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Bioactive Natural Products from Monarda for Control of Tomato Disease

A Thesis
Presented for the
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Degree
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Sharon Elizabeth Greene
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Dedication

This thesis is dedicated to my family and friends.

It is especially for

Marvin, Mae Lou, and Angie Goins

who encouraged and believed in me.

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ABSTRACT

Environmentally-friendly methods to control plant disease are needed in order to reach the goal of sustainability in agriculture. Because diseases caused by soil-borne organisms significantly reduce crop yields, identifying environmentally-friendly methods for control of these diseases is imperative. The purpose of the research described in this thesis was to determine the impact of bioactive natural products on disease control in tomato production. The specific objectives were: 1) to determine the effect of adding herbage (dried and ground leaves and flowers) of three *Monarda* cultivars to greenhouse growth media on seedling losses caused by *Rhizoctonia solani*, and 2) to evaluate biological pesticides (alone and in various combinations) for control of *Pythium* disease. Experiments were designed as factorials with two rates of herbage, 0 or 10% (v/v) and two rates of *R. solani* inoculum, 0 or 2% (v/v) with 20 replicates in a randomized complete block design. In a second set of experiments, seedlings were transplanted into a substrate-based hydroponic system, and commercial production methods were used. The impact of herbage from three *Monarda* cultivars, one isolate of a commercial plant growth promoting rhizobacteria preparation, and one isolate of the pathogen *Pythium myriotylum* (all of which were substrate additives), as well as two isolates of *Beauveria bassiana* (seed treatment), alone and in combination was tested. Amending germination mix with herbage from 'Marshall's Delight' increased seedling height and germination above that of control regardless of *R. solani* infestation. Amendment with 'Sioux'

did not protect against *R. solani*. In experiments with *P. myriotylum*, the pathogen rarely impacted fruit quantity or weight. Herbage did not increase fruit yield over nontreated controls, and treatment with *B. bassiana* reduced yield. However, there were significant interactions among treatments; for example Grade 1 tomatoes, treatment with *P. myriotylum* and *B. bassiana* increased yield above that of treatments with *P. myriotylum* alone. Neither treatment was greater than control. Although additional research is needed, based on these results, these environmentally-friendly methods hold promise for disease control in tomatoes.

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Chapter 1

Literature Review

Rhizoctonia

Rhizoctonia solani Kühn (teleomorph – *Thanatephorus cucumeris* (Frank) Donk) is a notorious soil-inhabiting plant pathogen that is capable of attacking a wide range of host plants worldwide. *Rhizoctonia solani* causes damage on more than 142 host species worldwide including many agricultural and horticultural crops (Sneh et al., 1991). Diseases caused by *R. solani* include seed decay, damping-off of seedlings; stem canker (soreshin), root rot, and basal stem rot (foot rot). Damping-off is the most common symptom caused by *Rhizoctonia* on most plants it affects (Agrios 1997). Serious economic losses of young seedlings of several horticultural and vegetable crops have been found in both greenhouse and field production systems (Howard et al., 1994). Classification of *Rhizoctonia* has been difficult because these fungi do not produce conidia and only rarely produce basidiospores. The concept of 'hyphal anastamosis' to characterize and identify *Rhizoctonia* was reintroduced in 1969 by J. R. Parameter (Sneh et al., 1991).

Rhizoctonia solani is a common soil inhabitant and can survive as a saprophyte effectively colonizing most types of dead plant material. Environmental conditions, such as pH, temperature, moisture, competitive ability, and soil factors influence fungal survival and inoculum potential (McCarter, 1991). *Rhizoctonia solani* produces symptoms in tomatoes that are dependent on plant growth stage. Damping-off is a common problem in greenhouse production

of tomato transplants. Germinating seedlings are often killed before or soon after they emerge above the soil line. Infected seeds become soft and spongy turning brown to black in color and will eventually decay. Seeds that have germinated and become infected develop water-soaked lesions that enlarge and turn brown. The infected tissues collapse, resulting in death of the seedling. The penetration and death of seedlings before emergence is termed preemergence damping-off; this occurs mostly in cool, wet soils. This pathogen also attacks older seedlings after they have emerged (post-emergence damping-off) usually at or below the soil line but invasion is limited to the outer cortical tissues and results in a reddish brown lesion that may enlarge and girdle the stem, eventually killing the plant. The stem is constricted (i.e., wire stem) by the attack, weakened, and the plant falls over and dies. On some hosts, including tomatoes, *Rhizoctonia* can cause fruit decay and foliage blight especially when these plant parts contact the soil.

This concept implies that isolates of *Rhizoctonia* that have the ability to recognize and fuse with each other are genetically related and isolates without this ability are unrelated. Hyphal anastamosis criteria have been used extensively to place isolates of *Rhizoctonia* into taxonomically distinct groups called anastamosis groups.

Pythium

With more than 120 species distributed worldwide, the genus *Pythium* is well known as a pathogen of many economically important plants. Members of this genus are no longer considered to be true fungi. Modern biochemical and molecular analyses suggest these organisms are more closely related to algae

and higher plants and therefore are now classified in the newly established and extremely diverse kingdom Stramenopila (Paul, 2001). Species of *Pythium* are placed within the Phylum Oomycota and are commonly referred to as oomycetes.

All *Pythium* species produce white, silken, coenocytic mycelium. They reproduce asexually by sporangia; sizes and shapes of sporangia are species-dependent. *Pythium aphanidermatum* (Edson) Fitzp. and *P. myriotylum* Drechs. produce lobulate sporangia that arise from inflated lobed hyphae (McCarter, 1991). The sporangia may germinate directly to form a germ tube or may produce zoospores depending on species and environmental conditions. Zoospores are formed and released from the sporangium on the surface of the root and when released they swim in the hydroponic solution, or water surrounding the substrate, and then move toward root exudates (chemotaxis). When the zoospores contact a root, they attach, lose their flagella, encyst, form a germ tube, and penetrate the root.

Damping-off disease caused by *Pythium* spp. in vegetable crops is economically very important worldwide (Whipps and Lumsden, 1991). Most *Pythium* species infect mainly immature or succulent tissues, thus restricting their parasitism to seedlings, feeder roots, or root tips of older plants, and stem tissues or watery fruits (Hendrix and Campbell, 1973). *Pythium* species cause preemergence damping-off as they attack the seed or emerging radicals. These fungal-like organisms also infect newly emerged seedlings at the soil line, causing them to disintegrate or fall over, a common symptom of post-emergence

damping-off. According to Hendrix and Campbell (1973), if plants are attacked at a later stage (i.e., after cells of stems and main roots have developed secondary thickenings), infection is restricted to feeder roots, causing seedlings to become stunted and chlorotic. This early root rot results in decreased yields since plants often do not recover even if conditions become unfavorable for further disease development.

Pythium myriotylum is a common pathogen in the southeastern United States (Csinos and Hendrix, 1978). In the United States, *P. myriotylum* was originally described from tomato (van der Plaats-Niterink, 1981). *Pythium myriotylum* causes disease on a wide range of plant species including tomato, bean, cucumber, wheat, oats, rye, ryegrass (McCarter and Littrell, 1968) and peanut (Bell and Minton, 1973). A toxin was reported in *P. myriotylum* that caused leaf necrosis and stunting of tomato plants (Csinos and Hendrix, 1978), but there has been no confirming study. *Pythium myriotylum* and *R. solani* are antagonistic (Garren, 1970). Experiments have shown synergistic effects on plant disease between *P. myriotylum* and *Fusarium solani* (Mar.) Sacc. or *Meloidogyne arenaria* (Neal) Chitwood (Garcia & Mitchell, 1975). Infection by *P. myriotylum* is influenced by a range of factors including inoculum density, moisture, temperature, pH, and light intensity. The favorable temperature range for *P. myriotylum* is from 5 to 40 C with an optimum at 37 C.

In the past few decades, vegetable production in soilless culture has become increasingly popular worldwide (Jensen, 1999). Avoidance of root diseases was one of the main factors in the development of hydroponics, yet root

diseases still occur and disease losses can be greater than in soil (Stanghellini and Rasmussen, 1994). Inoculum is introduced into the greenhouse in soil on equipment or workers' shoes. Inoculum can be introduced on infected seed or propagation material. Peat may contain pathogens (Favrin et al., 1988). Reservoir water or surface water may contain zoosporic pathogens such as *Pythium* (Pickett-Pottorff and Panter, 1994).

Diseases caused by *Pythium* species have been described in a variety of plant species in soilless systems, including tomato (Jenkins & Averre, 1983). A wide variety of *Pythium* species have been described from greenhouse production systems (van der Plaats-Niterink, 1981). Among the most common species are *P. ultimum*, *P. aphanidermatum*, *P. irregulare*, *P. myriotylum*, *P. spinosum*, and *P. splendens* (Daughtery et al., 1995). Diseases caused by these pathogens can be destructive because of high plant densities and favorable environmental conditions for disease development. Growers have traditionally depended on preventative fungicide drenches to manage diseases in greenhouse crops caused by *Pythium* species, but no fungicides are registered for use in hydroponic systems.

Monarda didyma

The genus, *Monarda,* consists of 16 species distributed from the Rocky Mountains to the Atlantic coast and from Canada to central Mexico (Prather et al., 2002). Species are primarily perennial herbs, one species is shrubby and several are annuals. A popular species, *Monarda didyma,* is cultivated to make an herbal tea, hence, one of its common names, Oswego tea. Another common

name for *Monarda* is bee balm because the lovely fragrant flowers attract hummingbirds, bees, and butterflies in the summer. *Monarda* species also are valued for their essential oil content. There are multiple ethnobotanical uses for *Monarda* species (Vogel, 1970; Duke, 1992) (http://www.ars-grin.gov/duke), many of which are related to the bioactive properties (antibacterial, antifungal, and antioxidant) of the components of the essential oils. Some species produce high quantities of essential oils that are known to be fungicidal such as thymol, γ-terpinene, *p*-cymene, geraniol, citral linalool, and carvacrol (Mazza and Marshall, 1992). At least 56 phytochemicals with antifungal or herbicidal activity have been isolated from *M. didyma,* at least 36 have been recovered from *M. fistulosa,* at least 26 isolated from *M. punctata,* and at least 21 from *M. citriodora* (Duke, 2001).

Plant essential oils are well known for their antifungal properties. As a result, they have been proposed as natural, safe pesticides (Bauske et al., 1994; Deans, 1991; Tsao and Zhou, 2000; Thompson, 1989). Several key essential oils inhibit the growth of significant soilborne fungal pathogens. *Fusarium* and *Sclerotinia* (Bowers and Locke, 2000), *Pythium* (Bauske et al., 1994a), and *Rhizoctonia* and *Verticillium* (Pitzarokili et al., 1999) have all exhibited growth inhibition when exposed to various plant essential oils, many of which are present in *Monarda* spp.

Beauveria bassiana

Beauveria bassiana (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) is a soilborne fungal pathogen of insects. Isolates of this fungus are ubiquitous in nature and have a wide host range. This pathogen has been reported as a suppressive agent against European corn borer, *Ostrinia nubilalis* (Hübner) (Wagner and Lewis, 2000), sweet potato whitefly, *Bemisia tabaci* (Faria and Wraight, 2001), Mexican rice borer, *Eoreuma loftini* and sugarcane borer, *Diatraea saccharalis* (Legaspi et al., 2000).

Also, *B. bassiana* has the ability to colonize certain corn cultivars living in the vascular tissue as an endophyte. Tunnelling by the European corn borer is reduced in corn plants colonized by this fungus. The fungus can colonize the plant when applied as a granular formulation of conidia on foliage at whorl stage, moving internally in the plant, and persisting throughout the season to provide significant suppression of corn borers (Wagner and Lewis, 2000).

In addition to activity against insects it has been determined that *B. bassiana* 11-98 is endophytic in tomatoes (Leckie, 2002). It has also been shown that when applied as a seed treatment, *B. bassiana* effectively controls Rhizoctonia damping-off in tomato seedlings (Seth, 2001).

Induced Resistance in Plants

Induced resistance is an enhanced defense capability developed by a plant when stimulated by a necrotizing pathogen, plant-growth promoting rhizobacteria (PGPR), or other entity. Inducing a plant's own defense response is an area of growing interest for plant disease control industries. These methods

use organisms or chemicals that are environmentally benign to stimulate disease resistance. Plants acquire a state of general resistance in response to an initial stimulus; this phenomenon is termed systemic acquired resistance (SAR) (Metraux, 2001). Salicylic acid, a simple phenolic compound, is necessary for SAR regulation, but it is not the mobile signal as was once thought (Metraux, 2001). The SAR response requires a necrotizing response of the plant. Plant-growth promoting rhizobacteria are naturally occurring root-colonizing bacteria that can induce increased plant growth (Cleyet-Marcel et al., 2001; Kloepper, 1994; Glick, 1995), often with concomitant reductions in plant diseases. The PGPR induce resistance in distant portions of the plant; this is termed induced systemic response or ISR (Raupach and Kloepper,2000; Jetiyanon and Kloepper, 2002). In ISR the response is independent of salicylic acid, but requires responsiveness to the plant growth regulators, jasmonic acid and ethylene. The beneficial effects of PGPR for disease control have been reported for many crops and pathogens (Raupach et al., 1996; Raupach and Kloepper, 1998; Reddy et al., 1999; Reddy et al., 2000). Much research has been devoted to combinations of PGPR to optimize disease control, since control by any single strain is usually less than that of fungicides. "Bioyield" (Gustafson LLC, Plano, Texas) (Becker Underwood, Ames, IA) is a commercial preparation of PGPR. The PGPR products are incorporated into the planting mix used to grow transplants and contain species of spore-forming *Bacillus* strains. Treated transplants show increased shoot and root growth leading to more rapid development than untreated transplants. An ISR response is frequently

observed. Insect herbivory is altered by PGPR colonization of some plants; colonization may lead to shifts in host metabolism and alteration of defense compounds (Zehnder et al., 2001). PGPR treatment also leads to enhanced growth. Simultaneous activation of ISR and SAR results in synergistic additive protection (Pieterse et al., 2001). Some PGPR produce salicylic acid and effectively induce resistance (Audenaert et al., 2001).

Chapter 2

Introduction

In the United States, farm value and consumption of tomato (*Lycopersicon esculentum*) are second only to potato (*Solanum tuberosum*) (Lucier, et al., 2000) among vegetable crops. Greenhouse-produced tomato consumption by American consumers has grown at an explosive rate over the past ten years (DeGiglio, 2003). There are roughly 850 acres of greenhouse tomatoes grown in the United States, accounting for six percent of total tomato production. Tomato is the most important vegetable produced in greenhouses. The two major categories of tomato crops in the U.S. are fresh and processing tomatoes. Tomato cultivars are bred to serve the application of either the fresh or the processing markets. Processing tomatoes are grown to make ketchup, sauces, and tomato paste, while fresh market tomatoes are sold on the open market. With large production cost and market uncertainty, fresh market tomato prices are higher and more variable than those for processing tomatoes. California is the leading producer of all tomatoes in the U.S. with approximately a third of the fresh market and 95% of the processing tomato output. Other important tomato-producing states are Ohio, Virginia, Tennessee, South Carolina, North Carolina, and Georgia (http://www.ers.usda.gov/Briefing/Tomatoes/background.htm).

Because tobacco (*Nicotiana tabacum*) producers are decreasing or ceasing production due to quota reductions and decreased profitability, many farmers in Tennessee need opportunities to produce high-value crops. Field-grown tomatoes are an alternative for tobacco producers because growth

requirements of the two crops are similar, and they require similar equipment. Greenhouse tomatoes are also an attractive alternative for some farmers. Although soil and soilless culture are both systems used for greenhouse tomato production, in this document the term greenhouse tomatoes will be used to mean those grown in modified hydroponic culture. Greenhouse tomatoes can be grown at a time when supplies are low, and therefore, are an excellent source of high cash receipts.

Tomatoes grown in field and greenhouse systems are different. Field tomatoes have either determinate or semi-determinate growth habit. They require very little care, have a predetermined number of clusters, and inputs are low, but both quality and yields are not very high. The fruit from field tomatoes are irregular in shape. Greenhouse tomatoes have an indeterminate growth habit. They are very labor-intensive, do not have a predetermined number of flower clusters, have excellent quality, are more consistent in shape and size, and can be grown year-round.

Greenhouse tomato production is dependable and high quality products are available for extended periods of time. Greenhouse tomatoes were first introduced from the Netherlands in the 1980s creating a market that has grown from 1% of the entire fresh tomato market to over 16% today (DeGiglio, 2003). Consumers of locally grown greenhouse tomatoes are loyal consumers who demand quality and are willing to pay a higher price for better produce. Growth of farmers' markets reflects consumer preferences for farm fresh produce. The volume of produce sold at farmers' markets is small, less than 2% of overall U. S.

sales, yet the number of U.S. farmers' markets has increased by 79% since 1994 to more than 3,100 in 2002 (Kremen et al., 2004).

In 1974, 70% of U.S. production was based on soil culture and 30% on soilless culture. By 1988 a significant shift to soilless culture occurred with soil systems making up only 40% of the acreage (Hickman, 1988). One reason for the increase in popularity of soilless culture is due to the pending elimination of methyl bromide as a soil fumigant for control of soilborne pathogens. Large increases in yield of tomatoes under soilless culture over that of soil may be due to several factors such as the absence of competing weeds, more control over the environment, and the ability to space plants closer together. In areas where the soil lacks nutrients or has poor structure, soilless culture is beneficial. Hydroponics systems that use only a nutrient solution are considered water culture or solution culture. If the nutrient solution is used in combination with a solid inert substance such as rockwool, perlite, sand, or clay granules to physically support root systems and hold the nutrient solution it is considered a substrate or aggregate culture. An aggregate culture can be inert, organic, or mixed. Organic aggregate culture contains peat, sawdust, hardwood bark, or rice hulls, while a mixed culture would be peat-perlite, peat-sand-hardwood bark, or peat-clay granule mixtures.

One of the principal forces underlying the development of hydroponics was the avoidance of root diseases (Stanghellini and Rasmussen, 1994). Diseases affecting tomatoes in the field can also damage tomatoes in the greenhouse. Several characteristics of a soilless system can increase disease

potential (Paulitz, 1997). First, with monoculture the plants can be uniformly susceptible, and dense planting may favor the movement of pathogens from infected to healthy plants. Second, the physical environment may be favorable for the pathogen, especially temperature and moisture. Third, pathogens can be easily spread from one plant to another in closed systems with recirculating water. A small amount of contamination can lead to considerable infection and disease loss. Finally, growth media used in aggregate culture lacks the microbial diversity found in natural soils; therefore, the pathogen may quickly become established and cause severe disease.

The purpose of the research described in this thesis was to determine the impact of bioactive natural products on greenhouse tomatoes. The specific objectives were: 1) to determine the effect of adding herbage (dried and ground leaves and flowers) of three *Monarda* cultivars ('Elsie's Lavender', 'Marshall's Delight' and 'Sioux'), to greenhouse growth media on seedling losses caused by *Rhizoctonia solani*, and 2) to evaluate biological pesticides (alone and in various combinations) for control of *Pythium* disease in the above system.

Chapter 3

Materials and Methods

Monarda Evaluation Garden

A *Monarda* Evaluation Garden is maintained as part of The University of Tennessee Gardens. This garden contains four replicated blocks of 52 *Monarda* species or cultivars. The majority of the plants are *Monarda didyma, M. fistulosa* or hybrids of these species; a few other species (e.g., *M. citriodora*, *M. punctata*) are also planted in the garden. These plants were sampled monthly for three growing seasons to monitor essential oil content and composition, which varies with cultivar, season, plant growth stage, and plant part. Protocols designed to separate known isomers had been developed. Cultivars were evaluated on the basis of total hexane-extractable essential oils, composition of the essential oils, as well as, known antifungal activity of the essential oil components. Based on chemistry of herbage, three *Monarda* cultivars were selected for this study - 'Sioux' (collected July 4, 2001); 'Marshall's Delight' (collected June 28, 2001) and 'Elsie's Lavender' (collected August 1, 2001).

Control of *Rhizoctonia solani* with Bioactive Herbage

Inoculum was prepared according to Seth (2001); cornmeal: sand (9:300 w/w) in 500-ml Erlenmeyer flasks. Flasks containing the mix were autoclaved for 1 h on two consecutive days. One-week-old cultures of *R. solani* were flooded with sterile deionized water (6 mL). The mycelium was scraped loose, and the fungal suspension from one potato dextrose agar (PDA) plate was added to a 500-ml Erlenmeyer flask containing the cornmeal sand mix. An additional 10 ml

of sterile deionized water was added to each flask. The inoculum was incubated in a growth chamber at 28^0 C for 12 days prior to addition into the germination mix. Treatments were mixed to a final concentration of herbage, *R. solani* inoculum, and germination mix (BM2) (Berger Peat Moss, Saint-Modeste, Quebec, Canada) (10:4:86 v/v/v). Treatments were transferred to 20 x 10 plug trays (Blackmore Company, Belleville, MI). Each *Monarda* cultivar was evaluated in separate experiments. Experiments were designed as 2x2 factorials with two rates of *Monarda* herbage, 0 or 10% (v/v), and two rates of *R. solani* inoculum, 0 or 4% (v/v) with 20 replicates in a randomized complete block design. Data collected included percent germination and seedling height at seven days. After the data from 'Marshall's Delight' were collected, it was determined that a disease index should be included. The following disease index, adapted from Seth (2001), was used: 1 = no symptoms (healthy seedling), 2 = living, but diseased, 3 = emerged, then died, 4 = did not emerge. The data were analyzed for significance with the Mixed Procedure of PC-SAS; significant effects were further analyzed with a F-protected Least Significant Difference (LSD) test at P = 0.05.

Commercial Tomato Production

Greenhouse. The greenhouse used in this study was a 270-m^2 plastic structure located on the Plant Sciences Unit of the Knoxville Experiment Station. This house was equipped with a trellis support system for the growth of indeterminate cultivars of greenhouse tomatoes. The greenhouse was equipped to contain 10 rows (5 double rows), spaced 122 cm apart on center, allowing 35

to 40 cm between stems of plants. Plant density for this greenhouse was 720 plants. The greenhouse was outfitted with an automated fertigation system in which five crops of tomatoes have been produced in hydroponic aggregate culture using perlite (bag culture).

Pesticides are not used in this greenhouse because impact of diseases and insects has been minimized by exclusion, sanitation, and environmental management aimed at reducing initial inoculum. Insect screens (mesh) were used to exclude insect vectors of plant disease. Clean, disease-free seed (certified seed) were used for all experiments. Sanitation activities that eliminated or reduced the amount of inoculum present and thereby reduced the spread of the pathogen to healthy plants were practiced. Environmental sensors and data loggers were used to monitor and adjust the microenvironment in favor of the host.

Tomatoes. 'Trust' (De Ruiter Seed Inc, Columbus, Ohio), a Dutch hybrid cultivar bred for greenhouse production, was used in this experiment. Seeds were planted and maintained for six weeks in greenhouses at the Tobacco Experiment Station, Greenville, TN. At six weeks, seedlings (ca. 15-20 cm in height) were transplanted into the bag culture system described above. The experiments were conducted simultaneously. Fruit were harvested from April 23 to June 15, 2004 (Table 1)(all tables in appendix). Effect of row on yield was analyzed with Proc Mixed Procedure of PC-SAS, Version 9.0 (SAS Instiutute, Cary, NC).

Biological Pesticides. Bioactive herbage was harvested from three cultivars in the *Monarda* Evaluation Garden as described. Two sources of *B. bassiana* were used; isolate Bb11-98 was obtained from B.H. Ownley, The University of Tennessee, and Botanigard ® was obtained from BioAgriculture Corporation (Butte, MT). BioYield ® was obtained from Gustafson LLC (Plano, Texas).

Experiments.

1. ***Effect of herbage and Pythium on yield of greenhouse tomatoes.*** The experiment was designed as a 2 x 4 factorial with pathogen (untreated, *Pythium*) and herbage (untreated, 'Marshall's Delight', 'Elsie's Lavender', 'Sioux') treatments. *Monarda* herbage (6.75 g) was packaged in commercial tea bags (GMBH, Hamburg, Germany). A crevice was created in the perlite by hand; the tea bag was inserted into the crevice and covered by the displaced perlite. A suspension of *P. myriotylum* zoospores (15 mL) was added directly adjacent to the stem. Each row served as a replicate, and each treatment was replicated eight times. Tomatoes were grown under standard greenhouse conditions (Ray, 2004). At harvest, tomatoes were counted, graded, and weighed (Table 2); culls were defined as fruit that were smaller than Grade 5, off-color, severely blemished or damaged. Data were categorized into eight harvest dates (Table 1). Total harvest data were analyzed with the Proc

Mixed Procedure of PC-SAS (Version 9.0, SAS Instiutute, Cary, NC).

2. ***Effect of herbage, Pythium, and Beauveria on yield of greenhouse tomatoes.*** This experiment was designed as a 4 x 2 x 3 factorial with herbage (untreated, 'Marshall's Delight', 'Elsie's Lavender', 'Sioux'), pathogen (untreated, *Pythium*), and *B. bassiana* (untreated, Bb11-98, Botanigard). Each row served as a replicate, and each treatment was replicated eight times. Seeds were treated with *B. bassiana* prior to seeding. In brief, seed treatments containing conidia of *B. bassiana* (10^6 colony forming units per ml were mixed with methyl cellulose and air dried prior to planting (Seth, 2001). Herbage and pathogen treatments were applied as described. Data collection and analysis were as described.

3. ***Effect of herbage, Pythium, and Bb11-98 on yield of greenhouse tomatoes.*** This experiment was designed as a 4 x 2 x 2 factorial with herbage (untreated, 'Marshall's Delight', 'Elsie's Lavender', 'Sioux'), pathogen (untreated, *Pythium*), and *B. bassiana* (untreated, Bb11-98). Each row served as a replicate, and each treatment was replicated eight times. Seeds were treated with *B. bassiana* prior to seeding, and herbage and pathogen treatments were applied at transplanting as described. Data collection and analysis were as described.

4. ***Effect of herbage, Pythium, and BotaniGard® on the yield of greenhouse tomatoes.*** This experiment was designed as a 4 x 2 x 2 factorial with herbage (untreated, 'Marshall's Delight', 'Elsie's Lavender', 'Sioux'), pathogen (untreated, *Pythium*), and *B. bassiana* (untreated, BotaniGard®) treatments. Each row served as a replicate, and each treatment was replicated eight times. Seeds were treated with *B. bassiana* prior to seeding, and herbage and pathogen treatments were applied at transplanting as described. Data collection and analysis were as described.
5. ***Effect of herbage, Pythium, and on yield of greenhouse tomatoes***. This experiment was designed as a 4 x 2 x 2 factorial with herbage (untreated, 'Marshall's Delight', 'Elsie's Lavender', 'Sioux'), pathogen (untreated, *Pythium*), and PGPR (untreated, BioYield®). Each row served as a replicate, and each treatment was replicated eight times. BioYield® (10mL) was applied directly adjacent to the stem opposite to where *Pythium* was applied. Herbage and pathogen treatments were applied at transplanting as described, and data collection and analysis were as described.
6. ***Effect of herbage and herbage rate on the yield of greenhouse tomatoes infested with Pythium***. This experiment was designed as a 4 x 2 factorial with herbage (untreated, 'Marshall's Delight', 'Elsie's Lavender', 'Sioux') and rate (high, low) treatments. *Monarda* herbage was packaged in commercial tea bags. In high-

rate treatments, 6.75 g were added to each tea bag, and for the low rate, 3.33 g were added. The treatments were applied at transplanting as described.

Chapter 4

Results

Monarda Evaluation Garden

At least three chemotypes of *Monarda* were identified in these studies (Table 3). 'Marshall's Delight' was identified as a carvacrol chemotype because carvacrol is the primary active essential oil. 'Elsie's Lavender' contained more carvacrol (approximately 10-fold) than did 'Marshall's Delight' but is identified as a carvacrol: thymoquinone chemotype because of it contains the highest amount of thymoquinone found in any of the cultivars. 'Sioux' is a thymol chemotype.

Control of *Rhizoctonia solani* with Bioactive Herbage

Amending greenhouse germination mix with herbage from 'Marshall's Delight' increased germination (Figure 1) and seedling height (Figure 2) above that of controls regardless of *Rhizoctonia solani* infestation. For 'Elsie's Lavender', shoot height and germination were reduced in treatments containing only herbage but no reduction occurred in those containing herbage + *R. solani* (Figures 1 and 2). The disease index of 'Elsie's Lavender' herbage or herbage + *R. solani* was less than pathogen alone but greater than uninfested, no herbage control. Amendment with 'Sioux' herbage did not protect against *R. solani* (Figure 3).

Commercial Tomato Production

Effect of herbage (high rate only) and Pythium on yield of greenhouse tomatoes. The main effect of herbage was significant for the number and weight of Grade 1, tomatoes (Table 4). 'Elsie's Lavender' and

control produced greater numbers and weight of fruit than 'Marshall's Delight' (Table 5). The main effect of *Pythium* was not significant for any of the measured variables. The interaction of herbage and *Pythium* was significant for the number of Grade 4 tomatoes (Table 4). 'Marshall's Delight' without *Pythium*, 'Elsie's Lavender' with *Pythium*, and control without *Pythium* were greater than 'Marshall's Delight' with *Pythium* (Table 5).

Herbage had a significant effect on the number and weight of fresh market and total marketable tomatoes (Table 4). 'Elsie's Lavender' and control had greater numbers and weight of fresh market tomatoes than 'Marshall's Delight'. 'Elsie's Lavender' had greater numbers of total marketable fruit than 'Marshall's Delight'. All treatments produced greater weight of total marketable tomatoes than MD. Herbage had no significant effect on processing tomatoes.

Effect of herbage, Pythium, and Beauveria (Bb11-98 and Botanigard) on yield of greenhouse tomatoes. For Grade 1 tomatoes there was a significant interaction effect between *Pythium* and *Beauveria*. Plants treated with Bb11-98 and inoculated with *Pythium* had greater weight of fruit than those inoculated with *Pythium* without Bb11-98, though neither treatment was different from the uninfested controls (Table 7). There were no significant differences in the number or weight of Grade 2 tomatoes. The main effect of *Beauveria* was significant for the number and weight of Grade 3 and 5 tomatoes (Table 6). The number and weight of Grade 3 and 5 tomatoes was greater in control than for those treated with Bb11-98 (Table 7). The main effect of *Pythium* was significant for the number and weight of Grade 4 tomatoes (Table 6). *Pythium* reduced both

the number and weight of Grade 4 tomatoes (Table 7).There was also a significant interaction effect between herbage and *Pythium* for both the number and weight of Grade 4 tomatoes (Table 6). Treatments with Bb11-98 produced a greater weight of tomatoes than either *Pythium* or Bb11-98 alone (Table 7).

There were no significant differences in either the number or weight of total fresh market tomatoes. The main effect of *Beauveria* was significant for both the number and weight of processing tomatoes and total marketable tomatoes (Table 9). For processing tomatoes number and weight of control was greater than either *Beauveria* treatment. For total marketable tomatoes, control was greater than Bb11-98 (Table 10).

Effect of herbage, Pythium, and Beauveria (Bb11-98) on yield of greenhouse tomatoes. P-values are summarized in Table 11. For the Grade 1 tomatoes there was a significant interaction effect between *Pythium* and Bb11-98. Plants treated with Bb11-98 and inoculated with *Pythium* had greater numbers and weights of tomato fruit than *Pythium* without Bb11-98, though neither treatment was different than controls (Table 12). Treatment with *Pythium* or Bb11-98 also had a greater weight of tomato fruit than the untreated with Bb11-98. For Grades 2 and 3, number and weight of tomatoes were greater in the untreated than in those treated with Bb11-98. The interaction between herbage, *Pythium,* and Bb11-98 was significant Table 11. For the *Grade* 4 tomatoes there was a significant interaction between herbage and *Pythium* for number of tomato fruit. 'Marshall's Delight' without *Pythium* was significantly greater than 'Sioux' without *Pythium* and 'Marshall's Delight' with *Pythium.* For

weight of tomatoes there was a significant interaction between herbage, *Pythium,* and *Beauveria.* 'Elsie's Lavender' plus *Pythium* without Bb11-98 and 'Marshall's Delight' without *Pythium* plus Bb 11-98 were significantly greater than 'Sioux' without *Pythium* plus Bb 11-98 and 'Elsie' Lavender' plus *Pythium* plus Bb11-98 (Table 13). For the Grade 5 tomatoes the untreated plants produced more fruit and greater weight of fruit than *Beauveria* treated plants (Table 12).

Isolate Bb11-98 had a significant effect on processing and total marketable tomatoes (Table 14). The numbers and weight of tomatoes were greater in the untreated than those treated with isolate Bb11-98 (Table 15). There was a significant interaction of herbage and *Pythium* for number of fresh market tomatoes (Table 14). The control without *Pythium* produced greater numbers of tomatoes than treatments with 'Elsie's Lavender' without *Pythium*, and 'Marshall's Delight' with or without *Pythium* (Table 15).There was a significant interaction between herbage and isolate Bb11-98 in fresh market tomatoes and total marketable tomatoes (Table 14). Plants treated with 'Marshall's Delight' produced lower numbers and weight of tomatoes than those treated with 'Marshall's Delight' and Bb11-98 and controls (Table 15). There was a significant interaction between the pathogen and isolate Bb11-98 for weight of fresh market and total marketable tomatoes (Table 14). Plants treated with *Pythium* plus Bb11-98 and control produced greater weight of tomatoes than those treated only with Bb11-98 (Table 15).

Effect of herbage, Pythium, and BotaniGard® on the yield of greenhouse tomatoes. There were no significant differences for Grades 1or 2

or for fresh market tomatoes. The main effect of BotaniGard® was significant for the number and weight of processing and Grades 4 and 5 tomatoes; the effect was also significant for the weight of Grade 3 and the number of total marketable tomatoes (Table 16). Plants treated with BotaniGard® produced fewer Processing tomatoes and Grades 4 and 5 tomatoes than untreated (Table 17). The main effect of *Pythium* was significant for the number and weight of Grade 4 processing tomatoes (Table 16); treatment with *Pythium* reduced yield (Table 17). There was also an interaction effect of herbage and *Pythium* for the number and weight of Grade 4 processing tomatoes (Table 16). Plants treated with 'Marshall's Delight' and *Pythium* produced lower number and weight of Grade 4 tomatoes than those treated with 'Marshall's Delight' alone or controls (Table 17).

Effect of herbage, Pythium, and Bioyield on yield of greenhouse tomatoes. There were no statistically significant differences in Grades 1, 2, 4 or 5; there were also no significant differences for the number or weight of fresh market, or processing tomatoes. There was an interaction effect of herbage and *Pythium* for both the number and weight of the Grade 3 processing tomatoes (Table 18). 'Elsie's Lavender' plus *Pythium* treatments were greater than 'Elsie's Lavender' without *Pythium* (Table 19). The main effect of herbage was significant for the number and weight of total marketable fruit (Table 18). Plants treated with 'Sioux' produced greater number and weight of tomatoes than those treated with 'Marshall's Delight' (Table 19). There was also a significant interaction effect of herbage and PGPR for the weight of total marketable fruit

(Table 18). Addition of BioYield to the 'Marshall's Delight' treatments negated the negative impact of 'Marshall's Delight' (Table 19).

Effect of herbage and herbage rate on the yield of greenhouse tomatoes infested with Pythium. There were no significant differences for Grades 2, 4 or 5 or for processing tomatoes. The main effect of herbage was significant only for number and weight of Grade 3 tomatoes (Table 20). Plants treated with 'Sioux' had greater yield than those treated with 'Marshall's Delight' (Table 21). There was a significant interaction of herbage and herbage rate for both the number and weight of Grade 1 and fresh market fruit as well as the weight of total marketable fruit (Table 20). 'Marshall's Delight' at the low rate and 'Elsie's Lavender' at the high rate were significantly greater than Marshall's Delight at the high rate (Table 21).

Chapter 5

Discussion

Plant essential oils have been proposed as natural, safe pesticides (Bauske *et al.*, 1994; Deans, 1991; Tsao and Zhou, 2000; Thompson, 1989). *Monarda didyma* is an excellent source of essential oils. The objective of these studies was to determine if dried herbage could be used as a delivery system for antifungal essential oils; relative disease control was evaluated for two economically important pathogens that are known to be sensitive to essential oils present in the herbage or have closely related species that are sensitive. At least three chemotypes of *Monarda* were used in these studies: 'Marshall's Delight' (a carvacrol chemotype), 'Sioux' (a thymol chemotype), and 'Elsie's Lavender' (a carvacrol:thymoquinone chemotype) (Table 4). 'Marshall's Delight' and 'Elsie's Lavender' contained high concentrations of hexane-extractable components. 'Marshall's Delight' was identified as a carvacrol chemotype because carvacrol was the only extractable essential oil with antifungal activity. 'Elsie's Lavender' contained more carvacrol than did 'Marshall's Delight', but because it contained the highest amounts of thymoquinone, it was designated a carvacrol: thymoquinone chemotype. 'Sioux' was selected because of its relatively low concentration of total essential oils. The primary essential oil of 'Sioux' was thymol. Chemotype classifications were developed in order to simplify classification of the essential oil profiles of plants in the Monarda Evaluation Garden, but it is essential to remember that the complex chemistries of natural essential oils do not lend themselves to simple definition.

The individual components of essential oils are acutely toxic to many plant pathogens; toxicity can be potentiated in complex mixtures so that the activity of the mixture is higher than would be expected by the additive effects of the individual components. The synergistic effect of one compound in minor percentage in these complex mixtures of essential oils has to be considered. Reduction of growth of *Botrytis cinerera, Fusarium solani,* and *Clavibacter michiganensis* was greater in colonies treated with rosemary oil than with sage oil that contained twice the amount of eucalyptol (the primary antifungal ingredient) (Daferera, 2003).

Essential oil of *Salvia fruticosa* containing 1,8-cineole and camphor as the main components was effective in inhibiting the growth of *Rhizoctonia solani* at a concentration of 2000 µL/L (Pitarokili *et al.*, 2003). The oil of *Neptea hindostana* inhibited *Pythium debaryanum* [minimum inhibitory concentration (MIC) = 550 ppm] and *R. solani* (MIC = 1000 ppm) (Kishore and Dwivedi, 1992). The essential oils of *Thymbra spicata* and *Satureja thymbra* were effective in inhibiting mycelial growth of *Fusarium moniliforme, Rhizoctonia solani, Sclerotinia sclerotiorum,* and *Phytophthora capsici* with MIC between 400 and 800 µg/mL medium (Muller-Riebau *et al.*, 1995); toxicity against these fungi was most likely due to different concentrations of the phenolic fraction (especially thymol and/or carvacrol) in the essential oils.

Compounds found in *Monarda* herbage (e.g., carvacrol, thymol, thymoquinone) are active against *R. solani.* Antifungal activity against three agricultural pathogens *Pythium ultimum, Rhizoctonia solani,* and *Fusarium*

sambucinum were evaluated using essential oils of *Pistacia* species (Duru *et al.*, 2003). The major components of essential oils of *Pistacia* spp. were rich in α-pinene, β-pinene, limonene, terpinene-4-ol, and γ-terpineol. Results from this study showed growth of *R. solani* was somewhat inhibited at less than 40% and none of the oils were effective against *Pythium ultimum* or *Fusarium sambucinum.* In this study, germination mix amended with herbage from *Monarda didyma* cultivars 'Elsie's Lavender' and 'Marshall's Delight' reduced the incidence of *Rhizoctonia* seedling disease of tomato. Although not directly comparable to the studies described above, taken collectively these data support the hypothesis that the disease reduction in herbage-amended treatments was due to the presence of carvacrol and thymoquinone.

No literature exists on the sensitivity of *P. myriotylum* to essential oils, but there are several reports of essential oils controlling or inhibiting other species. Damping-off disease of tomato caused by *Pythium aphanidermatum* and *Pythium debaryanum* were controlled with essential oils extracted from fresh leaves of *Hyptis suaveolens* (Labiatae) (Pandey and Dubey, 1994). Seeds treated with essential oils were selectively fungitoxic without evidence of phytotoxicity. Essential oils from *H. suaveolens*, *Murraya koenigii* (Rutaceae), and *Ocinum canum* (Labiatae) controlled damping-off disease of tomato up to 83, 67, and 50% respectively in soil infected with *P.aphanidermatum* and 86, 71, and 43% respectively in soil infected with *P. debaryanum.* Combinations of acetone soluble extracts of *Hyptis suaveolens, Murraya koenigii, Ocinum canum* were

active at lower concentrations against *Pythium aphanidermatum* and *Pythium debaryanum* when combined than when used alone (Pandey and Dubey, 1997).

Plant pathogens are estimated to cause yield reductions in crops of almost 20% worldwide (Oerke *et al.*, 1994) so disease control methods are essential in modem agriculture. There is increased public concern regarding the use of pesticides that are damaging to human health or the environment. Such concerns are driving the search for more environmentally-friendly methods to control plant disease that will contribute to the goal of sustainability in agriculture. Large demands for fungicides exist in agriculture, food protection and medicine. Also, consumer demand for organically-grown produce is increasing annually; farmers in 48 states dedicated 2.3 million acres of cropland and pasture to organic production systems in 2001. Over 1.3 million acres were used for growing crops. California, North Dakota, Minnesota, Wisconsin, Iowa, Montana, and Colorado had the most organic cropland (http://www.ers.usda.gov).

Bioactive natural products have the potential to control disease in greenhouses without synthetic pesticides. However, fundamental research in natural products is lacking, and there is a lack of consistency among labs involved in natural product research. Efforts have to be made to standardize test procedures in order to increase reproducibility between laboratories. One difficulty in standardization is the differences in the types of laboratories in which this type of research is being performed (e.g., the approach of researchers trained primarily in food chemistry is different than those trained fundamentally in plant pathology). Antifungal activities are often reported as MIC values which

usually denote the minimum inhibitory concentration of the test compound. Although reproducible with yield values expressed in µg/mL, MICs are still a function of the conditions set by the tester (Rex *et al.*, 1997). Standardization should apply to all research fields employing antifungal susceptibility testing including natural product research, ecotoxicology and phytopathology.

Essential oils are difficult to standardize because there are many influences on essential oil composition in the plant. Many factors affect the constituents of essential oils. Intra-specific variation can occur within plants as a result of differing soil conditions, altitude, climatic conditions and other environmental factors. In some cases, different chemotypes may occur as a result of the above factors. Parameters such as the time of day and stage of growth when the plant is picked, what part(s) of the plant are distilled, the length of distillation, method of distillation, whether the plant is distilled immediately or whether it is dried first, and storage conditions if dried plant parts are used will all affect the constituents of essential oils and hence their quality and chemical effects.

Conclusions based on the chemistry of extracted essential oils may be in error because important compounds may not be extracted by the solvent or distillation system. The hexane extraction used in this research is a nonstandard method, but it was employed because of its simplicity, rapidity and cost-effective nature. Supercritical fluid extraction with carbon dioxide is a milder extraction method than steam distillation and avoids the degradative heat processes, hydrolysis, isomerization and racemization (Lemberkovics et al., 2003). In

general, the supercritical fractions of essential oils have been found to be richer in monoterpene-ester components than the steam distilled oils, regardless of the plant-source (Lemberkovics et al., 2003).

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APPENDIX

FIGURES

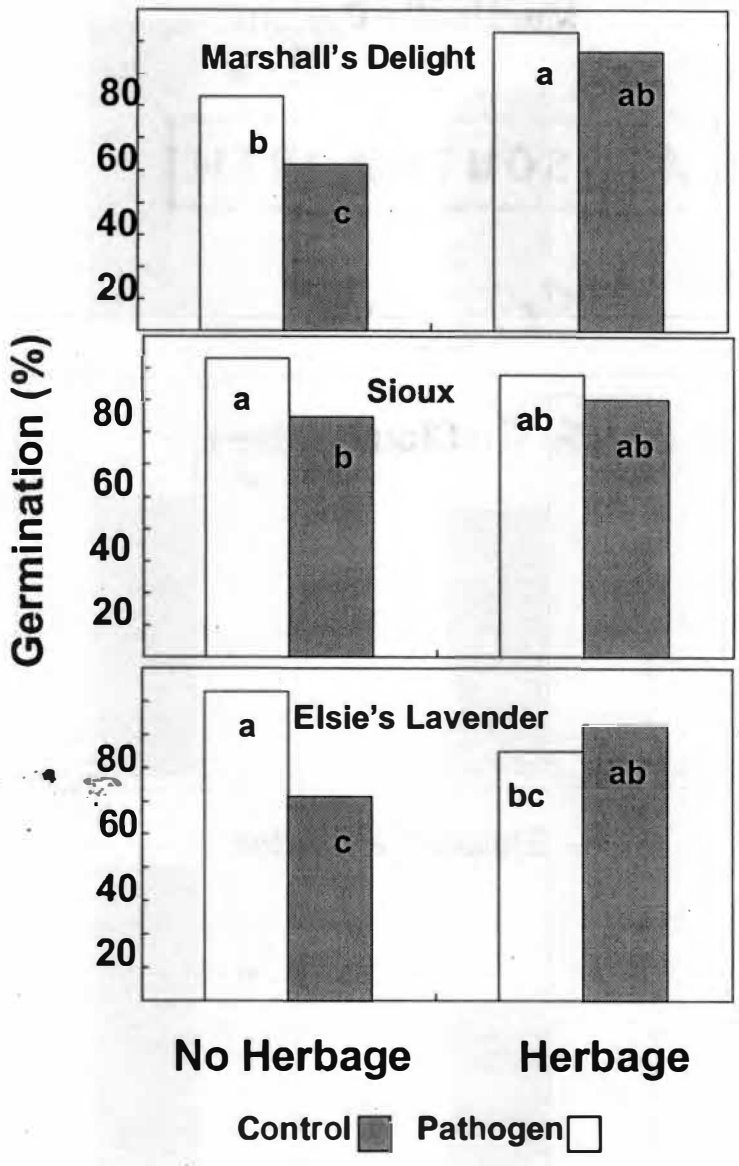

Figure 1. Percentage germination of tomato seeds with and without herbage amendment and with and without *Rhizoctonia solani*. Herbage and *R. solani* inoculum were mixed with commercial germination mix prior to seeding. Herbage was obtained from three cultivars of *Monarda didyma*, 'Marshall's Delight', 'Elsie's Lavender', and 'Sioux'. Bars with the same letter are not significantly different according to a F-LSD at $P = 0.05$.

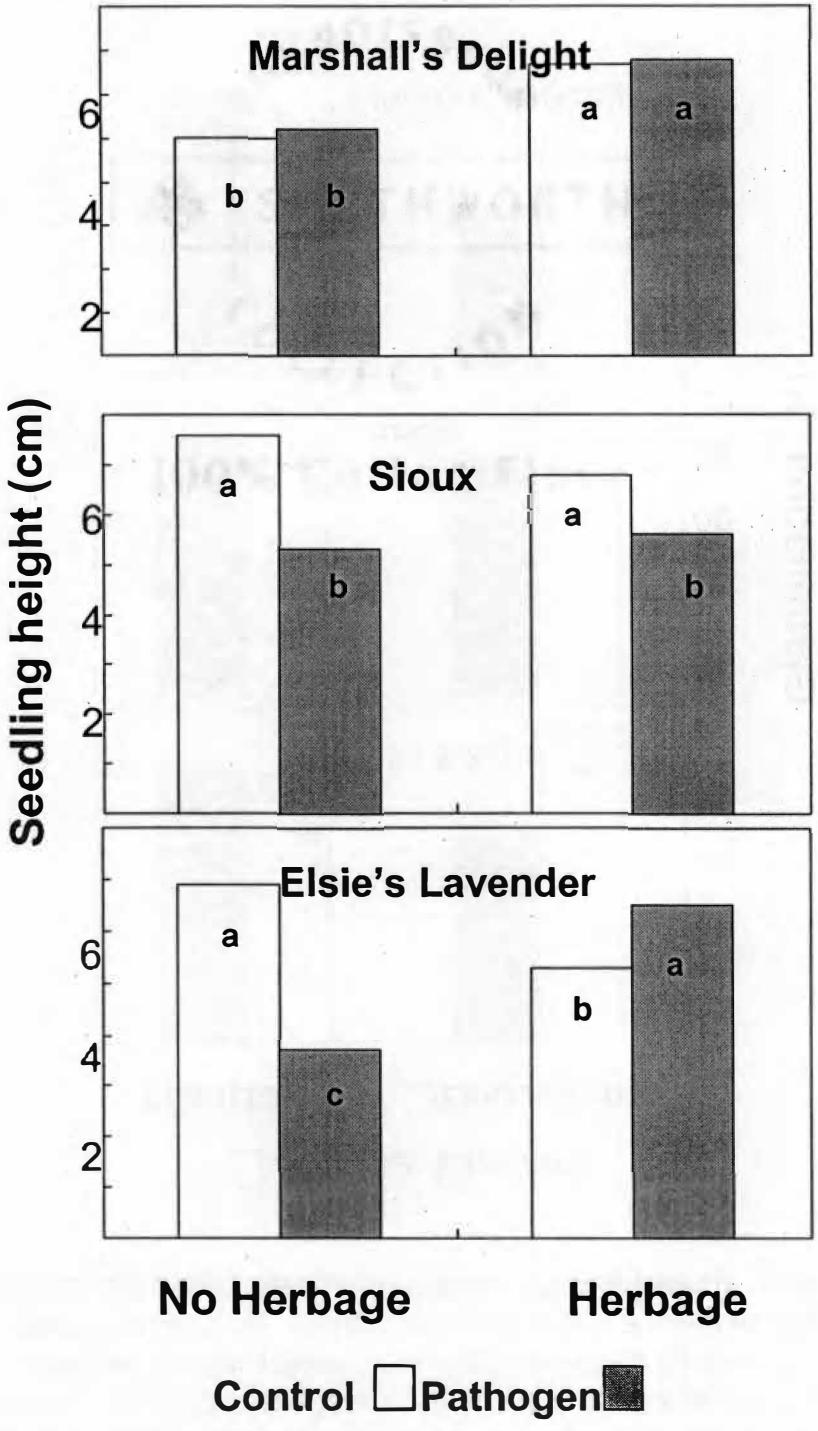

Figure 2. Effect of *Monarda* herbage on disease losses due to *Rhizoctonia solani*. Tomato seeds were planted in greenhouse germination medium or medium amended with herbage from a *Monarda* cultivar. Treatments were control or amended with *Rhizoctonia* inoculum. Seedling height was determined after 7 days.

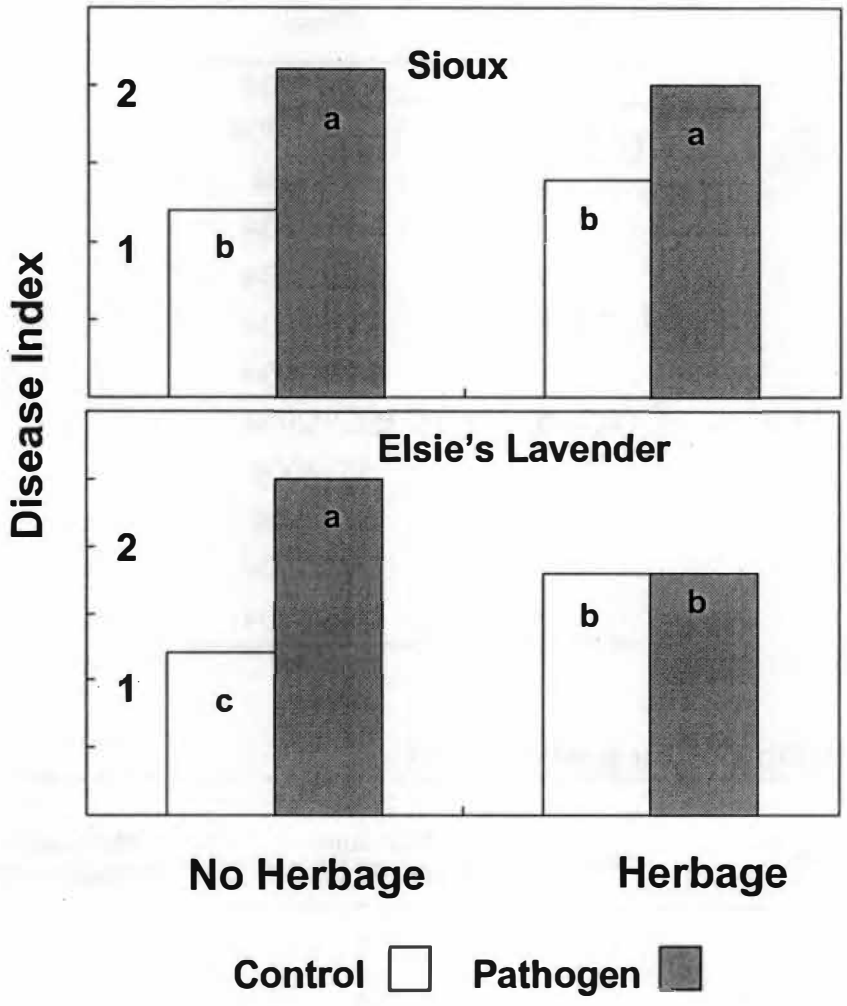

Figure 3. Effect of treatment on disease severity of tomato. Herbage and R. *solani* inoculum were mixed with commercial germination mix prior to seeding. Herbage was obtained from three cultivars of *Monarda didyma*, 'Marshall's Delight', 'Elsie's Lavender', and 'Sioux'. Bars with the same letter are not significantly different according to a F-LSD at $P = 0.05$.

TABLES

Table 1. Tomato fruit harvest dates

Harvest	Week
1	4/23/2004
	4/30/2004
2	5/5/2004
3	5/11/2004
4	5/18/2004
5	5/21/2004
	5/25/2004
6	5/28/2004
	6/2/2004
7	6/4/2004
	6/11/2004
8	6/15/2004

Table 2. Size classification for grading tomatoes

Size classification [z]	Grade	Minimum diameter (cm)	Maximum diameter (cm)
Jumbo	1	8.2	--
Extra Large	2	6.5	8.2
Large	3	5.8	6.5
Medium	4	5.4	5.8
Small	5	4.6	5.4

[z] Fresh market tomatoes = Grades 1-2. Processing tomatoes = Grades 3-5. Marketable tomatoes = Grades 1-5.

Table 3. Concentrations (μM) of essential oils extracted from Monarda cultivars. Herbage was extracted in hexane and analyzed by GC/MS. Concentrations were determined by comparison to standard curves

essential oils	'Marshall's Delight'	'Elsie's Lavender'	'Sioux'
Borneol	37.9	ND [z]	ND
Bornyl Acetate	18.4	ND	ND
Carvacrol	255.1	2,574	19.4
Cineole	114.9	7.25	ND
Cymene	87.8	670.8	601.3
Linalool	2.5	ND	3.9
Limonene	62.0	17.5	13.9
Myrcene	16.4	47.6	31.6
1-Octen-3-ol	ND	294.2	69.4
Pinene	30.3	23.2	15.3
γ-Terpinene	72.7	211.1	187.2
α-Terpineol	61.4	ND	ND
Thymol	0.5	10.8	788.3
Thymoquinone	3.78	493.4	59.2

z ND = not detected

Table 4. Effects of herbage and the interaction of herbage x *Pythium* on Grades 1, and 4, fresh market and marketable tomato

Treatment	Grade 1		Grade 4		Fresh Market		Marketable	
	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)
Herbage	0.0958 [z]	0.0569	-	-	0.0417	0.272	0.0791	0.0160
Herbage x *Pythium*	- [y]	-	0.0216	-	-	-	-	-

[y] - = Not significant.

[z] = All numbers are *P* values calculated using the Proc Mixed procedure of PC-SAS, Version 9.0, SAS Institute, Cary, N.C.

Table 5. Effects of herbage on the number and weight of Grade 1, fresh market, and marketable tomatoes and interaction of herbage x *Pythium* on the number of Grade 4 tomatoes

Treatments [x]	Grade 1		Grade 4		Fresh Market		Marketable	
	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)
Elsie's Lavender	4.8 a [z]	2.32 a	-	-	9.0 a	3.77 a	17.1 a	5.21 a
Control	4.6 a	2.20 a	-	-	8.8 a	3.60 a	15.4 ab	4.77 a
Sioux	4.0 ab	1.97 ab	-	-	7.8 ab	3.28 ab	16.3 ab	4.83 a
Marshall's Delight	2.8 b	1.29 b	-	-	5.9 b	2.37 b	13.2 b	3.58 b
MD	- [y]	-	3.8 a	-	-	-	-	-
EL + P	-	-	3.8 a	-	-	-	-	-
Control	-	-	3.4 a	-	-	-	-	-
Sioux	-	-	3.0 ab	-	-	-	-	-
Sioux + P	-	-	2.9 ab	-	-	-	-	-
EL	-	-	2.6 ab	-	-	-	-	-
Control + P	-	-	2.5 ab	-	-	-	-	-
MD + P	-	-	1.5 b	-	-	-	-	-

[y] - = Not significant.

[z] = within each column, numbers followed by the same letter are not significantly different at $P = 0.05$ according to an F-LSD test.

[x] = MD = 'Marshall's Delight', EL = 'Elsie's Lavender', P = *Pythium*

Table 6. Effects of herbage, *Pythium* and the interaction of these factors on the number and weight of fresh market, processing, and marketable fruit

Treatments	Fresh Market (1 – 2)		Processing (3 – 5)		Marketable (1 – 5)	
	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)
Herbage	0.042 [z]	0.027	-	-	0.079	0.016
Pythium	- [y]	-	-	-	-	-
Herbage x *Pythium*	-	-	-	-	-	-

[y] - = Not significant.

[z] = All numbers are P values calculated using the Proc Mixed procedure of PC-SAS, Version 9.0, SAS Institute, Cary, N.C.

Table 7. Effects of herbage, *Pythium*, and Beauveria and the interactions of herbage x *Pythium* and *Pythium* x *Beauveria* on the number and weight of Grades 1, 3, 4, and 5 tomatoes

Treatments	Grade 1		Grade 3		Grade 4		Grade 5	
	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)
Pythium	- [y]	-	-	-	0.0381	0.0687	-	-
Beauveria	-	-	0.0130	0.0094	-	-	0.0183	0.0424
Herbage x *Pythium*	-	-	-	-	0.0067	0.0325	-	-
Pythium x Beauveria	-	0.0889 [z]	-	-	-	-	-	-

[y]

- = Not significant

[z] =All numbers are P values calculated using the Proc Mixed procedure of PC-SAS, Version 9.0, SAS Institute, Cary, N.C.

Table 8. Effects of *Pythium* on the number and weight of tomato fruit, Grades 1- 5. Effects of *Beauveria* (Bb 11-98 and BotaniGard®), the interaction of herbage x *Pythium* and the interaction of *Pythium* x *Beauveria* on the number and weight of tomato fruit

Treatment [x]	Grade 1		Grade 3		Grade 4		Grade 5	
	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)
Control	- [y]	-	-	-	2.9 a	0.45 a	-	-
Pythium	-	-	-	-	2.2 b	0.35 b	-	-
Control	-	-	2.9 a	0.71 a	-	-	1.8 a	0.17 a
BotaniGard®	-	-	2.3 ab	0.55 ab	-	-	1.1 ab	0.12 ab
Bb 11-98	-	-	1.9 b	0.46 b	-	-	0.9 b	0.10 b
MD	-	-	-	-	4.0 a	0.62 a	-	-
P	-	-	-	-	2.9 ab	0.45 ab	-	-
EL	-	-	-	-	2.8 ab	0.43 ab	-	-
Control	-	-	-	-	2.7 abc	0.43 ab	-	-
Sioux x P	-	-	-	-	2.2 bc	0.36 b	-	-
EL x P	-	-	-	-	2.2 bc	0.35 b	-	-
Sioux	-	-	-	-	1.9 bc	0.32 b	-	-
MD x P	-	-		-	1.4 c	0.27 b	-	-
P x Bb 11-98	-	2.45 a [z]	-	-	-	-	-	-
P x BotaniGard®	-	2.20 ab	-	-	-	-	-	-
Control	-	2.09 ab	-	-	-	-	-	-
BotaniGard®	-	2.03 ab	-	-	-	-	-	-
Pythium	-	1.80 b	-	-	-	-	-	-
Bb 11-98	-	1.78 b	-	-	-	-	-	-

[y] - = Not significant.

[z] = within each column, numbers followed by the same letter are not significantly different at $P = 0.05$ according to an F-LSD test.

[x] = MD = 'Marshall's Delight', EL = 'Elsie's Lavender', P = *Pythium*, Bb = *Beauveria bassiana* (Bb 11-98)

Table 9. Effect of and *Beauveria* on the number and weight of processing and marketable tomatoes

Treatment	Processing		Marketable	
	Num	Wt (g)	Num	Wt (g)
Beauveria	0.0014 [y]	0.0030	0.0077	- [z]

[z] - = Not significant.
[y] = All numbers are *P* values calculated using the Proc Mixed procedure of PC-SAS, Version 9.0, SAS Institute, Cary, N.C.

Table 10. Effect of *Beauveria* (Bb 11-98 and BotaniGard®) on the number and weight of processing fruit and the number of marketable fruit

Treatments	Processing		Marketable
	Num	Wt (g)	Num
Control	7.6 a [z]	1.34 a	15.47 a
BotaniGard®	5.8 b	1.05 b	13.92 ab
Bb 11-98	5.2 b	0.93 b	12.88 b

[z] = Within each column, numbers followed by the same letter are not significantly different at *P* = 0.05 according to an F-LSD test.

Table 11. Effects of Beauveria bassiana 11-98 (Bb) and the interaction of herbage x *Pythium*, *Pythium* x Bb and herbage x *Pythium* x Bb on the number and weight of Grade 1, 2, 3, 4, and 5 tomatoes

Treatment	Grade 1		Grade 2		Grade 3		Grade 4		Grade 5	
	Num	Wt (g)	Num	Wt(g)	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)
Bb	-[y]	-	0.0697	0.0383	0.0046	0.0038	-	-	0.0148	0.0210
Herbage x Pythium	-	-	-	-	-	-	0.0295	-	-	-
Pythium x Bb	0.0372[z]	0.0277	-	-	-	-	-	-	-	-
Herbage x Pythium x Bb	-	-	-	-	-	0.0950	0.0245	0.0541	-	-

y - = Not significant.

z = All numbers are P values calculated using the Proc Mixed procedure of PC-SAS, Version 9.0, SAS Institute, Cary, N.C.

Table 12. Effects of *Beauveria bassiana* (Bb11-98) on the number and weight of Grade 2, 3, and 5 greenhouse tomatoes, interaction of herbage x *Pythium* for the number of Grade 4 tomatoes, interaction of *Pythium* x Bb11-98 on Grade 1 tomatoes, and interaction of herbage x *Pythium* x Bb11-98 on the weight of Grade 3 and the number and weight of Grade 4 greenhouse tomatoes

Treatment	Grade 1		Grade 2		Grade 3		Grade 4	Grade 5	
	Num	Wt	Num	Wt	Num	Wt	Num	Num	Wt
Untreated	-[y]	-	3.8 a	1.31 a	2.9 a	0.71 a	-	1.8 a	0.17 a
Bb 11-98	-	-	3.2 b	1.05 b	1.9 b	0.46 b	-	0.9 b	0.10 b
MD	-	-	-	-	-	-	3.7 a	-	-
MD + P	-	-	-	-	-	-	1.7 c	-	-
EL	-	-	-	-	-	-	3.1 ab	-	-
EL + P	-	-	-	-	-	-	2.6 abc	-	-
Sioux	-	-	-	-	-	-	2.1 bc	-	-
Sioux + P	-	-	-	-	-	-	2.8 abc	-	-
Control	-	-	-	-	-	-	2.4 abc	-	-
P control	-	-	-	-	-	-	3.0 abc	-	-
P + Bb	5.2 a[z]	2.44 a	-	-	-	-	-	-	-
Pythium	3.8 b	1.80 b	-	-	-	-	-	-	-
Control	4.3ab	2.09 ab	-	-	-	-	-	-	-
Control + Bb	3.8 ab	1.77 b	-	-	-	-	-	-	-

y - = Not significant.
z = Within each column, numbers followed by the same letter are not significantly different at P = 0.05 according to an F-LSD test.
x = MD = 'Marshall's Delight', EL = 'Elsie's Lavender', P = Pythium, Bb = Beauveria bassiana (Bb 11-98)

Table 13. Interaction of herbage x *Pythium* x *Beauveria bassiana* (Bb11-98) on the weight of Grade 3 and the number and weight of Grade 4 greenhouse tomatoes

Treatment [y]	Grade 3	Grade 4	
	Wt (g)	Num	Wt (g)
MD	0.52 bc [z]	3.8 a	0.54 ab
MD x Bb 11-98	0.54 bc	3.6 a	0.56 a
MD x P	0.69 abc	1.5 bc	0.31 ab
MD x P x Bb 11-98	0.39 c	1.9 abc	0.32 ab
EL	0.92 ab	2.6 abc	0.42 ab
EL x Bb 11-98	0.34 c	3.6 a	0.54 ab
EL x P	0.61 abc	3.8 a	0.59 a
EL x P x Bb 11-98	0.64 abc	1.5 bc	0.22 b
Sioux	0.75 abc	3.0 abc	0.48 ab
Sioux x Bb 11-98	0.59 abc	1.3 c	0.24 b
Sioux x P	1.02 a	2.9 abc	0.45 ab
Sioux x P x Bb 11-98	0.45 c	2.6 abc	0.44 ab
Pythium	0.65 abc	2.5 abc	0.40 ab
Control	0.53 bc	3.4 abc	0.52 ab
Bb 11-98	0.47 bc	1.4 abc	0.21 ab
P x Bb 11-98	0.30 c	3.4 abc	0.50 ab

z =Within each column, numbers followed by the same letter are not significantly different at P = 0.05 according to an F-LSD test.
y = MD = 'Marshall's Delight', EL = 'Elsie's Lavender', P = Pythium, Bb = Beauveria bassiana (Bb 11-98)

Table 14. Effect of *Beauveria bassiana* 11- 98 (Bb) and the interaction of herbage x *Pythium*, herbage *x Bb* and *Pythium* x Bb on the number and weight of fresh market, processing and total marketable tomatoes

Treatment	Fresh Market		Processing		Marketable	
	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)
Bb	-	-	0.0010	0.0017	0.0016	0.0473
Herbage x *Pythium*	0.0692 y	-	-	-	-	-
Herbage x Bb	0.0403	0.0440	-	-	0.0493	0.0172
Pythium x Bb	- z	0.0656	-	-	-	0.0933

[z] - = Not significant.

[y] = All numbers are *P* values calculated using the Proc Mixed procedure of PC-SAS, Version 9.0, SAS Institute, Cary, N.C.

Table 15. Effects of *Beauveria bassiana* (Bb11-98) on the number and weight of processing and marketable fruit, interaction of herbage x *Pythium* for the number of fresh market tomatoes, interaction of herbage x Bb11-98 on the number and weight of fresh market and marketable tomatoes, interaction of *Pythium* x Bb11-98 on the weight of fresh market and marketable tomatoes

Treatment [x]	Fresh Market		Processing		Marketable	
	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)
Untreated	- [y]	-	7.6 a	1.34 a	15.5 a	4.6 a
Bb 11-98	-	-	5.2 b	0.93 b	12.9 b	4.1 b
MD	**6.9 bc** [z]	-	-	-	-	-
MD + P	6.9 bc	-	-	-	-	-
EL	6.4 c	-	-	-	-	-
EL + P	9.0 ab	-	-	-	-	-
Sioux	7.6 abc	-	-	-	-	-
Sioux + P	7.9 abc	-	-	-	-	-
Control	9.6 a	-	-	-	-	-
P control	7.5 abc	-	-	-	-	-
MD	**5.9 b**	**2.37 c**	-	-	**13.2 bc**	**3.58 c**
MD + Bb 11-98	8.0 ab	3.14 abc	-	-	13.3 bc	4.12 bc
EL	9.0 a	3.77 a	-	-	17.1 a	5.21 a
EL + Bb 11-98	6.4 b	2.63 bc	-	-	11.8 c	3.60 c
Sioux	7.8 ab	3.28 abc	-	-	16.3 a	4.83 ab
Sioux + Bb 11-98	7.7 ab	3.34 ab	-	-	12.3 c	4.26 bc
Control	8.8 a	3.60 a	-	-	15.4 ab	4.77 ab
Control + Bb 11-98	8.4 ab	3.47 abc	-	-	14.3 abc	4.44 abc
P + Bb	-	**3.45 a**	-	-	-	**3.45 a**
Pythium	-	3.09 b	-	-	-	3.09 ab
Control	-	3.42 a	-	-	-	3.42 a
Control + Bb	-	2.85 b	-	-	-	2.85 b

y = not significant

z = Within each column, numbers followed by the same letter are not significantly different at $P = 0.05$ according to an F-LSD test.

x = MD = 'Marshall's Delight', EL = 'Elsie's Lavender', P = *Pythium*, Bb = *Beauveria bassiana* (Bb 11-98)

Table 16. Effects of *Pythium*, BotaniGard® and the interaction of herbage x *Pythium* on Grade 3, 4, 5, processing and marketable tomatoes

Treatment	Grade 3		Grade 4		Grade 5		Processing		Marketable	
	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)
BotaniGard®	-[y]	-	0.0082	0.0282	-	-	-	-	-	-
Pythium	-	0.0758 [z]	0.0114	0.0412	0.0585	0.0896	0.0054	0.0119	0.0579	-
Herbage x *Pythium*	-	-	0.0105	0.0374	-	-	-	-	-	-

[y] - = Not significant.
[z] = All numbers are P values calculated using the Proc Mixed procedure of PC-SAS, Version 9.0, SAS Institute, Cary, N.C.

Table 17. Effect of *Pythium* on number and weight of Grade 4 greenhouse tomatoes. Effect of BotaniGard® on Grade 3 weight, Grades 4 and 5 number and weight, number and weight of processing tomatoes, and the number of marketable tomatoes, and interaction of herbage x *Pythium* for Grade 4 greenhouse tomatoes

	Grade 3	Grade 4	Grade 5		Processing		Mkt	
Treatment [x]	Wt (g)	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)	Num
Pythium	- [z]	2.1 b	0.34 b	-	-	-	-	-
Untreated	-	3.0 a	0.47 a	-	-	-	-	-
BotaniGard®	0.53 b [y]	2.1 b	0.34 b	1.1 b	0.12 b	5.5 b	1.00 b	13.7 b
Untreated	0.70 a	3.0 a	0.47 a	1.8 a	0.18 a	7.7 a	1.34 a	15.4 a
MD	-	4.2 a	0.68 a	-	-	-	-	-
MD + P	-	1.2 c	0.23 c	-	-	-	-	-
EL	-	2.3 bc	0.35 bc	-	-	-	-	-
EL + P	-	2.4 bc	0.38 bc	-	-	-	-	-
Sioux	-	2.2 bc	0.35 bc	-	-	-	-	-
Sioux + P	-	2.0 bc	0.32 bc	-	-	-	-	-
Control	-	3.3 ab	0.51 ab	-	-	-	-	-
P control	-	2.7 b	0.43 bc	-	-	-	-	-

z - = Not significant.
y = Within each column, numbers followed by the same letter are not significantly different at P = 0.05 according to an F-LSD test.
x = MD = 'Marshall's Delight', EL = 'Elsie's Lavender', P = Pythium, Mkt = Marketable

Table 18. Effect of herbage and the interaction of herbage x *Pythium* and herbage x BioYield® on Grade 3 and marketable tomatoes

	Grade 3		Marketable	
Treatment	**Num**	**Wt (g)**	**Num**	**Wt (g)**
Herbage	- [y]	-	0.0827	0.0470
Herbage x *Pythium*	0.0202 [z]	0.0185	-	-
Herbage x Bioyield®	-	-	-	0.0861

[y] - = Not significant.

[z] = All numbers are P values calculated using the Proc Mixed procedure of PC-SAS, Version 9.0, SAS Institute, Cary, N.C.

Table 19. Effects of herbage on the number and weight of marketable tomatoes. Interaction of herbage x *Pythium* on the number and weight of Grade 3 greenhouse tomatoes and the interaction of herbage x BioYield® on the weight of marketable tomatoes

	Grade 3		Marketable	
Treatment	**Num**	**Wt (g)**	**Num**	**Wt (g)**
Sioux	- [y]	-	16.2 a	4.83 a
Elsie's Lavender	-	-	15.6 ab	4.76 a
Marshall's Delight	-	-	13.7 b	3.97 b
Marshall's Delight	2.1 b [z]	0.50 b	-	-
Marshall's Delight + *Pythium*	3.1 ab	0.77 ab	-	-
Elsie's Lavender	3.4 a	0.81 a	-	-
Elsie's Lavender + *Pythium*	2.1 b	0.50 b	-	-
Sioux	2.9 ab	0.72 ab	-	-
Sioux + *Pythium*	3.4 a	0.84 a	-	-
Marshall's Delight	-	-	-	3.58 b
Marshall's Delight + BioYield	-	-	-	4.36 ab
Elsie's Lavender	-	-	-	5.21 a
Elsie's Lavender + BioYield	-	-	-	4.31 ab
Sioux	-	-	-	4.83a
Sioux + BioYield	-	-	-	4.82 a

[y] - = Not significant.
[z] = Within each column, numbers followed by the same letter are not significantly different at P = 0.05 according to an F-LSD test.

Table 20. Effects of herbage, herbage rate and the interaction of herbage x herbage rate on Grade 1, 3, fresh market, and marketable tomatoes

Treatment	Grade 1		Grade 3		Fresh Market		Marketable	
	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)	Num	Wt (g)
Herbage	- [y]	-	0.0387	0.0904	-	-	-	-
Herbage rate	-	-	-	-	-	-	-	-
Herbage x Herbage rate	0.0110 [z]	0.0141	-	-	0.0465	0.0397	-	0.0952

[y] - = Not significant.

[z] = All numbers are P values calculated using the Proc Mixed procedure of PC-SAS, Version 9.0, SAS Institute, Cary, N.C.

Table 21. Effects of herbage for the number and weight of Grade 3 greenhouse tomatoes, interaction of herbage x herbage rate for Grade 1, 3 and fresh market tomatoes, and interaction of herbage x herbage rate for the weight of marketable fruit

	Grade 1		Grade 3		Fresh Market		Mkt [x]
Treatment	Num	Wt (g)	Num	Wt (g)	Num	Wt(g)	Wt(g)
Sioux	- [y]	-	3.7 a	0.90 a	-	-	-
Elsie's Lavender	-	-	2.6 ab	0.63 ab	-	-	-
Marshall's Delight	-	-	2.2 b	0.57 b	-	-	-
Marshall's Delight x high rate	2.3 b [z]	1.11 b	-	-	5.1 b	2.21 b	3.38 b
Marshall's Delight x low rate	5.6 a	2.58 a	-	-	8.6 a	3.61 a	4.70 ab
Elsie's Lavender x high rate	5.4 a	2.55 a	-	-	9.6 a	3.99 a	5.38 a
Elsie's Lavender x low rate	3.5 ab	1.61 ab	-	-	7.8 ab	3.00 ab	4.33 ab
Sioux x high rate	3.8 ab	1.88 ab	-	-	7.8 ab	3.24 ab	4.90 ab
Sioux x low rate	3.9 ab	1.91 ab	-	-	6.6 ab	2.81 ab	4.36 ab

[y] - = Not significant.
[z] = Within each column, numbers followed by the same letter are not significantly different at P = 0.05 according to an F-LSD test.
[x] = Mkt = Marketable

Vita

Sharon Elizabeth Fitzpatrick Greene was born and raised in Anderson, SC. She graduated from McDuffie High School and Anderson School of Practical Nursing. Later she married and has one son, Alan Jeffery Greene Jr. She enjoyed nursing and went on to attend Greenville Technical College in Greenville, SC and practiced nursing for a number of years prior to moving to Louisville, TN in 1987. After working in nursing in Tennessee for a few years Sharon decided on a career change and earned her B.S. in Agriculture at the University of Tennessee in 2002 and a M.S. in Entomology and Plant Pathology in May 2005.

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