium spp.*)	antibiosis, competition	Seed dressing or root dipping	Oili, 1992; Yehia et al., 1981
T.harzianum	T-35	FCRR	competition, mycoparasitism	Inoculation to growing medium, seed coating	Cherif and Benhamou, 1990; Chet, 1990; Sivan et al., 1987
T.harzianum	MTR-35D	FCRR	antibiosis, competition	Inoculation to growing medium	Bochow, 1989; Van Steekelenburg, 1991
T.harzianum Rifaii. Aggr.		FCRR, Rhizoctonia fruit rot, Southern blight (*Sclerotium rolfsii)*	competition, mycoparasitism	Inoculation to soil and fruit	Baker, 1970; Elad et al., 1982a; Marois et al., 1981; Strashnov et al., 1985
Trichoderma asperellum		Fusarium wilt		Inoculation to growing media	Cotxarrera et al., 2002
Trichoderma hamatum	Tm-23, TRI-4	Rhizoctonia fruit rot	competition, mycoparasitism	Inoculation to soil	Lewis et al., 1990
T.harzianum	RootShield ®	Stem canker (*B. cinerea*)	Competition	Foliar spray	Utkhede et al., 2001
T. harzianum	KRL-AG2, Th2, T-22	FCRR	competition, mycoparasitism	Inoculation to growing medium	Hartman and Fletcher, 1991; Datnoff et al., 1995; Nemec et al., 1996

Biocontrol agent	Isolate / Strain	Disease/Pathogen	Mechanism	Application	References
Trichoderma harzianum	RootShied™	FCRR		Inoculation to soil	Datnoff and Pernezny, 1998
Trichoderma koningii		*Rhizoctonia solani* (Kuhn) Damping-off	Mycoparasitism	Inoculated to soil	Marouli and Tzavella-Klonari, 2002
Trichoderma various spp.	T-39, 1295-22	Grey mold	competition, mycoparasitism	Seed coating, Inoculation to soil	Gullino, 1992; Elad et al., 1994 and 1995; Fokkema , 1995; Lamboy, 1997; Shtienberg and Elad, 1997
Verticillium chlamydosporium	IACR Vc 10	*Meloidogyne javanica*	Mycoparasitism	Soil drench	Verdejo-Lucas et al., 2002
α-tomatine (Tomatinase)		Fusarium wilt	Induced Resistance	Inoculation to plant	Lairini et al., 1997

The Genus *Fusarium* Link:Fr. with emphasis on *F. oxysporum* (Schlectend.Fr.)

Taxonomy and Historical Overview

Fusarium is a genus of hyphomycetes, formerly classified in the Deuteromycetes, and now widely considered an anamorphic genus affiliated with the Hypocreales (Ascomycetes) (Seifert, 1996). *F. oxysporum* is a widespread soilborne plant pathogen which causes diseases such as vascular wilt and crown and root rot (Booth, 1971; Jarvis, 1988). The species delimitation has been defined according to morphological and physiological characteristics; with strains of F. *oxysporum* classified into formae speciales on the basis of pathogenicity on a particular host plant and races based on differences in virulence to given host cultivars. There are over 120 described formae speciales and races (Armstrong and Armstrong, 1981; Correll, 1991). It has been observed that the host range of formae speciales sometimes overlap. However, various formae speciales seem to be limited to one host species; in the case of F. *oxysporum* f.sp. *lycopersici* the tomato plant is the unique host (Beckman, 1987). Telemorphs of the fungus are unknown; however, because of its close similarity to *E. moniliforme* (section Liseola) it may be related to the fungus *Gibberella* (Messiaen and Gassini, 1981). Both species can be distinguished mainly by formation of chlamydospores such as observed in *F. oxysporum* (Nelson et al., 1983). Most modern *Fusarium* taxonomy is based on cultural characters.

The genus *Fusarium* may represent one of the earliest fungi become established on the earth (Snyder, 1981). Atkinson (1892) first described *Fusarium oxysporum* on cotton; a gummy substance clogging the vascular system of cotton with the key diagnostic characteristics of microconidia and conidiogenous cells. However, the genus

was not named until 1940 by Snyder and Hansen. They revised the genus into nine species, which is now used by most researchers.

General Characteristics

The genus *Fusarium* contains many species that are well adapted to life on earth and can be found in soil, water, and air, on subterranean and aerial plant parts, as well as in plant debris and other organic substrates (Burgess, 1981; Snyder, 1981; Burgess et al., 1985; Marasas et al., 1985). Fusarium species are even found in extreme environments ranging from the desert to the arctic (Joffe and Palti, 1977; Nelson 1990). Fusarium consists of both soil-borne and airborne species. Some soil-bone species are even adapted to dispersal in the atmosphere (Burgess, 1981). Fusarium consists of four main groups: plant pathogens (including mycoparasites), insect pathogens, saprophytes, and soil inhabitants (Booth, 1971) A few species bridge the gap attacking both plant and insects (Price, 1984)

Fusarium spp. are higher fungi whose sexual stage is unknown (Agrios, 1997). Due to the great variabilty within this genus, it is one of the most difficult of all fungal groups to distinguish taxonomically (Alaexopoulos and Mims, 1996). Conidia can be divided into three groups: microconida, macroconidia, and chlamydospores. Microconidia are generally abundant, variable, oval-ellipsoid cylindrical, one celled, straight to curved, and measure 5 to 12 μm in length and 2.2 to 3.5 μm in width. Macroconidia are thin-walled, generally 3 to 5 septate, and pointed at both ends. Some species produce spores with rounder ends. A septate spore, in the range of 27 to 46 μ in length and 3 to 4.5 μm in width, is most commonly found. The shapes of these spores are used to differentiate between the different species (Nelson et al., 1981). Both

macroconida and microconidia are produced from phialides. Chlamydospores, both smooth- and rough-walled, are generally abundant, and form both terminally and intercalary on older mycelium, and generally solitary but occasionally form in pairs or chains (CMI, 1973; Agrios, 1997). Some *Fusarium* spp., including *F. oxysporum* and *F. solani*, produce chlamydospores. Chlamydospores are capable of surviving in soils, soil debris, or other substrates for more than 10 or 20 years (Kucharek et al., 2000). *Fusarium* spp. can also survive by colonizing roots or stems of so-called non-host plants. For example, roots of barley and nutsedge can serve as colonization sites for the Fusarium wilt pathogen of cotton. Brazilian pepper, cudweed, and carpet weed will continually support populations of the fungus that causes crown rot in tomato (Kucharek et al., 2000).

In solid media culture, such as potato dextrose agar (PDA), the different special forms of *F. oxysporum* can have varying appearances. In general, the aerial mycelium first appears white, and then may change to a variety of colors - ranging from violet to dark purple - according to the strain (or special form) of *F. oxysporum.* If sporodochia are abundant, the culture may appear cream or orange in color (Nelson et al., 1981; Smith et al., 1988).

Types of Plant Diseases Caused by Fusarium spp.

Members of the genus Fusarium are some of the most important plant pathogens in the world (Nelson et al., 1983). They attack a wide range of major food and fiber crops which include wheat (Burgess et al., 1975), grain sorghum (Burgess and Trimboli, 1986; Nelson et al., 1987), millet (Marasas et al., 1987), sweet corn (Lawrence et al., 1981), field corn, rice, banana, potato, cotton, tomato, cucurbits, etc (Nelson et al., 1981; Burgess et al., 1981; Agrios, 1997; Zitter, 1998; Kucharek et al., 2000).

Fusarium or vascular wilt is the major disease caused by this pathogen on several different hosts Fusarium wilt diseases, caused by the fungus *Fusrium oxysporu*m, lead to significant yield losses of horticultural and agricultural crops. The pathogen infects the roots and colonizes the vascular tissue, leading to wilting and finally death of the plant (Peterson and Pound, 1960; MacHardy and Beckman, 1981). *Fusarium* spp. also cause root rots, stem rots, crown rots and fruit rots, corm rots under field or greenhouse conditions; and pink or yellow molds of fruits during post-harvest storage. The post-harvest diseases occur on vegetables and ornamentals, and especially on root crops, tubers, and bulbs.

Disease Cycle

Since *Fusarium* spp. cause a variety of diseases in plants Fusarium wilts will be used as a model in explanation of disease cycle. *Fusarium spp.* that cause vascular wilts can be spread in soil, dust, and irrigation water (Smith et al., 1988). Rain, farm equipment, and decaying plant tissue can also help to spread the fungus. Wind-blown dispersal of spores of *Fusarium* spp. may occur. For example, air-borne spores (conidia) of the crown rot pathogen of tomato can re-contaminate nearby fumigated sites in the field or the greenhouse. However, for most diseases caused by *Fusarium* spp., soilborne chlamydospores are generally regarded as the primary source of inocula (Kucharek et al., 2000).

Fusarium is a soil inhabitant that overwinters between crops in infected plant debris as mycelium and in its three spore forms. It can remain in the soil for long periods of time, including fallow periods. Chlamydospores are capable of surviving in soils, soil debris, or other substrates for more than 10 or 20 years (Agrios, 1997; Kucharek et al.,

2000). Healthy plants can become infected through their root tips; either directly, through wounds, or at the point of formation of lateral roots (Agrios, 1997). The fungus grows as mycelium through the root cortex intercellularly, ultimately advancing to the vascular tissue. As the mycelium continues to grow - usually upward toward the stem and crown - it branches and produces microconida. The proliferation of fungal growth in the plant's vascular tissue eventually causes the plant to wilt and die. The fungus can continue to grow on the decaying tissue where it can sporulate profusely. At this point, the spores can be spread to other plants or areas by wind, water, or through the movement of soil (Agrios, 1997). On occasion, the fungus can reach the fruit and contaminate the seed. This occurs when the soil moisture is high and the temperature is relatively low (Agrios, 1997).

In addition the vascular wilting, the fungus can infect other parts of the plant close to the soil to induce root, stem, and corm rots. When seedlings are infected with Fusarium, damping-off may occur. If harvested fruits are contaminated with the fungus, postharvest diseases such as "pink or yellow molds" on vegetables and ornamentals can develop. This is especially important on root crops (tubers and bulbs), as well as on low-lying crops like cucurbits and tomatoes (Agrios, 1997).

Management

Because *F. oxysporum* and its many special forms affect a wide variety of hosts, the management of this pathogen is discussed in more detail for a specific Fusarium disease in the respective literatures. In general, however, some effective means of controlling *F. oxysporum* include: disinfestation of the soil and planting material with fungicidal chemicals, soil solarization, crop rotation with non-hosts of the fungus, using

resistant cultivars, biological control methods, or integration of available control methods (IPM) (Jones et al., 1982; Smith et al., 1988; Sivan and Chet, 1993; Agrios, 1997; Larkin and Fravel, 1998).

Fusarium Crown and Root Rot of Tomato

Fusarium crown and root rot (FCRR) caused by *Fusarium oxysporum* Schlect f. sp. *radicis-lycopersici* Jarvis and Shoemaker (FORL) is one of the most damaging soil-borne pathogens of tomato and becoming more common in greenhouse tomato production in the U.S. The disease was initially reported in 1974 in Japan (Yamamoto et al., 1974) and soon afterwards identified in North America (Sonoda, 1976). FCRR has also occurred in Canada, Mexico, Israel, Japan, many countries in Europe (Jarvis, 1988) and other states in the U.S. including California, New Jersey, New York, New Hampshire, Ohio, Pennsylvania, and Texas (Roberts et al., 2001). The disease was also identified in Colorado in 1998. The disease occurs in both greenhouse and field worldwide and causes significant losses in tomato production. In closed systems, with recirculation of nutrient solution and rock wool as a growing medium, crown and root rot of tomato can cause serious problems (Hartman and Fletcher, 1991; Rattink, 1992). It is reported that FCRR affects 40% of the surveyed acreage in Florida. Commercial yields have been reported to be reduced 15-65 percent (Sonoda, 1976; Anonymous, 1999).

Symptoms

A wide range of symptoms are associated with FCRR. The fungus invades susceptible plants through wounds and natural openings created by newly emerging roots (Roberts et al., 2001). Early symptoms caused by FORL in tomato seedlings include stunting, yellowing, and premature abscission of cotyledons and lower leaves. A

pronounced brown lesion that girdles the hypocotyls, root rot, wilting, and seedling death are advanced symptoms (McGovern et al., 1993a). Typically, the first symptom in the mature plants is a yellowing along the margins of the oldest leaves when the first fruit is at or near maturity. Yellowing is soon followed by necrosis and collapse of the leaf petiole. Symptom development progresses slowly upward on successively younger leaves. Some plants may be stunted and wilt quickly and wither. Older plants may wilt slowly and still be alive at the end of the harvest (Jones et al., 1991). Wilting first occurs during the warmest part of the day, and plants appear to recover at night. Infected plants may be stunted, totally wilt and die, or persist in a weakened state, producing reduced numbers of inferior fruit (Roberts et al., 2001)

As disease progresses dry brown lesions develop in the cortex of the tap or main lateral roots and taproot often rots away (Roberts et al., 2001). Chocolate brown lesions develop at or near the soil line and extend into the vascular system. When diseased plants are sectioned lengthwise, extensive brown discoloration and rot are evident in the cortex of the crown and roots. This brown vascular discoloration typically does not extend more than 25-30 cm above the soil line, which helps to distinguish this disease from Fusarium wilt, where discoloration may extend 1 meter high. Stem cankers may develop at or above the soil line. Following rains and during fogs, the pink sporulation of the pathogen can be profuse on exposed necrotic lesions (Jones et al., 1991; Gabor and Wiebe, 1997: Davis, 2002). The fungus produces masses of white mycelium and yellow to orange spores in necrotic stem lesions on dead and dying plants (Roberts et al., 2001).

Biology, Ecology and Epidemiology

The causal fungus produces three types of spores: macroconidia, microconidia and chlamydospores. Two of these spores figure prominently in the survival and spread of FORL. Microconidia form in great abundance in necrotic tissue and has been implicated in the recolonization of sterilized soil in greenhouses through aerial dispersal (Rowe, 1977; Davis, 2002). They readily reinfest soil sterilized by heat or broad-spectrum biocides such as fumigants. Chlamydospores have thicker walls and enable the fungus to survive in the soil and wooden stakes for long periods in the soil (McGovern and Datnoff, 1992; Roberts et al., 2001).

FCRR severity varies widely by site and season and is favored by cool temperatures (McGovern et al., 1995). It grows best from 10°C to 20°C, which is lower than the optimum for the fungus that causes Fusarium wilt disease on tomato. The disease affects the plants in early crops and those located in the cooler areas of the greenhouse. The optimum temperature for disease development is 21°C. Low soil pH, ammoniacal nitrogen, and waterlogged soil also exacerbate the disease (Kucharek et al., 2000; Roberts et al., 2001).

FORL is a polycyclic soilborne pathogen. Lateral spread of the pathogen from plant to plant during a growing season is via root-to-root contact. Large numbers of FORL macroconidia are produced on stem surface of diseased plants and aerially disseminated in fields and greenhouses. Infection occurs either via soil infestation and subsequent root infection or by a direct infection of the foliage (Rekah et al., 1999, 2000, 2001). Movement of the fungus in the soil in the absence of roots is minimal (< 2.5 cm). Long-range dissemination of FORL can occur through infected transplants and perhaps

via chlamydospores in soil particles on contaminated shoes, plant stakes, farm machinery, transplant trays, and other equipment (Roberts et al., 2001; Davis, 2002).

The host range of the pathogen includes some non-solanaceous plants, including spinach, beets legumes, cucurbits, and other solanaceous plants, such as pepper and eggplant, but not potatoes (Roberts et al., 2001; Davis, 2002). The fungus has also been isolated either naturally or experimentally from the roots of a number of weeds, including Brazilian pepper (*Schinus terebinthifolius*), carpet weed (*Mollugoverticillata*), hickweed (*Stellaria media*), corn spurry (*Spergula arvense*), cudweed (*Gnaphatium sp.*), curly dock (*Rumex crispus*), narrow leaved plantain (*Plantago lanceolata*), redroot pigweed (*Amaranthus retroflexus*), *Scoparia* sp., Shepard's purse (*Capsella bursa-pastoris*), and wild buckwheat (*Polygonum convolvulus*) (Roberts et al., 2001), and saltcedar plant (*Tamarisk nilotica*) (Rekah et al., 2001). Monocots, such as corn, are not susceptible.

Management Options

Several control procedures have been attempted for managing FCRR in the greenhouse and field including use of resistant varieties, cultural practices, pesticides, fumigants etc. but losses are still substantial. Control programs should be put in place before planting, because there are no rescue treatments. Once the disease is present, avoiding stress is an important part of minimizing losses (Nesmith, 1998). Up to date, no treatment is effective during the crop cycle once infection established. Four management options; cultural control, chemical control, biological control and integrated control will be discussed in the following sectios for managing FCRR in a greenhouse.

Cultural Control

Aggressive sanitation programs are very important, starting with a very clean house and all the items to be used. The worse cases of this disease have been associated with attempts to reuse items without sanitizing them, especially items that come in direct contact with the soil mix (Nesmith, 1998). Crown rot is likely to occur with a higher frequency where direct seeding is used, instead of healthy transplants, and where the soil contains high levels of chloride salts. The utmost sanitation production scheme for transplants in greenhouses should be used so that individuals or equipment used within or around the transplant site do not become contaminated with disease-causing organisms from the field. Finally, transplants should be transported, pulled, and set without tissue damage as damaged tissues are likely to be sites for infection (Kucharek et al., 2000). Transplanting should be done when soil or media is 20°C or above (Dodson et al., 2002). The selection and application of fertilizers can significantly influence the disease development. For example, increasing soil pH and minimizing use of ammoniacal nitrogen help controlling FCRR (Kucharek et al., 2000). Dead tomato plants need to be completely removed (Blankard, 1994).

One of the most important components in an integrated disease control program is the selection and planting of cultivars that are resistant to pathogens. The term *resistance* usually describes the plant host's ability to suppress or retard the activity and progress of a pathogenic agent, which results in the absence or reduction of symptoms (Koike et al., 2000). Some resistant varieties are available for FCRR for mostly greenhouse production. The use of crown rot resistant cultivars is increasing, but is currently not widely accepted due to horticultural characteristics that make these varieties less competitive than

standard varieties (Anonymous, 1999). The following are some cultivars with resistance: Trend, Trust, Medallion, Match, Switch, and Blitz for greenhouse production; Charleston and Conquest for field production. Resistance to Fusarium crown and root rot is conferred by a single dominant gene and already has been incorporated into commercially available cultivars (Chellemi, 1997).

Crop rotation is a historical method of crop production that reduces soil pest problems by removing susceptible plants from an infested area for a period of time long enough to reduce pest populations to tolerable levels. Rotation away from tomatoes may be necessary on fields with a recurring crown rot problem. Avoid rotation with eggplant or pepers; use lettuce instead, which is not sensitive to FORL (Blankard, 1994; Pernezny, 1997). Crop rotation as a control strategy may be limited in controlling FCRR because the fungus can survive in soil many years. Capital field improvements such as irrigation systems, water permitting requirements, and the availability of suitable land also limit adoption of crop rotation as a pest control strategy. Once a grower has invested in an irrigation system for a piece of land, the grower is less likely to rotate to a lower value crop.

Another cultural method in controlling FCRR is soil solarization. Solarization is a non-chemical soil disinfestation method, first developed for soilborne disease control in Israel and California during the 1970s (Katan, 1981; DeVay and Stapleton, 1997). Soil solarization, namely, heating the soil by solar energy resulting in both physical and biological processes to control pathogens and other soil pests (Katan, 1987; DeVay and Katan, 1991). Solarization depends on solar energy to heat the soil to temperatures which are lethal to these organisms. This is accomplished by covering moist soil with a clear

plastic film or mulch during a 2 to 8 week period with plentiful solar radiation. Most soilborne pests and plant pathogens are mesophilic and are killed at temperatures between 40 and 60°C. At these elevated temperature, disfunction of membranes and increased respiration are responsible for death. However, death depends on the thermal dose, a product of temperature and exposure time (Pinkerton, 1998). Exposure to long periods of sublethal temperatures may effectively control diseases by reducing the ability of propagules to geminate, increasing the susceptibility to biological control organisms, and decreasing the ability to infect the host (Pinkerton, 1998).

Soil solarization has been demonstrated to control FCRR. Cultural practices consisted of soil solarization using three different types of plastic film: clear, low density polyethylene; a photo-selective, low density polyethylene; and a clear, gas impermeable film consisting of low density polyethylene co-extruded with nylon. Soil solarization reduced populations of *Fusarium oxysponzm f.sp. radicis-lycopersici* down to a depth 5 cm (Chellemi et al., 1994).

Crown rot incidence was significantly reduced by Metam Sodium (29%), solarization + Metam Sodium (51%) and by Methyl bromide + chloropicrin (50%), while disease severity was significantly reduced (74%) by both the latter two treatments. No significant differences in marketable yield were observed among the treatments (McGovern et al., 1996). Preliminary studies carried out in the open field showed at 12 days soil solarization reduced survival of FORL propagules significantly. The effectiveness of pathogen control was improved by combing solarization with manure, or extending the solarization treatment to 27 days. In a closed greenhouse, solarization and

biofumigation with bovine manure proved effective in reducing the viability of FORL chlamidospores, disease incidence, and in increasing commercial yield (Cartia, 2002).

Grafting is also used in control of crown and root rot of tomato. Resistant rootstocks, provide excellent control of many tomato soilborne pathogens and particularly *Fusarium oxysporum* f. sp. *lycopersici*., *F. oxysporum* f. sp. *radicis-lycopersici*, *P. lycopersici* and *Meloidogyne* spp. This technique, which initially was considered too expensive, is now widely used at a commercial level in many Mediterranean countries and North America. In general, without grafting, the tomato plant density per hectare is about 18,000 plants. When grafted plants are used, the same yield could be obtained with half plant population (9,000 plants/ha). In addition to controlling some soilborne pathogens, tomato grafting promotes growth, increases yield, increases plant tolerance to low temperature, extends the growth period and improves fruit quality (Besri, 2000). Susceptible tomato plants grafted on FCRR-tolerant hybrid rootstock (He-man), even cropped in a severe FORL infested soil, remained healthy during the growing season and gave a profitable yield (Di Primo and Cartia, 2001).

Chemical Control

Chemical control of FCRR in steam sterilized soil by using a captafol drench proved effective in preventing resinfestation by airborne FORL conidia (Rowe and Farley, 1981). Mihuta-Grimm (1990) reported that the application of benomyl at 0.090 g a.i./L on a 21-day schedule to plants growing on rockwool productions slabs resulted in optimum FCRR control. Yield from infected transplants treated with benomyl, however, was not significantly different from that of control plants. Other candidate fungicides proved to be phytotoxic at levels needed to control FCRR (Jarvis, 1988). Although

fungicides such as benomyl or captafol have been demonstrated to be effective, captafol is no longer labeled for usage, and there is an imminent possibility of fungicide resistance.

Fumigation with methyl bromide (MBr) + chloropicrin formulations have been the most commonly used pre-plant practice for control of fusarium crown and root rot in tomatoes (McGovern et al., 1988; McGovern et al., 1996). Application of methyl bromide + chloropicrin significantly reduces the incidence and severity of the disease (McGovern et al., 1988). Methyl bromide: chloropicrin (67:33, by volume) reduced populations of FORL to a depth of 35 cm (Chellemi et al., 1994). However, Fusarium crown and root rot incidence is still very high. Even with the use of methyl bromide as a preplant fumigant, epidemics of Fusarium crown and root rot have occurred in commercial production fields (Chellemi, 1997). MBr is a powerful soil fumigant providing effective control of a wide range of soil-borne pathogens and pests, including fungi, bacteria, nematodes, insects, mites, weeds and parasitic plants. It is also relatively economical and convenient in its use. Despite these major advantages, the use of MBr has been associated with major problems, including the depletion of the ozone layer (EPA, 1997). Because of this, its production and use will be phased out on a worldwide scale, by 2005 in the U.S. and E.U. and other developed countries and by 2015 in the developing countries (Rowlands, 1993). An estimated 22.2 million kilograms of methyl bromide are applied annually for preplant soil fumigation in the United States (Ferguson et al., 1994). Many strawberry and tomato growers have depended on methyl bromide for control of nematodes, weeds, and fungal pathogens. The elimination of methyl bromide in accordance with the U.S. Clean Air Act poses a critical challenge to these growers,

whose crops are valued at more than $2.5 billion annually (USDC, 1996). Although fungicides such as benomyl or captafol have been demonstrated to be effective, captafol is no longer labeled for usage, and there is an imminent possibility of fungicide resistance.

The ban on methyl bromide production and use has prompted the study of new chemical alternatives for the control of soil-borne pests. However, these materials tend to provide a narrower spectrum of control than MBr, have less predictable efficacy, and may have their own problems with environmental pollution and safety (Stapleton et al., 2000). Five soil fumigants (1,3-dichloropropene, chloropicrin, dozamet, fosthiazate, and sodium methyldithiocarbamat), a contact nematicide and several combinations with pebulate herbicide were compared to methyl bromide/chloropicrin (98 and 2%, respectively) for control of nutsedge, Fusarium wilt and crown rot, and nematodes in tomato. Fusarium crown rot was reduced by methyl bromide and 1,3 dichloropropene + chloropicrin in the spring, but in the fall all chemical treatments, except those containing SMDC, provided better crown rot control than methyl bromide (Gilreath et al., 1994). McGovern et al. (1996) reported that Crown rot incidence was significantly reduced by Metam Sodium (29%), solarization + Metam Sodium (51%) and by Methyl bromide + chloropicrin (50%), while disease severity was significantly reduced (74%) by both the latter two treatments. No significant differences in marketable yield were observed among the treatments.

A fresh market tomato study comparing metam sodium and methyl bromide fumigation to an untreated control reported that yields and fruit quality obtained with metam sodium were equivalent to those achieved with methyl bromide fumigation (Cook

and Keinath 1994). Metam sodium has been demonstrated to significantly reduce crown rot incidence and when combined with solarization, control was equivalent to methyl bromide + chloropicrin (McGovern et. al. 1996). Metam sodium could reduce Fusarium crown rot only when thoroughly incorporated in the planting bed, such as through application to the soil prior to bed formation (McGovern et al., 1996).

Plantpro45^{TM}, a new low risk iodine-based compound, was investigated as a potential alternative in controlling FCRR. Plantpro45^{TM} provided significant control of Fusarium crown rot of tomato in naturally infested fields. Under greenhouse conditions, soil drench with Plantpro 45^{TM} at 80 ppm a.i. followed by planting 21 days later and a foliar application at 80 ppm one week after planting increased root and shoot weight, and improved root condition of tomato when grown in field soil naturally infested with FORL. Final disease incidence ratings revealed that plots pretreated with Plantpro45 were comparable to methyl bromide for control of FCRR Adams et al., 2000, 2001).

Biological Control

Research has demonstrated that biological control of FCRR has been successful in some instances under greenhouse and field conditions. The fungus *Trichoderma,* a natural soil-inhabiting species, has been used successfully to control Fusarium crown rot and root rot of tomatoes. The potential of *T. harzianum, Aspergillus ochraceus* and *Penicillium funiculosum* in controlling of FCRR of the field tomatoes was shown (Marois et al., 1981). Sivan and Chet (1993) used *T. harzianum* in combination with soil sterilization and reduced rates of methyl bromide to obtained significant control of tomato crown and root rot in the field with transplants colonized by *T.harzianum* during greenhouse propagation.

Datnoff et al. (1995) conducted field experiments in Florida to evaluate commercial formulation of two fungi, *T. harzianum* and *Glomus itraradices*, for control of FCRR of tomato. Compared to controls, significant reduction in disease incidence was obtained with treatment of biocontrol agents. The interaction between *Glomus intradices* and FORL and its effect on tomato plants were investigated. Caron et al., (1985 and 1986) reported that tomato crown and root rot was decreased with *Glomus intradices*. However, there was no growth response of tomato plants to the inoculation of the biocontrol agent. *T. harzianum* applied, as a peat-bran preparation to the rooting medium at the time tomatoes were transplanted, decreased fusarium crown rot significantly through the growing season in field conditions and yield increased as much as 26.2% over the controls in response to the treatment (Sivan et al., 1987). Nemec et al. (1996) evaluated some biocontrol agents; *T. harzianum, Glomus intraradices*, and *Streptomyces griseverdis*, for controlling root diseases of vegetable crops and citrus. At the end of the study, they found that all biological control agents reduced FCRR of tomato in field. In particularly, *T. harzianum* and *B.subtilis* were the most effective biocontrol agents. *Paenibacillus macerans* and *T. harzianum* were evaluated for promoting plant growth and suppressing Fusarium crown and root rot (FCRR) under fumigated and non-fumigated field conditions. *Trichoderma harzianum* and *Paenibacillus macerans* significantly reduced severity of FCRR. *Trichoderma harzianum* reduced the severity of FCRR by 12% and *P. macerans* 9% in comparison to the untreated control in the nonfumigated treatments. No differences were observed between the biologicals and the untreated control in the methyl bromide treated plots (Datnoff and Pernezny, 2001). They also reported that *T. harzianum* and *P. macerans* alone or in combination significantly

affected the growth of tomato transplants in the greenhouse and after outplanting into the field 30 days later.

The potential of *T. harzianum* as a biocontrol agent in soilless culture system was investigated with tomato plants infected with FORL. The application of *Trichoderma* reduced the incidence and spread of FCRR in tomatoes on an artificial growing medium (Van Steekelenburg, 1991). Marois and Mitchell (1981) reported that in greenhouse and growth chamber experiments, the fungal amendment significantly reduced the mean lesion length and the incidence of FCRR. Under greenhouse conditions, the incidence of crown rot of tomato was reduced by up to 80% 75 days after sowing when *T. harzianum* T35 was applied as either seed coating or a wheat bran-peat preparation (Sivan *et al.,* 1987). Cherif and Benhamou (1990) showed that a strain of *Trichoderma* displayed the ability to produce chitinase and reduced FCRR of tomato by inhibiting growth of the pathogenic fungus.

Much research has been done on the potential of nonpathogenic *F. oxysporum* for control of FCRR. Louter and Edgington (1985) and Brammall and Higgens (1987) used isolates of avirulent F. oxysporum and isolates of F. solani respectively, to reduce the effects of FORL on tomato plants. It was suggested that the fungi acted through either cross protection (Louter and Edmington, 1985) or competition for infection court (Brammall and Higgens (1987) sites.

Alabouvette and Couteadier (1992) studied the efficacy of nonpathogenic *F. oxysporum* strain Fo47 and fungicide himexazol for control the root diseases of tomato in the greenhouse. Both treatments gave a good control of the FCRR; the yield was slightly higher with biological treatment and cost was less than the cost of the chemical product.

Under greenhouse conditions, FCRR of tomato can be achieved by introduction of either starin Fo47, or fluorescent *Pseudomonas* strain C7, or by the association of both into the growing medium (Lemanceau and Alabouvette, 1991). Four nonpathogenic isolates of *F. oxysporum* (26B, 43A, 43AN1 and 43AN2) and *T. harzianum* (Th2) were found to be effective in protecting tomato seedlings from FCRR. However, *T. harzianum* was less effective reducing disease (Hartman and Fletcher, 1991).

There are also some bacteria, especially *Pseudomonas spp.,* that have been shown to be effective in controlling FCRR. *Pseudomonas fluorescens* strain CHA0 suppressed crown and root rot of tomato (Duffy and Défago 1997). M'Piga et al. (1997) reported that *P. fluorescens* colonizes and grows in the outer root tissues of whole tomato plans and sensitizes them to respond rapidly and efficiently to FORL attack in addition to exhibiting an antimicrobial activity in *planta.* Chin-A-Woeng (2000) showed that *Pseudomonas chlororaphis* strain PCL1391 efficiently controls tomato foot and root rot caused by FORL. *P. chlororaphis* PCL1391 produces the antifungal metabolite phenazine, carboxamide, which is a crucial trait for it suppressive activity besides its excellent root colonizing ability. *Pseudomonas chlororaphis* PCL1391 controls tomato foot and root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici.* The production of phenazine-1-carboxamide (PCN) is crucial for this biocontrol activity (Thomas et al., 2001).

Streptomyces griseoviridis (Mycostop^{TM}) has been tested against *F. oxysporum* crown rot in Israel and in the UK. A clear reduction of the disease was observed, but complete control was not achieved by using Mycostop^{TM}. Mycostop^{TM} is a live formulated strain of the bacterium *Streptomyces griseoviridis* that was discovered in

Finnish peat. It is labeled for use on greenhouse tomato, and is available from at least two suppliers in the US. (Lahdenpera, 2000). *Streptomyces* sp. Di-944, a rhizobacterium from tomato, suppressed Rhizoctonia damping-off and Fusarium root rot in plug transplants when applied to seeds or added to potting medium. Antibiosis was suspected as a key mechanism of biocontrol (Sabaratnam et al., 2001).

Among the most promising bioactive oligosaccharides is chitosan (poly-N-glucosamine), a mostly deacetylated derivative of chitin occurring in the cell walls of several fungi, which is readily extracted from the chitin of crustacean shell wastes (Hadwiger et al., 1988). Oligomers of chitosan (poly-N-glucosamine), which are likely to be released by the action of plant encoded-chitosanase from walls of invading fungi, can protect tomato roots against *Fusarium oxysporum* f.sp. *radicis-lycopersici* when applied to the seed or roots (Benhamou and Theriault, 1992; Benhamou et al., 1994). Chitosan, derived from crab-shell chitin, was applied as seed coating and substrate amendment prior to infection with the fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Experiments were performed either on a mixture of peat, perlite, and vermiculite or on bacto-agar in petri dishes. In both cases, a combination of seed coating and substrate amendment was found to significantly reduce disease incidence. The potential of *Bacillus pumilus* strain SE 34, either alone or in combination with chitosan, for inducing defense reactions in tomato plants inoculated with the vascular fungus, FORL, was studied by light and transmission electron microscopy. Treatment of the roots with *B. pumilus* alone or in combination with chitosan prior to inoculation with FORL substantially reduced symptom severity of FCRR as compared with controls. Although some small, brownish

lesions could be occasionally seen on the lateral roots, their frequency and severity never reached levels similar to those observed in control plants (Benhamou et al., 1998).

Oligandrin, the elicitin-like protein produced by the mycoparasite *Pythium oligandrum*, crab shell chitosan and crude glucans, isolated from *P. oligandrum* cell walls were applied to decapitated tomato plants and evaluated for their potential to induce defence mechanisms in root tissues infected by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Benhamou et al., 2001). A significant decrease in disease incidence was monitored in oligandrin- and chitosan-treated plants as compared to water-treated plants whereas glucans from *P. oligandrum* cell walls failed to induce a resistance response. In root tissues from oligandrin-treated plants, restriction of fungal growth to the outer root tissues, decrease in pathogen viability and formation of aggregated deposits, which often accumulated at the surface of invading hyphae, were the most striking features of the reaction. In chitosan-treated plants, the main response was the formation of enlarged wall appositions at sites of attempted penetration.of the reaction.

Lettuce residue soil amendments and lettuce intercropping were considered for biological control (Jarvis and Thorpe, 1981, 1983). Co-planted lettuce and T. harzianum strain Th2 provided protection from naturally occurring FCRR in a commercial tomato crop (Hartman and Fletcher, 1991).

Integrated Management

At present, Fusarium crown and root rot is difficult to control in field-grown tomatoes because the pathogen rapidly colonizes sterilized soil and persists for long periods. However, an integration of the following management procedures may help to reduce the impact of crown and root rot (Roberts et al., 2001):

1. Use disease-free transplants. Transplant houses should not be located near tomato production fields. Avoid over watering, which makes the transplants more susceptible to crown and root rot. Disinfect transplant trays by steaming before reuse.
2. Use a pre-plant fumigant. The soil should be of good tilth and adequately moist for at least two weeks prior to fumigation. Use an appropriate chisel spacing and depth, and immediately cover the bed with plastic mulch following fumigation.
3. Optimize cultural practices in the field. Avoid injuring transplants when they are set in the field. Physical damage and injury from excessive soluble salts may make young plants more susceptible to crown and root rot. The use of water drawn from wells rather than ditches for watering-in transplants may help to prevent recontamination of fumigated soil. Avoid ammoniacal nitrogen and maintain the soil pH at 6 to 7. Rapidly plow in crop debris following final harvest. Disinfest tomato stakes before reuse, or use new stakes.
4. Rotate with a non-susceptible crop. Incomplete knowledge of the host range of FORL makes precise recommendations in this area difficult. Current research data suggests that leguminous crops should be avoided in favor of corn and similar crops. Rotation and intercropping with lettuce had reduced FORL in greenhouse-grown tomatoes.
5. Significant progress has been made in breeding for resistance to Fusarium crown and root rot in field-grown tomatoes. Although the commonly used commercial cultivars do not have resistance, some resistant cultivars, such as Conquest, are available for field use and Trust for greenhouse use.
6. Additional management strategies under investigation include the use of biological control, cover crops, and soil solarization alone or in combination with fumigants.

Integration of different management methods have been shown to be effective in controlling FCRR. McGovern (1994) indicated that Fusarium crown and root rot of tomato can be effectively managed by integrating the use of pathogen-free transplants and stakes, resistant cultivars and pre-plant fumigation. A commercial tomato field in southwest Florida, naturally infested with FORL was used to compare the effectiveness of methyl bromide: chloropicrin, 67%:33% (Terr-O-Gas 67, 336 kg/ha), metam sodium (Vapam, 935 l/ha), composted sewage sludge (Florida Organix, 5.5 MT/ha), soil solarization and combinations of solarization and Vapam or Florida Organix in reducing FCRR. Fusarium crown rot incidence was significantly reduced by Vapam (-29%), solarization plus Vapam (-51%) and by Terr-O-Gas (-50%), while disease severity was significantly reduced (-74%) by both the latter two treatments (McGovern et al., 1996).

Minuto et al. (2000) reported that the combination of soil solarization with reduced dosage of Dazomet and of MB controls *Fusarium* and *Verticillium* wilts and *Fusarium* crown rot on tomato. Preliminary studies carried out in the open field showed at 12 days soil solarization reduced survival of FORL propagules significantly. The effectiveness of the pathogen's control was improved by combing solarization with manure, or extending the solarization treatment to 27 days. In a closed greenhouse, solarization and biofumigation with bovine manure proved effective in reducing the viability of FORL chlamidospores, disease incidence, and in increasing commercial yield (Cartia, 2002).

Under field conditions, the combination of *T. harzianum* with soil solarization or with a reduced dose of methyl bromide resulted in significant disease control of FCRR of tomato induced by FORL (Sivan and Chet, 1993). Combination of biocontrol agent

Pseudomonas fluorescens with mineral element zinc significantly reduced disease severity of FCRR of tomato, however, *P. fluorescens* strain CHA0 alone was only moderately effective (Duffy and Defago, 1997).

Trichoderma spp. with an emphasis on *Trichoderma harzianum*

Taxonomy and Historical Overview

Trichoderma are among the most common saprophytic fungi. They are within the subdivision Deuteromycotina, which represents the fungi lacking or having an unknown sexual state. Further, it is part of the form class Hyphomycetes (Spain, 2002).

Trichoderma was named in 1794 and introduced to the literature by Persoon (1794) who established four species within the genus. These four species are now considered to be unrelated to each other with only one, *T.viride*, still within the original *Trichoderma* genus. Harz (1971) was the first to emphasize microscopic characteristics in separating the genus and primarily used phialides to distinguish the different species. Oudemans and Koning (1902) were the first to isolate a *Trichoderma* from the soil and designated the isolate as *T. koningi*. Bisby (1939) investigated the variability of species, and concluded that *Trichoderma* was a monotypic genus (Rifai, 1969). Prior to the genus revision by Rifai (1969), soilborne *Trichoderma* with small, globose, subglobose or short obovoid, and smooth-walled phialospores, produced on short phialides were referred to as *T. lignorum.* After the revision, isolates exhibiting these characteristics were referred to as *T. harzianum* (Rifai, 1969). Rifai (1969) introduced the concept of species aggregate, differentiating groups primarily by conidiophore branching patterns and conidium morphology. The 1969 revision of *Trichoderma* includes nine species and has been well accepted by most workers (Cook and Baker, 1983). Traditional taxonomy was

based upon differences in morphology, primarily of the asexual sporulation apparatus, but more molecular approaches are now being used. Consequently, the taxa recently have gone from nine to at least thirty-three species (Harman, 2001).

Ecology, Biology and Morphology

Trichoderma is a genus of filamentous *Deuteromycetes* that is ubiquitous in the environment. Its members are generally found in all soils including forest humus layer as well as in agricultural and orchard soils (Roiger et al., 1991; Samuels, 1996). In soil, they frequently are the most prevalent culturable fungi (Harman, 2001). *Trichoderma spp.* are likely to perform well at any pH below 7. In fact, the fungus seems to do well even in basic soil if there are high concentrations of carbon dioxide or bicarbonate available (Papavizas 1985). *Trichoderma* can utilize many different food sources from the seed or from the soil, including carbohydrates, amino acids, and ammonia (Papavizas, 1985).

Trichoderma is also vulnerable to a lack of iron. Iron deficiency was a problem, for instance, when the fungus was introduced into a New York soil with many *Pseudomonas sp.* bacteria that had used their sideophores to sequester most of the soluble iron (Hubbard et al., 1983). They are favored by the presence of high levels of plant roots, which they colonize readily. Some strains, such as T22 and T95, are highly rhizosphere competent. According to Harman (1992) "Rhizosphere competent organisms are those capable of colonizing the root surface or rhizosphere when applied as a seed or other point source at the time of planting (e.g. a tuber or in-furrow granule) in the absence of a bulk flow of water". Ahmad and Baker (1987) was the first to show benomyl mutant of *Trichoderma harzianum* strain T95 is rhizosphere competent However, most strains

lack this ability. In addition to colonizing roots, *Trichoderma* spp. attack, parasitize and otherwise gain nutrition from other fungi (Harman, 2001).

Trichoderma species are rarely reported to occur on living plants and have not been found as endophytes of living plants (Samuels, 1996). However, one aggressive strain has been found that causes a significant disease of the commercial mushroom (Seaby, 1998). While wild strains are highly adaptable and may be heterokaryotic, strains used for biocontrol in commercial agriculture are, or should be, homokaryotic (Harman, 2001). There is a great deal of variability among the different *Trichoderma* species and isolates in their tolerances to a wide range of environmental conditions (Tronsmo and Hjeljord 1998). *T. harzianum* has been characterized as more characteristic of warm climates; however, it is evident from the field study and published literature that cold tolerant strains, such as *Trichoderma atroviride*, do exist (Bissett, 1992; McBeath, 2001).

Trichoderma harzianum has no known sexual stage and is believed to be mitotic and clonal. Colonies are pale, very fast growing, 5 - 8 cm on PDA at 7 days, thinly cottony, soon giving rise to white sporodochial tufts which turn green as conidia develop (Samuels, 1996). *Trichoderma harzianum* has a temperature optimum for growth at 30°C, but strains effective at temperatures near 0°C, have been found (Tronsmo, 1989).

Life cycle

The organism grows and ramifies as typical fungal hyphae, 5 to 10 μm in diameter. *T. harzianum* conidiophores (typically 3 to 5 μm in diameter) form distinct, continuous dark green ring-like zones in culture which arise in loose tufts (Rifai, 1969; Harman, 2001). Conidia are released in large numbers. Intercalary or terminally resting chlamydospores are also formed on the media (Cook and Baker, 1983), these also are

single celled, although two or more chlamydospores may be fused together (Harman, 2001). *Trichoderma spp.* can also produce chlamydospores on natural substrates, such as oat kernels placed in sterile and natural soils. These structures may play an important role in the survival of this genus in the soil (Henis and Papavizas, 1983). High numbers of chlamydospores have been reported to form within plant tissue (Cohen et al., 1983).

Biological Control with *Trichoderma spp.* with emphasis on *T. harzianum*

Several species of *Trichoderma* have been extensively studied for their biological control effects against fungal plant pathogen (Cook and Baker 1983; Sivan et al., 1984 and Coley-Smith et al., 1991; Papavizas, 1985; Harman, 1996). In fact, the antifungal abilities of these beneficial microbes have been known since the 1930s, and there have been extensive efforts to use them for plant disease control since then (Samuels, 1996). Weindling (1932, 1934) and Weindling and Fawcett (1936), perhaps, were the first investigators to demonstrate the potential of *Trichoderma spp.* to control plant disease.

Many *Trichoderma* strains have been identified as having potential applications in biological control of plant pathogenic fungi on many crops including strawberries, beans, peas, cucumbers, tomatoes, radishes, sugar beets, cotton, and a partial list of plant pathogenic fungi affected by *Trichoderma* includes: *Armillaria*, *Botrytis*, *Chondrostereum*, *Colletotrichum*, *Dematophora*, *Diaporthe*, *Endothia*, *Fulvia*, *Fusarium*, *Fusicladium*, *Helminthosporium*, *Macrophomina*, *Monilia*, *Nectria*, *Phoma*, *Phytophthora*, *Plasmopara*, *Pseudoperonospora*, *Pythium*, *Rhizoctonia*, *Rhizopus*, *Sclerotinia*, *Sclerotium*, *Venturia*, *Verticillium*, and wood-rot fungi (Tronsmo and Dennis, 1977; Harman et al., 1980; Elad et al., 1980; Elad et al., 1993; Sutton and Peng, 1993; Datnoff et al., 1995; Monte, 2001).

T. harzianum, a member of the fungal genus *Trichoderma,* has been extensively studied as biological control agent (Lewis and Papavizas, 1991; Elad, 2000) due to its ability to successfully antagonize other fungi including plant pathogenic species. It has been commercially produced to prevent development of several soil pathogenic fungi. Strains of *T. harzianum* are marketed in a number of products; PlantShield® / RootShield® from U.S., Trichodex® from Israel, Binab T® from Sweden, and Supresivit® from the Czech Republic (Paulitz and Belanger, 2001).

Trichoderma spp. have evolved numerous mechanisms for attacking other fungi and for enhancing plant and root growth. These meachanisms are competition for space and nutrients (Elad et al., 1999), mycoparasitism (Haran et al., 1996; Lorito et al., 1996a; Lorito et al., 1998), production of inhibitory compounds (Sivasithamparam and Ghisalberti, 1998), inactivation of the pathogen's enzymes (Roco and Perez, 2001), and induced resistance (Yedidia et al., 1999; Kapulnik and Chet, 2000). These mechanisms are going to be discussed in detail with examples.

Competition is one of the mechanisms of biological control activity of *Trichoderma spp.* against phytopathogenic fungi. *Trichoderma* species are generally considered to be aggressive competitors (Samuels, 1996). Competition can take place between the antagonist and the pathogen for iron through production of siderophores, for colonization sites and nutrients supplied by seeds and roots. *Trichoderma* spp. are often very fast growing and rapidly colonize substrates, thus excluding pathogens such as *Fusarium* spp. (Papavizas, 1985). Rhizosphere competence by biocontrol agents, described in previous section, is important in the mechanism of competition, especially with seed treatments. This is the zone where protection against pathogens is critical. It is

important because a biocontol agent can not compete for space and nutrients if is unable to grow in the rhizosphere (Howell, 2003). *Trichoderma* species, either added to the soil or applied as seed treatments, grow readily along with the developing root system of the treated plants (Ahmad and Baker, 1987; Harman, 2000; Harman, 2001; Howell, 2003).

Soil treatments with *T. harzianum* spores suppressed infestations of *Fusarium oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *melonis* mainly by competitive interaction for nutrients (Sivan and Chet 1989b). The study by Utkhede et al. (2001) demonstrated that applications of RootShield ® (*T. harzianu*m) and yeast strain (S33) of *Rhodosporidium diobovatum* applied as a postinoculation foliar sprays were effective to control tomato stem canker caused by *B. cinere*a. They concluded that the mechanism could be competition for space. Competition between *Trichoderma* and *Botryris* is especially active during the colonization of floral debris, and supply of the antagonist at the end of the flowering is of prime importance (Duboss, 1987). Competition at atmosphere level as biocontrol mechanism in *Trichoderma* spp. was suggested by Marchetti et al. (1992). In *in vitro* experiments *Rhizoctonia solani*, *Pythium ultimum* and *Chalara elegans* were strongly inhibited by *Trichoderma* while *Fusarium oxysporum* and *Cytospora* sp. showed tolerance to the antagonistic activity of 4 species of *Trichoderma*. Both the pathogens and the *Trichoderma* were grown in pairs on the same agarized medium in a confined environment on separate media. The biocontrol efficacy of *Trichoderma* seems to perform not only at medium, but also at atmosphere level. The observed inhibiting action of *Trichoderma* was associated with a high rate and extent of CO_2 accumulation. The plant pathogenic fungi that were characterized by slow rates of CO_2 production were more sensitive to the antagonists.

Many isolates of *Trichoderma spp.* produce volatile and nonvolatile antibiotics (Dennis and Webster, 1971a, 1971b). Howell and Stipanovic (1983) isolated and described a new antibiotic, glovirin from *Gliocladium (Trichoderma) virens* that was strongly inhibitory to *Pythium ultimum* and *Phytophthora* species. Sivan et al. (1984) reported that growing *Trichoderma*, which is antagonistic *Pythium aphanidermatum,* on a cellophane membrane placed on agar. By removing the membrane and inoculating the agar with *Pythium* they showed that the growth pf the pathogen was partially inhibited by substances produced by the *Trichoderma.* Liftshitz et al. (1986) showed that control of Pythium species on peas by *T. harzianum* atrain T12 and *T. koningii* strain T8 was not due to either competition or mycoparasitism. They ascribed biocontrol to the production of a toxic factor by the biocontrol organism in the spermosphere, which inhibited growth of the pathogens. However, most attempts to correlate in vitro antibiosis by *Trichoderma* against fungal pathogens with what actually happened in natural systems have failed (Bell et al., 1982). Examination of the literature has shown that *Trichoderma spp.* secrete a number of antifungal antibiotics including pyrones, isocyanates, peptides, and trichothenes. Whether or not these antifungal metabolits are relative to biocontrol under field conditions is a point of speculation (Ghisalberti and Sivasithamparam, 1991). Another antibiotic that is produced by *T. harzianum* is peptaibols (Schirmbock et al., 1994).

Other mechanism involved in the antagonistic activity of members of the genus *Trichoderma* against phytopathogenic fungi, is mycoparasitism. *Trichoderma spp.* are active as mycoparasites and, therefore, can serve as potential biocontrol agents. The mode hyphal interaction and parasitism of *Trichoderma spp.* with several soilborne

pathogenic fungi has been reported (Chet et al, 1981; Dennis and Webster, 1971a; Elad et al., 1983; Lifshitz et al., 1986). *Trichoderma* grows tropically toward hyphae of other fungi, coil about them in a lectin-mediated reaction, and degrade cell walls of the target fungi by the secretion of different lytic enzymes. This process (mycoparasitism) limits growth and activity of plant pathogenic fungi. *Trichoderma* attaches to the host hyphae via coiling, hooks and appressorium like bodies, and penetrate the host cell wall by secreting lytic enzymes. The interaction is specific and not merely a contact response. *Trichoderma* recognizes signals from the host fungus, triggering coiling and host penetration (Sivan and Chet, 1989a; Tronsmo, 1996). These enzymes have been reported mainly in isolates of *T. harzianum* (Sivan and Chet, 1989a; Lorito et al., 1993; Lorito et al., 1996b).

Trichoderma harzianum is known to produce relatively high concentrations of cell-wall degrading enzymes as β-1,3-glucanases and different chitinolytic enzymes. These enzymes have been suggested as the key enzymes in mycoparasitism (Cherif and Benhamou, 1990; Elad et al., 1982b). Several enzymes have been purified and characterised, and their ability to inhibit the germination of spores and elongation of the hyphae belonging to the pathogenic fungi has been shown in vitro (Lorito et al., 1993). For instance, scanning electron microscopy and fluorescence microscopy showed that both *T. harzianum* and *T. hamatum* were mycoparasites of both Sclerotium rolfsii and Rhizoctonia solani. The antagonist attached to the pathogen and secreted glucanase and chitinase enzymes that ate through the sell wall (Elad et al., 1983). T. harzianum strain T24 showed a potential for control of the pyhtopathogenic basidiomycete Sclerotium rolfsii. Inhibition of S. rolfsii correlated with both chitanase and β-1,3-glucanase

activities in the culture filtrate of T. harzianum strain T24, suggesting the involvement of thsese enzymes in the biocontrol process (El-Katatny et al., 2001).

Trichoderma produces cellulose, β-1,3-glucanase, and chitinase and degrades the glucans in the walls of *Pythium spp.* and the chitin and glucans in the walls of *Rhizoctonia solani* (Harman et al., 1980; Chet and Baker, 1981). Papavizas et al. (1982) reported that seed treatment with *T. harzianum* reduced Pythium seed tot of pea and Rhizoctonia damping-off of cotton. Application of *Trichoderma lignorum* as a wheat-bran preparation, conidial suspension, or seed coating greatly decreased the number of infested seeds by *R. solani* as well as damping-off percentages and hence controlling the fungal disease (Aziz et al., 1997). Varaschin et al. (2000) reported that *Trichoderma* spp. strains, SC164, SC167 and SC168, selected *in vitro* were good biocontrol agents against the disease caused by *R. solani* in tomato under greenhouse conditions. One of the strains improved plant growth. Lewis and Lumsden (2001) showed *T. hamatum* and *T. virens* reduced damping-off of eggplant, zinnia, pepper, cucumber and cabbage caused by *R. solani.* On the other hand, some controversial experimental results have also been reported with regard to the potential role attributed to chitinases, since no correlation was found between antagonism against R.solani and the hydrolic activity of trichoderma sp.p. (Kohl and Schlosser, 1992).

Specific strains of fungi in the genus *Trichoderma* colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense response, which in the end leads to induced systemic resistance (ISR) in the entire plant. *Trichoderma* species have been reported to induce systemic resistance in plants. Application of *Trichoderma harzianum* Rifai to bean

roots resulted in a 25 to 100% reduction in the severity of the foliar disease, gray mold, caused by *Botrytis cinerea* (De Meyer et al., 1998a). Biocontrol fungus *T. harzianum* T39 and a chemical BTH (benzothiadiazol) were tested for induction of resistance in tomato to *B. cinerea*. In these experiments it became clear that resistance inducing strains stopped the fungus in a very early stage, and the number of spreading lesions declined with about 30 % (Audenaert et al., 1998). The involvement of locally and systemically induced resistance was demonstrated with *T. harzianum* isolate T39. Cells of the biological control agent applied to the roots, and dead cells applied to the leaves of cucumber plants induced control of powdery mildew. Enzymes of *Botrytis cinerea*, such as pectinases, cutinase, glucanase and chitinase, were suppressed through the action of T39 secreted protease on plant surfaces (Elad et al., 1999). The activation of plant defense systems in association of roots with *T. harzianum* strain T-203 was suggested by Yedidia et al. (1999). The roots of cucumber plants inoculated with T-203 exhibited higher activities of chitinase, β –1,3-glucanase, cellulase and peroxidase when compared to an untreated control 72 hours post inoculation. Scanning electron microscopy revealed typical fungal structures previously associated with mycoparasitic interactions of *Trichoderma* spp. Treatment of cucumber plants with 2,6-dichloroisonicotinic acid, an inducer of the plant defense response, displayed responses that were similar but not identical to those of plants inoculated with *T. harzianum.* Khan et al. (2001) reported that *T. hamatum* 382 (T382) inoculated into a composted cow manure-amended potting mix (compost mix) that T382 induced systemic resistance in cucumber against Phytophthora root and crown rot as well as leaf blight. *Pseudomonas aeruginosa* 7NSK2 and *Trichoderma harzianum* T39 induced systemic resistance against *B. cinerea* on bean and

tomato and stopped spread of the pathogen at a very early stage. When the infection pressure was very high, however, *B. cinerea* spread could not be controlled effectively by induced resistance (De Meyer et al., 1998b).

Inactivation of the pathogen's enzymes is another biocontrol mechanism by *Trichoderma spp.* The *in vitro* biocontrol ability of *Trichoderma harzianum* on the phytopathogen *Alternaria alternata* was investigated in the presence of the growth regulators. *A. alternata* is a pathogenic fungus that can secrete endo-polygalacturonase (endo-PG) and pectate lyase (PL) activities. These enzymes are responsible for the hydrolysis of pectic components of the plant cell wall. The presence of *T. harzianum* decreased endo-PG secretion of *A. alternata* by about 50%. This inhibitory effect was independent of the presence of growth regulators (Roco and Perez, 2001).

The strains of *T. harzianum* are marketed in a number of products. Commercial products currently on the open market or under registration include (Monte, 2001):

- Bio-Fungus (Belgium) against *Sclerotinia*, *Phytophthora*, *Rhizoctonia solani*, *Pythium* spp., *Fusarium*, *Verticillium*
- Trichodex (Israel) against *Botrytis* of vegetables and grapevines
- Binab-T (Sweden) for control of wound decay and wood rot
- Root Pro (Israel) against *R. solani*, *Pythium* spp., *Fusarium* spp., and *Sclerotium rolfsii*
- RootShield (also sold as Bio-Trek T-22G) (USA) against *Pythium* spp., *R. solani*, *Fusarium* spp.
- SoilGard (formerly GlioGard) (USA) for damping-off diseases caused by *Pythium* and *Rhizoctonia* spp.

- Supresivit (Denmark) against various fungi
- Trichoject, Trichopel, Trichodowels and Trichoseal (New Zealand) for control of *Armillaria*, *Botryosphaeria*, *Chondrosternum*, *Fusarium*, *Nectria*, *Phytophthora*, *Pythium*, *Rhizoctonia*
- TUSAL (Spain) for damping-off diseases caused by *Pythium*, *Phoma* and *Rhizoctonia* species, rhizomania disease of sugar beet and drop of lettuce
- Trichoderma 2000 (Israel) against *R. solani*, *S. rolfsii*, *Pythium* spp., *Fusarium* spp.
- Trieco (India) against *Rhizoctonia* spp., *Pythium* spp., *Fusarium* spp., root rot, seedling rot, collar rot, red rot, damping-off, *Fusarium* wilt.

Adverse effect of *Trichoderma spp.* in Biological Control

Although *Trichoderma harzianum* is an effective biocontrol agent against several fungal soilborne plant pathogens, possible adverse effects of this fungus on arbuscular mycorrhizal (AM) fungi might be a drawback in its use in plant protection. AM fungi are obligate biotrophic endosymbionts in roots of most herbaceous plants. These fungi grow from the roots out into the surrounding soil, forming an external hyphal network which increases uptake of mineral nutrients (Smith and Read, 1997) and consequently promotes plant growth. The results from pot experiments suggest that *Trichoderma* species suppress AM root colonization (Wyss et al., 1992; McAllister et al., 1994; Siddiqui and Mohmood, 1996). Green et al. (1999) examined the interaction between *Glomus intraradices* and *T. harzianum* in soil. The presence of *T. harzianum* in root-free soil reduced root colonization by *G. intraradices*. The external hyphal length density of *G. intraradices* was reduced by the presence of *T. harzianum* in combination with wheat

bran. On the other hand, adverse effects of AM fungi on the population density of *Trichoderma koningii* have also been observed (McAllister et al., 1994).

CHAPTER III

Evaluation of *Trichoderma Harzianum* Strains to Control Crown and Root Rot of Greenhouse Fresh Market Tomatoes

INTRODUCTION

The root and crown diseases of tomato can be more devastating than foliar diseases, because they can kill plants outright. They are also more difficult to control. These diseases can intensify from season to season, especially if the growing substrates (rockwool slabs, coir slabs or perlite bags) are reused from crop to crop.

Fusarium crown and root rot (FCRR) caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* Jarvis & Shoemaker (FORL) is one of the most prevalent soilborne diseases of tomato. The disease occurs in both greenhouse and field worldwide and causes significant losses in tomato production. Especially in closed systems, with recirculation of nutrient solution and rock wool as a growing medium, crown and root rot of tomato can cause serious problem (Raitink, 1992; Hartman and Fletcher, 1991). The disease was initially reported in 1974 in Japan (Yamamoto et al., 1974) and soon afterwards identified in North America, and in the1980's in several countries of Europe (Jarvis, 1988). FORL forms very resistant spores that can survive for a long period in the growing media. The fungus invades susceptible plants through wounds and natural openings created by newly emerging roots (McGovern and Datnoff 1992; Roberts et al., 2001). Disease development is favored by moderate (20°C) soil temperatures. An airborne phase of this disease has been reported, an unusual occurrence for a *Fusarium* pathogen (Rekah et al., 2000). A wide range of symptoms are associated with FCRR, but

a general yellowing moving from the bottom up on individual plants, stunting, premature loss of cotyledons and lower leaves are usually involved. Overt symptoms, such as mid-day wilting, are often not evident until about the time of first harvest. In the interior of the stems from ground level up to about 25 cm definite vascular browning can be seen in diseased plants, which helps to distinguish this disease from Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* (Gabor and Wiebe, 1997; Roberts et al., 2001).

The use of Fusarium-resistant tomato cultivars can provide some degree of control of FCRR, but the occurrence and development of new pathogenic races is a continuing problem, and currently there are no commercially acceptable cultivars with adequate resistance to FORL (Jarvis, 1988; Jones et al., 1991; McGovern et al, 1993). FCRR is generally controlled in tomato by pre-plant soil fumigation with methyl bromide (MBr). Tomatoes represent the largest single-crop use of MBr in the United States, accounting for 25% of the total MBr use for soil fumigation (UNEP, 1994). However, fumigation with MBr is expensive and not always an effective measure due to rapid colonization of growing media by FORL (Rowe et al., 1978; Gabor and Wiebe, 1997). In addition to other potential health, safety, and environmental risks, concerns over the ozone-depleting properties of MBr has led to announcements of phase-out schedules The Montreal Protocol schedule for reducing MBr production and importation for the U.S. and other developed countries is 25% in 1999, 25% in 2001, 20% in 2003, and complete phase out in 2005. In developing countries, consumption will be frozen in 2002 at 1995-98 average levels, followed by 20% reduction in 2005 and complete phase out in 2015 (Anonymous, 2000). Therefore, alternative control measures are necessary and need to be made available as soon as possible.

Biological control is a potential alternative to the use of chemical pesticides. Biological fungicides may act to suppress the population of the pathogenic organism through competition with pathogenic organisms, stimulate plant growth which may allow plants to quickly outgrow any pathogen effects, or damage or destroy the pathogen by means of toxins produced (Cook, 2000; Gilreath, 2002). A variety of soil microorganisms have demonstrated activity in the control of various soilborne plant pathogens, including Fusarium wilt pathogens. Of the fungi used for control of soilborne pathogens, various species of *Trichoderma* spp have received the most attention. *Trichoderma harzianum* is a fungal biocontrol agent that attacks a range of economically important phytopathogenic fungi. *T. harzianum* alone or in combination with other *Trichoderma* species can be used in biological control of several plant diseases (Papavizas, 1985; Chet 1987; Samuels 1996). It has been also shown to be effective in controlling Fusarium crown and root rot under greenhouse and field conditions. Datnoff et al. (1995) demonstrated that using selective commercial microorganisms such as *T. harzianum* and *G. intraradices* alone or in combination were effective for controlling FCRR. Application of *T. harzianum* reduced the incidence and spread of FCRR in rockwool-grown tomatoes (Van Steekelenburg, 1991). Marois and Mitchell (1981) reported that in greenhouse and growth chamber experiments, the *T. harzianum* significantly reduced the mean lesion length and the incidence of FCRR. Under greenhouse conditions, the incidence of crown rot of tomato was reduced by up to 80% 75 days after sowing when *T. harzianum* T35 was applied as either seed coating or a wheat bran-peat preparation (Sivan et al., 1987). *T. harzianum* applied, as a peat-bran preparation to the rooting medium at the time tomatoes were transplanted, decreased Fusarium crown rot significantly through the

growing season in field conditions and yield increased as much as 26.2% over the controls in responce to the treatment (Sivan et al., 1987). Cherif and Benhamou (1990) showed that a strain of *Trichoderma* displayed the ability to produce chitinase and reduced FCRR of tomato by inhibiting growth of the pathogenic fungus. Under field conditions, the combination of *T. harzianum* with soil solarization or with a reduced dose of methyl bromide resulted in significant disease control of FCRR of tomato induced by FORL (Sivan and Chet, 1993). Nemec et al. (1996) evaluated some biocontrol agents; *T. harzianum, Glomus intraradices*, and *Streptomyces griseverdis*, for controlling root diseases of vegetable crops and citrus. They found that all biological control agents reduced FCRR of tomato in field. In particularly, *T. harzianum* and *B.subtilis* were the most effective biocontrol agents. Although *Trichoderma spp* is ubiquitous, the type of the soil can affect growth, proliferation and effectiveness as biocontrol agent. Because soil ecology is complex, and since there are year-to-year fluctuations in climate and growth conditions, treatments with microbials are sometimes inconsistent (Quarles, 1993).

The objective of this study was to evaluate efficacy of existing biocontrol strains of *Trichoderma harzianum* including commercial formulations for controlling FCRR of tomato under greenhouse conditions.

MATERIALS AND METHODS

Cultivar

FORL-susceptible Tomato *(Lycopersicon esculentum* Mill.) cultivar Caruso was used in the experiments. Caruso is an older beefsteak cultivar of tomato still popular with market gardeners because of its good flavor. It matures to a rich red color with an average weight of 180-225 g. Caruso, which has sparse foliage, is best adapted to fall cropping

and may produce yellow-shouldered fruit under high light intensities. The seeds were provided by Hydro-Gardens, Colorado Springs, CO, U.S.A.

Growing media

Rock wool slabs and Coir slabs were used in this study as growing media. Rock wool (Grodan® Rockwool Talent) and coir slabs were provided by SunBlest Farms LLC., Peyton, CO, U.S.A. Rockwool is manufactured by melting basaltic rock at high temperature and spinning the melt into fibers. Immediately following spinning, a binder is added to the fibers and they are compressed and cured into large slabs. By adjusting the amount of pressure, the density of the media is adjusted. The large slabs can be cut into smaller slabs and propagation blocks for easy handling. The spun fibers are also formed into a granulated (flocked) product, which can be handled in a manner similar to bales of peat (Dowgert, 2002). It has been widely used for many years in the horticultural industry. Soil fungi are not present in rockwool but may be introduced by irrigation water and/or airborne deposition and can cause considerable damage (Alabouvette et al., 1996).

On the other hand, coir slab is relatively new and has been used in greenhouse tomato production as an alternative growing media to rockwool. In fact the use of coconut fiber in European greenhouse production is well accepted as new technology. Coir slabs consist of a waste product of the coconut industry in countries such as Sri Lanka, India, Malaya, Mexico as well as countries in West Africa (Drakes et al., 2001). The use of coconut fiber as a growing media for tomato has been investigated. Teo and Tan (1993) found that a mixture of coconut fiber and charcoal dust (2:1; v:v) produced the greatest plant height, number of fruit, total fruit weight per plant and the largest mean weight and fruit diameter (Teo and Tan, 1993).

Pathogen isolate and preparation of inoculum

The isolate of *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) used in this study was isolated from naturally infected tomato plants grown in a commercial greenhouse in Colorado, U.S.A. The isolate of *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) used in this study was isolated from naturally infected tomato plants grown from a commercial tomato greenhouse. Samples collected from diseased plants were thoroughly washed with tap water; roots and crowns were removed. The crown and root pieces were surface sterilized by immersion for 2 min. in 3% bleach (sodium hypochlorite) solution followed by two rinses in sterile deionized water. The blotted tissue was placed on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI, U.S.A.) and incubated at 25°C for 5 days. Any developing fungus was isolated on new PDA plates, purified and identified according to Nelson et al., (1983). Pathogenicity was confirmed for FORL in the test with tomato cv. Caruso. A spore suspension (10^6 conidia + mycelial fragments/ml) was prepared by blending 14-day old cultures of FORL grown on PDA at 25°C with sterile distilled water and filtering the suspension through cheesecloth. Conidial densities in the suspension were determined by use of a hemacytometer under a light microscope. The Pathogen inoculum was added to rockwool blocks.

Strains of biocontrol agent and preparation of cultures

Three strains of biocontrol fungi were evaluated in this study. *T. harzianum* strain T95 (T95), a rhizosphere-competent strain of *T. harzianum* originally isolated from a Columbian soil, was kindly provided by Suzanne M. Nemeth, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins,

CO, U.S.A) *T. harzianum* Rifai strain KRL-AG2 (PlantShield™, $1x10^7$ colony forming units/g as a wettable powder) was supplied by Bioworks Inc., Geneva, N.Y., U.S.A.) *T. harzianum* Rifai strain 1295-22 (T22) was derived from 14-day old cultures grown on PDA plates incubated at 25°C. PlantShield™ and T22 have the same active ingredient; only difference in this study was the preparation of the strains for inoculum. T22 and T95 were maintained on PDA and kept at 4°C. PlantShield™ was maintained in the container provided by the manufacturing company and kept at 4°C. Fungal inoculum of strain T22 and T95 was prepared by blending 2-week old PDA-grown cultures of the fungus with sterile distilled water, straining the suspension through cheesecloth. The *T. harzianum* strains T95 and T22 (10^6 and 10^7 conidia/ml) were inoculated either by soaking the rockwool cubes at sowing or by soaking the rockwool blocks at transplanting.

Greenhouse Experiment

Experiments were established in the greenhouse facilities of the W.D. Holley Plant Environment Research Center, Colorado State University, Fort Collins, CO, U.S.A. in 2001 and 2002. Greenhouse structural components and irrigation systems were surface sanitized prior to and between experiments with quaternary ammonia. The three *T. harzianum* strains were compared in their ability for controlling FORL attack to greenhouse tomato plants grown in two different soilless media. Seeds of tomato *(Lycopersicon esculentum* Mill., cultivar Caruso) surface-sterilized by immersion in 1% sodium hypochlorite for 30 min. and rinsed were sown in rockwool cubes (4cm x 4 cm x 4cm) placed into a propagation flat and holes were filled with vermiculite. The tomato seedlings were transferred to rockwool blocks after two weeks. The seedlings were watered by hand on daily basis and complete nutrient solution was applied with each

watering. The nutrient solution consisted of CHEM-GRO™ tomato formula (Hydro-Gardens, Colorado Springs, CO, U.S.A), calcium nitrate and magnesium sulfate. CHEM-GRO™ tomato formula contains 4% N, 18% P_2O_5, 38% K_2O, 0.80 % Mg, 0.20, B, 0.05% Cu, 0.40% Fe, 0.40% Mn, 0.01% Mo, 0.05% Zn, and 2% Cl. Electrical conductivity of the solution was maintained between 1.5-2.0 mS/cm. Nutrient solution was adjusted to pH 6.2-6.5. Transplanting to slabs took place 5 weeks later. Each slab contained three tomato plants spaced about 30 cm apart. Each plant was irrigated by a single drip irrigation emitter after transplanting. Temperature was maintained at 18°C night and 25°C day. Irrigation and temperature were controlled by Microstep/SA Computer System (Wadsworth Control Systems, Inc., Arvada, CO, U.S.A.). Tomato plants were trained to a single stem and supported by twine to an overhead wire. All lateral branches or suckers were removed when they are 3 cm to 7 cm long. The above cultural practices emulate commercial practice in Colorado. Yellow sticky cards were used to detect insects in the greenhouse. Insects, such as western flower thrips and greenhouse white fly, were controlled using biological control agents. *Encarsia Formosa* (Hymenoptera, Aphelinidae) was used to control white flies. Biological control with the parasitoid *E. formosa* is now used commercially in 90% of the tomato growing areas in the Netherlands and in many other countries (Van Roermund et al., 1997). Conserve SC (0.5 ml/L) was used to control thrips and some other insects. Conserve, a spinosyn insecticide, is the newest, and most effective, thrips control product for the greenhouse market. Spinosad is originally isolated from an organism found in soil samples taken in 1982. In 1988, the organism was identified as a new bacterium species, *Saccharopolyspora spinosa* and belongs to the order Actinomycetales (Anonymous,

1998). The name "spinosad" comes from the active chemicals in the insecticide known as spinosyns. The insecticide is not readily absorbed across the insect integument. Its main effect then is through ingestion, carrying with its effective use all the requirements of good spray coverage (Salgado, 1997). The actual mode-of-action of spinosad has recently been determined. It acts on the insect nervous system at the nerve synapse (Salgado, 1997).

Spore suspensions of *T. harzianum* strains T95 and T22 (10^6 and 10^7 conidia/ml) , prepared as previously described, were inoculated once, either by soaking the rockwool cubes at sowing or by soaking the rockwool blocks at transplanting when the seedlings were 5 weeks old. PlantShiled^{TM} was applied once to rockwool cubes at sowing in the amount and concentration (0.5-1.0g/L) as per the label. Treatments were summarized in Table 3.1.

Table 3.1.Treatments

Treatment	Description
1	*T. harzianum* T95 (10^6 conidia/ml) at seeding + FORL.
2	*T. harzianum* T95 (10^7 conidia/ml) at seeding + FORL
3	*T. harzianum* T95 (10^6 conidia/ml) at transplanting + FORL.
4	*T. harzianum* T95 (107 conidia/ml) at transplanting + FORL
5	*T. harzianum* T22 (10^6 conidia/ml) at seeding + FORL.
6	*T. harzianum* T22 (107 conidia/ml) at seeding + FORL
7	*T. harzianum* T22 (10^6conidia/ml) at transplanting + FORL.
8	*T. harzianum* T22 (107 conidia/ml) at transplanting + FORL
9	PlantShield + FORL
10	*T. harzianum* T95 alone
11	*T. harzianum* T22 alone
12	PlantShield alone
13	FORL alone
14	Untreated control

The pathogen, *F. oxysporum* f.sp. *radicis-lycopersici*, was added to the rockwool blocks in inoculation level of 10^6 conidia/ml two weeks after transplanting. Plants were observed regularly for visible disease symptoms of the disease. In diseased tomato plants, symptoms worsened over the time, but none of the plants died. The populations of the biocontrol agents were not monitored over time. However, samples of drainage water (100ml) from each slab inoculated with the pathogen were collected three weeks after inoculation for determination of the presence of the pathogen in the effluent. FORL of tomato was evaluated 9 weeks after the inoculation of the pathogen for disease incidence and disease severity. Disease incidence was calculated as follows:

Incidence (%) = (No. of plants infected by the pathogen / No. of total plants) x 100

Disease severity was determined by using a rating scale of 0 to 3 where 0=no disease and 3=50 to 100% internal necrosis of root system 10 to 15 cm up the stem from the crown (Datnoff et al., 1995). The mean percentage of severity for each numerical rating was used for estimating the differences between treatments. Yield was also recorded to measure response of the tomato plants to the biological control agents. Fruits were harvested when approximately 60-90% of the fruits were pink to red, and the fruits were sized and graded according to the U.S. standard of small (54-58 mm), medium (58-64 mm), large (64-73 mm) and extra-large (73-88 mm) (Jones, 1999).

In vitro evaluation of mycelium growth and of biocontrol activity

All fungi were grown on potato dextrose agar (Difco Laboratories, Detroit, Mich.) at 25°C for 3 days until the mycelium reached the edge of the agar plate. A 9-mm plug of mycelia from one-week old pure cultures of FORL or *T. harzianum* strains T22 and T95 was removed from the outer edge of the mycelium with a sterilized cork borer, and the

plug was transferred to the center of a fresh potato dextrose agar plate. Measurements were taken each day of the distance from the plug to the edge of the mycelium. Growth rate of the fungi was expressed as mm/72h. When biocontrol activity was tested, both FORL and *T. harzianum* strains were seeded in the same dish at opposite sides (dual cultures), and their growth was evaluated as above. Controls were performed seeding each fungus against itself. Results correspond to the mean of two different experiments with three replicates. At the end of the incubation period, radial growth was measured from the edge of the plug to the edge of the growing colony. Radial growth reduction was calculated in relation to growth of the control as follows:

$$\frac{C}{T} \times 100 = \%\, Inhibition of\ radial\ mycelial\ growth$$

Where C is radial growth measurement of the pathogen in control and T is radial growth of the pathogen in the presence of *T. harzianum* strains (Sivakumar et al. 2000).

Experimental design and data analysis

The design for efficacy tests was a factorial design with three replicates of each treatment. Rockwool and Coconut coir were the main plots. Each subplot consisted of a slab of growing medium (rock wool or coconut-fiber) with 91cm long x 18cm wide x 7.5 cm deep encased in opaque plastic film. Three tomato seedlings were planted to each slab. Table 1 describes the different treatments employed in the greenhouse experiment. Data were analyzed by analysis of variance (ANOVA) and the means were separated by using Fisher's LSD tests at alpha values of 0.05. Statistical analyses were conducted using the general linear models procedure of SAS Version 8e (SAS Institute Inc., Cary, NC, U.S.A.).

RESULTS

Greenhouse Experiment

Inoculation of tomato plants caused symptoms similar to natural infections described by Gabor and Wiebe (1997). The analysis of variance of data resulted in significant differences in treatment effects at $P \leq 0.05$. In this study we found all three strains of *T. harzianum* tested gave significant control of FCRR. In general, the treatments applied at transplanting resulted in more disease control than those applied at seeding. In 2001 experiments, when *T. harzianum* strains were applied at transplanting, Fusarium crown and root rot incidence of greenhouse-grown tomatoes was reduced up to 79% in coir slabs and up to 73% in rockwool slabs (Table 3.2). The highest mean incidence of FCRR in tomato plants grown in coir and rockwool slabs (61%, 70% respectfully) was observed in the control seedlings grown in the presence of the pathogen alone. The effects different treatments on disease severity 9 weeks after inoculation with the pathogen are illustrated in Table 3.4. *T. harzianum* strains significantly reduced ($P \leq 0.005$) disease severity in tomato plants grown in coir and rockwool slabs (45%, 48% respectfully) compared with untreated plants. Maximum disease control was obtained with T22 and T95 applied at transplanting.

Treatments that resulted in disease control also produced a significant yield increase ($P \leq 0.05$). The highest yield improvement was recorded in plots where *T. harzianum* strains have been applied at transplanting at the inoculum level of 10^7conidia/ml. T22 and T95 increased fruit yield 37% for coir and 25% for rockwool on tomato control in the presence of the pathogen with untreated control (Table 3.6). There was no significant fruit yield difference between rockwool and coir slabs (Table 3.8).

In 2002 experiments, when *T. harzianum* strains were applied at transplanting, Fusarium crown and root rot incidence of greenhouse-grown tomatoes was reduced up to 75% in coir slabs and up to 69% in rockwool slabs (Table 3.3). The highest mean incidence of FCRR in tomato plants grown in coir and rockwool slabs (72%, 77% respectfully) was observed in the control seedlings grown in the presence of the pathogen alone. The effects different treatments on disease severity 9 weeks after inoculation with the pathogen are illustrated in Table 3.5. *T. harzianum* strains significantly reduced ($P \leq 0.005$) disease severity in tomato plants grown in coir and rockwool slabs (49%, 52% respectfully) compared with untreated plants. Maximum disease control was obtained with T22 and T95 applied at transplanting as in the previous year.

Treatments that resulted in disease control produced a significant yield increase ($P \leq 0.05$) in the year of 2002 too. The highest yield improvement was recorded in plots where *T. harzianum* strains have been applied at transplanting at the inoculum level of 10^7conidia/ml. T22 and T95 increased fruit yield 35% for coir and 29% for rockwool on tomato control in the presence of the pathogen with untreated control (Table 3.7). There was no significant fruit yield difference between rockwool and coir slabs (Table 3.12 and 3.13) in both years. Yields of fruit in all size categories were influenced by biological control treatments (Table 3.8, 3.9, 3.10, and 3.11). In both growing media, differences between all treatments were detected for small and medium fruit sizes in presence and absence of the pathogen. In rockwool, percentage of large and extra-large fruit was higher with biological control treatments in the presence of the pathogen, but not in the absence of the pathogen in both years (Table 3.10 and 3.11). Same result was observed with the coir slabs in the year of 2001; however, percentage extra-large fruit was higher

with biological control treatments in the presence and absence of the pathogen in 2002 (Table 3.8 and 3.9). This finding suggests that *T. harzianum* strains used in this experiment act not only biological control agents, but also growth promoters.

The experiment was repeated the following year, in the same greenhouse. Disease development in Fusarium-treated plots was more rapid than during the previous year, but final disease indices were similar in both experiments (Table 3.2 and 3.3). In General, the results in 2002 were consistent with the results of the experiments carried out in 2001.

In vitro experiment

In the antagonism tests using *Trichoderma spp.* against FORL in culture, *Trichoderma* completely filled the plate and surrounded the FORL colony in all cases. None of the *T. harzianum* strains tested significantly developed an antagonistic zone against FORL compared with the control. The pathogen and biocontrol agents have reached to each other and stop at that point. It appears that the *T. harzianum* strains tested here were faster growing than FORL at 25°C, and out-compete it for space and food resources (Figure 3.1 and Figure 3.2).

Table 3.2. Effect of *Trichoderma harzianum* strains on incidence of Fusarium crown and root rot tomato plants grown in coir and rockwool slabs, 2001

Treatment	Disease incidence[1] (%)	
	Coir	Rockwool
T. harzianum T95 (106 conidia/ml) at seeding + FORL.	38.58 bc[2]	44.31 bc
T. harzianum T95 (107 conidia/ml) at seeding + FORL	38.37 bc	44.81 b
T. harzianum T95 (106 conidia/ml) at transplanting + FORL.	38.17 c	22.97 d
T. harzianum T95 (107 conidia/ml) at transplanting + FORL	19.41 e	19.58 d
T. harzianum T22 (106 conidia/ml) at seeding + FORL.	43.03 b	37.98 c
T. harzianum T22 (107 conidia/ml) at seeding + FORL	31.08 d	49.70 b
T. harzianum T22 (106 conidia/ml) at transplanting + FORL.	15.72 ef	20.95 d
T. harzianum T22 (107 conidia/ml) at transplanting + FORL	13.12 f	19.24 d
PlantShield + FORL	38.45 bc	44.15 bc
T. harzianum T95 alone	0.00 g	0.00 e
T. harzianum T22 alone	0.00 g	0.00 e
PlantShield alone	0.00 g	0.00 e
FORL alone	60.96 a	70.13 a
Untreated control	0.00 g	0.00 e

[1] Incidence was determined based on the number of plants exhibiting symptoms of FCRR.

[2] Numbers in a column followed by the same letter are not significantly different (P =0.05) according to Fisher's LSD test.

Table 3.3. Effect of *Trichoderma harzianum* strains on incidence of Fusarium crown and root rot tomato plants grown in coir and rockwool slabs, 2002

Treatment	Disease incidence[1] (%)	
	Coir	Rockwool
T. harzianum T95 (10^6 conidia/ml) at seeding + FORL.	56.3 b	65.0 b
T. harzianum T95 (10^7 conidia/ml) at seeding + FORL	60.3 b	64.0 b
T. harzianum T95 (10^6 conidia/ml) at transplanting + FORL.	44.0 c	27.7 c
T. harzianum T95 (10^7 conidia/ml) at transplanting + FORL	19.0 e	26.7 c
T. harzianum T22 (10^6 conidia/ml) at seeding + FORL.	65.0 ab	73.3 a
T. harzianum T22 (10^7 conidia/ml) at seeding + FORL	60.0 b	75.0 a
T. harzianum T22 (10^6 conidia/ml) at transplanting + FORL.	28.7 de	30.0 c
T. harzianum T22 (10^7 conidia/ml) at transplanting + FORL	27.3 de	23.7 c
PlantShield™ + FORL	36.7 cd	26.0 c
T. harzianum T95 alone	0.0 f	3.3 d
T. harzianum T22 alone	0.0 f	0.0 d
PlantShield™ alone	0.0 f	0.0 d
FORL alone	71.7 a	77.0 a
Untreated control	3.3 f	3.3 d

[1] Incidence was determined based on the number of plants exhibiting symptoms of FCRR.
[2] Numbers in a column followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD test.

Table 3.4. Effect of *Trichoderma harzianum* strains on severity of Fusarium crown and root rot tomato plants grown in coir and rockwool slabs, 2001

Treatment	Disease severity[1] (1-3)	
	Coir	Rockwool
T. harzianum T95 (106 conidia/ml) at seeding + FORL.	1.21 ab[2]	1.17 bc
T. harzianum T95 (107 conidia/ml) at seeding + FORL	1.16 abc	0.99 d
T. harzianum T95 (106 conidia/ml) at transplanting + FORL.	1.10 bc	1.02 cd
T. harzianum T95 (107 conidia/ml) at transplanting + FORL	0.99 cd	0.79 e
T. harzianum T22 (106 conidia/ml) at seeding + FORL.	1.26 ab	1.31 ab
T. harzianum T22 (107 conidia/ml) at seeding + FORL	1.23 ab	1.32 ab
T. harzianum T22 (106 conidia/ml) at transplanting + FORL.	0.79 de	0.88 de
T. harzianum T22 (107 conidia/ml) at transplanting + FORL	0.74 e	0.81 e
PlantShield + FORL	1.06 bc	0.89 de
T. harzianum T95 alone	0.00 f	0.00 f
T. harzianum T22 alone	0.00 f	0.00 f
PlantShield alone	0.00 f	0.00 f
FORL alone	1.34 a	1.41 a
Untreated control	0.00 F	0.00 f

[1] Disease severity was recorded 9 weeks after the inoculation of the pathogen, using a scale from 1 to 3 (see Materials and Methods).

[2] Numbers in a column followed by the same letter are not significantly different (P =0.05) according to Fisher's LSD test.

Table 3.5. Effect of *Trichoderma harzianum* strains on severity of Fusarium crown and root rot tomato plants grown in coir and rockwool slabs, 2002

Treatment	Disease severity[1] (0-3)	
	Coir	Rockwool
T. harzianum T95 (10^6 conidia/ml) at seeding + FORL.	1.47 a-c	1.47 b-d
T. harzianum T95 (10^7 conidia/ml) at seeding + FORL	1.30 b-e	1.37 b-e
T. harzianum T95 (10^6 conidia/ml) at transplanting + FORL.	1.16 b-e	1.20 c-e
T. harzianum T95 (10^7 conidia/ml) at transplanting + FORL	0.90 e	1.13 de
T. harzianum T22 (10^6 conidia/ml) at seeding + FORL.	1.53 ab	1.67 ab
T. harzianum T22 (10^7 conidia/ml) at seeding + FORL	1.40 a-d	1.60 a-c
T. harzianum T22 (10^6 conidia/ml) at transplanting + FORL.	1.03 de	1.33 b-e
T. harzianum T22 (10^7 conidia/ml) at transplanting + FORL	0.97 e	0.97 e
PlantShield™ + FORL	1.10 c-d	1.10 de
T. harzianum T95 alone	0.00 f	0.20 f
T. harzianum T22 alone	0.00 f	0.00 f
PlantShield™ alone	0.00 f	0.00 f
FORL alone	1.76 a	2.00 a
Untreated control	0.20 f	0.23 f

[1] Disease severity was recorded 9 weeks after the inoculation of the pathogen, using a scale from 0 to 3 (see Materials and Methods).

[2] Numbers in a column followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD test.

Table 3.6. Effect of *Trichoderma harzianum* strains on yield of tomato plants grown in coir and rockwool slabs, 2001

Treatment	Yield (kg/plant)	
	Coir	Rockwool
T. harzianum T95 (106 conidia/ml) at seeding + FORL.	2.42 bc*	2.22 abc
T. harzianum T95 (107 conidia/ml) at seeding + FORL	2.51 bc	2.30 abc
T. harzianum T95 (106 conidia/ml) at transplanting + FORL.	2.75 abc	2.27 abc
T. harzianum T95 (107 conidia/ml) at transplanting + FORL	2.31 bc	2.75 abc
T. harzianum T22 (106 conidia/ml) at seeding + FORL.	2.04 c	2.13 c
T. harzianum T22 (107 conidia/ml) at seeding + FORL	2.06 c	2.19 bc
T. harzianum T22 (106 conidia/ml) at transplanting + FORL.	2.78 abc	2.40 abc
T. harzianum T22 (107 conidia/ml) at transplanting + FORL	2.99 ab	2.71 abc
PlantShield + FORL	2.47 bc	2.28 abc
T. harzianum T95 alone	2.60 bc	2.65 abc
T. harzianum T22 alone	2.40 bc	2.69 abc
PlantShield alone	3.45 a	3.04 ab
FORL alone	2.17 c	2.16 c
Untreated control	2.73 abc	3.06 a

* Values of each column followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD test.

Table 3.7. Effect of *Trichoderma harzianum* strains on yield of tomato plants grown in coir and rockwool slabs, 2002

Treatment	Yield (kg/plant)	
	Coir	Rockwool
T. harzianum T95 (10^6 conidia/ml) at seeding + FORL.	1.96 cd	2.02 b-d
T. harzianum T95 (10^7 conidia/ml) at seeding + FORL	2.04 b-d	2.19 b-d
T. harzianum T95 (10^6 conidia/ml) at transplanting + FORL.	2.11 a-d	2.23 a-d
T. harzianum T95 (10^7 conidia/ml) at transplanting + FORL	2.42 a-c	2.27 a-d
T. harzianum T22 (10^6 conidia/ml) at seeding + FORL.	1.96 cd	1.83 cd
T. harzianum T22 (10^7 conidia/ml) at seeding + FORL	1.99 cd	1.85 cd
T. harzianum T22 (10^6 conidia/ml) at transplanting + FORL.	2.28 a-d	2.21 b-d
T. harzianum T22 (10^7 conidia/ml) at transplanting + FORL	2.30 a-d	2.35 a-c
PlantShield™ + FORL	2.24 a-d	2.27 a-d
T. harzianum T95 alone	2.56 ab	2.48 ab
T. harzianum T22 alone	2.60 a	2.44 ab
PlantShield™ alone	2.41 a-c	2.75 a
FORL alone	1.79 d	1.82 cd
Untreated control	2.42 a-c	2.31 a-c

* Values of each column followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD test.

Table 3.8. Effects of *T. harzianum* strains on fruit size of tomato grown in coir slabs, 2001

Treatment	Fruit size distribution[1] (%)			
	Small (54-58 mm)	Medium (58-64 mm)	Large (64-73 mm)	X-large (73-88 mm)
1	18.7 d	26.7 ab	29.6 de	25.0 abc
2	22.4 bc	27.1 ab	28.2 e	22.3 c
3	18.0 d	24.3 abc	32.3 cde	25.3 abc
4	18.9 cd	18.7 de	38.9 de	23.5 bc
5	22.6 b	25.0 abc	29.0 cde	23.3 bc
6	18.8 cd	21.5 cde	34.5 bcd	25.3 abc
7	16.7 d	19.9 de	37.8 abc	25.5 abc
8	18.0 d	19.9 de	39.6 ab	22.5 c
9	16.0 de	21.8 cd	36.4 bc	25.8 abc
10	11.8 f	18.0 e	43.5 a	26.6 ab
11	13.1 ef	23.7 bc	39.8 ab	23.4 bc
12	10.2 f	19.1 de	42.5 a	28.3 a
13	26.3 a	27.9 a	27.7 e	18.1 d
14	12.9 ef	19.1 de	40.0 ab	28.0 a

[1] Percent fruit size distribution was calculated dividing number of small, medium, large or x-large fruits by total number of fruit from each treatment and multiplying the result by 100.

[2] Values of each column followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD test.

Table 3.9. Effects of *T. harzianum* strains on fruit size of tomato grown in coir slabs, 2002

Treatment	Fruit size distribution[1] (%)			
	Small (54-58 mm)	Medium (58-64 mm)	Large (64-73 mm)	X-large (73-88 mm)
1	22.6 bcd	27.7 abc	32.0 efg	17.8 ef
2	23.9 abc	28.9 ab	29.0 fg	18.2 ef
3	18.5 efg	25.2 cd	35.4 cde	20.9 cde
4	19.0 efg	17.9 fg	39.5 abcd	23.6 abc
5	24.7 ab	29.0 ab	27.0 g	19.3 def
6	23.2 bc	23.6 de	34.1 def	19.1 def
7	16.0 gh	25.8 bcd	36.4 cde	21.8 bcd
8	16.7 fgh	20.8 ef	37.7 bcd	24.8 ab
9	19.6 def	20.0 f	36.5 cde	23.9 abc
10	13.8 h	16.0 g	44.5 a	25.6 a
11	16.5 fgh	17.5 fg	42.3 ab	23.7 abc
12	21.0 cde	16.0 g	39.6 abc	23.4 abc
13	27.1 a	29.9 a	26.9 g	16.1 f
14	10.4 i	28.1 abc	40.6 abc	20.8 cde

[1] Percent fruit size distribution was calculated dividing number of small, medium, large or x-large fruits by total number of fruit from each treatment and multiplying the result by 100.

[2] Values of each column followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD test.

Table 3.10. Effects of *T. harzianum* strains on fruit size of tomato grown in rockwool slabs, 2001

Treatment	Fruit size distribution[1] (%)			
	Small (54-58 mm)	Medium (58-64 mm)	Large (64-73 mm)	X-large (73-88 mm)
1	23.7 ab	24.3 bc	32.4 def	19.6 def
2	19.7 cd	25.1 bc	34.9 cde	20.3 cdef
3	16.5 efg	23.3 cd	38.8 abc	21.4 bcde
4	17.9 efd	19.0 f	39.5 abc	23.6 abc
5	19.0 cde	24.1 bc	36.3 bcd	20.5 cdef
6	21.1 bc	25.0 bc	33.7 cdef	20.2 cdef
7	16.0 efg	22.8 cde	36.4 bcd	24.8 ab
8	15.1 fg	18.4 f	43.8 a	22.7 abcd
9	24.9 a	27.3 ab	29.7 ef	18.2 ef
10	13.4 g	18.2 f	44.7 a	23.7 abc
11	13.8 g	17.0 f	44.5 a	24.6 ab
12	16.1 efg	19.3 ef	41.9 ab	22.7 abcd
13	25.6 a	28.9 a	28.6 f	16.9 f
14	15.2 fg	20.1 def	39.4 abc	25.2 a

[1] Percent fruit size distribution was calculated dividing number of small, medium, large or x-large fruits by total number of fruit from each treatment and multiplying the result by 100.

[2] Values of each column followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD test.

Table 3.11. Effects of *T. harzianum* strains on fruit size of tomato grown in rockwool slabs, 2002

Treatment	Fruit size distribution[1] (%)			
	Small (54-58 mm)	Medium (58-64 mm)	Large (64-73 mm)	X-large (73-88 mm)
1	16.0 ef	21.8 a	36.4 def	25.8 a
2	19.4 bcd	21.7 a	36.1 def	22.8 ab
3	20.1 bc	19.7 ab	34.1 ef	26.2 a
4	12.5 gh	16.1 c	46.1 ab	25.3 a
5	16.9 ed	21.1 a	38.4 cdef	23.6 ab
6	19.0 cd	17.9 bc	39.5 bcde	23.6 ab
7	22.1 b	15.9 c	36.1 def	25.9 a
8	12.8 gh	17.7 bc	46.4 a	23.0 ab
9	12.0 gh	21.5 a	42.9 abcd	23.6 ab
10	13.8 fgh	16.0 c	44.5 abc	25.6 a
11	11.3 hi	16.0 c	47.3 a	25.4 a
12	14.8 efg	20.3 ab	38.2 cdef	26.7 a
13	28.4 a	19.6ab	31.9 f	20.1 b
14	9.0 i	16.5 c	49.3 a	25.2 a

[1] Percent fruit size distribution was calculated dividing number of small, medium, large or x-large fruits by total number of fruit from each treatment and multiplying the result by 100.

[2] Values of each column followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD test.

Table 3.12. Effect of the growing media on tomato yield, 2001

Treatment	Yield (kg/plant)	Yield (t/ha)
Coconut coir slabs	2.9 a*	171.5 a
Rockwool slabs	2.8 a	167.5 a

* Values of each column followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD test.

Table 3.13. Effect of the growing media on tomato yield, 2002

Treatment	Yield (kg/plant)	Yield (t/ha)
Coconut coir slabs	2.50 a*	150.0 a
Rockwool slabs	2.45 a	147.0 a

* Values of each column followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD test.

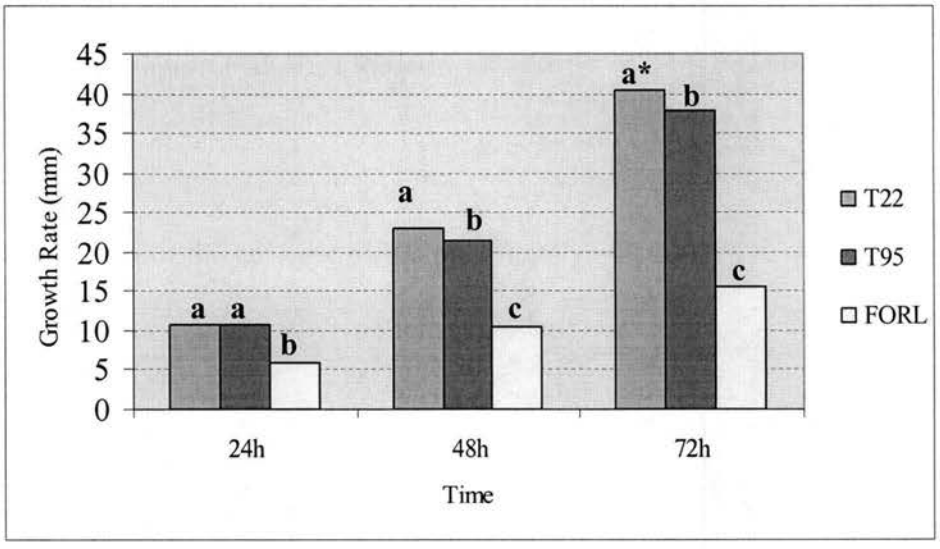

Figure 3.1. Radius of colonies of *T. Harzianum* strains and FORL grown on PDA.
* Treatments followed by the same letter are not significantly different (P=0.05) according to Fisher's LSD test.

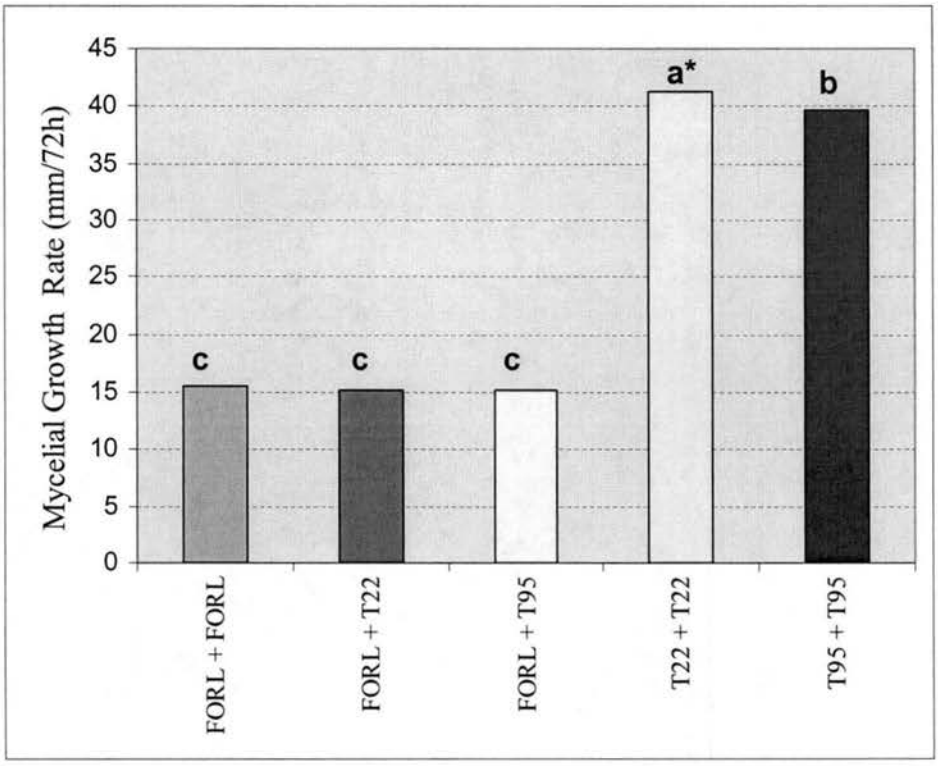

Figure 3.2. Mycelium growth and biocontrol activity of *T. harzianum* strains.
* Treatments followed by the same letter are not significantly different *(P*=0.05) according to Fisher's LSD test.

DISCUSSION

Fusarium crown and root rot has been one of the most serious soilborne diseases of tomato in the U.S for the last couple of decades. *Fusarium oxysporum f.* sp. *radicis-lycopersici* colonizes soil most aggressively when competing microorganisms have been eliminated through soil sterilization making it an excellent candidate for managemant through biological control (Marois and Micthel, 1981). In the recent years there has been an increasing interest in the use of *Trichoderma spp.* for controlling root diseases caused by number of plant pathogens (Sivan et al., 1984; Chet, 1987; Jin et al., 1991; Coley-Smith et al., 1991; Datnoff et al., 1995), especially in the greenhouse systems. The controlled environmental conditions, the increasing restrictions on the use of chemical

pesticides, and the high commercial value of the commodities provide favorable circumstances for the use of biocontrol strategies in greenhouse production systems (Coley-Smith et al., 1991; Naegley, 1997).

The objective of this study was to evaluate efficacy of existing biocontrol strains of *Trichoderma harzianum* including commercial formulations for controlling FCRR of tomato under greenhouse conditions. The biocontrol efficacy of *T. harzianum* strains against Fusarium crown and root rot of tomato was assessed in greenhouse micro-plots consisted of either rockwool slabs or coir slabs. Results of the present study demonstrated that susceptible tomato plants treated with *T. harzianum* strains gained increased protection against crown and root rot caused by FORL in both soilless growing media. The *T. harzianum* strains were effective in reducing the incidence and severity of Fusarium crown and root rot of tomato plants grown in rockwool and coir slabs throughout the experiments carried out in 2001 and 2002. These results are in agreement with the earlier studies. The effect of *Trichoderma* on reduction of the crown and root rot disease and on yield of tomatoes has been investigated. When *T. harzianum* T35 was applied as seed coating, crown and root rot incidence of greenhouse-grown tomatoes was reduced up to 80% by 75 days after sowing (Sivan et al., 1987). Van Steekelenburg (1991) showed that *T. harzianum* reduced the incidence and spread of Fusarium crown and root rot in rockwool-grown tomatoes. Sivan and Chet (1993) used *T. harzianum* in combination with soil sterilization and reduced rates of methyl bromide to obtained significant control of tomato crown and root rot in the field with transplants colonized by *T.harzianum* during greenhouse propagation. *T. harzianum* and *Paenibacillus macerans* significantly reduced severity of FCRR (Datnoff and Pernezny, 2001).

Mechanisms of biocontrol documented for *T. harzianum* include mycoparasitism via production of chitinases, β-1-3 glucanases and β-1-4 glucanases (Lorito et al, 1996a, 1996b), antibiotics (Sivasithamparam and Ghisalberti, 1998), competition (Elad et al., 1999), solubilization of inorganic plant nutrients (Altomare et al., 1999), induced resistance (Bailey and Lumsden, 1998), and inactivation of the pathogen's enzymes involved in the infection process (Elad et al., 1999; Elad and Kapat, 1999). Our experiments were not intended to clarify specific mechanisms by which the *T. harzianum* strains protected the host from the diseases caused by FORL. We did, however, find that none of the *T. harzianum* strains tested significantly developed an antagonistic zone against FORL compared with the control *in vitro* experiments. The results obtained from *in vitro* experiments and those reported by Louter and Edgington (1990) suggest that antibiosis does not seem to be involved in the biocontrol activity, but is has not been ruled out in the rhizosphere. We did not observed any direct contact between *T. harzianum* strains and the pathogen or apparent hyphal coiling in dual cultures. However, Cherif and Benhamou (1990) reported that a *Trichoderma sp.*, isolated from a commercially available sample of peat, was able to produced several extracellular chitinases, to degrade cell wall chitin of FORL, and ultimately to induce pathogenic cell death.

The severity of an epidemic of Fusarium crown and root rot of tomato is dependent upon the rapid proliferation of the pathogen in the treated growing media (Rowe et al., 1977). *T. harzianum* competes well with the soil microflora (Harman et al., 1989). When the *T. harzianum* strains were applied to growing media before recolonization by the pathogen could occur, they were be able to effectively occupy the

space and infection sites (Marois and Micthell, 1981). The preoccupied space and infection court sites were rendered unavailable to the pathogen. The decrease in the growth of the pathogen was due to its inability to compete in growing media recolonized by antagonists. In our study, we observed that the isolates of *T. harzianum* tested *in vitro* assays grew much faster than FORL at 25ºC. In the line of earlier studies (Rowe et al., 1977; Marois and Micthell, 1981; Marois et al., 1981; Sivan et al., 1987), the results of this investigation suggest that competition between pathogen and biocontrol agents may have played an important role in the biocontrol of FCRR. Ahmad and Baker (1987) reported that the rhizosphere competence of Trichoderma spp. was directly correlated with competitive saprophytic ability. Competition between *T.harzianum* and *F. oxysporum* on rhizosphere colonization was also demonstrated in other plants such as melon and cotton (Sivan and Chet, 1989b).

Effectiveness of microorganisms used for biocontrol to reduce a disease such as FCRR should translate into increased plant yield. *T. harzianum* strains increased yield in the presence of measurable disease. Reduction of disease by the use of *T. harzianum* strains had improved tomato yields between 6% and 37% in coir and between 2% and 29% in rockwool. However, they had no significant effect on yield in the absence of the disease compared with untreated and uninoculated control. While this result confirms some of the previous studies it is also in disagreement with others. Marois et al. (1981) were the first to demonstrate successful biological control of Fusarium crown and root rot on tomatoes with *T. harzianum.* They showed the potential of a multifungus conidial suspension including *T.harzianum* in controlling the disease in fumigated soils under field conditions. The antagonist reduced disease incidence but had no effect on tomato

yield. On the other hand, Sivan et al. (1987) reported that application of *T. harzianum* protected tomato plants against Fusarium crown and root rot and the total yield of tomatoes in the plots treated with *T.harzianum* was increased as much as 26.2% over the controls. Reduction of FCRR by the use of *T. harzianum* and *G. intraradices* had improved tomato yields between 4 to 25%, although not significantly (Datnoff et al., 1994; Datnoff et al., 1995). Similar observations were made by Nemec et al. (1996), the number and weight of large to extra-large fruit in the first harvest increased between 31 to 48 for the bacterial strain and *T. harzianum* over the control in the fumigated plots. On the other hand, tomato plants grown in coconut coir slabs resulted in same fruit yield with the plants grown in rockwool slabs in both years. The results suggest that coconut coir is a potential alternative to the rockwool as growing medium in soilless tomato culture because of its environmentally friendly feature.

In conclusion, the use of biological control agents has the potential to be useful component of integrated disease management strategy for tomatoes grown in soilless system. Biological control offers an environmentally friendly approach to the management of plant disease and can be incorporated into cultural and physical controls and limited chemical usage for an effective integrated pest management (IPM) system. Biological control can be a major component in the development of more sustainable agriculture systems.

CHAPTER IV

The Effect of the *Trichoderma harzianum* Strains on the Growth of Tomato Seedlings

INTRODUCTION

Tomato is one of the most important vegetable crops in the U.S.A. According to the agricultural statistics in 1997, total area in which tomato has been grown is185, 353 hectares. Total production of fresh and processing tomatoes is approximately 13 million tons and the estimated value of production is $1.6 billion in 1996 (Anonymous, 1997). In tomato production, the USA ranks second in the world after China. Dollar value of the production could be much higher than the amount above if we can reduce the losses due to poor growing media, poor seedlings, plant diseases and the cost for chemicals to control diseases. Plant diseases, especially root diseases, cause significant losses in tomato production. For example, soil-borne plant pathogens cause seed rot, damping-off, root rot, wilt and fruit rot, which result in an annual $4-5 billion in the United States alone (Jewell, 1987). To remain competitive with the leading countries in tomato production, growers in United States must increase yields and offset production costs.

Growing quality tomato transplants offers a number of benefits, in more economic production and convenience, to both commercial vegetable growers and home gardeners. To produce and market profitable crops, growers often depend on earliness, which can be achieved by setting out well-grown and properly aged transplants. Transplant production in containers using potting media reduces plant mortality during field establishment and gives early and uniform crop yields (McKee, 1981). By using quality transplants,

producers can insure a good stand of vegetable plants without the uncertainty of direct seeding (Courter et al., 1984).

Adding biocontrol agents into a planting mix or applying directly to the roots of transplants is an efficient, inexpensive means to provide a more vigorous transplant with disease protection when it is transplanted to the field (Nemec et al, 1996). Many saprotrophic fungi, particularly certain isolates of *Trichoderma* species, can provide plant growth promotion in the absence of any major pathogens (Whipps, 1997; Inbar et al., 1994). *Trichoderma spp.* are common inhabitants of the rhizosphere and are biological control organisms against a wide range of soilborne pathogens (Chet, 1987). Beside their biocontrol activity, *Trichoderma spp.* were found to enhance plant growth. The application of *Trichoderma* strains to the soil as biological control agents, in the greenhouse and under field conditions, not only resulted in reduced disease incidence and severity but also enhanced plant growth (Chang et al., 1986; Inbar et al., 1994; Ousley et al., 1993 Datnoff et al., 1995 and 1998; Harman and Bjorkman, 1998). Increased plant growth by *Trichoderma spp.* was also demonstrated, in the absence of pathogens, in experiments conducted autoclaved soil rooting medium (Windham et al., 1986; Kleifeld and Chet, 1992). The purpose of current experiment was to determine the effects of *Trichoderma harzianum* strains on growth of tomato seedlings under greenhouse conditions

MATERIALS AND METHODS

An experiment was conducted at in the greenhouse facilities at W.D. Holley Plant Environment Research Center, Colorado State University, Fort Collins, CO, U.S.A. to test the effect of three known *T. harzianum* strains on tomato transplant growth.

Plant material

Tomato *(Lycopersicon esculentum* Mill.) cultivar Caruso was used in the experiment. Caruso is an older beefsteak cultivar of tomato still popular with market gardeners because of its good flavor. It matures to a rich red color with an average weight of 180-225 g. Caruso, which has sparse foliage, is best adapted to fall cropping and may produce yellow-shouldered fruit under high light intensities. The seeds were provided by Hydro-Gardens, Colorado Springs, CO, U.S.A. Experiments were carried out in the greenhouse facilities of the W.D. Holley Plant Environment Research Center, Colorado State University, Fort Collins, CO, U.S.A.

Preparation of fungal inoculum

Three strains of biocontrol fungi were evaluated in this experiment. *T. harzianum* strain T95 (T95) was kindly provided by Suzanne M. Nemeth, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO. *T. harzianum* Rifai strain KRL-AG2 (PlantShield^{TM}, 1x10^7 colony forming units/g as a wettable powder) was supplied by Bioworks Inc., Geneva, N.Y.) *T. harzianum* Rifai strain 1295-22 (T-22) was derived from 14-day old cultures grown on Potato Dextrose Agar (PDA) plates incubated at 25°C. Plantshield and T22 have the same active ingredient; only difference in this study was preparation of the strains for inoculum. T22 and T95 were maintained on PDA and kept at 4°C. PlantShield^{TM} was maintained in the container provided by the manufacturing company and kept at 4°C. Fungal inoculum (10^7conidia/ml) of strain T22 and T95 was prepared by blending 2 week-old PDA-grown cultures of the fungus with sterile distilled water, straining the suspension through cheesecloth. Conidial densities in the suspension were determined by use of a

hemacytometer under a light microscope. PlantShiled™ inoculum was applied according to company protocol (0.5-1.0g/L). Inoculation was performed by dipping the roots in the appropriate microbial suspension for 30 min.

Plant growth conditions and treatments

Tomato seeds (*Lycopersicon esculentum* Mill., cultivar Caruso) sterilized in a 1% solution of (NaOCl) for 30 min and rinsed thoroughly in sterile distilled water. The seeds were then soaked in a 50 ml of suspension (10^7 conidia/ml) of each *T. harzianum* strains (T95, T22 and PlantShield™) and incubated 30 min. Control seeds were soaked in an equal volume of distilled water. Treated and untreated control seeds were directly sown into plug trays filled with Pro-Mix™ BX planting mix. Plug trays were placed on a bench in greenhouse. Seedling emergence was monitored for 14 days after seeding to determine biocontrol agent's effects on germination. 18 day-old tomato seedlings from each treatment were removed from plugs and potting mix was gently washed off of the root system. Transplant dip solution from each *T. harzianum* strain was prepared to a concentration of 10^7conidia/ml. Bare tomato transplant roots were fully submerged in the solution for 30 minutes and immediately planted into 10 cm x 10 cm square plastic pots filled with Pro-Mix™ BX planting mix. An untreated control was included to experiment. Untreated seedling roots were dipped in distilled water for 30 min. Five tomato seedlings were grown for each treatment/replication. The seedlings were watered by hand on daily basis and complete nutrient solution was applied with each watering. The nutrient solution consisted of CHEM-GRO™ tomato formula (Hydro-Gardens, Colorado Springs, CO, U.S.A), calcium nitrate and magnesium sulfate. CHEM-GRO™ tomato formula contains 4% N, 18% P2O5, 38% K2O, 0.80 % Mg, 0.20, B, 0.05% Cu,

0.40% Fe, 0.40% Mn, 0.01% Mo, 0.05% Zn, and 2% Cl.. The effects of *T. harzianum* strains on the growth of tomato seedlings were evaluated after 6 weeks from sowing. Five tomato seedlings from each treatment were removed from pots and planting mix was gently washed off of the root system. Number of leaves, shoots height, stem caliper at the soil line, shoot fresh weight, and shoot dry weight, root fresh weight, and root dry weight of tomato seedlings were recorded. Plant heights were measured from the soil line to shoot apices. Shoots and roots were dried 43°C for four days to obtain dry weight determinations (McGovern, et al., 1992).

Root colonization by the *T. harzianum* strains

Root colonization by *T. harzianum strains* (T22, T95, and PlantShiled^{TM}) was estimated in a separate experiment conducted in greenhouse again. Tomato seeds *Lycopersicon esculentum* Mill., cultivar Caruso) sterilized in a 1% bleach solution (sodium hypochloride) for 30 min and rinsed thoroughly in sterile distilled water were directly sown into 20 cm x 4 cm plastic tubes (designed especially for colonization studies) filled with Pro-Mix^{TM} BX planting mix inoculated with a spore suspension (10^7conidia/ml) of each *T. harzianum* strains prepared as previously described. An untreated control was included to the experiment too. Experiment was terminated when seedlings were 4 weeks old. Root systems were rinsed with tap water to remove potting mix particles. Root samples collected cut into small fragments (1cm-long). Surface-disinfested root fragments were transferred onto acidic PDA (5 fragments/plate), and incubated at 25°C for 5 days. The percent *Trichoderma* root colonization was recorded from the number of roots yielding at least one colony of the target organism.

Experimental design and data analysis

All tests were repeated once and included three replicates per treatment. The treatments were arranged in a randomized complete block design with five-seedling plots with three replicates of each treatment. Data were analyzed by analysis of variance (ANOVA) and the means were separated by using Fisher's LSD tests at alpha values of 0.05. Statistical analyses were conducted using the general linear models procedure of SAS Version 8e (SAS Institute Inc., Cary, NC, U.S.A.).

RESULTS AND DISCUSSION

Root colonization

There was no significant difference statistically among the strains in colonizing roots of 4-week old tomato seedlings. Root colonization of tomato seedlings grown in 10 cm x 10 cm plastic pots filled with Pro-Mix™ BX planting mix by *T. harzianum* strains T22 and T95 was usually at 100% and 93% by Plantshield™ (Table 4.1). Control plant roots had no colonization by any of the strains.

Table 4.1. Percentage root colonization of tomato seedlings by *T. harzianum* strains

Treatment	Colonization[1] (%)
Control	0 b[2]
PlantshieldTM	93 a
T. harzianum T22	100 a
T. harzianum T95	100 a

[1] The percent *Trichoderma* root colonization was recorded from the number of roots yielding at least one colony of the target organism (see Materials and Methods).
[2] Numbers in a column followed by the same letter are not significantly different (P =0.05) according to Fisher's LSD test.

One of the most important characteristics necessary for acceptance and effectiveness of biocontrol agents is their ability to survive in the environments other than their origin and colonize plants roots during certain period of time to control plant pathogens (Nemec et al., 1996). Colonization of the surface of the seeds or roots has frequently been seen to be a desirable trait for biocontrol activity (Kleifeld and Chet, 1992; Harman and Bjôrkman, 1998). In this study all three strains have maintained their populations at high levels after inoculation in the period of 4 weeks. This validates the other studies (Sivan and Chet, 1993; Nemec et al., 1996; Datnoff, and Pernezny, 1998).

Transplant growth

The potential of *Trichoderma harzianum* strains to induce increased growth of tomato transplants eas evaluated. The analysis of variance of data resulted in significant differences in treatment effects at $P \leq 0.05$. PlantShield™ significantly affected seedling emergence increasing 17% compared with control. However, T. harzianum T22 and T95 had no significant effect on emergence of tomato seedlings (Figure 4.1). The biocontrol agent strains were not better or worse than each other in the effect on number of true leaves. All the strains significantly increased true leaf number and shoot height of the seedlings compared with control. PlantShield™ did not affect stem caliper while T22 and T95 significantly increased stem caliper of tomato seedlings (Figure 4.3). T22 and T55 increased shoot fresh and dry weights (Figure 4.5 and Figure 4.6). None of the *T.harzianum* strains had a significant effect on root fresh and dry weights (Figure 4.7 and Figure 4.8). Most of these results are in aggreemet with earlier studies while some of the results (root fresh and dry weights) are in disagreement with previous studies (Windham et al., 1986; Datnoff and Pernezny, 2001).

The concept of adding biocontrol agents into a planting mix or applying directly to the roots of transplants is an efficient, inexpensive means to provide a more vigorous transplant with disease protection when it is transplanted to the field (Nemec et al., 1996). In addition to their bicontrol activities, *Trichoderma spp*. have been reported promote plant growth (Chang et al, 1986; Inbar et al., 1994; Yedidia et al., 2001). *T. harzianum* and *Paenibacillus macerans* alone or in combination significantly affected the growth of tomato transplants in the greenhouse and after outplanting into the field 30 days later. In the greenhouse, petiole numbers were increased between 6 to 9%, heights 8 to 18.8%, stem caliper 10 to 13.6%, leaf area 7 to 21%, petiole fresh weight 25 to 38% and root fresh weight 50%. In the field, petiole numbers were increased between 3 to 5%, heights 2 to 8% and stem caliper 1 to 7% (Datnoff and Pernezny, 2001).

Possible explanation of this phenomenon includes; control of minor pathogens leading to stronger growth a nutrients uptake (Ousley, et al, 1993), solubilization of insoluble minor nutrients in soil (Altomare et al., 1999) and production of growth hormones (Windham et al., 1986). *Trichoderma spp*. enhances plant growth by increasing the solubility of zinc, copper, iron, and manganese ions, all plant nutrients with low solubility. *T. harzianum also* increases plant nitrogen efficiency (BARD, 2000). *T. harzianum* 1295–22 was shown to solubilize phosphate and micronutrients that could be made available to provide plant growth (Altomare et al., 1999). Yedidia et al. (2001) reported that an increase of 90% in phosphorus (P) and 30% in iron (Fe) concentration was observed in *T. harzianum* inoculated cucumber plants. They concluded that the improvement of plant nutritional level might be directly related to a general beneficial growth effect of the root system following *T. harzianum* inoculation. The results of present study in the line of earlier studies indicated that *T. harzianum* strains had a positive effect on tomato transplant growth.

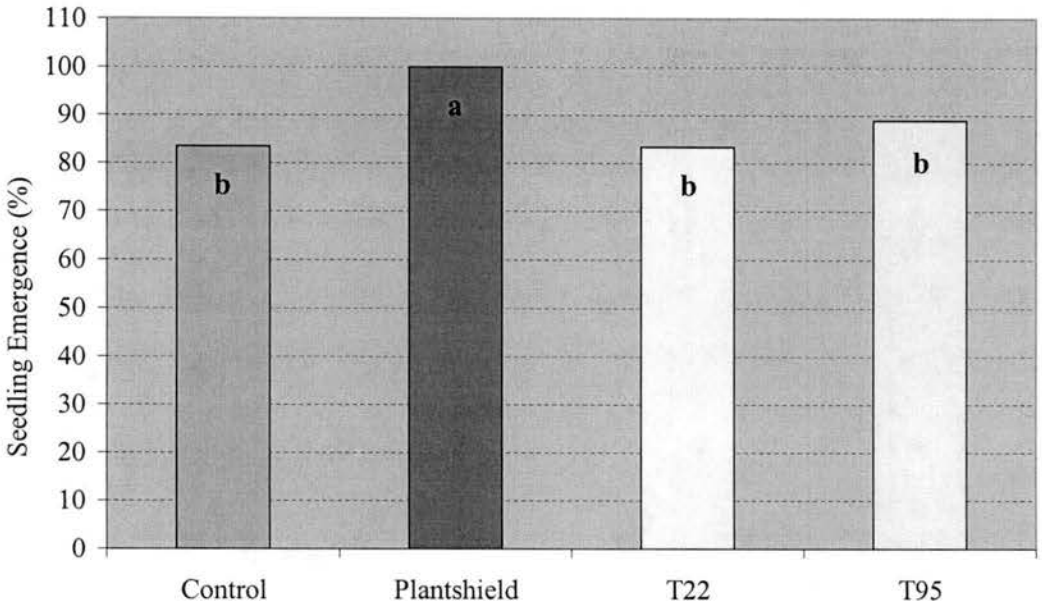

Figure 4.1. Effect of biological treatments on tomato seedling emergence. Treatments followed by the same letter are not significantly different *(P* =0.05) according to Fisher's LSD test.

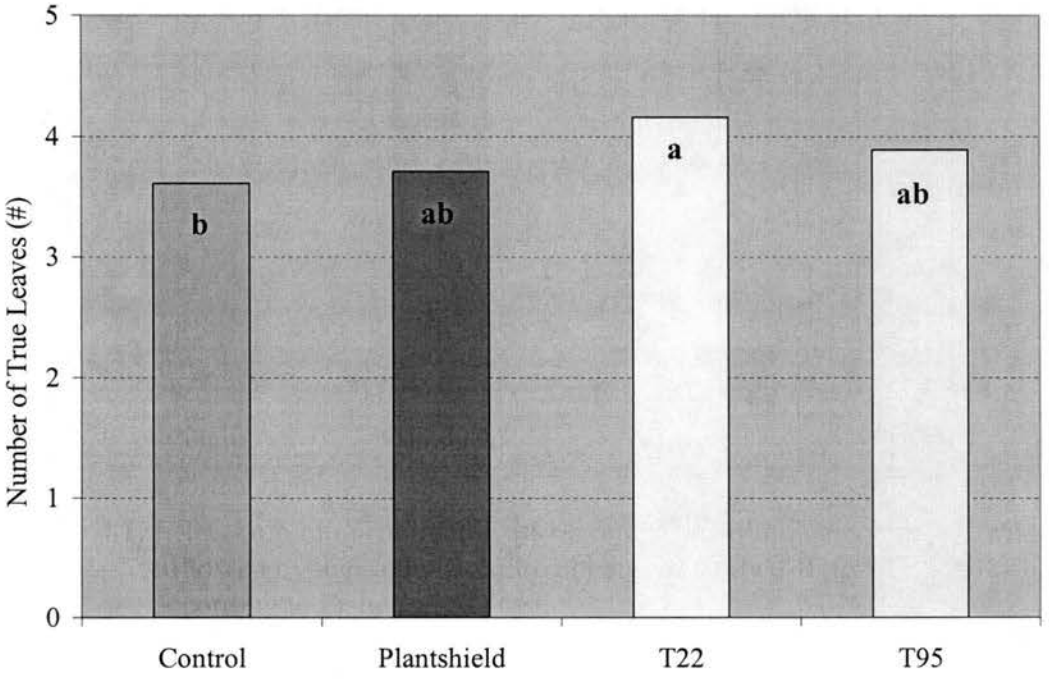

Figure 4.2. Effect of biological treatments number of true leaves of six-week old tomato transplants. Treatments followed by the same letter are not significantly different *(P*=0.05) according to Fisher's LSD test.

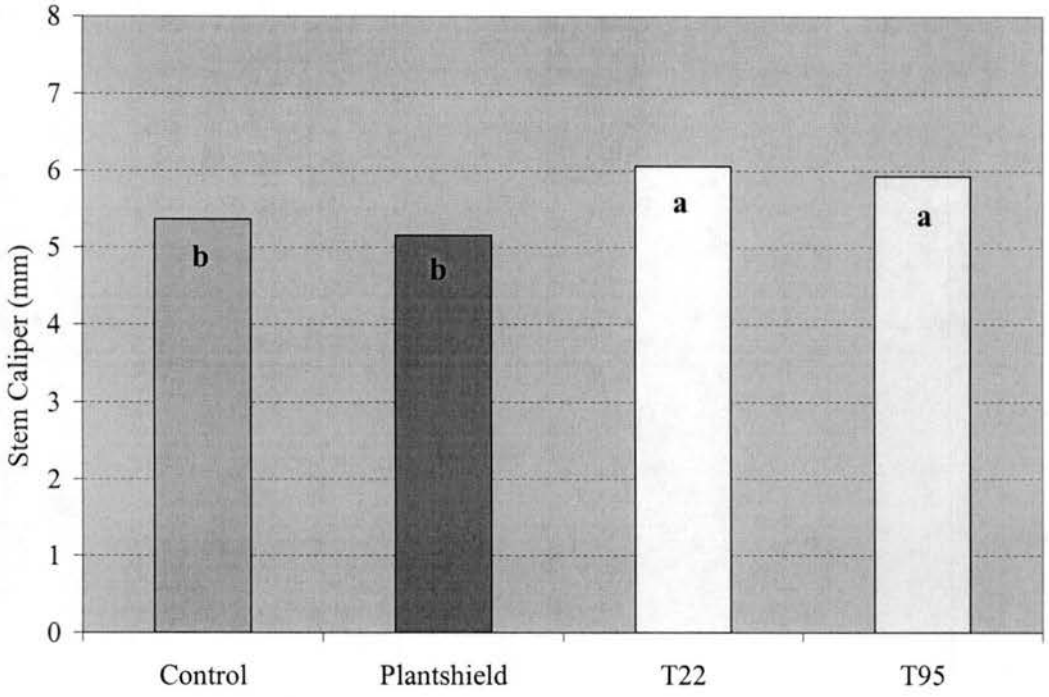

Figure 4.3. Effect of biological treatments on stem caliper of six-week old tomato transplants. Treatments followed by the same letter are not significantly different *(P*=0.05) according to Fisher's LSD test.

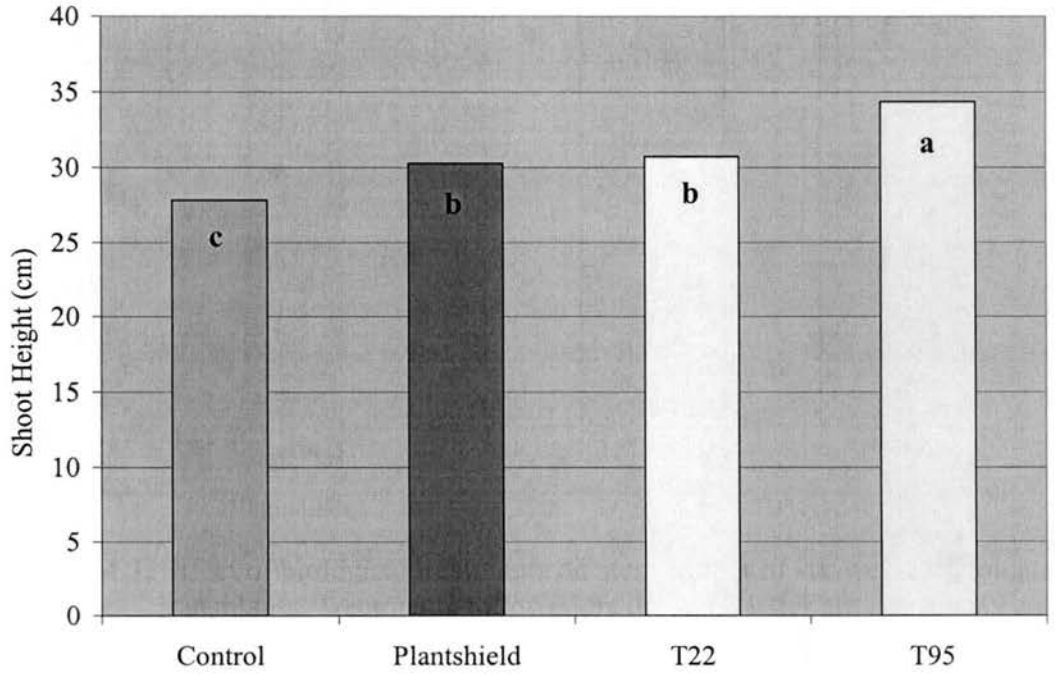

Figure 4.4. Effect of biological treatments on shoot height of six-week old tomato transplants. Treatments followed by the same letter are not significantly different *(P*=0.05) according to Fisher's LSD test.

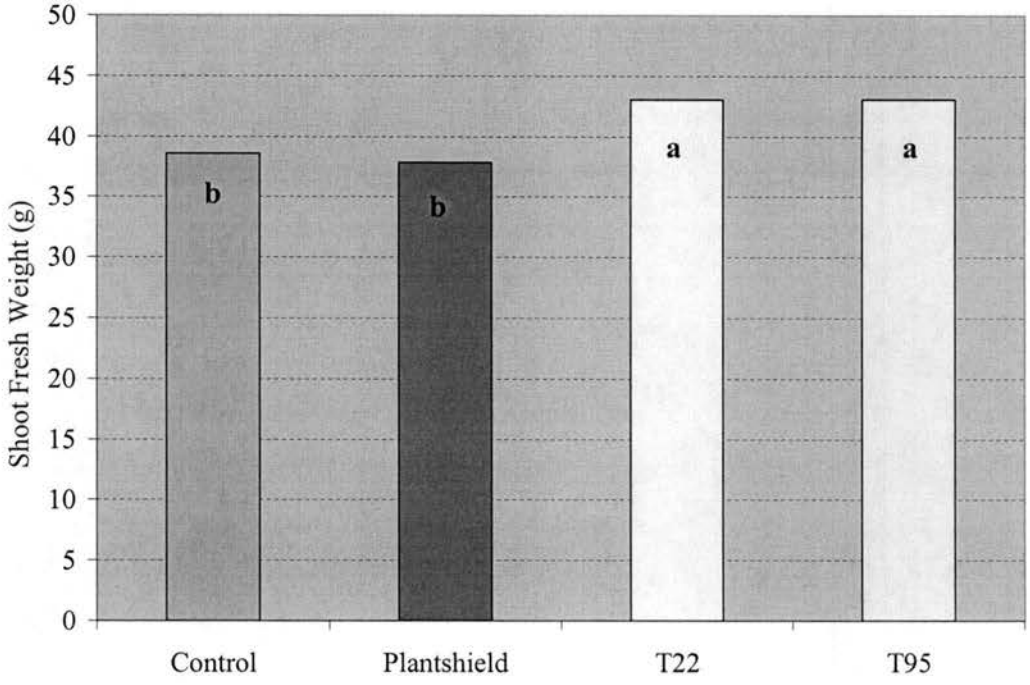

Figure 4.5. Effect of biological treatments on shoot fresh weight of six-week old tomato transplants. Treatments followed by the same letter are not significantly different *(P*=0.05) according to Fisher's LSD test.

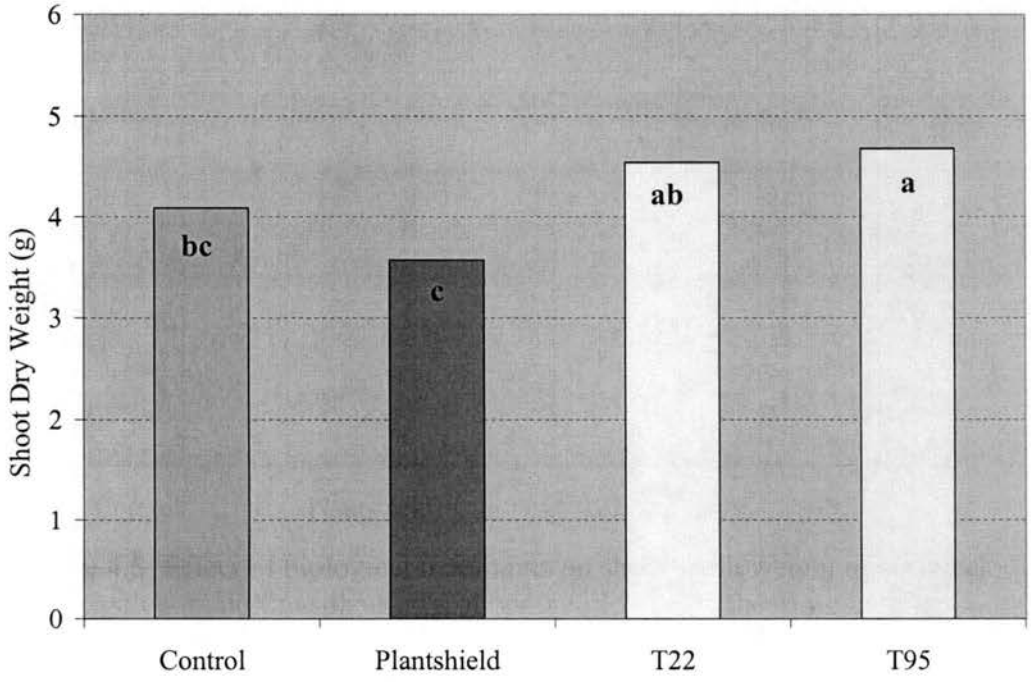

Figure 4.6. Effect of biological treatments on shoot dry weight of six-week old tomato transplants. Treatments followed by the same letter are not significantly different *(P*=0.05) according to Fisher's LSD test.

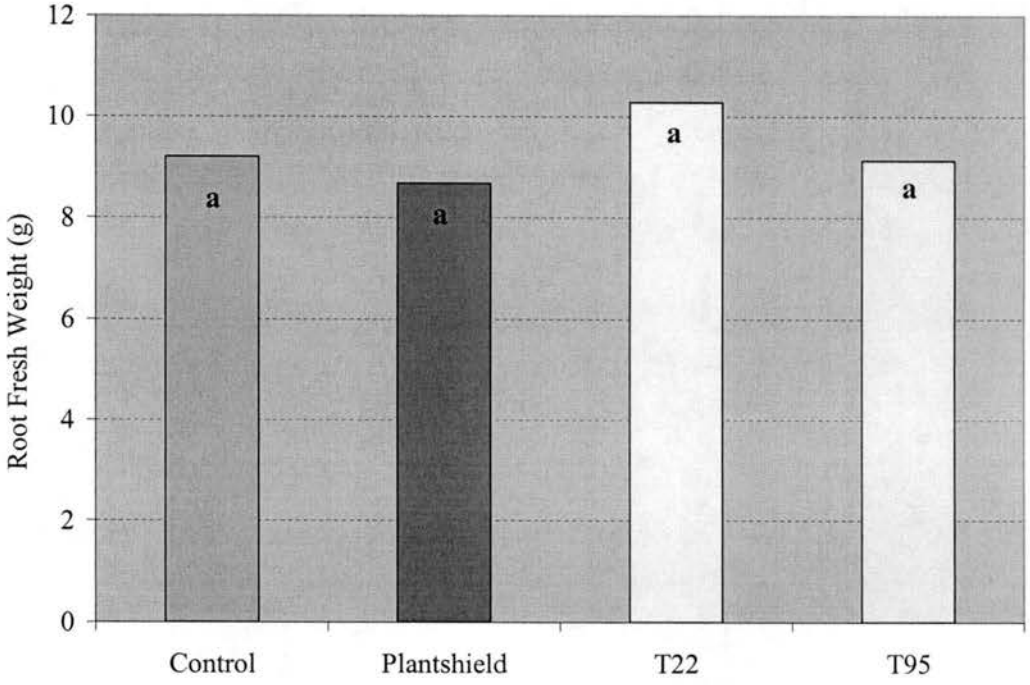

Figure 4.7. Effect of biological treatments on root fresh weight of six-week old tomato transplants. Treatments followed by the same letter are not significantly different *(P*=0.05) according to Fisher's LSD test.

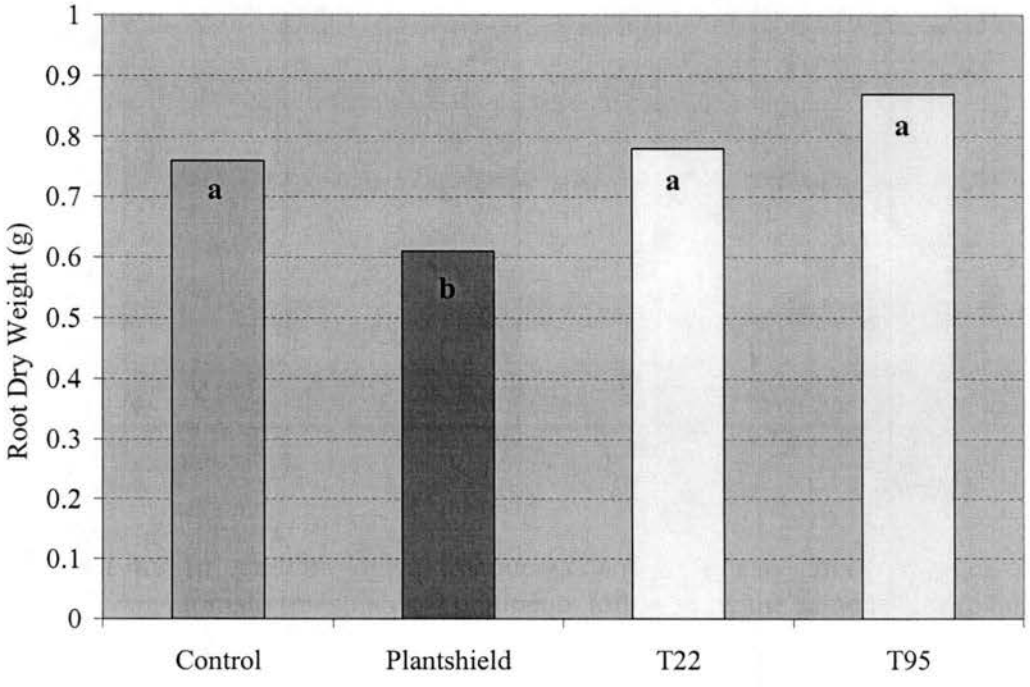

Figure 4.8. Effect of biological treatments on root dry weight of six-week old tomato transplants. Treatments followed by the same letter are not significantly different *(P*=0.05) according to Fisher's LSD test.

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