

To the Graduate Council:

I am submitting herewith a dissertation written by Martin Terrence Lyons entitled "Composts, Biofumigation, Dazomet, and Integrated Treatments Influence Southern Blight, Yield, and Microbial Community Composition in Plasticulture Tomato Production." I have examined the final paper copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant and Soil Sciences.

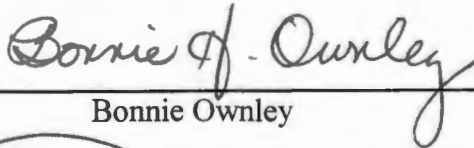


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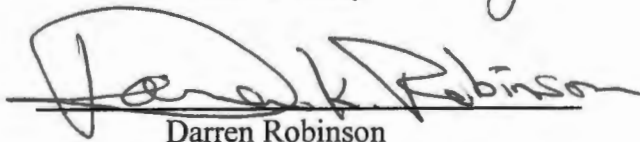
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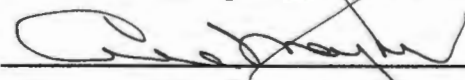


Bonnie Ownley



Darren Robinson

Acceptance for the Council:



Vice Provost and Dean of
Graduate Studies

**COMPOSTS, BIOFUMIGATION, DAZOMET, AND INTEGRATED
TREATMENTS INFLUENCE SOUTHERN BLIGHT, YIELD, AND
MICROBIAL COMMUNITY COMPOSITION IN
PLASTICULTURE TOMATO PRODUCTION**

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DEDICATION

This dissertation is dedicated to Scott Taylor

Let us continue for you

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ABSTRACT

Plasticulture vegetable production is an important agricultural industry in the United States. Advantages of utilizing plastic mulch in combination with trickle irrigation and fertigation include weed control, water conservation, erosion prevention, and avoidance of disease by restricting contact of vegetables with contaminated soil.

Southern Blight, caused by the phytopathogenic fungus *Sclerotium rolfsii* Sacc., represents one of the major disease threats to tomato crops in the southeast United States. Integrated pre-plant soil treatments that can reduce pathogen propagule numbers and re-establish a balanced soil microecology have potential as effective, sustainable disease control strategies.

The soil treatments investigated in this study were biofumigation with incorporated Brassica cover crops, spent mushroom and poultry waste composts, solarization, and the commercial fumigant dazomet. Integrated treatments were also investigated.

Compost amendments enhanced yields (36, 91, and 97% in 2000, 2001, and 2002, respectively) and suppressed disease in all three years (5, 7, and 33% diseased plants in 2000, 2001, and 2002, respectively). Dazomet fumigation controlled Southern Blight in all three years (8, 5, and 24% disease in 2000, 2001, and 2002, respectively). When a full Brassica crop stand was established, as in 2002, the biofumigation treatment was effective at controlling disease and improving yields (52% fruit yield above control and 39% diseased plants in 2002). Lethal temperatures were not generated by the fall and spring solarization applications, hence, disease suppression did not occur.

Integrated treatments displayed good potential as effective pre-plant strategies. Reduced dose dazomet (199 kg/ha) plus compost treatments controlled Southern Blight to 1% and increased yields 79% above controls in 2001.

Phospholipid fatty acid (PLFA) analysis indicated that compost-amended plots had a higher microbial biomass than controls. Signature lipid biomarker quantification revealed that the compost treatments had higher relative populations of Gram-negative bacteria and lower soil microeukaryote/fungal populations than controls.

Hierarchical cluster analysis of all PLFA biomarkers indicated that all compost-based treatments shared similar microecological profiles. Ordination modeling established a close link between improved tomato yields, high Gram-negative bacteria populations, and compost-based soil treatments.

The potential for biologically based soil treatments to be integrated into sustainable agricultural production systems is supported by the results in this research study. Both enhanced yields and lower disease incidence were observed in compost and certain biofumigation treatments. Those treatments that promote beneficial microbial community compositions were linked with improved production.

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Part I

INTRODUCTION

The botanical classification of the tomato is:- Kingdom: Plantae, Phylum: Tracheophytes, Class: Angiosperm, Order: Scrophulariales, Family: Solanaceae, Genus: *Lycopersicon*, Species: *esculentum*. Species from the family Solanaceae are generally found in temperate to tropical geographical ranges with the greatest diversity in Central and South America.

The tomato is acknowledged to originate in Peru but its domestication occurred in Mexico. It was introduced into Europe in 1544. It was not immediately popular because of its relationship to nightshades and belladonnas and was thought to share their poisonous characteristics.

Tomatoes are cropped as annuals but are actually short-lived perennial plants. Freezing temperatures will kill outdoor tomatoes, however, indeterminate greenhouse plants can be cropped for two years or more. In determinate cultivars many side shoots arise from the primary shoot leading the plant to develop a bushy appearance. Each shoot terminates in a flower cluster. Shorter determinate cultivars are often used for fresh market production because they are easier to stake and the concentrated maturity of the fruit reduces the harvest period.

The use of raised-bed plasticulture systems for fruit and vegetable production is prevalent within the United States. Advantages include increased crop performance by conserving moisture and nutrients, stabilizing soil temperature, reducing some diseases, reducing or eliminating weeds, and increasing early-harvest yields (Jaworski et al., 1981). This production method is commonly used for field tomatoes (*Lycopersicon esculentum* Mill.) that are an economically important agricultural industry in many states. In the U.S., the 127,700 acres were planted for fresh market tomatoes in 2002, with total fruit

production of 37,302,000 *cwt*. In Tennessee 4,200 acres of fresh market tomatoes were in production in 2002, with 1,326,000 *cwt* of fruit produced with a farm value of \$43,758,000 (USDA-ERS, 2003).

Often grown without rotations, tomato fields can develop high pathogen inoculum densities. Southern Blight, caused by the phytopathogenic fungus *Sclerotium rolfsii* Sacc., represents one of the major disease threats to tomato crops in the southern U. S. Although accurate figures for losses due to Southern Blight in tomato are not available, loss estimates for soybeans were 6,000 mt in 1996, 7,000 mt in 1997, and 9,000 mt in 1998 (Wrather et al, 2001). This pathogen is also a hazard to many other crops throughout the U.S. Thus, the investigation of pre-plant soil treatments for plasticulture tomato production under pressure from Southern Blight is an economically important area of research.

The disease termed Southern Blight is recognized to affect at least 100 families and 500 species of plants. Many commercial vegetable crops can become infected with the soilborne fungus *S. rolfsii*. Vegetable crops grown in regions that have predominantly warm, humid growing seasons are particularly susceptible to this disease. The disease spreads most effectively between 25-30C. Significant economic losses have been attributed to Southern Blight in tomato, green beans, cantaloupe, peppers, and potatoes. The initial symptoms can easily be confused other fungal wilt diseases such as those caused by *Fusarium* and *Verticillium* spp. However, woody-stemmed plants, such as tomato and pepper can quickly develop symptoms of decay at the soil surface line with a distinctive mycelial/sclerotial formation. After plant death, *S. rolfsii* grows abundantly

on decaying plant debris and will overwinter saprotrophically (Aycock, 1966; Punja and Rahe, 1992).

Methyl bromide fumigation has routinely been heavily relied upon for pathogen management in modern raised-bed plasticulture production systems. However, the control of soilborne diseases through the use of chemical fumigation has several inherent problems. Some commercially available synthetic fumigants have potential for toxicity and persistence in soil with the resulting threat of groundwater contamination. The gaseous by-products of fumigants such as methyl bromide have been targeted as class-I ozone depleters and will be phased-out by 2005 (UNEP, 2000). Concerns for the safety of farm workers that handle such compounds is an important issue, as is elevated costs of application (MBTOC, 1998).

In this study the efficacy of the fumigant dazomet, a recognized alternative to methyl bromide was investigated. The commercially available soil fumigant Basamid utilizes dazomet as its primary active ingredient. Methyl isothiocyanate (MITC) is the major product evolving from the breakdown of dazomet. This compound accounts for most of the fumigant activity, but the by-products formaldehyde, hydrogen sulfide, and carbon disulfide are also formed. MITC is degraded through hydrolysis and photolysis (BASF, 1999).

Fumigants act to sterilize the soil, however, either residual or re-introduced pathogens can quickly re-populate the substrate in subsequent weeks and months. Rather than depending on the corrective measures of chemical pesticides alone, disease control in integrated management systems relies on the principles of avoidance, monitoring, and suppression of pest populations. The importance of prevention is emphasized. It can be

defined as the practice of preventing a pest population from infesting a crop or field. The monitoring of pathogen populations should be performed as the basis for any prophylactic activities. Elevated pathogen counts would indicate that avoidance tactics were not successful and active containment will become necessary to avoid economic loss (Burn et al., 1987).

A multiple-strategy approach can lead to more prolonged suppression and be sustainable for the grower and the environment. Chemical pesticides are effective at rapidly reducing pathogen populations, however, there are many advantages to be gained by integrating these compounds with biocontrol measures. Production costs can escalate to inhibitive levels as pesticide prices rise, especially if increasing quantities of a formulation are required to achieve consistent levels of disease control. Biologically based control techniques such as recycled organic materials or phytoactive cover crops are often less expensive. Stabilizing the population density of a pest at a low, economically tolerable level is a more practical goal. Pathogen control may occur through competition with the non-pathogenic microbial community (Cook, 1993).

Integrated pest management systems that monitor microbial populations could greatly decrease reliance on methyl bromide and other full-dose fumigants. Establishing and maintaining the microbial community structure in a disease suppressive state may obtain sustainable pest control in the soil. Such a state has been established when populations of pathogens are prevented from increasing beyond a threshold that results in significant economic yield loss. However, current agricultural practices permit little time for the establishment of microbial equilibriums. Detailed knowledge of the ecology of disease suppression is paramount to the maintenance of healthy fields and high yields.

There is an accumulation of experimental data supporting the concept that many non-chemically based pest management strategies are effective in controlling diseases caused by soilborne pathogens. The mode of action of many of these treatments also has been described (EPA, 1995; Lambrada and Fornasari, 2001).

Composting is an ecologically sound way to recycle organic farm wastes, such as animal manures and plant debris. Composting avoids the environmental hazards associated with burning, burying, or spreading these agricultural by-products on fields. These methods can lead to problems such as increased particulate matter in the air, leaching and ground water pollution, and toxic excesses of phosphorous and nitrogenous compounds in the soil. Composts improve plant health and productivity due to enhanced soil nutrient levels and improved physiochemical properties (increased bulk density, porosity, aeration) and increased water-holding capacity (Corti et al., 1998). Many researchers have noted disease suppression due to advantageous changes to the microbial composition of the soil, including increases in the populations of known biological control agents (*Trichoderma*, *Bacillus*, and *Pseudomonas* spp.), (Hoitink and Boehm, 1999).

Macerated tissues from *Brassica* cover crops are known to release biocidal compounds such as isothiocyanates (ITCs). When incorporated into the soil ITCs act as an effective **biofumigant**, reducing populations of pathogenic fungal species (*Sclerotium*, *Rhizoctonia*, and *Pythium*), nematodes, weeds, and certain insect species (Kirkegaard and Sarwar, 1998; Charron and Sams, 1999; Harvey et al., 2002).

Solarization is a technique that elevates soil temperatures beneath a clear plastic layer to reduce soilborne pests. The lethal temperatures often generated by effective

solarization reduce pathogen numbers by deactivating their propagules (Katan and Devay, 1991). The sub-lethal temperatures generated in many regions of the southeast U.S. may lead to an improvement in the mode of action of other sustainable treatments, such as biofumigation and composting.

Sustainable control of soilborne phytopathogenic fungi is likely to be achieved through the enhancement of alternate control methods via the integration of multiple techniques (Cook, 1990). Sub-lethal doses of chemical fumigants can act to weaken pathogen propagules making them more susceptible to the actions of the microbial antagonists that can be delivered through compost applications. Similarly, the heating of soilborne sclerotia via solarization increases their chance of colonization by bacteria (Lifshitz et al., 1983). The combination of biofumigation and chemical fumigation is structured to take advantage of any Allyl ITC/Methyl ITC broad-spectrum effects.

It is clear that a fundamental attribute of an effective integrated system is its ability to establish a soil microecology suppressive to pathogens. **Phospholipid fatty acids (PLFA)** are major lipid components in the cell membranes. They are not found in cellular storage lipids, and are labile upon cell death. PLFA analysis provides information about the microbial community biomass, metabolic status and community structure (White et al., 1996). Microorganisms can be categorized based on their unique composition relative to the fatty-acid synthetic pathways (Kaneda, 1991). If the specific PLFA components can be related to certain subsets of the microbial community, then the PLFA patterns can be used to define changes in the community composition (Ringelberg et al., 1989).

Utilizing the analytical techniques of lipid analysis it is possible to identify numerous signature lipid biomarkers (SLB) that are common within the microbiological structure of disease suppressive soils. Baker and Cook (1974) recognized that important mechanisms of biocontrol could be described via the actions of general suppression and specific suppression (of pathogens). Total viable microbial biomass, a crucial component of general suppression can be accurately measured by PLFA analysis of agricultural soils (Lyons et al., 2001). Total biomass is closely linked to the ability of soils to resist the rapid increases in populations of opportunistic pathogens that can occur following sterilization with fumigants. PFLA analysis may be a useful technique for describing the mechanisms of general suppression, as well as identify biomarkers present in disease suppressive soils.

The primary objectives of this study were to determine the effectiveness of chemical, biologically based, and integrated pre-plant soil treatments to enhance yields and control soilborne disease in plasticulture tomato production. Secondly, PLFA analysis was utilized to assess and quantify changes in the soil microbial community composition due to the application of these treatments.

Part II

LITERATURE REVIEW

Plasticulture Vegetable Production

Plasticulture production is an agricultural technique that uses plastic mulch, raised beds and drip irrigation for vegetable farming. Production acreage that utilizes plasticulture is increasing. In 1999 one-third of Florida vegetables, including all tomatoes, strawberries, peppers, eggplants, and most melons, were produced on polyethylene-mulch. This represents 124,000 acres (USDA, 1998). Many vegetable growers use plasticulture because they believe that this system permits high quality, superior yields and early spring produce, thus, making the grower more competitive in today's vegetable markets (Wien et al., 1988). First used as a mulch in the late 1950s, polyethylene plastic film accelerates plant growth by increasing soil temperature and stabilizing soil moisture (Emmert, 1957; Courter and Oebeker, 1964). Properly scheduled drip (trickle) irrigation used in conjunction with plastic mulch helps maintain optimum soil moisture, aids plant establishment and promotes crop growth throughout the season (Jaworski et al., 1981; Bogle et al., 1989). Plastic mulch also helps protect vegetables from decay by preventing contact with contaminated soil. Additionally, black plastic mulches can reduce the problem of excessive weeds by decreasing the opportunity for growth (Wien et al., 1988).

Numerous advantages to plastic mulching have been described. These include earlier plant growth and maturity due to increased in soil temperature, and a one to two week earlier spring vegetables harvest (Bonanno and Lamont, 1987). Plastic mulch can improve vegetable quality by preventing direct contact of the edible product with the soil, thus, reducing decay. A plastic-mulch covered bed prevents rainfall from percolating through the soil and moving nutrients below the rootzone (leaching). Reduced leaching

conserves fertilizer, reduces costs and helps protect ground water from contamination (Bogle et al., 1989). The non-transmission of light through black plastic mulch prevents the growth of many weeds, reducing herbicide use (Wien et al., 1988). Soil water evaporation is reduced under plastic mulch. As a result, the soil moisture supply is more uniform and irrigation is needed less frequently. Soil under the plastic mulch tends to remain loose and well aerated compared with no-raised and non-mulched beds. Roots have an adequate oxygen supply and microbial activity is enhanced (Bogle et al., 1989). Plastic mulch improves the effectiveness of soil fumigation by slowing the escape of fumigants from the soil (Sumner et al., 1986). The major disadvantages of plastic mulch are removal and disposal problems, and increased initial production costs (Wien et al., 1988).

In plasticulture production, drip irrigation is often used in conjunction with plastic mulch. Advantages compared to non-mulched beds are: 1) increased water use efficiency - water loss is minimized due to decreased evaporation, runoff and deep percolation. (When drip irrigation is used in combination with plastic mulch, a crop's water requirements are supplied with only 30 to 50 percent of the water applied by conventional sprinkler irrigation) (Emmert, 1957), 2) fertilizer efficiency is increased due to the more precise application and placement of controlled quantities, 3) disease pressure is reduced as a consequence of the foliage not staying wet, hence, a diminished opportunity for the development and spread of many diseases, 4) weed problems are reduced since water is applied only in the row, thus, buffer zones remain drier and are less conducive to weed growth, and 5) increased yields and fruit quality due to more precise management of water and fertilizer (Phene et al., 1990; Blatt and McRae, 1986).

Some disadvantages of drip irrigation include: 1) higher annual cost due to the purchase of disposable drip tube, above-ground mains and submains, valves, layflat, filters, etc., and 2) require clean, low-particulate content water (emitters may become clogged, hence, water must be filtered to remove particulate matter and lines periodically flushed) (Bhella, 1988). Initial costs per acre are usually less for drip irrigation systems than sprinkler irrigation, however, longterm usage is thought to be more expensive (Deyton, 2003).

Tomato Production

Taxonomy: Kingdom: Plantae, Phylum: Tracheophytes, Class: Angiosperm, Order: Scrophulariales, Family: Solanaceae, Genus: *Lycopersicon*, Species: *esculentum*.

Production Methods: Tomato production is best suited to well-drained soil with high organic matter and a pH of 6.5. A pre-plant application of 400 to 600 lbs per acre of 10-10-10 is recommended. Rows are 4 to 6 ft apart and determinate plants are usually spaced 18 to 24 inches apart. Black plastic mulch is a preferred production method as it speeds maturity and increases yield. Irrigation and fertigation via trickle tape helps increase yield and reduce blossom end rot. The “Florida Weave” staking and tying system is commonly utilized (Sanders, 2001).

Production Statistics: In 2002 worldwide acreage of tomato in production was 9,879,799. China was the largest producer with 2,407,944 acres, followed by India, Turkey, Egypt, and the United States (436,700,000). In terms of fruit produced in 2002, the United States with 270,438,000 *cwt*, was second behind China (561,433,000 *cwt*). Total world production was 2,380,374,000 *cwt*. In 2001 the United States was the world’s fifth largest exporter of tomatoes, with 205,486 mt and the world’s leading

importer 823,541 mt. Tennessee production in 2002 was 3,900 acres harvested with a production of 1,326,000 *cwt* of fruit. Total farm value was \$43,758,000.

A detailed 1997 USDA study on tomato production costs in southwest Florida calculated operation costs of \$4,244 per acre and fixed costs of \$2,730 for a total pre-harvest cost of \$6,975 per acre. Overall production costs were calculated at \$11,973 per acre, or \$8.55 per carton. A 1996 study of operating costs at a New Jersey organic tomato farm calculated total cost at \$4,568 per acre (USDA, 2003). The average U.S. monthly retail price in 2002 ranged from \$1.45 lb in January to \$1.16 lb in September.

Soilborne Plant Pathogens

By definition a pathogen is a biotic agent that causes disease. Plant diseases caused by soilborne pathogens are the result of that organism affecting continued irritation to the plant and eventually initiating injurious alterations to the plant's metabolism. Plant cells and tissues are destroyed but the pathogen subsists obtaining nutrition from the host. Plant susceptibility and pathogen activity are the major factors that determine the degree of infection and damage (Agrios, 1997). Soil is the source of many phytopathogens, including bacteria, fungi, nematodes, protozoa, and viruses, as well as plant pests such as insects and mites.

Plant roots are important pathways for pathogen entry. The rhizosphere is the narrow zone of soil surrounding plant roots that contains root exudates, sloughed root remains, and large populations of soil microorganisms. Bulk soil, outside of the rhizosphere, has relatively smaller populations of microorganisms (Kluepfel, 1993).

Soil Agroecology

Changes to the soil environment are generally slow, producing a relatively stable habitat to which microorganisms become adapted. Slight changes in soil environmental conditions may have profound effects on populations of all microorganisms present.

Alterations due to agricultural practices tend to be varied, rapid, and ongoing, which permits little time for re-establishment of the biological equilibrium (Pahl-Worst, 1995). Intensive use of fertilizers, pesticides, tillage, irrigation, and other crop management practices can result in increased severity or incidences of plant disease. The introduction of monoculture often produced conditions that acted to shift the biological equilibrium toward the development of plant disease epidemics. The occurrence of an epidemic indicates that: 1) the pathogen is in high numbers, 2) the soil environment is favorable for the pathogen, and 3) inhibition by other microorganisms is not taking place. Control measures are then required, whether they are chemical, cultural, or biological (Baker and Cook, 1974; Killham, 1994).

Southern Blight

Southern blight, caused by the phytopathogenic fungus *Sclerotium rolfsii* Saccardo, represents one of the major disease threats to tomato crops in the southeast U.S. This pathogen is also a threat to many other crop species throughout the U.S.

Sclerotium rolfsii was first identified as the causal agent of “tomato blight” by Peter Henry Rolfs in 1892. He reported losses as high as 70% in contaminated fields. The general distribution of this pathogen is in tropical and sub-tropical regions. It is prevalent in Central America, the Caribbean, South America, and the southern U.S. There have been reports of serious crop losses due to this fungus from Maryland through

to the Gulf States of Texas and Florida. In Florida there have been reports of the disease year round and in all types of soil. *Sclerotium rolfsii* has a diverse host range. Its hosts are as varied as cotton in Arizona, sugar beets in California, and peanuts in Texas. Sporadic reports have been recorded in apple nurseries near Indianapolis and some horticultural crops in the northwest states of Oregon and Washington (Aycock, 1966).

Although difficult to assess, the economic losses caused by *S. rolfsii* can be quite severe due to the possibility of total destruction of the crop in some species. Five percent of the annual loss of crops in the southern U.S. could be attributed to this disease-causing organism (Jenkins and Averre, 1986). Damage to solanaceous vegetable crops seems to average between 25-30% of the total number of plants in the field (Aycock, 1966).

When conditions are favorable, *S. rolfsii* is able to infect any part of a susceptible plant, however, it is most commonly known as a parasite of stems. Tomato plants are most vulnerable to attack at ground level, usually between the first lateral root and the stem. The reason for the localization of the fungal development on this particular part of the plants is likely due to favorable conditions for mycelial growth due to the availability of moisture, oxygen, and nutritional needs. Hyphal growth rarely extends more than a few inches above ground level (Smith et al., 1986). Other plants parts (e.g. the fruit of non-plasticultured field tomatoes) that are in contact with contaminated soil can also become infected. Maturing fruit are particularly susceptible to invasion. The epidermis of the fruit at first appears sunken and yellowish. The skin tends to rupture when the diameter of the lesions extends 2 cm or more (Punja and Rahe, 1992).

Yellowing and wilting of leaves is often the first indicator of *S. rolfsii* infection. Nighttime recovery from wilt may occur initially but after several days the wilt becomes

permanent. Pronounced color change and dryness will follow. Lesions become apparent at the soil surface and under favorable conditions the stem becomes girdled. The cortex becomes decayed at the base of the stem and white mycelia become visible. Shortly thereafter the characteristic sclerotial development begins. Spherical fuzzy bodies appear which develop into smoother, more distinct sclerotia, adopting light tan to dark brown coloring (Aycock, 1966).

The temperature range for hyphal extension and dry weight production is between 8 and 40C, however, maximum growth and sclerotial formation occur at 27-30C (Punja et al., 1985). In culture, agar plates become covered by a silky white mycelial mat as soon as one day after inoculation. Similar growth rates can occur in sterile soil. In as few as four days, or after the growth media has been covered, the fungus begins to form sclerotia. They become mature within 2-3 weeks. At first, these structures are white but then assume the more familiar tan to dark brown coloring. They range in diameter from 0.5 - 2 mm and in mature-form contain a melanized outer rind two to four cell-layers thick (Punja et al., 1985). Underlying the rind is a cortical layer and then an innermost medullar region that is composed of loosely interwoven hyphae. These resistive structures contain amino acids, sugars, fatty acids, and lipids. The walls of the sclerotia contain chitin and laminarin (Punja et al., 1985). Studies have shown that in soil, sclerotial germination rates decrease below 2.5 cm and are almost negligible below 8 cm soil depth (Punja, 1985).

Integrated Disease Control

Benefits from the reduced use of potentially hazardous chemicals include, lowering the possible risk of pesticide residues on produce; alleviating negative environmental ramifications such as contamination of the soil, groundwater, rivers and lakes, or the atmosphere; non-promotion of resistant pathogens populations, and a reduction in health risks to farmworkers (Burn et al, 1987). Many of these factors do not directly affect the grower, however, the ultimate objective of an integrated control approach is to produce the maximum economic return/yields when all possible costs and effects are accounted, including the long-term preservation of the environment.

In conventional production, various control methods have been utilized to lessen the incidence of pytopathogens in commercial vegetable fields. Fumigation generally acts to eliminate populations of both pathogens and their antagonists. Reduced-dose applications will not result in a biological vacuum and may reduce the potential for a rapid reintroduction of opportunistic pathogens. The addition of composts to agricultural fields serves to reintroduce antagonistic fungal and bacterial species to the soil microcommunity (Baker, 1987). Combining fumigation with the addition of biocontrol agents can contribute to the creation of a new microbial balance in the soil (Gamliel and Stapleton, 1997).

Some of the limitations of biologically based pre-plant treatments can be overcome by combining control methods such as reduced dosage fumigation, biofumigation, solarization, or composts. The viability of the sclerotia of *S. rolfsii* was completely eliminated after seven days in organic amended solarized soil (Gamliel and Stapleton, 1993). Elad et al. (1980) demonstrated that combining solar heating with

applications of *Trichoderma* was more effective than either treatment alone. Punja (1985) contended that by altering the composition or activities of soil microflora, including the addition of biocontrol agents such as antagonistic microorganisms, there was a potential for disease control. *Trichoderma*, which has been described as a destructive mycoparasite that competes aggressively against other soil fungi, is acknowledged to suppress Southern Blight when introduced to field soils (Jenkins and Averre, 1986). The growth of *Trichoderma* is enhanced with solar heating (Jenkins and Averre, 1986). *Pseudomonas*, *Bacillus*, and *Streptomyces* species have also been shown to survive solarization and contribute to the biological control of plant pathogens (Antoniou et al., 1995).

Composts

The pre-plant application of composts has many advantages. They have the ability to provide biological control of plant diseases while simultaneously improving physical soil properties (Corti et al., 1998). Composts improve plant health and productivity due to enhanced soil micronutritional levels and improved physiochemical properties (increased bulk density, porosity, aeration) and increased water-holding capacity (Stratton et al., 1995). Application of more easily decomposable organic residues also helps to form humic colloids and collapse soil structures. In well-structured soils, the plant's ability to uptake nutrients is greatly enhanced compared to soils with low organic matter content. Many researchers have noted that disease suppression is due to the advantageous changes to soil microbial composition, including increases in the populations of known biological control agents (*Trichoderma*, *Bacillus*, and *Pseudomonas* spp.) (Stratton et al., 1995; Hoitink and Boehm, 1999; Lyons et al., 2001).

Composted soil amendments act to create a suppressive environment in the soil that can biologically control soilborne pathogens through microbial competition, antagonism, antibiosis, hyperparasitism, and possibly induce systemic resistance in plants (Hoitink and Grebus, 1994).

Composted materials are often derived from agricultural waste products and, hence, serve to “recycle” these compounds. Composting is an ecologically sound way to recycle organic farm wastes, such as animal manures and plant debris. It avoids the environmental hazards associated with burning, burying, or spreading of bulky agricultural by-products on fields. These methods can lead to problems such as increased particulate matter in the air, leaching and ground water pollution, and toxic excesses of phosphorous and nitrogenous compounds in the soil (Stratton et al., 1995). Potential problems with compost as a pre-plant treatment include using a product that has not been adequately matured (Hoitink and Fahy, 1986). Compost maturity levels can be gauged by measuring C:N ratios and pathogen levels.

The composting process can be divided into three phases. In the initial phase, easily biodegradable substances are metabolized as temperatures climb rapidly. The second phase is longer lasting and is characterized by high temperatures and the degradation of cellulose-based substances. The third, or curing phase, ultimately leads to the recolonization of the substrate with mesophilic microflora and microfauna (Hoitink and Fahy, 1986). Microorganisms commonly found in composts include: *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp. *Streptomyces* spp., *Trichoderma* spp., and *Gliocladium* spp. (Hoitink and Grebus, 1994). Some of these species are recognized to exhibit properties of biological control. Biological control of plant pathogens has been

observed and studied for many years. As defined by Baker (1987), biocontrol is “the action of parasites, predators, or pathogens in maintaining another organism’s population at a lower average than would occur in their absence.”

Biofumigation

Biofumigation is the use of volatile plant chemicals for control of soilborne pests. The compounds (E)-2-hexenal, hexanal, (Z)-3-hexenal, and (Z)-3-hexenol are often produced by crushed green plant material, including *Brassica* leaves (Buttery, 1981). These products have been shown to suppress the pathogens *Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Didymella lycopersici*, and *Cladosporium fulvum* (Urbasch, 1984). In addition, macerated *Brassica* tissues release isothiocyanates (ITCs) - particularly effective pesticidal plant chemicals. Although the fungicidal properties of ITCs have been identified since at least 1937 (Walker et al., 1937), only recently has the practice of using *Brassic*as for pest control been termed “biofumigation” (Angus et al., 1994). Volatiles from several *Brassica* species have been shown to suppress the growth of the tomato pathogens *Pythium ultimum*, *R. solani*, and *S. rolfsii* (Charron and Sams, 1998; Charron and Sams, 1999; Harvey et al., 2002).

The biocidal activity of *Brassica* residues against fungal pathogens, nematodes, weeds, and insects is frequently attributed to the ITCs from *Brassica* tissues. ITCs are breakdown products of glucosinolates, a class of botanic compounds containing a thioglucose moiety, a sulphonated oxime, and a side chain (R-group). Sorensen (1990) reviewed the chemistry of glucosinolates. Among cultivated crops, glucosinolates are characteristic of the *Brassicaceae* family. Enzymatic hydrolysis of glucosinolates yields compounds including ITCs, thiocyanates, nitriles, epithionitriles, oxazolidinethiones, and

amines. In six soils tested, the predominant breakdown product of the glucosinolate sinigrin was allyl ITC (Borek et al., 1994). This result is important for two reasons: 1) allyl ITC is an effective, broad-spectrum pesticide (Mithen et al., 1986; Isshiki et al., 1992), and 2) substantial quantities of it can be produced for field application. Black mustard (*B. nigra* L. W. Koch) and Indian mustard (*B. juncea* L. Czern and Coss.) produce high levels of allyl ITC (Tollsten and Bergström, 1988) and could be utilized in a biofumigation cropping system.

ITCs inhibit numerous pathogens (Delaquis and Mazza, 1995). Canola (*Brassica napus* L.) roots release methyl ITC (Angus et al., 1994), which is used commercially as a fungicide and which was fungitoxic to *Helminthosporium solani*, the causal agent of potato silver scurf (Vaughn et al., 1993). In laboratory studies, the naturally occurring compounds allyl ITC, β -phenylethyl ITC, benzyl ITC, and 3-methylsulfonylpropyl ITC suppressed the fungi *Aspergillus niger*, *Penicillium cyclopium*, and *Rhizopus oryzae* (Drobnica, 1967). In an *in vitro* study, allyl ITC, p-hydroxybenzyl ITC, and 4-methylsulphinybut-3-enyl ITC completely inhibited the conidial germination of *B. cinerea*, *Monilinia laxa*, *Mucor piriformis*, *Penicillium expansum*, and *Rhizopus stolonifer* (Mari et al., 1993). In a study, the presence of glucosinolate degradation products from rapeseed correlated strongly with toxicity of rapeseed meal toward *Aphanomyces euteiches*, the cause of *Aphanomyces* root rot of peas (Smolinska et al., 1997). Mayton et al. (1996) reported that volatiles from the Indian mustard cultivar 'Cutlass' were fungicidal to *Verticillium dahliae*, *V. albo-atrum*, *Colletotrichum coccodes*, *P. ultimum*, and *R. solani*. When compared with other Brassica species, Indian

mustard was found to be the most effective at reducing radial mycelial growth of both *P. ultimum* and *R. solani* (Charron and Sams, 1999).

Indian mustard residues also inhibited the growth of *S. rolfii* (Harvey et al., 2002). Volatile compounds of *Brassica oleracea* suppressed the bacterial pathogen *Pseudomonas marginalis* in laboratory experiments (Charron and Sams, 2002).

In field studies, *Aphanomyces* root rot of peas was decreased when soil was amended with residues from *Brassicaceae* such as cabbage (*B. oleracea* L. var. *capitata*), kale (*B. Oleracea* L. var. *acephala*), rapeseed, and white mustard (*Sinapsis alba* L.) (Chan and Close, 1987; Muehlchen, 1990). Dried cabbage and broccoli amendments also effectively reduced the number of viable microsclerotia of *V. dahliae* in laboratory soil experiments (Morgan and Michailides, 1992). In experiments examining the relationship of soil temperature and broccoli amendments to *V. dahliae* microsclerotia, the maximum number of microsclerotia was reduced within 15 days at all temperatures tested (10, 15, 20, 25, 30, and 35 °C). It was also determined that the effectiveness of broccoli amendments increased with soil temperature (Subbarao and Hubbard, 1996).

Solarization

Solarization is a technique that elevates soil temperatures beneath a clear plastic layer to reduce soilborne pests. The capacity of soil solarization to suppress propagule numbers of soilborne pathogens relies on numerous factors. The temperatures obtained in the moistened soil covered by the transparent sheeting and the exposure time of the organisms to elevated temperatures, are both important characteristics of this pre-plant soil treatment (Katan and Devay, 1991). Solarization has been effective for disease control in many geographical locations around the world. It is most successful in regions

with the appropriate meteorological parameters, such as high air temperatures and extended periods of high solar radiation (Katan and Devay, 1991).

In the U.S., successful solarization studies have been conducted in several states. In a study in California, solarization alone increased strawberry yield 12% over non-treated plots (Hartz et al., 1993). In northern Florida, soil solarization decreased densities of *Phytophthora nicotianae* at a depth of 25 cm and *Ralstonia solanacearum* at a depth of 15 cm (Chellemi et al., 1994). In an Alabama study, temperatures attained via solar heating were 48C at the soil surface and 34C 20 cm below the surface (Himelrick and Dozier, 1991). In Tennessee, temperatures of 49.5C at 5 cm depth and 46.0C at 15 cm depth have been recorded under solarization treatments (Price, 1999).

Lethal temperatures are unlikely to be reached in many regions of the U.S., however, solarization may still be effective when combined with other pest management strategies such as biofumigation and composting. Katan and Devay (1991) stated that microbial processes induced by solarization may contribute to disease control beyond the physical effect of heating. Both the vulnerability of soilborne pathogens to mycoparasitic attack and the activity of beneficial microorganisms could be enhanced by solarization. In particular, Lifshitz et al. (1983) showed that sclerotia of *S. rolfsii* became heavily colonized by bacteria under the influence of the sub-lethal temperatures generated by solarization. Cracking of the rind and increased leakage of organic compounds from the sclerotia was reported. Hence, the germinability of these propagules was compromised.

The re-introduction of biocontrol agents such as *Trichoderma* spp. and *Bacillus* spp. for the control of soilborne diseases may be more effective when integrating solarization with biofumigation and composts than either treatment alone. Populations of

these two microbial antagonists increased relative to other microorganisms in solarized soil (Stapleton and DeVay, 1986).

Soil Fumigation with Dazomet (Basamid®)

Basamid is the commercial name of a chemical soil sterilant registered in the U.S. for use on turf, plus nursery and tobacco seedbeds. It is considered to be a chemical alternative to methyl bromide and consists of the compound dazomet (99.0%) and inert ingredients (1%). When applied to moist soils, the pesticide's active ingredient (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) breaks down into methyl isothiocyanate (MITC). Other commercially available soil fumigants, such as Vapam, also produce MITC upon volatilization (Meister et al., 1984). Basamid is granular in formulation and stays inert until application. With a half-life of less than 24 hours under favorable conditions, degradation is more rapid than methyl bromide. It is not considered an ozone-depleting substance and is non-persistent in the environment (BASF, 1999).

Soil preparation is an important factor when using dazomet, especially pertaining to moisture and temperature. The soil temperature should be above 10C and soil must be moist and finely cultivated. Phytotoxicity can result if the fumigated soil is not properly aerated after Basamid application (Pennington, 1995).

When dazomet is tilled into moist soil it is quickly broken down to several strong irritant products. Methyl isothiocyanate, accounts for most of the fumigant activity, but the toxic compounds formaldehyde, monomethylamine, hydrogen sulfide and carbon disulfide (in acid soils), are also formed. The compounds are gases, or volatile liquids, that diffuse upward through the spaces in the soil with the toxic compounds killing living organisms that they contact (Juzwik, 1994).

When applied correctly dazomet has broad-spectrum effectiveness against soilborne pests including nematodes, fungi and weeds (McElroy, 1985). It can successfully control root rots, damping off, and wilt diseases caused by soilborne fungi such as *Aphanomyces* spp., *Fusarium* spp., *Phytophthora cactorum*, *Pythium* spp., *Rhizoctonia* spp., *Thielaviopsis basicola*, and *Verticillium* spp. (Harris, 1990).

The breakdown of dazomet is greatly dependent on soil moisture and temperature, as well as soil type. Low temperatures and higher alkalinity of the soil reduce the rate of breakdown and decrease the efficacy of dazomet, while sandy soil, moisture, and warm conditions favor breakdown. The breakdown products, which have half-lives of several days to a few weeks depending on soil conditions, are primarily lost from the soil through volatilization (Juzwik, 1994). MITC is primarily broken down by hydrolysis and photolysis and has a relatively short half-life but may persist longer under low temperatures and moist conditions (Labrada and Fornasari, 2001). Dazomet is unstable in water with a half-life ranging from 1.46 to 8.6 hours, depending on pH. This rapid breakdown reduces the likelihood that dazomet will leach into groundwater (Juzwik, 1994).

Basamid can control a number of soilborne pests. These include root-knot nematodes (*Meloidogyne incognita*), black root rot caused by *Thielaviopsis basicola*, and black shank (*Phytophthora parasitica* f.sp. *nicotianae*) (Miner and Worsham, 1990). Suppression of the phytopathogenic fungus *Fusarium oxysporum* has also been shown (Littke, 1994).

In 1985 McElroy demonstrated that Basamid was effective, compared to methyl bromide/chloropicrin, in controlling *Phytophthora*, *Pythium* and *Fusarium*, reducing

populations to 2.3 propagules/gram of soil, compared to the control level of 243. Seedling mortality and overall plant quality were increased compared to controls (McElroy 1985). MITC based fumigants have been shown to reduce the build up of *cylindrocladium* sp. in fields of groundnuts (*Arachis hypogaea* L) (Cline and Beute, 1986). This control was later confirmed (Phipps, 1990).

Soil Microecology and Signature Lipid Biomarkers

The great majority of soil microbes have not been cultured to date and their direct microscopic analysis in the soil, or attempts at quantitative release from the soil matrix, are time consuming and ineffective (Pace, 1996). PLFA is a quantitative method to define the rhizosphere/soil micro biota. Research has established that: 1) signature lipid biomarker analysis (SLB) is a quantitative measure of viable biomass, community composition, and nutritional/physiological status of the in situ microbiota in soil; and 2) SLB analysis enables the detection of changes in microecology of agricultural soils exposed to different treatments (White et al., 1996).

Lipids are among the cellular components used for the analysis of the microbiota without isolation. Lipids are ubiquitous components of the cytoplasmic membranes that surround all living things. The plasma membrane consists of phospholipids and proteins organized into two layers. The phospholipid bilayer is the basic structural unit of all membranes and serves as a permeability barrier to most water-soluble substances. Each phospholipid molecule consists of two hydrophobic “tails” and a hydrophilic “head,” and is therefore an amphipathic molecule. The fatty acid “tails” of the membrane lipid are usually 14 to 24-carbon atoms in length, with 16 and 18-carbon fatty acids especially common. Membrane thickness is primarily dictated by the chain length of the fatty acids

required for bilayer stability (Fig. 1). In addition to differences in length, the fatty acids also vary considerably in the presence and number of double bonds. For example linoleate is an 18-carbon unsaturated fatty acid with two double bonds (Becker et al., 1995). The distinctive profile created by the fatty acid composition of a membrane makes lipid biomarkers unique to a specific group of organisms. Each microorganism has a characteristic pattern of lipid composition that can be used as a diagnostic characteristic and, thus, lipids can serve as signatures to define community structure (White et al., 1996). Published microbial lipid components facilitate community lipid profile analysis (Ratledge and Wilkinson, 1988).

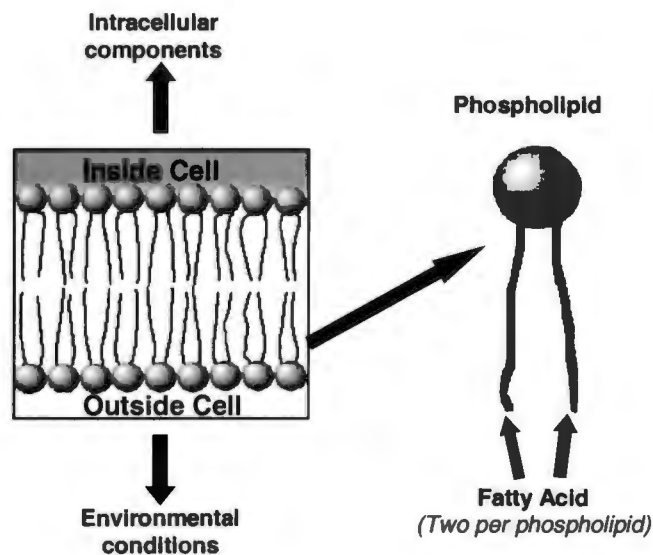


Figure 1. Diagram of plasma membrane displaying location of phospholipid fatty acids as well as basic structure of lipid components (Microbial Insights, 2003).

Signature lipid biomarker analysis provides quantitative insight into three important attributes of microbial communities: viable biomass, community structure, and nutritional/physiological status (White et al., 1997).

Viable Biomass

Traditional measures of biomass can be problematic. The detachment of microorganisms from soil particles is selective and often not quantitative (Pinkart et al., 2000). The determination of total phospholipid ester-linked fatty acids provides a quantitative measure of the viable, or potentially viable biomass. Viable microbes have an intact membrane that contains PLFA. Cellular enzymes hydrolyze the phosphate group within minutes to hours of cell death (White et al., 1979). In soil studies, the viable biomass as determined by PLFA was equivalent (but with a much smaller standard deviation) to that estimated by intercellular ATP, cell wall muramic acid, and acridine orange direct counts (AODC) (Balkwill et al., 1988). Conversion factors between lipid analysis and cell numbers have been developed (Pinkart et al., 2000). Assays that are dependent on enzymatic activities, growth, or respiration to determine microbial biomass may be less reliable because metabolic activity does not necessarily correlate with microbial biomass (Pinkart et al., 2000). The accurate repeatability of direct counts is very low and error is large. Also, abundance is not biomass (Montaga, 1982). PLFA measurements are more constant (White et al., 1996).

Community Composition

SLB analysis cannot be used to detect every species of microorganism in a sample because many species have similar PLFA patterns. However, SLB analysis provides a quantitative definition of the microbial community structure. Specific groups of

microbes often contain characteristic fatty acid profiles (White et al., 1996). Hydroxy fatty acids are predominantly found in Gram-negative bacteria such as *Pseudomonas* spp. Whereas Gram-positive bacteria, such as *Bacillus* spp., often contain terminally branched saturated PLFA (Ratledge and Wilkinson, 1988). Although they have been reported in cyanobacteria, polyenoic PLFA generally indicate the presence of microeukaryotes, with the fatty acid 18:2 ω 6 prominent in fungi. Hierarchical cluster analyses of PLFA patterns of total microbial communities can be used to quantitatively define relatedness between different microbial communities.

Nutritional Status

Specific patterns of PLFA can indicate physiological stress (White et al., 1997). Cyclopropane fatty acids (cy17:0 and cy19:0) are formed preferentially over the monoenoics 16:1 ω 7c and 18:1 ω 7c as bacteria switch from log to stationary phase growth (Findlay et al., 1989; Guckert et al., 1986; White et al., 1996). The formation of poly β -hydroxyalkanoic acid (PHA) in bacteria relative to the PLFA provides a measure of nutritional status. For example, bacteria grown with adequate carbon and terminal electron acceptors form PHA when they cannot divide because an essential component is missing (phosphate, nitrate, trace metal, etc.). The PHA/PLFA ratio in rhizosphere microorganisms from *Brassica napus* grown in a nutritional deprived sand media was <0.0001. Ratios greater than 0.2 usually indicate unbalanced growth (Pinkart et al., 2000). The proportion of triglyceride relative to PLFA provides a measure of the nutritional or physiological status of microeukaryotes.

Response to environmental changes, such as desiccation or exposure to toxic environments (e.g. pesticides), can lead to minicell formation and a relative increase in

specific *trans* monoenoic PLFA compared to the *cis* homologues. When exposed to a toxin, *Pseudomonas putida* increased the proportion of *trans* monoenoic PLFA, however, a decreased proportion of monoenoic to saturated PLFA was more closely linked to decreased permeability (Pinkart et al., 2000). *Trans/cis* ratios of greater than 0.1 have been shown to indicate starvation in bacterial isolates. Starvation of bacterial species also leads to the conversion of monoenoic PLFA to cyclopropane PLFA. Prolonged exposure to conditions sustaining stationary growth phase induce the formation of the cyclopropane PLFA (Gucket et al., 1987).

Increasing the specificity of lipid analysis by extracting intact lipids, fractionating them into major lipid classes (usually neutral lipids, glycolipids and polar lipids) and derivatizing by use of a mild alkaline transesterification provides a sufficiently selective analytical system for the direct analysis of soil lipid extracts (White et al., 1979). When polar lipid fractions are examined for patterns of fatty acids it proves possible to readily detect differences in soil microbiota as a result of different agricultural practices (Peacock et al., 2001; Zelles et al., 1992). Zelles et al. (1992) found that when crop rotation is implemented the ratio of monoenoic to normal fatty acids favors the latter. He also found a lower microbial biomass in soils that had been cultivated and treated with copper-based fungicides.

Analytical Sensitivity

Colorimetric analysis of lipid fractions is relatively insensitive and requires large sample populations (White et al., 1979). PLFA analysis using GC/chemical ionization/MS produces fewer ion fragments than MS and so increases sensitivity (Tunlid et al., 1989). The implementation of rapid sequential high pressure/temperature

extraction to provide the biomarker fractions suitable for high performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC/ESI/MS/MS) expanded the scope, comprehensiveness and sensitivity of the PLFA analysis. With HPLC/ESI/MS/MS the precursor ions of each phospholipid molecule species can be selected in the first quadrupole for fragmentation and the product ions scanned in the third quadrupole. The limits of quantification for GC/MS are $\sim 10^5$ bacterial cells whereas with HPLC/MS it is $\sim 10^4$ (Fang and Barcelona, 1998). Unlike GC, the components for HPLC analysis need not be volatilized. The coupling of electrospray ionization (ESI) increases the ionization of the analytes to essentially 100%, thereby increasing the sensitivity of the method several fold (Fang and Barcelona, 1998). Utilizing the tandem MS/MS, specific parent ions can be selected for fragmentation and specific product ions can be detected, decreasing the chemical noise and increasing the signal to noise ratio significantly. The limit of quantification of specific fatty acids using HPLC/ESI/MS/MS was found to be two orders of magnitude lower than GC/MS when examining phospholipid samples (Lytle et al., 2000).

Organic Agriculture

In October of 2002 the USDA fully implemented the National Organic Program Standards (NOPS) (USDA, 2002). The result is a clarification of the certification for organically farmed produce allowing consumers to easily identify foods grown via organic methods. National certification is likely to increase consumption and demand for organic produce. Organic farming has been identified as an emerging agricultural sector and as such, an alternative to traditional, high chemical input crop production (Lockeretz, 2000; Koenig, 2002).

The USDA has identified certain non-synthetic, biological disease management inputs as being necessary for aggressive pathogen control. The use of botanical, or plant-derived compounds, are recognized. Biofumigation is such a method. The USDA standards also recognize the benefits of applying mature, well-produced composts in preference to raw animal manures (NOPS, sec. 205.203). Identifying soil microbial community profiles that are linked with disease suppression also has been recognized as a priority area of investigation by the USDA (Koenig, 2002).

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Part III

Composts, Biofumigation, Solarization, and Dazomet Influence Tomato Yields in Plasticulture Production.

Abstract

Fresh market tomato production in the southeast states has a retail value of \$587,836,000 from 54,700 acres harvested. Sales in Tennessee are \$44 million annually. Production utilizing plastic mulch, drip irrigation and fertigation is a preferred technique. The objective of this study was to investigate how biologically based soil treatments and the fumigant dazomet effect tomato fruit yield.

Amendments with substantial quantities of organic matter alter the physiochemical and microbiological characteristics of the soil, impacting plant growth and fruit yield. Up to ten treatments were investigated throughout the three years of the study. The treatments were arranged by split plot with replication in three pH ranges throughout the field. The pre-plant treatments were biofumigation with incorporated Brassica cover crops, spent mushroom and poultry waste composts, solarization, and the commercial fumigant dazomet. Integrated treatments also were investigated.

Tomato fruit yields were impacted considerably by soil treatment. The compost based treatments improved yields significantly in each season compared to the control. In 2002, yields in this treatment were 97% greater than the control. Peak yields occurred in 2001 with 7.45 kg/plant of fruit in the compost treatments compared with 3.90 kg/plant in the control plants. Integrated treatments that utilized compost also had high yields, although they were not significantly higher than the compost alone in any year. The biofumigation treatment was impacted by loss of biomass due to freezing temperatures. As such, effective biofumigation did not occur in 2000. In 2002 successful biofumigation did occur and yields were improved. Dazomet fumigation improved yields in 2000 and 2002, but solarization was not effective in the two years it was implemented.

Introduction

Sustainable, organic and traditional methods for vegetable production.

In the twentieth century the widespread development and refinement of monoculture production and the associated increased mechanization of agriculture has resulted in high-yielding intensive systems. However, it has been recognized that high-input farming methods can lead to undesirable impacts in the ecosystem. A total system approach to crop production is regarded as being more sustainable with benefits extending to the entire community.

Establishing practices that manage, instead of disrupt, the soil's nutritional state and microecology act to produce a balanced, productive agroecosystem. Sustainable agricultural practices have been implemented at many sites throughout the world. There is evidence that yields can be maintained with a significant reduction in the use of chemicals when integrated approaches are combined with increased knowledge of the soil ecosystem (Lewis et al, 1997). The implications associated with increased attention to plant and soil health, and a more judicious use of farm resources, is a shift away from the formulated use of agricultural chemicals and toward a total systems approach.

Soil treatments should be chosen for their contribution to increased productivity and long-term sustainability. They should act beneficially on organic matter, soil structure, nitrogen production, soil microbial activity, nutrient enhancement, rooting action, weed suppression, as well as water and soil conservation. The use of cover crops and composted amendments satisfies many of these requirements (Sullivan and Diver, 2001). Also referred to as green manuring, cover crops can be used not only for the

prevention of soil erosion and weed deterrence but also for soil improvement and, in the case of cruciferous plants, for disease suppression.

Fertilizer requirements can be reduced through the use of a cover crop and/or composts. Nutrients such as N, P, K, Ca, Mg, and S are accumulated by the cover crop as it grows. The incorporation of a green manure crop into the soil can cause a rapid increase in microorganisms. Rodriguez-Kabana et al. (1987) noted increases in populations of actinomycetes, algae, bacteria, and fungi in organically amended soils. The microbial breakdown of the incorporated plant tissues releases nutrients and makes them available to the following crop (May, 1981). The slow release property of this action results in more efficient nutrient use. Factors such as soil moisture, temperature and C:N ratio influence microbial action.

When cover crops are used in conjunction with composts and solarization the parameters for microbial activity can be enhanced. Solarization will elevate soil temperatures; a leafy cover crop will add moisture to the soil, while composts will increase the water-holding capacity of the soil. The decreased bulk density of compost amended soils increases porosity, allowing air to permeate through the substrate and sustain microbial activity. Composted soils are more likely to maintain the optimum C:N ratio of between 15:1 and 25:1 for rapid decomposition of organic matter (McLeod, 1982).

The use of composts and cover crops will add organic matter and improve soil tilth. It has been documented that cover crops increase levels of total soil C and N (Hu et al., 1997). The addition of mucilaginous compounds results in greater soil aggregation. A well-aggregated soil tills easily, is well aerated, and has a high water infiltration rate

(Allison, 1973). Improved soil structure will aid the retention of moisture and improve crop performance (Blevins et al., 1971).

Alleopathic cover crops are an important method of weed control in sustainable agriculture. They act not only to shade, reduce space, and compete for light, water, and nutrients against weeds but also to inhibit weedy growth via the release of inhibiting toxins (Sullivan and Diver, 2001).

Tomatoes are an important vegetable crop with a high market value. In several studies, the improvement to soil physical and chemical properties through the addition of composts has resulted in significantly better plant growth and higher yields than commercial fertilizers alone. In Connecticut, Maynard (2000) documented that plots that were both composted and fertilized inorganically out-yielded either treatment alone. Maynard found that fertilizer rates could be halved in the second year of the study with no loss of yield in the composted plots. An experiment in Ohio documented that marketable yield increased by 33% in compost-amended organic plots (Abbasi et al., 2002). In North Carolina, fully organic, compost-amended systems produced equal yields to conventionally fertilized systems (Bulluck and Ristaino, 2002).

There are many benefits to the adoption of agricultural practices that utilize organic soil additions that enhance plant and soil health. Farm wastes products can be recycled and utilized as beneficial organic soil conditioners and sources of nutrition. The vegetable producer could become less reliant on expensive chemical inputs and the organic farmer could enhance overall production.

The objective of this research was to evaluate the effects of biologically based pre-plant soil treatments on the yield of tomatoes grown in a plasticulture system. The

soil fumigant Basamid was evaluated as an alternate chemical control to methyl bromide. Treatment effects were assessed when implemented alone and when integrated with chemical treatments.

Materials and Methods

Field Experimental Design: 1999-2002

Tomato research plots were established on the Knoxville Experiment Station in fall, 1999 with the first fruit harvest occurring in the summer of 2000. Tomato production research was conducted on these plots for the next three years. The statistical design was a split plot arrangement of a randomized complete block design with pH whole plot treatments and the pre-plant amendments utilized as the subplot treatments. The field measured 76.3 m x 30.5 m in total. It was divided into thirty rows measuring 30.5 m x 1.5 m with 1.0 m alleys between the rows and 3 m buffer zones between the blocks of 10 rows. Four blocks were designated running north to south, thus, 120 separate replicates of 10 m x 1.5 m existed. Within each block, three whole plots were established, each 23.5 m x 10 m and containing 10 rows. Within each whole plot, the 10 subplots (replicates) were available for individual treatment assignment. Thus, each individual treatment could be replicated twelve times throughout the entire field – four times in each of the three pH ranges. The field was limed to maintain whole plot treatments of soil pH in the range of 5.5-6.5, 6.5 -7.0 and >7.0. When formed into plastic mulched raised beds, each replicate measured 7.3 m x 0.7 m with a 0.6 m buffer between each adjacent replicate. Within the three pH plots, ten subplot treatments were applied. One control treatment was utilized allowing for the comparison of seasonal yields.

Pre-plant Treatments

Biofumigation: In 2000, three Brassica treatments were implemented: 1) Indian mustard (*B. juncea* - PI 458928) seeded at 13 g/plot, 2) 'Fall Raab' (*B. campestris*) seeded at 8 g/plot, and 3) a mixture of Indian mustard and fall raab. In 2001 and 2002 'Florida Broadleaf' mustard (*B. juncea* L. Czerniak) was planted at 13g/row. The seeds were drilled in rows along the length of the plot. In 1999 all seeds were planted in September and the mature crop incorporated in May. In 2000 all biofumigation treatments were planted in September, however, the "fall" treatment was incorporated in December while the "spring" treatment was overwintered and incorporated in May. In 2001 all biofumigation treatments were planted in September and incorporated in December. The Brassica cover crop was mowed with a flail-mower and immediately tilled into the soil. Beds were raised directly after this step.

Composting: Two compost types were used: spent mushroom substrate (SMS) and poultry waste compost (PWC). The SMS was obtained from Monterey Mushrooms (Loudon, Tennessee). The PWC was obtained from Rollins Farms (Prospect, Tennessee). When packaged, the Rollins Farm PWC is known as "R-grow" and is sold as a soil conditioner. Both composts were applied at a rate of 11 mt/ha. This rate equates to 54 kg of compost per each 10 m row. The composts were evenly dispersed on top of each row and incorporated via tilling to a depth of 15 cm. In 2000, SMS and PWC were applied as separate treatments. In 2001 and 2002, the two composts were mixed (50% SMS:50% PWC) but applied at the same total rate of 11 mt/ha.

Solarization: Soil solarization was applied twice throughout the year: 1) late-summer/early fall -immediately following the removal of the previous seasons tomato

crop, and 2) late winter/early spring - prior to the transplant of the new seasons tomato seedlings. A 4-mm clear plastic tarp was laid over the 10-m rows. Ten-cm high blocks were used as spacers to create an airspace between the black plastic of the bed and the clear plastic tarp. The sides and ends of the tarp were sealed with loose soil in order to trap the heat.

Fumigation: Dazomet, in the form of the BASF product Granular Basamid[®], was used at the recommended rate of 398 kg/ha. The granules were measured and spread evenly over the bed. The soil was tilled to a depth of 15 – 20 cm. The beds were then either water-sealed or immediately raised and covered with black plastic mulch.

Combination Treatments: Dazomet with compost: Dazomet was incorporated into the moistened soil at least four weeks prior the anticipated date for bed formation. Overhead irrigation was used to water-seal the beds and keep them moist during the critical volatilization phase. After approximately four weeks, the beds were re-tilled to release any remaining irritant products. The compost was then applied and the plastic mulched beds formed.

Biofumigation with compost: Prior to seeding of the *Brassica* cover crop compost was applied at the same 11 mt/ha rate used in the compost only treatment. The same procedures for standard biofumigation were followed.

Compost with dazomet at 50%: The beds were amended with compost then a 50% dose of dazomet (199 kg/ha) was applied.

Solarization with compost: Adhering to the practices employed in the ‘solarization’ treatment, a clear plastic tarp was laid over the black plastic mulched bed that had been previously amended with compost.

Biofumigation with dazomet at 50%: After the Brassica cover crop was mowed, a 50% dose of dazomet (199 kg/ha) was distributed over the plot, immediately followed by tilling and bed formation.

General Production Practices

Tomatoes were grown utilizing commonly implemented plasticulture production methods (Rutledge et al., 1999). In the fall of the year prior to each growing season 10.8 kg/ha of 10-10-10 and 0.37 mt/ha of lime for pH maintenance were broadcast onto the field. The 0.7 m-wide rows were tilled to a depth of 15 – 20 cm. The beds were formed, black plastic laid, and trickle tape inserted in a one-pass operation.

The tomato cultivar ‘Celebrity’ was used. The transplants were planted through the plastic at 0.45 m intervals. Twelve seedlings per 7.3 m bed were planted. The experimental data was collected from the middle ten plants. For one week following the original planting date, dead seedlings were replaced as needed. The plants were staked using the Florida weave system after the first pruning (Rutledge et al., 1999).

Insecticides were applied as commercially recommended (Extension bulletin, 1999).

Weeds were controlled manually. During the growing season the plants received trickle irrigation approximately equal to 25 mm/week. Fertigation through the drip irrigation system consisted of the following: within one week of transplanting a starter solution of 9-45-15 at the rate of 8.1 kg/ha was applied. After initial flower set, CaNO_3 and KNO_3 were alternated weekly at 0.9 kg N/ha.

Harvesting was conducted twice weekly. The fruit from each replicate was harvested separately. USDA grading standards (1991) were used throughout the study. The grades (diameter) are small (5.40 – 5.79 cm), medium (5.72 – 6.43), large (6.35 –

7.04 cm), and extra large (>6.99 cm). Grading was performed by hand in 2000 and 2001 using grading boards. In 2002 a mechanical grading table was used. Harvest weights were first recorded on a worksheet and then transferred to a computer spreadsheet.

The specific treatments evaluated in each of the three years of the experiments are shown in Table 1. Treatments were changed in 2001 and 2002 to reflect results observed in the experiment of the previous year. The field plot design is shown in Figure 2.

Notes on Field Treatments

In 2000, PWC and SMS composts were applied separately at 11 mt/ha. In 2001 and 2002 all “compost” treatments consisted of a 50:50 mixture of PWC/SMS combination at 11 mt/ha total rate. In 2000, planting beds were formed in the spring. In 2001, all of the beds, apart from the ‘spring’ biofumigation treatment, were formed in the fall of the previous year. In 2002, all beds were formed in the fall. Non-Brassica treatments were sown with a rye cover crop in all years at a rate of 100 g per 7.3 m row.

Table 1. Pre-plant soil treatments for tomato production field experiment for 2000-2002.

Field Treatments			
TRT.	2000	2001	2002
1 ^x	Biofumigation (Ind.mstd) ^z	Biofumigation (spring) ^z	Biofumigation ^f
2	Biofumigation (fall raab) ^z	Biofumigation (fall) ^y	Biofumigation + daz.50% ^y
3	Biofumigation (mix) ^z	Biofumigation + compost ^y	Biofumigation + compost ^y
4	Compost (PWC)	Compost (PWC/SMS)	Compost (PWC/SMS)
5	Compost (SMS)	Solarization + compost	Solarization + compost
6	Control	Control	Control
7	Dazomet	Dazomet	Dazomet
8	Dazomet + compost	Dazomet + compost	Dazomet + compost
9	No treatment	Solarization	Solarization
10	No treatment	Compost + daz.50%	Compost + daz.50%

^z Brassica cover crop sown in September and incorporated the following spring.

^y Brassica cover crop sown in September and incorporated in December.

^x In 2000 all beds were formed in the spring. In 2000 and 2002 all beds, apart from “spring” biofumigation were formed in the fall of the year prior. All non-Brassica treatments were sown with a rye cover crop (100g/plot).

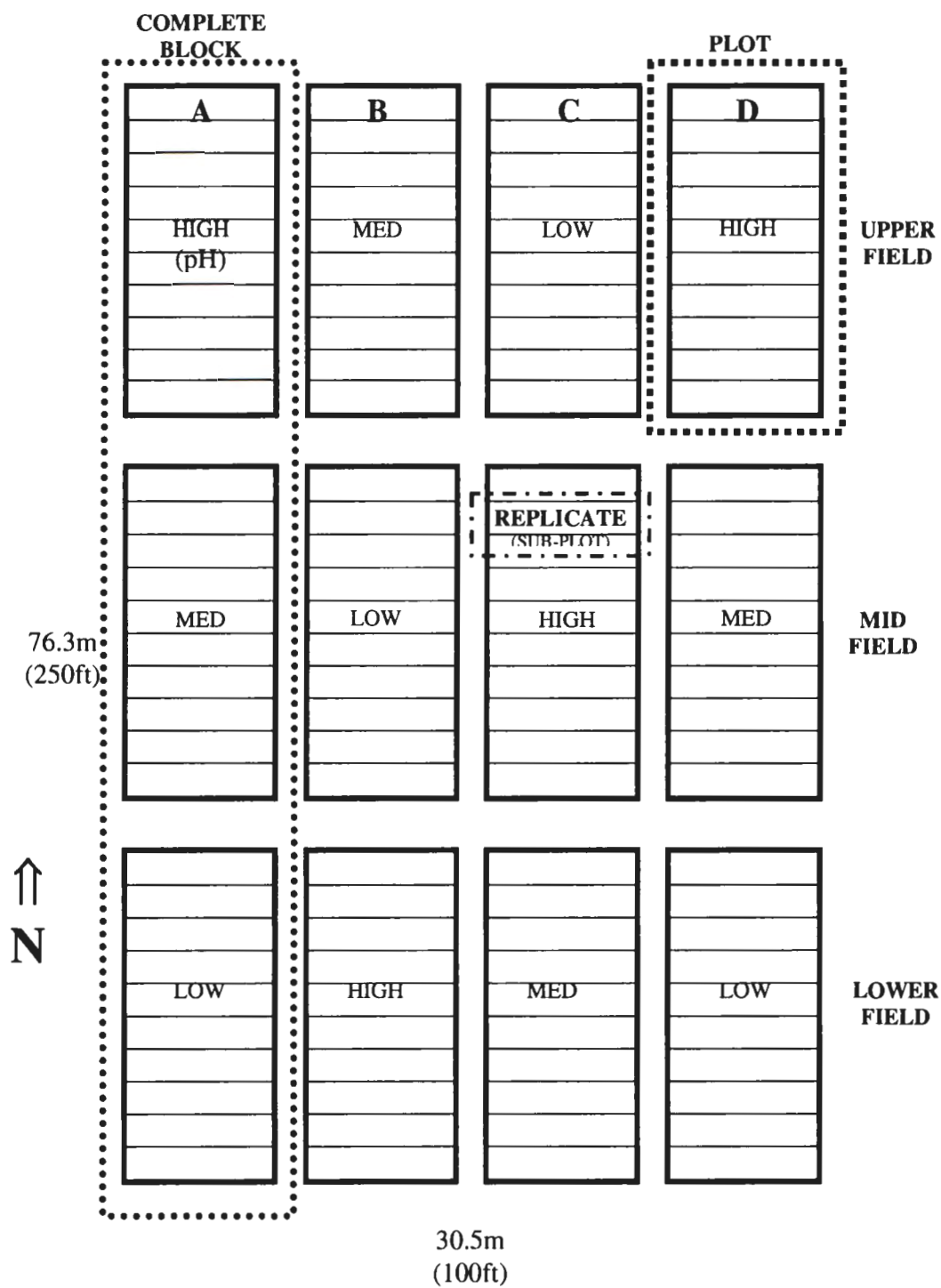


Figure 2. Field layout and experimental design for tomato experimental plots.

Results

Field Experiment: 2000

Tomato harvesting was conducted from August 1 to August 21, 2000 with seven separate harvests. The fruit from each replicate was kept separate and the quantity, weight, and size grade were determined for each replication of each treatment. Fruit defects also were noted. Marketable fruit were considered to be those with a diameter greater than 6.35 cm and having no defects.

In the growing season of 2000, all seven experimental treatments had higher yields than the controls (Table 2, Figure 3. All subsequent tables and figures are in the appendix). The increase above the control ranged from 34% for the “mixed” biofumigation (fall raab plus Indian mustard) to 83% for the dazomet plus compost treatment. However, only the fall raab biofumigation, poultry waste compost, dazomet and dazomet plus compost treatments were different from the control at $P<0.05$ (Tables 2 and 3).

Total yield of plants from all three of the biofumigation treatments were not different. The Indian mustard and mixed treatments produced 381 and 380 kg of fruit respectively, however, the fall raab treatment yielded 468 kg (23% more than the other two biofumigation treatments) and was statistically different from the controls. The poultry waste compost (PWC) resulted in higher yields than the spent mushroom substrate (SMS) (500 kg compared to 388 kg) and was significantly greater than the SMS and the controls ($P<0.05$). Plots with the highest yields were those fumigated with dazomet and amended with compost. Plants in these treatments produced a total of 516

kg of tomatoes. This was 110 kg more than produced by plants in the dazomet alone treatment. These two treatments were also significantly different ($P<0.05$).

The percentage of “marketable” fruit recorded was low for all treatments, ranging between 20 and 30% of the total yield. The compost treatments had the highest percentage of marketable fruit, 29% (Table 2).

The 6.5–7.0 pH treatments produced the largest yields, 959 kg compared with 786 kg for the 5.5–6.5 treatment. This difference was significant. The >7.0 treatment was not different from the other two pH treatments, yielding 911 kg for the season (Table 2).

Field Experiment: 2001

The 2001 field treatments are documented in Table 4. The two biofumigation treatments tested in 2001 were a fall incorporation of Florida broadleaf mustard and a spring incorporation of the same cultivar. Neither biofumigation treatment was significantly different from the control or from each other (Tables 4 and 5, Fig. 4).

Solarization was introduced into the study in 2001. Tomato yields from this treatment were not different from the control. However, when a compost incorporation preceded the solarization treatment, the yields were 270 kg higher, and were significantly different from the controls. All of the treatments that utilized compost had enhanced yields. The compost alone treatment yielded 894 kg of fruit, 91% higher than the controls. Compost plus a 50% dose of dazomet produced 837 kg of fruit. The full rate of dazomet plus compost treatment was not reapplied in 2001. Tomatoes were planted in the same beds that received this treatment in 2000 but with no additional treatment. The yields were not significantly different than that of the controls. When applied in 2000, the yields for this treatment were 82% above controls.

The relative volume of market grade fruit recorded was higher in 2001, ranging from 34% to 55% of the total yield. The composted treatments again produced higher relative quantities of marketable fruit compared to controls. All three soil pH ranges produced similar results in 2001.

Field Experiment: 2002

The overall yield in 2002 was 28% less than in 2001 (Tables 6 and 7, Fig. 5). The Florida broadleaf mustard biofumigation treatment yielded 393 kg of tomatoes compared to 258 kg for the control (Table 6). This was 52% higher than controls and was significantly different, just as the fall raab biofumigation treatment was in 2000 (Table 7). When combined with a 50% dose of dazomet, the biofumigation treatment increased yields to 433 kg, about 68% greater than controls. This yield was significantly different from the yield of the biofumigation alone treatment.

The solarization treatment did not significantly enhance yields. However, the compost plus solarization treatment yielded 386 kg of fruit and was significantly different from both the solarization alone treatment and controls. This treatment did, however, yield significantly less than the compost alone treatment.

The compost plus biofumigation treatment (455 kg) had 76% higher yield than the control. The most successful integrated treatments were those that combined dazomet with compost. After not being applied in 2001, the dazomet followed by compost treatment was reapplied in 2002. This combination produced the highest yields, 570 kg; 120% greater than controls. The compost plus 50% dazomet treatment produced similar results, yielding 553 kg of fruit for the season.

All of the treatments, apart from solarization, enhanced the relative proportion of marketable fruit to overall yield. Both dazomet plus compost combinations produced high percentages of marketable quality fruit, 13-14% higher than the control ratio.

The 5.5-6.5 pH plots had the lowest fruit yields and was different than the >7.0 plots, but not from the 6.5-7.0 pH plots (Table 7).

Three-year Totals: 2000 – 2002

Results of the three-year study are collected in Table 8 and Figure 6. When considering the effect of pH on yield over the length of the study, the high pH plots (>7.0) produced the greatest yields, 4752 kg of tomatoes. However, the medium pH blocks (6.5-7.0) produced a similar yield of 4613 kg of fruit over three years, whereas, the low pH blocks (5.5-6.5) had a lesser total yield of 4270 kg. None of the pH treatments were significantly different from each other.

Five treatments, including the control, were repeated over all three years (36 replicates each from 2000-2002). The three-year total yields for the control replicates were 1,009 kg of fruit. All of the three-year experimental treatments were higher. The biofumigation treatment totaled 1,348 kg and the dazomet treatment 1,444 kg of fruit. The highest yielding treatments were the compost-based treatments. The dazomet plus compost and the compost alone treatments were not different, yielding 1,658 kg and 1,904 kg of tomatoes over three years respectively.

Examination of the data for average fruit weight produced by each plant in the treatments (360 plants each) also reflects differences between the treatments. The average per plant fruit yield for the controls over three years was 3.00 kg (6.60 lbs) per

plant, whereas, the plants in the compost treatments produced 5.64 kg (12.41 lbs) per plant. The biofumigation treatment averaged 4.07 kg (9.11 lb) per plant.

Discussion

Throughout the duration of this study tomato plants grown in the treated plots consistently yielded more tomatoes, by number and weight, than those grown in the control treatments. However, not all of the differences in yield among the treatments and the controls were significant. Variability in the effectiveness of the application of the various treatments is an important factor when considering its ability to enhance fruit yields.

Biofumigation

The biofumigation treatments had varied success enhancing yields. The results obtained in this study corresponded to prior research that identified the parameters of cultivar selection, growth of the cover crop, and incorporation methods as being pivotal to the success of biofumigation.

Two Brassica cultivars were investigated in 2000, as well as a third treatment that consisted of a mixture of the two types. All three treatments were seeded in the fall of 1999, overwintered and incorporated in the spring of 2000. The stand estimates were low for each treatment. The Indian mustard stand averaged 57% of full coverage, fall raab averaged 20% and the mixed treatment averaged 23%. Tomato plants from the Indian mustard cultivar treated plots produced 381 kg of fruit compared to 283 kg in the controls. Tomato plants in the fall raab plots produced 468 kg of fruit. Tomatoes in the mixed biofumigation treatment produced 380 kg of fruit compared to 283 kg of fruit in the control plots. Plants from the control treatments produced 283 kg of fruit. Only the

raab biofumigation crop was significantly different from the control. Harsh conditions throughout the winter of 1999 and 2000 affected the Brassica stands and reduced the quantity of plant material that was plowed into the ground. The superior ability of the fall raab to enhance yields may be due to a greater ability to overwinter rather than an improved action upon ensuing incorporation into the soil.

Only one Brassica cultivar, 'Florida broadleaf' mustard, was used in 2001, however, two production methods were tested. All of the mustard treatments were seeded in the early Fall of 2000. The fall biofumigation treatment was incorporated into the soil during the first week in December. The production beds were raised and covered with black plastic. The spring mustard treatment was permitted to overwinter and incorporated in March. Winter freezes again affected the stand of the spring treatment and, as a consequence, less biomass was available for incorporation in March. The stand estimate for the fall treatment was 96% and the spring treatment 53%. Rainfall from September to December, 2000 was 289 mm and from January to March, 2001 was 369 mm.

Neither biofumigation approach was successful at significantly improving tomato yields in 2001. The tomato plants in the spring biofumigation treatment yielded 364 kg of fruit, whereas, the fall treatment yielded 487 kg and the control plants 468 kg and. The 26% difference between the spring and fall treatments is likely due to deterioration of the spring mustard cover crop due to climatic affects. The relative decrease in effectiveness of the 2001 biofumigation treatments, compared with 2000, could be due to either the cultivar type utilized or a disparity in incorporation methods between each season. For example, soil moisture levels may have been less in 2001, thus, compromising the

efficacy of the biofumigation treatments in that year. However, rainfall amounts at the Knoxville Experiment Station for January through April of 2000 and 2001 were 501 mm and 425 mm respectively. This would seem to be sufficient in both years for good plant growth and adequate soil moisture.

The fall biofumigation treatment was repeated in 2002. A good stand of mustard was grown from September through November of 2001. The stand estimate for all three biofumigation treatments averaged 86% of full cover. The mustards were plowed into the soil and the beds raised and covered in early December. The 2002 biofumigation treatment produced 52% more fruit than the controls – a result comparable to the 2000 fall raab biofumigation result, i.e. both yield totals were significantly different from the controls.

Harvey (2002) also found that incorporated Brassica cover crops improved yields compared with a rye control. In that study, significant yield increases of 40% above controls were reported; a comparable result to this study. Lazzeri and Maninici (2001) found that even though strawberry yields did not match those of the beds treated with methyl bromide, they did surpass yields from a conventional green manure (barley) and from untreated soils.

When all of the favorable parameters for effective biofumigation are realized, this treatment has the ability to improve yields in plasticulture tomato production. However, further identification and investigation of the factors and considerations required for this treatment to be consistently successful is needed.

Composted amendments

The cumulative total (1904 kg of fruit) for the best compost treatment over the three years of the study was 89% better than the total for the controls (1009 kg) (Table 8). This data suggests that composted amendments are effective at increasing tomato yields in plasticulture production at the 11 mt/ha rate employed.

The two compost types were in separate trials in 2000. Poultry waste compost (PWC) is a mature substrate consisting of chicken mortalities layered among spent litter aged for 12-18 months. The production beds that were augmented with PWC significantly (29%) out-yielded the beds amended with spent mushroom substrate (SMS). The SMS plots produced 37% more fruit than the control plots, even though only the PWC yields were found to be significantly different from controls. The tomato yields from the SMS treatment were similar to those from two of the biofumigation treatments (Indian mustard and “mixed”) in 2000.

In 2001 and 2002, a single compost treatment was used of equal amounts of PWC and SMS at a combined rate of 11 mt/ha. In 2001, the compost treated plots yielded 894 kg of fruit, the highest total yield of any treatment in the three years of the study. Each plant yielded 7.45 kg (16.4 lb), a 91% improvement above the control plants (3.90 kg/plant). Even though overall yield was not as high in 2002, the compost treatment still significantly out-yielded the controls by 97%. The increased yield from the compost treatment was also significantly different from the biofumigation and solarization treatments in regards to total fruit weight.

In each of the three years of the study the compost treatments significantly produced more fruit than the controls. This was the only three-year treatment where that

was observed. The enhanced yields were likely linked to an improvement in soil physiochemical properties and/or changes in microbial community structure. Many researchers, including Hoitink and Fahy (1986) and Corti et al. (1998), have described these factors. Other investigators have also noted improved yields due to compost amendments (Ozores-Hampton et al., 1994; Roe et al., 1994; Abdul-Baki and Teasdale, 1997; and Bulluck and Ristaino, 2002).

Dazomet (Basamid)

The primary function of treating the tomato production beds with dazomet was for the prevention of disease via soil fumigation. Some synthetic chemical amendments may deleteriously impact vegetable yields. The total tomato yields from the dazomet treated plots were higher than in control plots 44%, 22%, and up to 82% in 2000, 2001, and 2002, respectively. In 2001 the increase above control was not significant.

In 1995, McSorley found that MITC-based fumigants increased total tomato yields above non-treated plants by 42%. Harris (1991) found that dazomet treated beds yielded 36% more strawberries than untreated beds. The performance of Basamid as a pre-plant fumigant for disease control depends upon its satisfactory implementation in the field. Similarly, ineffectual or erroneous application is likely to impact yields independently. The primary reason Basamid might negatively impact production is via plant injury or metabolic interference. Residual granules may be prevented from volatilizing by inadequate soil moisture or low temperatures. Plant injury may result if biocidal compounds remain in soil after tomato transplanting has occurred. The success of Basamid in improving yields during this study would tend to indicate that the product was utilized effectively.

Solarization

Solarization treatments were implemented in 2001 and 2002. In neither year were there significant differences in yield from the controls. In 2001, the solarized beds produced 13% more fruit than the control. In 2002 the elevation was 12%. A study conducted by Chellemi et al. (1997) in Florida found that the temperatures generated during July and August by clear plastic solarization were able to alter soil conditions enough to result in tomato yields almost double that of non-solarized beds in a subsequent crop.

There was no difference in the sequence of fruit ripening i.e. solarizing the beds did not advance the harvest in comparison to the non-heated beds. The spring, pre-plant application of the clear plastic tarp did not directly change the long-term physiochemical properties of beds. Unlike the biofumigation and compost treatments, there was no addition of organic matter, or any other type of fertilizer or conditioner to the soil. Solarization without the corresponding addition of organic matter does not seem to have the ability to improve yields when put into practice during the spring or fall in east Tennessee.

Integrated Treatments

In all three years of the study combination treatments of soil fumigation, with the recommended rate of Basamid, followed by a PWC/SMS compost applications were employed. In 2001, four other combined treatments were implemented, and in 2002 five others were investigated. In 2001, all of the integrated treatments involved compost combined with another management technique.

The compost plus biofumigation strategy entailed cultivating the Brassica cover crop in plots already amended with compost. In 2001, this treatment increased tomato yields by 16% above controls, however, in 2002 the treatment significantly increased yields by 76%. The enhanced yields in 2002 may have been due to the accumulation of organic matter released by the breakdown of the Brassica plant tissue and compost additions over two seasons.

The compost plus solarization treatment improved yields by 58% and 50% in 2001 and 2002, respectively above the control. This is a substantial improvement over the solarization alone treatment in both years, however, the compost alone treatment significantly out-yielded the compost plus solarization treatment by 33% and 48% in 2001 and 2002, respectively.

Chellemi et al. (1997) also investigated integrating solarization with other pre-plant treatments. They found that combining solarization with a cabbage residue produced higher tomato yields than solarization alone. The compost treatment successfully improved yields in all years, whereas, the solarization treatment did not significantly enhance yields. It could, therefore, be stated that the constituent that contributed to the improved yields in the compost plus solarization treatment was the compost adjuncts to the soil before it was covered with the clear plastic tarp. There was no enhanced, affect of solarizing over an already compost treated bed. This effect was not evident at the temperatures generated during the fall and spring dates. It is possible that a compost/solarization regime conducted throughout the heat of the summer may enhance the effect and actually out-perform compost alone. A summer solarization plus biofumigation treatment may also be effective.

The integrated treatments that combined dazomet with composts were the most effective at improving tomato yields. In 2000, the treatment consisted of full rate (398 kg/ha) Basamid fumigation followed by compost incorporation (11 mt/ha). The compost was incorporated after two-weeks, thus permitting activation and dissipation of the MITC. In 2001 these beds were left undisturbed i.e. the treatment was not re-applied but the tomato crop was still grown. Our purpose was to assess the ability of this treatment to remain active over two seasons. In 2002, the treatment was re-applied in the same manner as in 2000. The significant improvement in yields in 2000 (82% above control) was not achieved in 2001 (22% above control). However, when the treatment was re-applied in 2002 the upsurge in yield (121%) above control was the largest of any experimental treatment in the three years of the study. Thus, not re-applying the treatment each season reduced its effectiveness. This outcome concurs with findings by Avnimelech et al. (1990) who found that the positive yield effects associated with compost applications were limited to only one growing season. It is likely that a rapid decomposition of the organic matter found in compost, into humic substances, occurs in the warm, moist, highly aerated conditions prevailing under summer plasticulture production. It can be concluded that the dazomet plus compost treatment should either be re-applied each season for best effect, or at least that the compost component of the treatment be applied each season.

In 2001 and 2002 a treatment was implemented that entailed amending the soil with the 11 mt/ha PWC/SMS rate and then fumigating those plots with 199 kg/ha Basamid (50% of the manufacturers recommended rate). This treatment was successful

at enhancing tomato yield by 79% and 114% above controls in 2001 and 2002, respectively.

The success of the reduced dose fumigation treatment in 2001 prompted the introduction of a biofumigation plus half-rate Basamid treatment in 2002. The granular Basamid was applied to the plots after the Brassica cover crop was mowed but before incorporation and beds formation. Thus, the raised beds contained both Brassica plant matter and a quantity of Basamid granules. Both materials would be expected to release volatile compounds as they decomposed under the plastic. Tomato growth in these treated soils significantly yielded more fruit than the biofumigation alone treatments (68% more than the control plots). Interestingly, the yields from reduced dose dazomet treatment were not different from the full dose treatment.

pH Effects

In all three seasons the plots in the low pH range (5.5-6.5) had the lowest cumulative yields, however, only in 2000 and 2002 were there significant differences from the other pH ranges. In 2001, yields from all three pH treatments were not different. The dazomet treatment was the only one that produced its highest mean replicate yields in the >7.0 range in each of the three seasons.

Fruit Defects and Marketable Yields

Grading tomatoes for physical defects is a subjective practice. The degree to which growth cracks, green shoulder, sunscald, and other blemishes are judged can vary. The criteria for assessment of the fruit is open to interpretation by each grader and the instructions given to the graders each season may differ. Subtracting defective and undersized quantities from total yields for that treatment derives marketable totals. Only

the size grading is non-subjective. According to the criteria utilized in 2000 only 24% of the total yield was marketable. This figure increased to 43% in 2001 and 73% in 2002. The variations in these figures are more likely due to changes in grading criteria than production conditions. The proportion of fruit that were undersized was 18% in 2000, 10% in 2001 and 20% in 2002. In all three years, the compost treatments produced a higher relative percentage of marketable fruit to overall yield than controls. Additionally, all of the integrated treatments that had a compost component also had higher percentage marketable yields than the controls each year. The biofumigation treatments were not as consistent.

The 'Celebrity' tomato cultivar selected for use during this study has been observed to have a tendency towards excessive cracking during ripening; especially in the ambient climatic conditions of east Tennessee. The large number of unmarketable fruit recorded during the study would tend to confirm this observation.

Conclusion

Amending production soils with the pre-plant treatments investigated in this research project advantageously affected fruit yields. Compost incorporations had the most extensive influence on tomato production. In all three years of the study, the compost treatments out-yielded controls by significant measures. The separation between the compost and control yields increased over the duration of the study, possibly indicating that the cumulative effect of successive additions of organic matter to the soil acts to enhance fruit production.

The biofumigation treatments were not as consistent. However, the production of the Brassica crop in 2000 and 2001 was greatly compromised. Effective biofumigation

did not occur with all of the treatments in those years. In 2000, only the fall raab significantly improved yields whereas in 2001 neither of the two treatments tested were significantly different from controls. However, by 2002 the biofumigation treatment did significantly affect yield. It may also be possible that an accumulation of organic matter in the biofumigation beds after three years of Brassica incorporation influenced the improved yields.

The dazomet-fumigated beds significantly improved yields in two of the three years. As with the biofumigation treatments, dazomet was less effective than in 2000 and 2001. Soil moisture and temperature levels at the time of application may have negatively influenced both of these treatments.

The solarization treatment did not enhance yields in either year it was implemented, however, when integrated with compost the improved yields above controls was significant. Biofumigation plus compost also produced similar yields to compost alone in 2002, just as occurred with solarization plus compost.

There were no instances in any of the three years of the study where an integrated treatment was superior to at least one of its components. For example, even though the dazomet plus compost treatment produced the highest weight of fruit per plant in 2002, the yields were not significantly different from the compost alone treatment. The conclusion, based on the data gathered in this field study, is that the improvement in yields over controls by the integrated treatments is primarily due to the positive influences of compost incorporations.

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Appendix

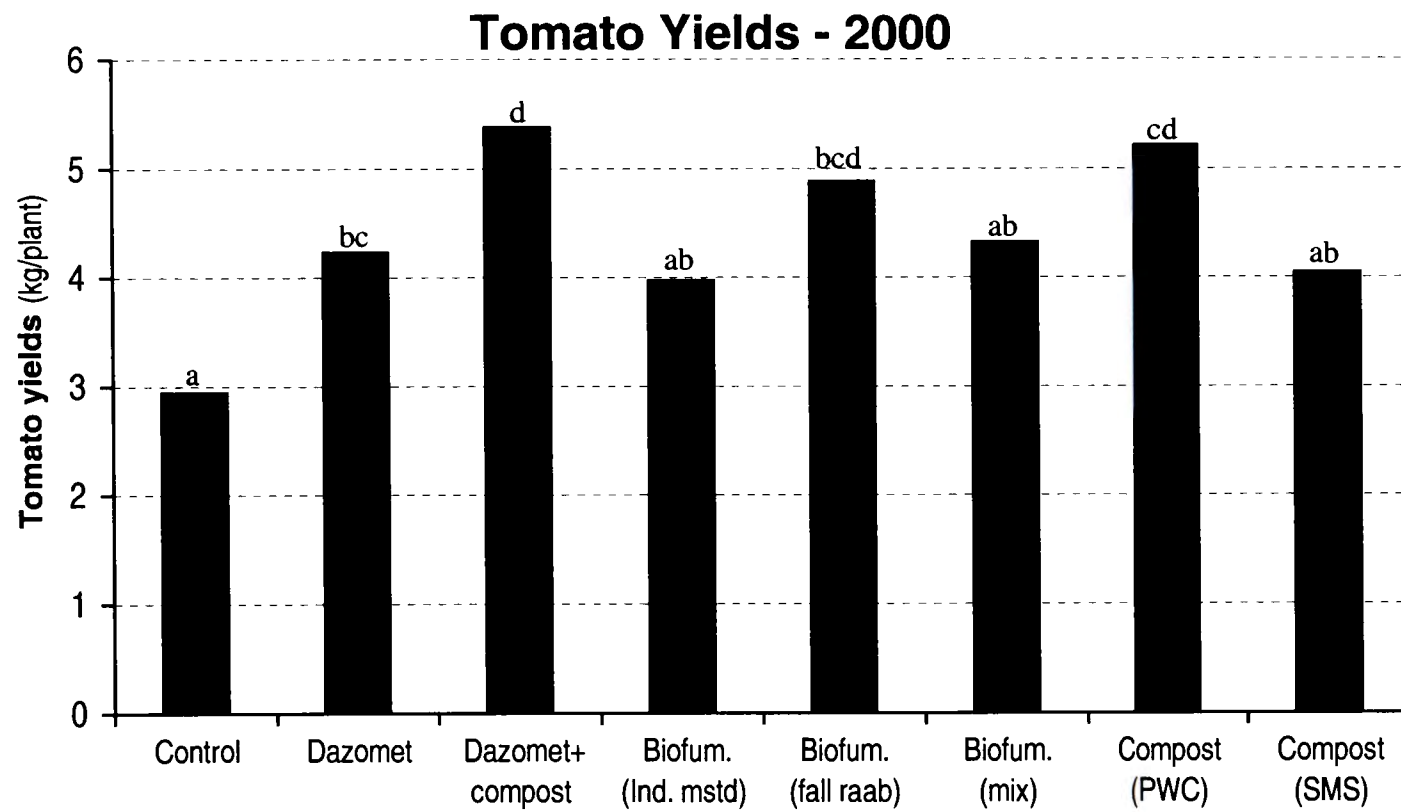


Figure 3. Tomato fruit yields (kg per plant) for all treatments in 2000. Bars with the same letters are not different at $P < 0.05$ based on LSD.

Table 2. Quantity (no.) and weight (kg) of tomato fruit harvested for all treatments and pH ranges in the tomato production experiment in 2000.

Tomato Yields - 2000								
TREATMENT	Total yield (no.)	Total yield (kg)	Mean yield per replicate (kg)	Mean yield per plant (kg)	Marketable ^y (% of total)	pH ranges		
						5.5-6.5 (kg)	6.5-7.0 (kg)	>7.0 (kg)
Control	1945	283 a ^z	23.6	2.94	20.5	75	89	62
Biofumigation (Ind. mstd.)	2276	381 ab	31.7	3.97	23.3	93	108	103
Biofumigation (fall raab)	2574	468 bcd	39.0	4.88	20.7	121	134	120
Biofumigation (mix)	2261	380 ab	31.6	3.96	20.8	99	118	86
Compost (PWC)	2996	500 cd	41.7	5.21	29.3	121	141	138
Compost (SMS)	2521	388 ab	32.3	4.04	28.9	92	113	106
Dazomet	2434	406 bc	33.8	4.23	22.7	65	114	146
Dazomet + compost	3014	516 d	43.0	5.38	24.7	121	142	149
TOTALS: ^x						786 a	959 b	911ab

^z Yields with same letters are not different at $P < 0.05$ based on LSD.

^y Marketable is all fruit >6.35 cm diameter, no physical blemishes, non-diseased.

^x Total yield for each pH range. The same letters are not different at an LSD at $P < 0.05$.

Table 3. Analysis of variance for tomato fruit yields for all treatments for 2000. Mean separation by LSD ($P < 0.05$).

ANOVA - Yields 2000								
TREATMENT	Mean - Replicate (kg)	Std. Dev.	% of control	Observations (no.)	Duncan grouping	pH ranges		
						5.5-6.5 (kg)	6.5-7.0 (kg)	>7.0 (kg)
Control	23.53	7.42		12	a	23.49	27.63	19.48
Biofumigation (Ind. mstd.)	31.73	9.01	134.8	12	a b	29.08	33.83	32.28
Biofumigation (mix)	31.64	8.86	134.5	12	a b	30.61	37.07	27.25
Compost (SMS)	32.33	10.84	137.4	12	a b	28.64	35.39	32.96
Dazomet	33.85	13.93	143.9	12	b c	20.20	35.67	45.67
Biofumigation (fall raab)	39.02	10.96	165.8	12	b c d	37.85	41.74	37.49
Compost (PWC)	41.66	12.05	177.1	12	c d	37.74	44.17	43.07
Dazomet + compost	43.03	15.06	182.9	12	d	37.84	44.61	46.65
pH: 5.5 - 6.5	30.68	9.58		32	a			
6.5 - 7.0	37.51	13.52		32	b			
>7.0	35.61	13.10		32	a b			

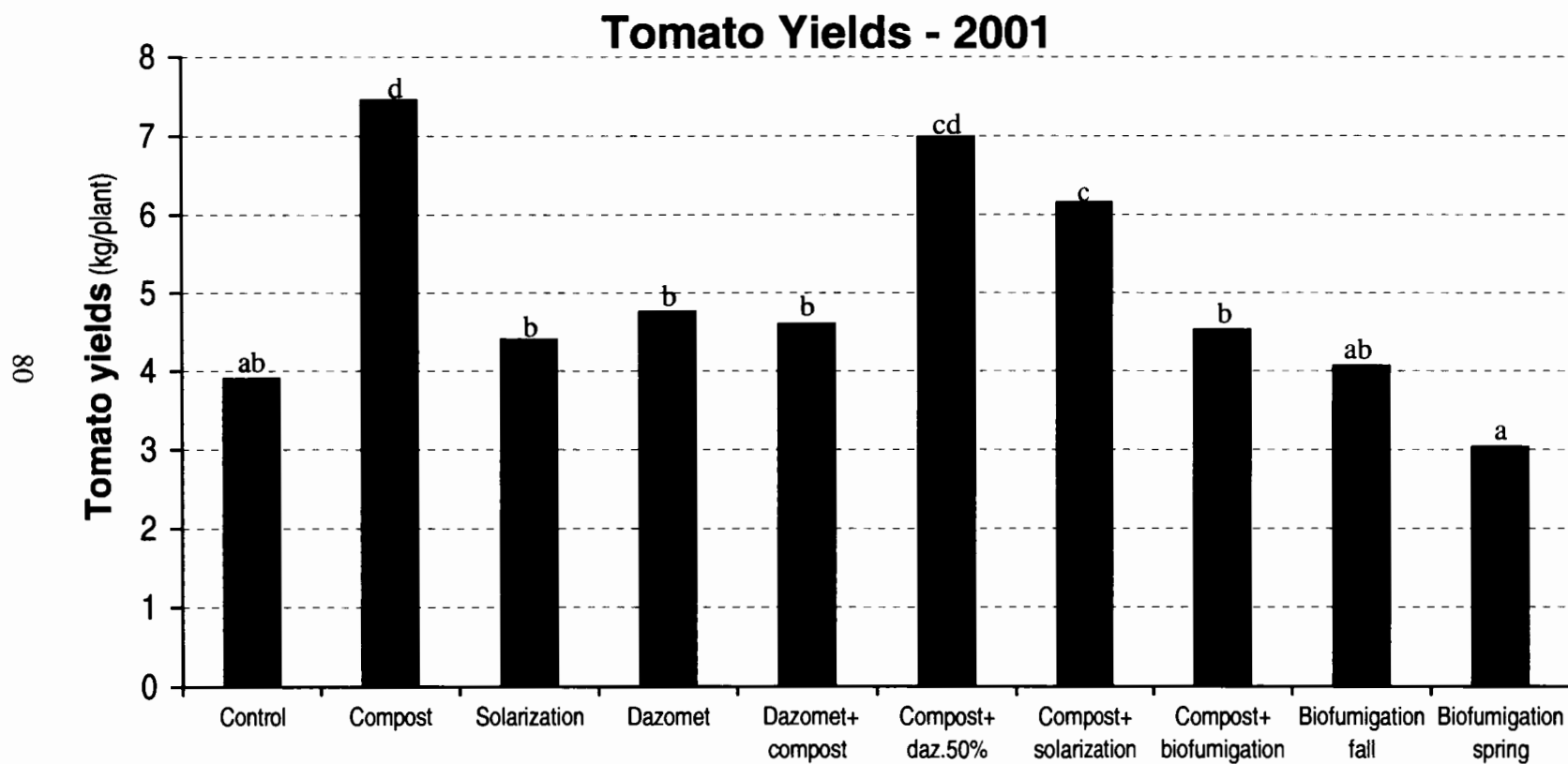


Figure 4. Tomato fruit yields (kg per plant) for all treatments in 2001. Bars with the same letters are not different at $P < 0.05$ based on LSD.

Table 4. Quantity (no.) and weight (kg) of tomato fruit harvested for all treatments and pH ranges in the tomato production experiment in 2001.

Tomato Yields - 2001								
TREATMENT	Total yield (no.)	Total yield (kg)	Mean yield per replicate (kg)	Mean yield per plant. (kg)	Marketable ^y (% of total)	pH ranges		
						5.5-6.5 (kg)	6.5-7.0 (kg)	>7.0 (kg)
Control	2575	468 ab ^z	39.0	3.90	37.5	143	144	181
Biofumigation (fall)	2812	487 ab	40.6	4.06	36.0	199	144	144
Biofumigation (spring)	2031	364 a	30.3	3.03	34.2	102	134	128
Compost	4091	894 d	74.5	7.45	54.8	309	309	277
Dazomet	3141	571 b	47.6	4.76	40.8	176	186	209
Solarization	2748	528 b	44.0	4.40	40.7	172	182	174
Dazomet + compost	3247	572 b	47.6	4.76	42.5	182	183	207
Compost + biofumigation	3147	542 b	45.2	4.52	42.5	177	190	174
Compost + solarization	3751	738 c	61.5	6.15	49.4	239	239	260
Compost + dazomet50%	4218	837 cd	69.8	6.98	48.4	251	250	336
TOTALS: ^x						1950a	1961a	2090a

^z Yields with same letters are not different at $P < 0.05$ based on LSD.

^y Marketable is all fruit >6.35 cm diameter, no physical blemishes, non-diseased.

^x Total yield for each pH range. The same letters are not different at an LSD at $P < 0.05$.

Table 5. Analysis of variance for tomato fruit yields for all treatments for 2001. Mean separation by LSD ($P < 0.05$).

ANOVA - Yields 2001								
TREATMENT	Mean - Replicate (kg)	Std. Dev.	% of control	Observations (no.)	Duncan grouping	pH ranges		
						5.5-6.5 (kg)	6.5-7.0 (kg)	>7.0 (kg)
Control	39.02	10.03		12	a b	35.68	36.12	45.25
Biofumigation (spring)	30.30	10.49	87.7	12	a	25.52	33.45	31.93
Biofumigation (fall)	40.59	18.14	104.0	12	a b	49.87	35.96	35.95
Dazomet	47.57	12.94	121.9	12	b	44.12	46.38	52.20
Solarization	44.01	16.69	112.8	12	b	42.90	45.61	43.53
Dazomet + compost	47.64	8.02	122.1	12	b	45.41	45.85	51.66
Compost + biofumigation	45.16	11.85	115.7	12	b	51.34	44.08	40.04
Compost + solarization	61.53	11.47	157.7	12	c	59.85	59.80	64.93
Compost + dazomet50%	69.79	14.54	178.9	12	c d	62.84	62.43	84.10
Compost	74.53	21.31	191.0	12	d	77.14	77.31	69.14
pH: 5.5 - 6.5	49.47	20.91		40	a			
6.5 - 7.0	48.69	17.17		40	a			
>7.0	51.87	19.36		40	a			

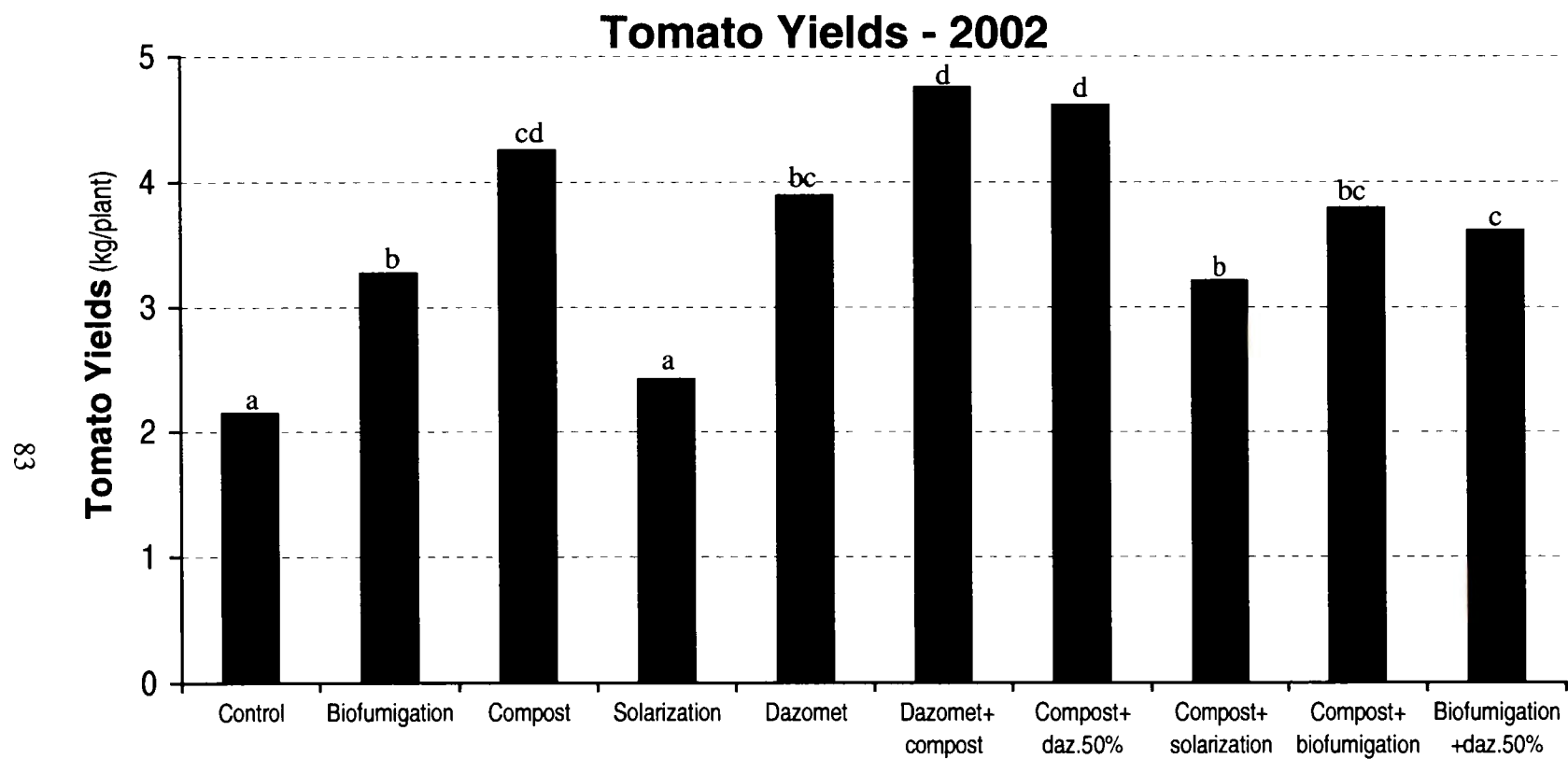


Figure 5. Tomato fruit yields (kg per plant) for all treatments in 2002. Bars with the same letters are not different at $P < 0.05$ based on LSD.

Table 6. Quantity (no.) and weight (kg) of tomato fruit harvested for all treatments and pH ranges in the tomato production experiment in 2002.

Tomato Yields - 2002								
TREATMENT	Total yield (no.)	Total yield (kg)	Mean yield per replicate (kg)	Mean yield per plant (kg)	Marketable ^y (% of total)	pH ranges		
						5.5-6.5 (kg)	6.5-7.0 (kg)	>7.0 (kg)
Control	1421	258 a ^z	21.5	2.15	64.7	98	71	89
Biofumigation	2266	393 b	32.7	3.27	73.6	95	131	167
Compost	2332	510 cd	42.5	4.25	76.9	171	191	148
Dazomet	2569	467 bc	38.9	3.89	75.3	157	147	163
Solarization	1509	290 a	24.2	2.42	63.4	85	80	125
Dazomet + Compost	3235	570 d	47.5	4.75	77.6	175	191	203
Compost + Biofumigation	2644	455 d	37.9	3.79	74.4	144	147	164
Compost + Solarization	1960	386 b	32.1	3.21	70.0	102	161	123
Compost + Dazomet50%	2785	553 d	46.1	4.61	78.6	161	201	191
Biofum. + Dazomet50%	2417	433 c	36.1	3.61	73.9	149	133	152
TOTALS: ^x						1337a	1452ab	1525b

^z Yields with same letters are not different at $P < 0.05$ based on LSD.

^y Marketable is all fruit >6.35 cm diameter, no physical blemishes, non-diseased.

^x Total yield for each pH range. The same letters are not different at an LSD at $P < 0.05$.

Table 7. Analysis of variance for tomato fruit yields for all treatments for 2002. Mean separation by LSD ($P < 0.05$).

ANOVA - Yields 2002								
TREATMENT	Mean - Replicate (kg)	Std. Dev.	% of control	Observ- ation (no.)	Duncan grouping	pH ranges		
						5.5-6.5 (kg)	6.5-7.0 (kg)	>7.0 (kg)
Control	21.53	11.80		12	a	24.44	17.82	22.34
Solarization	24.17	7.66	112.3	12	a	21.33	19.87	31.30
Compost + solarization	32.15	9.09	149.3	12	b	25.51	40.19	30.74
Biofumigation	32.71	10.10	151.9	12	b	23.64	32.81	41.68
Dazomet	38.90	4.80	180.7	12	b c	39.16	36.76	40.78
Biofum. + dazomet50%	36.06	9.74	167.5	12	c	37.20	33.12	37.87
Compost	42.49	9.36	197.4	12	c d	42.82	47.70	36.95
Compost + dazomet50%	46.08	6.08	214.0	12	d	40.21	50.29	47.75
Compost + biofumigation	37.95	10.27	176.3	12	d	36.12	36.72	41.01
Dazomet + compost	47.46	6.49	220.4	12	d	43.85	47.76	50.78
pH: 5.5 - 6.5	33.43	11.51		40	a			
6.5 - 7.0	36.31	12.79		40	a b			
>7.0	38.12	10.68		40	b			

Table 8. Summary of tomato fruit yields for selected treatments for 2000 – 2002 including separation by treatment type, complete block, and pH.

Tomato Yields 2000-2002								
TREATMENT	Fruit yield (kg/field)				Mean fruit yield per plant (kg)			
	2000	2001	2002	Total	2000	2001	2002	Avg.
Control	282.6 ^z	468.2	258.4	1009	2.94	3.90	2.15	3.00
Biofumigation	468.3	487.1	392.5	1348	4.88	4.06	3.27	4.07
Compost	500.3	894.3	509.8	1904	5.21	7.45	4.25	5.64
Compost + biofumigation	N/A	542.4	455.2	998 ^y	N/A	4.52	3.79	4.16
Dazomet	406.2	570.8	466.8	1444	4.23	4.76	3.89	4.29
Dazomet + compost	516.4	571.7	569.5	1658	5.38	4.76	4.75	4.96
				<u>Total</u>				
pH: 5.5 - 6.5	983	1950	1337	4270				
6.5 - 7.0	1199	1962	1452	4613				
>7.0	1139	2089	1525	4752				
				<u>Total</u>				
Block				<u>Total</u>				
A ^x	842	1460	1040	3342				
B	795	1489	1108	3392				
C	751	1574	1059	3384				
D	933	1478	1108	3519				

^z Total fruit weight for all reps of treatment in that year.

^y Two year total.

^x Three reps of each treatment – one per pH treatment.

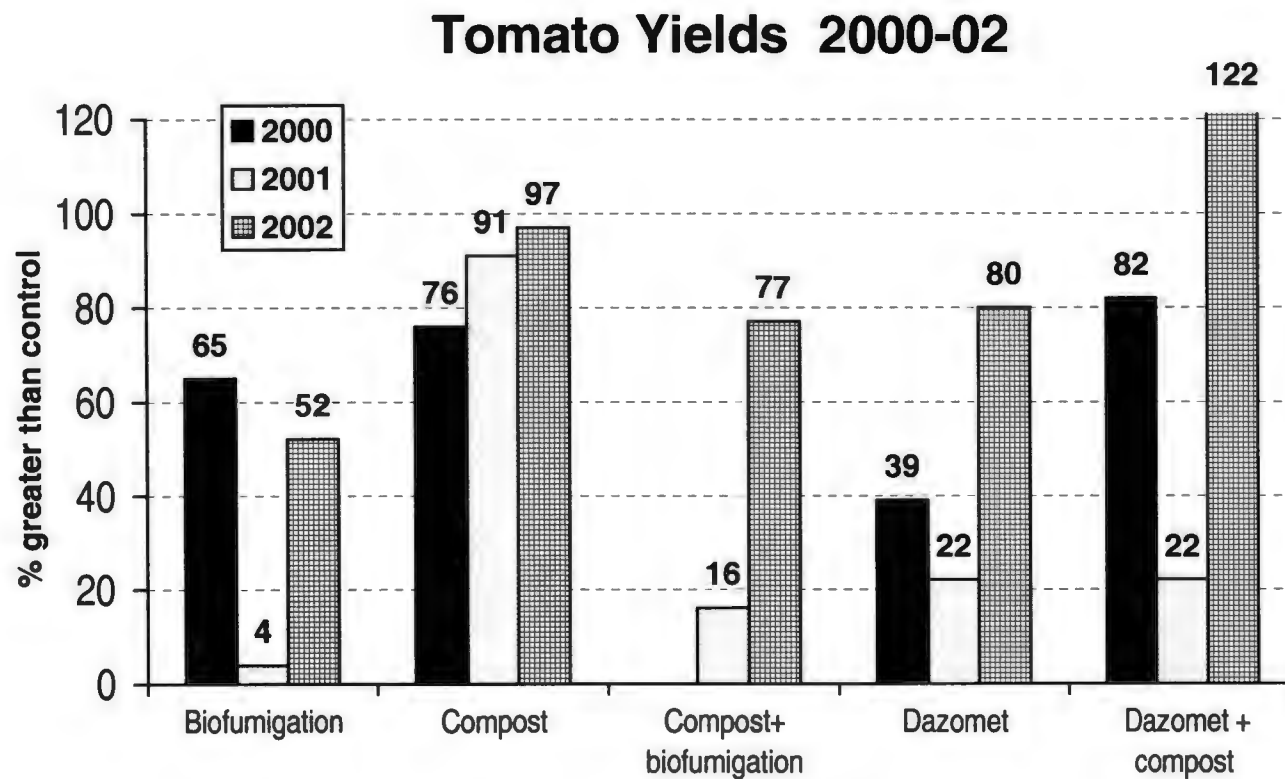


Figure 6. Tomato fruit yields for selected treatments for tomato production experiment from 2000-02. Data expressed as fruit yield greater than control (%) for each year.

Part IV

**The Suppression of Southern Blight by
Integrated Pre-Plant Treatments.**

Abstract

Commercial tomatoes crops are often grown without rotation and face pressure from many species of soilborne pathogens. Inoculum levels can quickly become elevated in production soils resulting in plant losses and compromised yields. The phytopathogenic fungus *Sclerotium rolfsii* is the causal agent of Southern Blight and is responsible for significant losses in many field crops throughout the southeast United States. Fumigation is the primary method of control of this and many other soilborne diseases.

After the removal of methyl bromide from the marketplace an integrated approach to disease management may provide growers with an economical production alternative. This research project investigated the soil fumigant dazomet as an alternative to methyl bromide. Chemical control can be expensive and may not eliminate all pathogen propagules from the soil. The resilient character of sclerotia of *S. rolfsii* favors the use of biologically based control methods. When implemented effectively biofumigation and solarization can reduce pathogen populations in the soil. Composted amendments introduce biocontrol agents such as *Trichoderma* and *Pseudomonas* spp. to the soil. Beneficial microorganisms can reduce disease in favorable environments.

The addition of spent mushroom and poultry waste compost to the soil reduced the incidence of Southern Blight in all three years of the study (4-33%). The level of disease suppression was similar to the fumigant, dazomet (4-20%). The biofumigation treatments had varied success. Fall raab in 2000 and Florida broadleaf mustard in 2002 reduced the incidence of Southern Blight significantly (9% and 18% respectively). The solarization treatment did not generate the temperatures required to disinfest soil.

Integrated treatments that utilized composts reduced Southern Blight in most instances. The reduced dose dazomet plus compost treatment decreased disease to 1% in 2001 and 16% in 2002.

Introduction

Widely used as a soil fumigant, methyl bromide is scheduled for removal from the marketplace, or at least severely reduced in its availability. An integrated approach to disease management may provide farmers with an economical production alternative for the control of soilborne diseases. A primary goal of this research project was to examine the effectiveness of utilizing both chemical and non-chemical methods to reduce the incidence of Southern Blight in plasticulture tomato production to economically manageable levels.

Accumulating evidence suggests that composted soil amendments (Hoitink and Fahy, 1986), solarization (Katan and Devay, 1976), and biofumigation with Brassica cover crops (Angus et al., 1994) have potential to reduce phytopathogenic fungal propagule numbers. Integrated disease management requires multi-level strategies. In fields extensively infested with *S. rolfsii*, the causal agent of Southern Blight, elevated propagule numbers need to be initially reduced either via a chemical fumigant, such as dazomet, or an effective biofumigant alternative. Solarization techniques that are able to generate high temperatures also have the potential to disinfest the soil. The subsequent introduction of a balanced microbial community will act to suppress the rapid repopulation of soil by pathogens (Baker, 1987). This goal can be achieved through the judicious application of composted amendments.

Southern Blight

Sclerotium rolfsii is a soilborne pathogen with an extensive host range of more than 500 plant species, including both dicotyledons and monocotyledons. The fungus causes “Southern Blight” (seedling damping-off, blight and stem rot) throughout the tropics, subtropics and in areas of the southern and southeastern U S. where high temperatures permit its growth and survival (Punja, 1985). *Sclerotium rolfsii* is one of a number of plant pathogenic fungi that form resistant, overwintering survival structures called sclerotia. Sclerotia are typically brown to tan in color and measure 0.5-mm to 2.0-mm in diameter. A sclerotium is comprised of a two to four cell-layers thick, melanized outer layer, a middle cortex and innermost medulla of loosely arranged filamentous hyphae (Punja, 1985).

Fumigation

Soil fumigation has been the primary method of chemical control of Southern Blight. Sclerotia can be resistant to methyl bromide and chloropicrin (Munnecke et al., 1982). However, biological control methods have been a successful alternative for the reduction of *S. rolfsii* in tomato production (Ristaino et al., 1991). Dazomet (Basamid) has been identified as a possible chemical alternative to methyl bromide. It is a granular soil fumigant that volatilizes within moistened soil to produce the irritant gas, methyl isothiocyanate (MITC). MITC has a significantly shorter half-life than methyl bromide and, subsequently, less persistence in the soil. It is also non-reactive in the atmosphere (Labrada and Fornasari, 2001).

In Florida, Locascio et al. (1997) investigated alternate fumigants in plasticulture tomato production and found that dazomet did not provide the same level of control of

soil pests as methyl bromide. In the UK, Harris (1990) saw a 99% reduction in strawberry diseases in dazomet-fumigated (metham-sodium and 1,3,-dichloropropene) fields compared to non-fumigated. In Savannah, Georgia, Rieger et al. (2001) found that dazomet treated fields produced strawberry yields similar to methyl bromide treatments. O'Brien and van Bruggen (1990) found that lettuce plots fumigated with 400 kg/ha of dazomet had 25-50% less corky root, however, half rate applications were not effective. In North Carolina, dazomet reduced bacterial wilt (*Ralstonia solanaceaium*) to 0.8% in plasticulture tomato production (Robinson and Lancaster, 1997). However, the fumigant was not effective against the tomato fungal pathogen, *Fusarium* sp. In contrast, Gilreath et al. (1994) demonstrated that 346 lb/acre of dazomet reduced *Fusarium* wilt to 2% plant mortality on plastic mulched tomato in Bradenton, Florida.

Biofumigation

One alternative non-chemical fumigant control measure is utilizing the pesticidal compounds released by macerated Brassica tissues. This control method has recently been referred to as biofumigation (Angus et al., 1994). The toxicity of Brassica tissues is derived from glucosinolates that are converted to isothiocyanates, organic cyanides, oxazolidinethiones, and ionic thiocyanates by the enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1). Allyl isothiocyanate (AITC) is the most toxic compound formed from allyl glucosinolate hydrolysis in *B. juncea* L., and possibly the most important for biofumigation (Sørensen, 1990).

For many years Brassicas, such as canola, have been utilized as windbreak crops and cover crops but only recently have they been investigated for their ability to suppress soilborne pathogens. Brown and Mora (1997) published a comprehensive review on the

impact of the breakdown products of glucosinolates on soilborne pests and concluded that allelochemicals have the potential to suppress a wide variety of soilborne pests.

Australian researchers Kirkegaard and Sarwar (1998) at CSIRO headquarters in Canberra, reported the toxicity of isothiocyanates to fungal pathogens including *Rhizoctonia* sp. and *Fusarium* sp. The growth of the tomato pathogens *Pythium ultimum*, *R. solani*, and *S. rolfsii* was suppressed by volatiles from several Brassica species (Charron and Sams, 1998; 1999; Harvey et al., 2002).

Field studies on the disease suppressive abilities of biofumigation have been less numerous. *Aphanomyces* root rot in peas (*Pisum sativum* L.) was controlled during the second year of a study when white mustard (*Brassica alba* L. Boiss) was chopped and incorporated into the soil (Muehlchen, 1990). Plots treated with broccoli (*Brassica oleracea* var *italica* Plenck) residue had reduced numbers of *Verticillium dahliae* propagules and the subsequent cauliflower (*Brassica oleracea* var. *botrytis* L.) crop was improved (Subbarao et al., 1999). *Verticillium* wilt severity was lowest in plots treated with a MITC-based fumigant. In a field study by Kirkegaard et al. (2000), Brassica biofumigation decreased inoculum levels of the take-all fungus in wheat, but did not reduce the expression of the disease or its impact on yield.

Composts

Diseases of belowground plant parts can be difficult to control by chemical approaches because large quantities of pesticides and repeated applications are often required to treat the soil volume occupied by plant roots. An advantage of introduced antagonists compared to chemical protectants is that microbial populations can grow from a small quantity of inoculum applied to the soil, such as is provided by compost

incorporations (Baker and Cook, 1974). Disease control with compost has been attributed to four possible mechanisms: 1) successful competition for nutrients by beneficial microorganisms, 2) antibiotic production by beneficial microorganisms, 3) successful predation against pathogens by beneficial microorganisms, and 4) activation of disease-resistant genes in plants by composts (Cook and Baker, 1983).

In the past three decades considerable research has been conducted investigating plant disease suppression using compost. Hoitink et al. (1977) found that incorporating a composted hardwood bark media suppressed *Phytophthora* in potting mixtures. The same media also suppressed other pathogens including *Rhizoctonia*, *Fusarium*, and *Pythium* (Hoitink et al., 1977). Composted cattle manure also reduced populations of *R. solani* and *S. rolfii* (Gorodecki and Hadar, 1990). Chellemi et al. (1992) found that bacterial wilt (*Erwinia tracheiphila*) in tomato crops could be reduced less than 20% disease incidence by spent mushroom and other composts. Bulluck and Ristaino (2001) investigated the effect of organic soil amendments, specifically on the occurrence of Southern Blight in tomatoes. They found disease incidences of 23% to 53% compared to 63% for non-amended plots.

Solarization

Mulching with clear polyethylene film to achieve soil disinfestation is known as soil solarization. This approach utilizes the sun's energy to heat moist soil. Transparent polyethylene film allows the solar radiation to be transmitted directly to the soil and reduces moisture loss from the soil through evaporation (Stapleton and DeVay, 1986). Moisture stimulates metabolic activity in resting structures, which increases pest and pathogen susceptibility to high temperature (Katan and Devay, 1991). Soil solarization

enhances the diurnal heating and cooling cycle of soil, resulting in daytime temperatures that are lethal to nematodes, fungal pathogens and weed propagules. Temperatures are highest near the soil surface and decrease with depth.

The use of soil solarization to elevate soil temperatures for soilborne pathogen control was detailed by Katan et al. (1976). By mulching soil with transparent polyethylene film in Israel during July or August, species of *Fusarium* and *Verticillium* were suppressed, there were fewer diseased plants, weeds were controlled, plant stand was improved, and plant growth and yield were increased (Katan et al., 1976). In a Florida study, Chellemi et al. (1994) recorded maximum temperatures of 39C under bare soil and 46C under solarization at 15 cm depth. He found significant decreases in the density of *Phytophthora* at 15 cm. In North Carolina, Ristaino et al. (1991) used solarization to control Southern Blight in tomato. Examining data from 1990, they recorded maximum temperatures of 49C at 10 cm and differences from control plots of 14C. They found a 62% reduction in sclerotia of *S. rolfsii* under solarization and a 77% reduced incidence of Southern Blight.

The objective of this study was to assess the capacity of the pre-plant soil treatments biofumigation, composts, solarization, and the fumigant dazomet to control Southern Blight in plasticulture tomato production. Physical and biological characteristics relating to the efficacy of each of the disease control techniques were examined. The results from each season influenced the refinement of existing treatments and the development of new integrated treatments for each subsequent year.

Materials and Methods

The experimental design and field production methods were identical to those described in chapter one. The fundamental soil treatments being investigated for disease suppressiveness were biofumigation with incorporated Brassica cover crops, spent mushroom and poultry waste composts, solarization, and the commercial fumigant dazomet. Integrated treatments also were investigated.

Southern Blight Assessment

During the growing seasons of 2000, 2001, and 2002 all plants were assessed for symptoms of Southern Blight. Visual inspections of each plant were made throughout the season. Plants were rated as diseased if distinctive lesions, hyphal growth, or sclerotia were observed on the stem. Results were analyzed by treatment.

Sclerotia Viability Assay

Sclerotial Production: *Sclerotium rolfsii*: The “paper culture” production method as described by Canaday (1989) was utilized to produce large quantities of sclerotia. The sclerotia were brushed from the corrugated paper, counted, and placed into nylon bags.

Rhizoctonia solani: A sample of 400 cm³ of screened sandy loam soil was placed into a 500 ml glass mason jar and autoclaved for one hour on two consecutive days. Fifty grams of chopped potato was added. The soil was inoculated with plugs of *R. solani* cut with a cork borer (15 mm) from the margins of an actively growing culture. The soil was incubated at 25C in darkness. The jars were periodically shaken to disperse the growing hyphae. After at least 30 days prolific sclerotia are formed. When required, the sclerotia are separated from the soil using wet-sieving, counted, and placed into nylon bags.

Mesh Bags: A 0.2-mm nylon mesh was used to produce the 4 x 4-cm bags. Ten sclerotia of each of the above species were placed into separate bags with 20 cm³ of sand to prevent clumping. The bags were sealed using a heat activated bag sealer.

Burial, Retrieval and Viability Assessment: The bags were buried at a depth of 5 cm in the plots immediately after the treatments were incorporated into the soil and the plastic mulched raised beds were formed. After three weeks the bags were retrieved and returned to the lab. The sclerotia were extracted from the opened bags, surface sterilization with dilute hypochlorite (5% for three min.), and placed onto potato dextrose agar plates. Sclerotia that germinated were counted as viable.

Soil Microbial Populations

Fifteen-cm-deep soil cores were collected from the bulk soil of each treatment at both tomato pre-plant and post-harvest. Six cores were taken and bulked into a composite sample. The soil samples were stored in a cooler at 5C until processed. Ten-fold serial dilutions onto selective media were used for analysis of microbial populations. Ten grams of soil in 90 ml of sterile 0.25% Bacto-agar solution formed the initial (10^{-1}) dilution. Transferring 10 ml of the solution to another 90 ml of 0.25% water agar solution formed subsequent dilutions. Dilutions up to 10^{-7} were made. Triplicate plates were used for each sample. Potato dextrose agar (PDA) with chloramphenicol (50 mg/L) was used for isolating fungal colonies and Difco Bacto-agar with cyclohexamide (50 mg/L) was used for bacterial isolations. *Trichoderma* spp. were isolated on a modified *Trichoderma*-selective media (Chen et al., 1988). Culturable bacteria were plated at 10^{-7} , total fungi at 10^{-3} , and *Trichoderma* spp. at 10^{-2} .

Populations of *Sclerotium rolfii* were enumerated by thinly spreading 100 g of the sampled soils in a disposable aluminum tray. The soil was misted with 1% methanol and after several days germinating sclerotia became visible and were counted (Rodriguez-Kabana et al., 1980).

Brassica Crop Stands and Biomass Assessments

Brassica plant stand/density was assessed via a visual rating of every plot. Plots that had a full coverage of plants with no apparent loss due to climatic conditions were assigned a 100% rating. The crop coverage of any compromised plots were assessed relative to the 100% standard.

The ratio of plant material to soil was determined. A large diameter auger was used to extract soil from the biofumigation plots within one day of incorporation of the Brassica cover crop. The samples were taken to the lab, weighed, and oven-dried overnight. Plant material was separated from soil particles. Each fraction was weighed; thus giving plant dry weight, soil dry weight, and the percentage of plant material present in each soil sample.

Soil Temperature Measurements

Sensors used to measure the temperature in the solarization treatments (Spectrum Watchdog, Spectrum Technologies Inc., Plainsfield, IL). The “0-cm” sensors were placed on top of the soil surface beneath the black plastic mulch and the clear plastic layer that covered the solarized beds. A second set of sensors was buried at a depth of 15 cm in close proximity to the “0-cm” sensors. The “control” sensors were placed at the same depths under black plastic. After the solarization treatment was concluded, the sensors were retrieved and the data downloaded into a computer (Spec5 Specware ver.

5.00.0029, Spectrum Technologies Inc., Plainsfield, IL). “Air temperature” data was obtained from temperature records taken from Knoxville Experiment Station sensors placed over bare soil.

Results

Rainfall totals at the Knoxville Experiment Station were: 2000 – 1251 mm, 2001 – 1179 mm, 2002 – 1426 mm”. During the growing season (May – August) the rainfall totals were: 2000 – 462 mm, 2001 – 460 mm, 2002 – 358 mm. Average temperatures during the growing season were: 2000 – max. 29.4C min. 17.2C, 2001 – max. 29.1C min. 16.9C, 2002 – max. 29.9C min. 17.1C.

Field Study: 2000

The incidence of Southern Blight in the production beds for all three years of the study, 2000-2002 is shown in Table 9 (All tables and figures are in Appendix). The percentage of plants infected with *Sclerotium rolfsii* is illustrated in Figure 7. The control beds developed 39 diseased plants out of a total of 120 (33%). Two Brassica cultivars were evaluated, Indian mustard and fall raab, as well as a third treatment consisting of a mixture of the two types. Of the three treatments, the fall raab and the ‘mixed’ (raab plus mustard) biofumigation significantly reduced disease (9% and 14% diseased plants, respectively using log transformed data at $P<0.05$). The 29% diseased plants in the Indian mustard treated plots were not significantly different from the controls.

Both composts types, poultry waste compost (PWC) and spent mushroom substrate (SMS) reduced disease, 4% and 6% diseased plants, respectively relative to the control and were not significantly different from each other. Interestingly, the number of diseased plants recorded in the compost-amended plots (5 and 7 plants for the PWC and

SMS, respectively) was lower than the dazomet fumigated plots (10 diseased plants). However, this difference was not significant. The dazomet plus compost integrated treatment was the same as the dazomet alone treatment, with 10 diseased plants.

Field Study: 2001

The number of plants in the control beds infected with Southern Blight was similar in 2001 (36) and 2000 (39) (Table 9 and Figure 8). The two biofumigation treatments differed in the timing of incorporation but not the cultivar used, i.e. 'Florida broadleaf' mustard. In 2001, the spring incorporation was not effective at controlling disease. Although, the fall Brassica incorporation plots had 42% less disease than the controls, the difference was not significant.

The synthetic fumigant, dazomet, was again effective at controlling Southern Blight. These plots experienced only 4% plant loss due to Southern Blight compared to 30% for the control. The compost amended plots (PWC plus SMS) were similarly effective with a disease incidence of 7%. The plants in the solarization plots developed 21% diseased plants, however, this was not significantly different from the control.

Four integrated treatments were tested in 2001. They developed less Southern Blight than the controls, however, only three of them were significantly different. With 28 diseased plants, the compost plus biofumigation treatment did not perform better than the fall biofumigation treatment. The compost plus solarization treatment had 50% of the disease of the controls and 19% less than the solarization alone treatment. The solarization treatment was not significantly different from the controls.

In 2001, the dazomet fumigation followed by compost treatment was not reapplied in order to evaluate its ability to control disease after an initial 2000

application. The treatment constrained disease to 50% of the controls, compared with 25% in 2000. The most effective treatment in 2001 was the compost plus 50% dazomet treatment. Only one plant of 120 developed Southern Blight.

Field Study: 2002

After completion of the third year of the study the incidence of Southern Blight throughout the field had increased. Out of the 1,200 plants in the study 496 became diseased. In the control plots, 89 out the 120 plants (74%) became infected with *S. rolf sii* (Table 9 and Figure 9). Due to its ineffectiveness in 2001, the spring biofumigation treatment was not reused in 2002. However, the fall incorporation of Florida broadleaf mustard was repeated. This biofumigation treatment had a 39% incidence of Southern Blight (47 diseased plants).

The dazomet fumigation was again effective at suppressing Southern Blight, although the quantity of diseased plants increased to 20% in 2002. The compost treatment (SMS plus PWC) had a disease incidence of only 33%, however, this was not as effective as the 7% in 2001 and 5% (avg.) in 2000. The solarization treatment was not effective at lowering disease; 76 of 120 plants in these plots developed Southern Blight.

Four of the five integrated treatments significantly reduced disease incidence. The compost plus biofumigation plots had 38% diseased plants. Biofumigation plus 50% dazomet treatment was added in 2002. This combination provided similar disease control (38%) as the biofumigation alone treatment (39%). The dazomet followed by compost treatment was reapplied in 2002. With 38 infected plants, the level of disease suppression was similar to compost alone (39) but not as good as dazomet alone (24). The most successful treatment was again the compost plus 50% dazomet combination.

Fewer plants became diseased (19) in these plots than the dazomet alone plots (24), but the differences were not significant.

Brassica Plant Stand and Biomass Incorporation

Pivotal to the efficacy of biofumigation is the quantity of plant biomass available for incorporation. Differing methods of production and cultivars brought diverse results. In 2000, the two separate Brassica cultivars, Indian mustard and fall raab, as well as the treatment that consisted of a mixture of the two types, were planted in the fall of the previous year (1999) and allowed to overwinter before incorporation in the spring. Most of the plots were affected appreciably by freezing temperatures. The average coverage immediately before incorporation for the Indian mustard was 57%, fall raab 20%, and the “mixed” plots were 23% (Table 10). The range of coverage was wide, with some plots rating as low as 5% while a small number of plots had enough plants survive to give full coverage.

In 2001, Florida broadleaf was the only Brassica cultivar used for biofumigation. All plots were seeded in the fall, however, the “fall” treatment was incorporated in late December and the “spring” treatment incorporated in April. Hence, the latter treatment overwintered as in 2000. Freezing temperatures again compromised the coverage of the spring treatment and by April these plots had an average coverage of 53%. The non-overwintering treatment had a greater canopy coverage (96%). The integrated Brassica plus compost treatment was also incorporated in the fall and had an average coverage of 92%. In 2002, the biofumigation treatment, plus the two integrated treatments, were plowed into the soil in December. Brassica coverage ranged from 70-100%.

In addition to canopy cover, the percentage of plant material that occupies the fumigated soil is central to successful biofumigation. In 2000, seven soil samples were taken directly after Brassica incorporation. The plant matter was separated from the soil and the percentage plant biomass calculated (Table 11). The percentages ranged from 0.7 to 4.0% plant material by dry weight. All of the samples were taken from plots that had at least 75% crop coverage. There was not a consistent correlation between measured plant material and coverage among these samples.

A total of 19 samples were taken in 2001 from the three biofumigation treatments (Table 12). The six samples taken from the “fall” incorporation had 100% Brassica coverage and the percentage plant material ranged from 1.9 to 3.5%. The Brassica crop grown in compost had many plots with 100% coverage (Table 13). The plant biomass in these samples ranged from 3.2 to 4.8%. The “spring” biofumigation treatment had a maximum coverage of 75% and a maximum plant material content of 2.9%. In 2002 all twelve samples had 100% Brassica coverage. The Brassica plus compost integrated treatment had the highest plant biomass levels, with three of the four samples being above 4%.

Solarization Temperatures

Numerous temperature sensors were either placed at the soil surface (0 cm) or buried in the soil (15 cm) under the solarization treatments in 2002. The range of temperatures recorded over a two-week period in April by three surface level sensors are shown in Figure 11. The “AIR” readings are on top of bare ground, “control” is under black plastic, and “solar” is under black plastic covered by clear plastic (including 10-cm air space between the plastic layers). The midday solarization temperature readings were

between 10-40C higher than the air temperatures. For example, on April 23, the peak daytime air temperature was 21C while the solarization reading was 50C. Daytime solarization temperatures were above or equal to 50C on nine of the 15 days of the sampling period. Nighttime lows for the solarization treatment were always within 10C of the air temperatures. In the second week, the nighttime solarization treatment temperatures had greater divergence from the overnight minimum air temperatures. Large differences were evident between daytime and evening temperatures under the clear plastic. For example, on April 18 the daytime solarization peak was 57C, however, by 6 am the following morning the temperature reading was 21C. Only small temperature differences occurred between the daytime highs for the solarization treatment and the recordings taken under black plastic, “control”. On April 22, for example, this difference was approximately 5C. However, the solarization temperatures were consistently warmer than the control (black plastic) temperatures during the night.

In Figure 12, data from the sensors buried at 15 cm depth and air temperatures on the bare ground are shown. Maximum soil temperatures at 15 cm were between 30 and 35C during the first week, dropping into the 20s during the second week. Soil temperatures in the solarization plots were generally 5-10C higher than air temperatures during the day and 7-15C warmer at night. The fluctuations in solarized soil temperatures were less than the maximum to minimum fluctuation on top of bare ground. The opposite was true in regards to the temperature readings from the solarization soil surface sensors (Figure 11). Under black plastic, the soil temperatures were generally 5C less during the day and 3C less at night than the solarization soil temperatures.

Solarized soil temperatures at 15 cm depth in compost amended beds and non-amended soils are shown in Figure 13. The daytime maximum temperatures were by 1-2C higher in the compost amended solarized beds. During the first week, the minimum temperatures were similar in both treatments, however, in the second week the solarization alone treatment had slightly higher readings than the solarization plus compost treatment.

Sclerotia Viability Assay

Sclerotia of *Sclerotium rolfsii* and *Rhizoctonia solani* were buried in beds each year and retrieved at a later date and assessed for viability (Table 14). *Sclerotium rolfsii* sclerotia retrieved from the control beds had high viability, i.e. when placed on a nutrient medium the sclerotia “germinated” and formed hyphae. The percentage viability of controls ranged from 96-99% during the three years of the study. The viability of the *R. solani* propagules retrieved from the control beds varied from 84-90%.

In 2000, the percentage viable sclerotia of *S. rolfsii* taken from the biofumigation plots averaged 63%, compared with 99% for the controls. *Rhizoctonia solani* propagules had a 46% survival rate compared to 84% for the controls. In 2001, the spring biofumigation treatment averaged 92% viability for *S. rolfsii* and 83% for *R. solani*. The fall incorporated Brassica treatment was more effective, 55% for *S. rolfsii* and 45% for *R. solani*. Sixty-three percent viable sclerotia of *S. rolfsii* and 52% of *R. solani* were retrieved from the biofumigation treatment (fall incorporation) in 2002.

The dazomet fumigation was the most effective treatment for killing buried sclerotia of both species. Only 18%, 15%, and 20% of the *S. rolfsii* sclerotia survived

dazomet treatment in 2000, 2001, and 2002, respectively. The *R. solani* sclerotia had only 4%, 2%, and 8% survival in 2000, 2001, and 2002, respectively.

The compost treatment did not kill the buried sclerotia to any significant degree using the technique implemented in this study. The viability of retrieved sclerotia for *S. rolfsii* varied from 88 to 94% from 2000 to 2002. The survival of *R. solani* sclerotia ranged from 78 to 83%.

The bags retrieved from 5 cm below the soil surface of the solarized plots contained 65% and 77% viable *S. rolfsii* sclerotia in 2001 and 2002, respectively. Survival of *R. solani* sclerotia varied from 67% in 2001, to 73% in 2002. Adding a compost amendment to the soil before solarizing reduced the percentage viability of both species. For *S. rolfsii* it was 60% in 2001 and 64% in 2002. *Rhizoctonia solani* viability was 46% in 2001 and 58% in 2002 for the solarization plus compost treatment.

A compost treatment plus a 50% dose of dazomet, reduced the viability of the retrieved sclerotia below that of the compost alone treatment, but not as low as the full rate dazomet plots, i.e. 47% viability for *S. rolfsii* and 30% for *R. solani* in 2001. In 2002, these values were 45% and 37% respectively. The biofumigation plus 50% dazomet treatment was somewhat more effective, with 40% and 28% viability for each species respectively.

In 2002, the compost plus 50% dazomet treatment had 45% and 37% viability for *S. rolfsii* and *R. solani* respectively. The biofumigation plus 50% dazomet treatment was somewhat more effective in 2002, with 40% and 28% viability respective to *S. rolfsii* and *R. solani*.

Sclerotial Germination - S. rolfsii

Estimates of the naturally-occurring propagule density of *Sclerotium rolfsii* in various treatment plots were made by observing and enumerating germination rates from a 100 g soil sample (Table 15). Total numbers increased from the first to the third year of the study. Sclerotial counts in the control samples ranged from 4.17 in 2000 to 10.55 in 2002. The number of sclerotia in the dazomet-fumigated plots was lower than those in the other treatments and had the least variability over the three years.

Microbial Enumeration

Total culturable fungi, *Trichoderma* spp., and bacterial counts for tomato pre-plant and post-harvest samples are displayed in Table 16. Generally, microbial counts were higher in the post-harvest soil samples than the pre-plant samples. For example, the bacterial counts in the compost plus 50% dazomet treatment increased from 1.56×10^8 CFUs in late April to 7.45×10^8 CFU per g soil in September.

Fungal propagule numbers were significantly higher in all compost amended treatments than in controls each year. *Trichoderma* and bacterial counts were also highest in the compost treatments. *Trichoderma* populations in the compost treatments were at least four times higher than in the controls. By contrast, in the dazomet fumigation treatment all microbial populations were below that of controls in the pre-plant samples. However, post-harvest bacterial counts became elevated in the fumigated beds and were higher than controls.

Although not always significant, pre-plant microbial counts from the biofumigation treatments were lower than the controls. *Trichoderma* populations were reduced by the biofumigation treatments. The pre-plant solarization samples had lower

fungi but higher bacterial counts than controls. *Trichoderma* populations were similar to controls.

Discussion

Southern Blight

The number of plants infected with *S. rolfsii* increased from 134 out of 960 plants in 2000, to 200 out of 1200 plants in 2001, to 496 out of 1200 plants in 2002. The control plots in 2002 had 89 diseased plants out of 120 total plants. All of the experimental plots also had higher disease incidence in 2002 than they did in the previous two years. The effectiveness of the treatments are best assessed by relating the number of plants that developed Southern Blight with numbers of diseased plants in the control plots for each year.

Biofumigation

The number of plants with Southern Blight in fall raab plots in 2000 was 9% of all plants for that treatment, whereas, the Indian mustard plots had a disease incidence of 29%. The disease incidence in these treatments did not correspond directly with crop coverage. The average canopy coverage for the Indian mustard crop was 57%, compared with 20% for the fall raab. The crop coverage rating was a subjective assessment of above ground plant material and, thus, did not measure root mass or biocidal activity of the plant biomass when incorporated. These latter two factors are also very important characteristics that affect the success of biofumigation.

Having a significant Brassica biomass available for mulching and incorporation is likely to enhance biofumigation. Allowing a cover crop to overwinter has many advantages, including the stabilization of the production area. However, winter and early

spring freezes compromised Brassica stands in 2000 and 2001. For the 2001 study, both biofumigation treatments were seeded with mustard in September of 2000. The spring incorporation of the Florida broadleaf mustard cultivar had an average coverage of 53% by mid-April 2001, with the highest single plot-rating of 75%. The same variety when incorporated in the preceding December produced 96% coverage on average with numerous plots at 100%. The spring-incorporated mustard averaged 2.1% plant material per dry weight of soil. However, the fall incorporation averaged 2.9% plant material. The increased volume of plant material in the soil would seemingly enhance the opportunity for effective biofumigation when the superior coverage of the fall crop is tilled into the plots. Disease incidence during the 2001 season supported this perception. The spring biofumigation treatment had a similar number of diseased plants as the control, 40 to 36, respectively. The fall biofumigation treatment, however, had 21 diseased plants, 58% of the controls. As a consequence of these results, the spring treatment was not continued in 2002, thus, eliminating the confounding component of winter freezing and reduced crop coverage. Even though the total number of diseased plants that developed in the biofumigation plots in 2002 was higher than the comparable treatment in 2001, the occurrence of Southern Blight relative to control was similar i.e. 53% in 2002 versus 58% in 2001.

Muehlchen (1990) found that mustard biofumigation was able to control disease caused by the soilborne pathogen, *Aphanomyces* sp. However, other studies did not find a decreased incidence of disease expression in plants following biofumigation, even though inoculum levels were reduced (Kirkegaard et al., 2000).

Composts

In all three years of the study the plots that had been amended with composts developed significantly less Southern Blight than control plots. The two types of composts being investigated, poultry waste compost (PWC) and spent mushroom substrate (SMS) were applied separately in 2000. The same rate of incorporation, 11 mt/ha, was used for both. They performed similarly, with 5 and 7 diseased plants for the PWC and SMS, respectively. These numbers were significantly different from the 39 diseased plants in the control plots but were not different from each other. Both composts have animal manures as a component of their parent material (PWC has poultry litter and SMS has discarded horse bedding and poultry litter, including wastes). This common component may influence the final characteristics of the compost and, hence, their performance as disease suppressive substrates.

As a consequence of their analogous performance in 2000, the two compost types were combined at a 1:1 ratio (11 mt/ha total loading rate) in 2001. The compost treatment was again very effective at disease suppression; with 8 plants infected with *S. rolfsii* compared with 36 in the control plots. The level of contamination was comparable to the dazomet fumigation in both 2000 and 2001. An increase in pathogen inoculum levels throughout the study was expressed as a high overall incidence of disease in 2002. The compost treatment did not curtail the disease pressure in 2002 as effectively as it did in the two previous years. Thirty-nine plants developed Southern Blight in the compost plots. This number was 44% of the control counts. In 2000 and 2001 this percentage was 15% (average) and 22%, respectively. The substantial increase in disease numbers in 2002 may indicate that environmental conditions were favorable for the advance of

Southern Blight in this field. Even though the annual rainfall was higher (250 mm more) than 2001, the summer months were drier in 2002. The average maximum temperature for April to August was higher by 0.8C in 2002 than 2001. The relative upsurge of disease in the compost plots compared to the controls may indicate that this treatment is less effective when disease pressure is high. However, countering this speculation is the observation that the dazomet plots also experienced an approximately 50% increase in diseased plants in 2002 versus 2001, compared to controls.

The reduced rate of Southern Blight in the compost-treated plots during the study agrees with the results of Gorodecki and Hader (1990) using composted cattle manure. Chellemi et al. (1992) observed a disease rate of 20% due to soilborne pathogens when SMS was applied to fields. Bulluck and Ristaino (2001) reported a 23-53% incidence of Southern Blight in tomato plants relative to controls grown in compost-amended soils.

Dazomet Fumigation

As with the compost treatment, the dazomet fumigation gave significant control of Southern Blight in all three years. The dazomet treatment had only 5 diseased plants in 2001. The disease incidence increased to 24 out of 120 plants in 2002. While the level of disease control was appreciable in all three years, dazomet was not able to keep the plots disease free. It is likely that pathogen inoculum survived in the soil between seasons and, consequently, was available for plant infection during the subsequent growing season. This was evident by the 24 dead plants in 2002. The effectiveness of the application of dazomet to agricultural soils is an ongoing issue. Parameters such as soil moisture and temperature, movement of the volatile MITC throughout the soil profile after activation, and the morphological state of the soilborne pathogens (sclerotia versus

hyphae) will affect performance and disease suppression. Economic thresholds for disease occurrence should be a consideration when choosing a fumigant such as dazomet.

In studies by Harris (1990) and Rieger et al. (2001) dazomet was more effective for disease suppression in strawberry production than for tomato production. O'Brien and van Bruggen (1990) reported a 25-50% decrease in the incidence of lettuce corky root when dazomet was used. In this study, there was a 73-86% decrease in disease incidence in dazomet treated plots compared to controls.

Solarization

In 2001 and 2002, the number of diseased plants in the solarization plots did not differ significantly from controls. In 2001, however, the incidence of Southern Blight in these plots was 21% compared to 30% for the controls. In 2002, under higher disease pressure, 76 out 120 plants became infected in the plots that had been subjected to solarization prior to planting. The temperatures generated throughout the soil zone that contains the pathogen propagules was not likely to be adequate to kill or deactivate them. Temperatures approaching 60C were recorded on the soil surface of the solarized beds. These temperatures are regarded as being suitable for pathogen suppression. However, for effective soil decontamination, these temperatures would need to occur down through the soil profile and be maintained for a longer period. Twenty to sixty days of effective solarization can enable successful disease control (Katan and DeVay, 1991). This phenomenon did not occur with the spring solarization regime implemented in this study. At a depth of 15 cm, temperatures up to 35C were generated during the daytime, dropping into the mid-20s overnight. If solarization is to be used as a solitary prophylactic

measure, mid-summer radiation in this region would seem to be required for lethal temperatures generation throughout the soil rootzone.

Studies that have used solarization to suppress plant disease effectively (Katan et al., 1976; Chellemi et al. 1994) have utilized the level of solar radiation usually only available during mid-summer. Katan and De Vay (1991) recorded temperatures of 47C at 10 cm soil depth, 42C at 20 cm, and 39C at 30 cm in July and August in Israel. Ristaino et al. (1991) had similar results for Southern Blight reduction as this study. They reported a disease rate of 77% for solarized treatments compared with the 69% in 2001 and 85% in 2002 reported here.

Integrated Treatments

Biologically based combinations, as well as synthetic plus biological disease control strategies were developed and tested throughout the duration of the study. Soil disinfestation in amalgamation with the re-introduction of a balanced soil microecology was expected to lead to disease suppression. Biofumigation, dazomet, and solarization were methods of reducing pathogen inoculum in the soil and were combined with compost amendments.

Several of the integrated strategies were effective at controlling Southern Blight. The statistical analysis, however, did not reveal a synergistic effect i.e. integrated treatments did not perform better than at least one of their components alone. For example, in 2002 the compost plus biofumigation treatment plots had 46 diseased plants; whereas, biofumigation alone had 47 and compost alone had 39. For all three treatments the disease incidence was similar, although they were all statistically different from the controls. An enhanced synergistic effect emanating from the combination treatment

would have resulted in significantly lower disease. Theoretical benefits from combining biofumigation with compost include: 1) growing the Brassica cover crop in compost amended plots may induce more vigorous plant growth and, hence, result in a greater biomass for incorporation, and 2) the volatile compounds acting on the composted soil could preferentially select a microbial population that was disease suppressive i.e. with reduced pathogen numbers and enhanced beneficial microorganisms. Either, or both, of these biological features may actually occur, however, the disease data collected during this study did not confirm an enhanced effect.

The compost plus solarization combination was selected with similar principles in mind as the other compost integrated treatments i.e. soil disinfestation plus microecological enhancement. The temperatures generated via trapping solar radiation under plastic would compromise the morphological structure of the pathogen propagules yielding them susceptible to attack from microbial biocontrol agents. The compost plus solarization treatment, however, was not significantly more effective than either treatment alone. As discussed previously, temperatures generated during the spring exposure period were not likely to be sufficient to significantly alter the existing microbial community structure. With increased soil temperatures, a compost plus solarization combination may indeed generate a sub-lethal, enhanced disease controlling effect.

From the results obtained in this study, management strategies that integrate composted amendments with synthetic fumigation seem to have potential as sustainable disease control techniques. The two theories, and subsequent methods, associated with combining synthetic fumigants with composts were: 1) an initial reduction in pathogen

populations via fumigation followed by the re-establishment of a balanced, suppressive soil microecology via the compost additions (this strategy is achieved by dazomet fumigation followed two weeks later by compost incorporation), and 2) an initial application of compost followed immediately by a reduced dose application of dazomet acts to compromise pathogen propagules, making them susceptible to the action of biocontrol agents (this strategy was implemented via the compost plus 50% dazomet treatment). Both of these techniques were successful at reducing the incidence of Southern Blight to varying degrees. In terms of numbers of diseased plants, the reduced dose integrated approach was the most successful of all of the experimental treatments in 2001 and 2002. In 2001, only one diseased plant was recorded out of 120 plants in all twelve replicates. Although not significantly different, the compost plus 50% dazomet plots had approximately 10% less disease than the dazomet alone treatment and 20% less than compost alone, when compared to control. In 2002, the reduced dose regime resulted in half the number of diseased plants than either the dazomet or compost plots.

The success of reduced dose fumigation in conjunction with an organic amendment in 2001 prompted the introduction of a biofumigation plus 50% dazomet treatment in 2002. It was proposed that the combined action of MITC, the active ingredient in dazomet, and AITC, one of the major biocidal volatile compounds known to be produced by Brassica biofumigation, could yield a more effective, broad-spectrum fumigation. The incidence of Southern Blight in these plots was very similar to biofumigation alone.

Sclerotia Viability

The technique of burying cultured sclerotia from known fungal species in permeable nylon bags permits the assessment, to some degree, of the soil treatments. The nylon bags enclosed the sclerotia while allowing volatile compounds to move through the mesh and interact with the fungal propagules.

Sclerotia of *S. rolfii* were hardier than those of *R. solani*. A higher percentage of viable *S. rolfii* sclerotia were retrieved from control plots than *R. solani*. For example, in 2000, 79 of 80 *S. rolfii* sclerotia retrieved from the controls were viable. This compared to 67 of 80 viable sclerotia for *R. solani*. The dazomet fumigation was the most effective at killing sclerotia from both species. For *S. rolfii*, the percentage viable was 18, 15, and 20% in 2000, 2001, and 2002, respectively. The viability of *R. solani* sclerotia was 4, 2, and 8% for each year, respectively. Even though these numbers are low, they do not represent a complete “sterilization” of the soil. This indicates that pathogen propagules do survive after fumigation and, hence, would be available for re-establishment in the production soils. Annual use of this fumigant would be required to control pathogen populations. The actual incidence of Southern Blight in the dazomet-fumigated plots was 10, 5, and 24% for 2000, 2001, and 2002, respectively. The disease ratings approximate the readings from the sclerotia viability assay. This would suggest that the “bag” test is a feasible method of assessing the potential effectiveness of a fumigant against pathogen propagules.

The 50% dazomet treatment resulted in a much higher percentage of viable sclerotia from both species. Full rate dazomet had 15–20% *S. rolfii* and 2–8% *R. solani* sclerotia viability. Although significantly different from the controls, the 47 and 40%

viability for *S. rolfii* and 30 and 28% for *R. solani* in 2001 and 2002 respectively, indicates that dazomet needs to be used at full rates to directly kill large proportions of the sclerotia. However, there is the possibility that even though the reduced dose fumigation did not kill as many sclerotia as the full dose, it may have caused physical damage to the propagules, thus, making them susceptible to microbial action. In conjunction with the viability data, the low incidence of disease in the compost plus 50% dazomet plots in 2001 and 2002 suggests that the suppressive action of this treatment is due to factors other than, or in concert with, the fumigant.

Some of the biofumigation treatments also reduced the number of viable sclerotia to levels significantly below controls. Biofumigation treatments from 2000 to 2002 reduced viable *S. rolfii* sclerotia to between 55 and 63% of controls. The spring 2001 biofumigation treatment was not effective at killing sclerotia, just as it was not effective at reducing Southern Blight in the field. This treatment had a low plant stand. Just as MITC is recognized as the compound responsible for the lethal action of dazomet fumigation, the volatile compounds released from Brassica crops are also active fumigants. Treatments that do not release volatile compounds as their primary mode of action were not as effective at reducing viable sclerotia numbers in this assay, even though they may have still been successful at suppressing disease in the field.

The compost treatments only reduced viable sclerotia between 88 and 94% of controls in the three years of the study. Colonization of bacteria on the surface of sclerotia, as well as the penetration of the rind by hyperparasitic fungal structures, are associated with biocontrol and compost applications (Lifshitz et al., 1983). Both of these phenomena could occur through the nylon mesh of the bags used in the assay. Longer

periods of time, or more favorable conditions may have been required for the compost treatments to produce lethal effects on the bagged sclerotia. The impacts of fumigation could be evident within a few days after application, however, the biological actions of the microorganisms in composts would likely need several weeks of warm, moist soil conditions for their actions to occur. These conditions are present during the growing season, but the bags containing sclerotia were removed from the soil before tomatoes were transplanted.

The reduction in viable sclerotia in the bags taken from the solarized plots was not significantly different from the controls in either year. The percentage of viable sclerotia was 65 and 77% for *S. rolfsii* and 67 and 73% for *R. solani* in 2001 and 2002, respectively. The bags were buried at a depth of 5 cm. Although not directly measured at this depth, it is feasible that the soil temperatures at this level may have caused, or at least contributed to the 23 to 35% sclerotial mortalities. The compost plus solarization treatment resulted in higher rates of sclerotial death than solarization alone. The elevated temperatures in these plots may have enhanced microbial biocontrol actions.

Microbial Populations and Sclerotia Counts

The concurrent increase in the number of diseased plants in the field in 2002 and the increase in germinated *S. rolfsii* propagules via the methanol spray assay confirmed the causal relationship. In particular, the high number of sclerotia found in the control samples correlates with the high number of diseased plants in these plots in 2002.

Numbers of total fungi or culturable bacteria did not increase to a considerable degree in the control plots from 2000 to 2002, however, the specific genera of these microorganism may have. The increase in sclerotia retrieved from these plots may indicate that higher

relative populations of pathogenic fungi may be replacing other species. This may be supported by the decrease in *Trichoderma* spp. in the control soils in 2002 compared with 2000.

The high population of fungi and bacteria in the compost treatments may indicate that this relates to the mechanism by which compost can suppress soilborne disease. General suppression is an important component of biocontrol. Competition for resources by other microorganisms may restrict the opportunity for pathogenic species to proliferate. The low incidence of Southern Blight in the composted plots may also be related to the elevated populations of *Trichoderma* recorded in the soil samples taken from those plots. Cook and Baker (1983) suggested that *S. rolfii* inhibition can be enhanced by specific suppression related to the presence of *Trichoderma* spp.

Dazomet fumigation effectively reduced microbial numbers in all years. However, by post-harvest sampling time fungal and bacterial populations had increased significantly. Populations of *Trichoderma* were still low in these plots. The solarization treatment reduced fungi numbers and increased bacteria compared to the control.

Conclusion

Composted amendments, biofumigation, and dazomet soil fumigation suppressed Southern Blight to varying degrees over the duration of the field study. Compared with 30-74% for the controls, tomato plants in the compost plots had a disease incidence of 4-33%. This compares favorably with the 4-20% incidence of disease among the plants in the dazomet fumigated plots. The effectiveness of the biofumigation treatments was linked to climatic conditions and subsequent plant biomass availability. Compromised crops, such as the Indian mustard treatment in 2000 and the “spring” Florida broadleaf

mustard treatment in 2001 were unsuccessful at controlling disease. Brassica crops that obtained full coverage suppressed Southern Blight in the range of 18-39%. These crops resulted in incorporations of up to 4.1% of plant material to soil (dry wt.).

The solarization treatment, conducted outside of the peak solar radiation months of June through August, was ineffective at controlling soilborne disease under the conditions implemented during this study. Even though temperatures of 59C were recorded at the soil surface, at 15 cm temperatures were closer to 35C. Sustained temperatures in the mid-40C range are required for disease control at this depth.

The most successful of the integrated treatments investigated was the dazomet/compost combinations. Plants in the compost plus 50% dose dazomet treatment had the lowest incidence of disease in 2001 and 2002.

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Appendix

Table 9. Incidence of Southern Blight in plasticulture tomato production experiment from 2000 – 2002.

Southern Blight 2000-02					
Treatment	Diseased Plants (no.)	% of treatment total	pH Treatments (no. diseased plants)		
			low	med	high
<u>2000</u>					
Control	39 a ^z	33	13	13	13
Biofumigation (Ind. mstd.)	35 ab	29	11	12	12
Biofumigation (mix)	17 bc	14	9	0	8
Biofumigation (fall raab)	11 c	9	4	0	7
Compost (PWC)	5 c	4	3	0	2
Compost (SMS)	7 c	6	1	5	1
Dazomet	10 c	8	1	4	5
Dazomet + compost	10 c	8	5	4	1
	<u>134</u>		<u>47</u>	<u>38</u>	<u>49</u>
<u>2001</u>					
Control	36 a	30	16	17	3
Biofumigation(spring)	40 a	33	10	8	22
Compost + biofumigation	28 ac	23	10	5	13
Solarization	25 abc	21	14	7	4
Biofumigation(fall)	21 ab	18	3	14	4
Compost + solarization	18 bc	15	8	7	3
Dazomet + compost(2 nd year)	18 cd	15	4	8	6
Compost (SMS+PWC)	8 bde	7	4	1	3
Dazomet (Basamid)	5 be	4	2	2	1
Compost + 50% dazomet	1 e	1	1	0	0
	<u>200</u>		<u>72</u>	<u>69</u>	<u>59</u>
<u>2002</u>					
Control	89 a	74	30	33	26
Solarization	76 a	63	35	33	8
Compost + solarization	72 ab	60	26	16	30
Biofumigation	47 bc	39	21	13	13
Compost + biofumigation	46 bd	38	17	20	9
Biofumigation + 50%dazomet	46 be	38	5	18	23
Compost (SMS+PWC)	39 cdef	33	13	13	13
Dazomet + compost	38 cdef	32	14	12	12
Dazomet (Basamid)	24 cdef	20	5	8	11
Compost + 50% dazomet	19 f	16	10	4	4
	<u>496</u>		<u>176</u>	<u>170</u>	<u>149</u>

^z Treatments with same letters are not different at $P < 0.05$ based on an LSD of log transformed data.

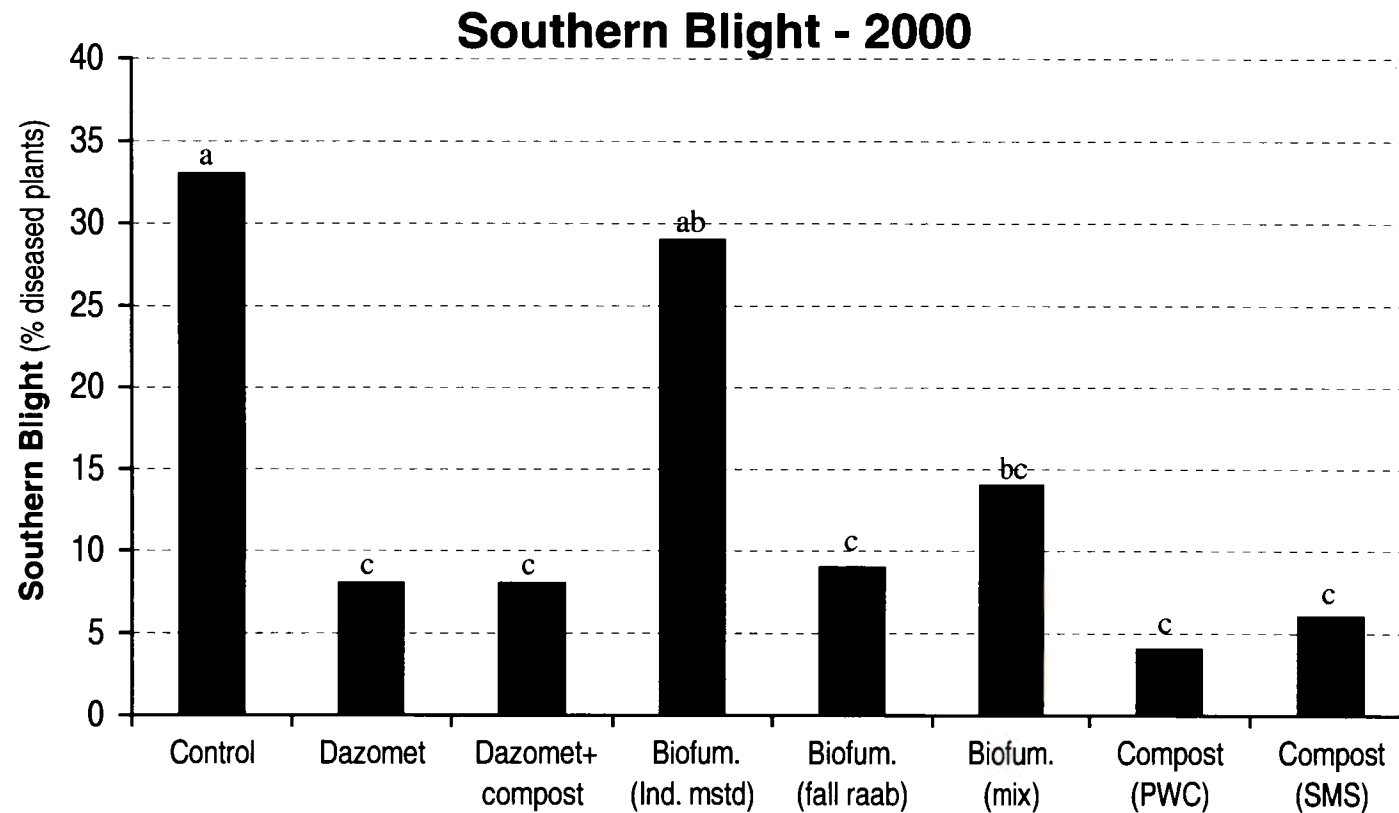


Figure 7. Incidence of Southern Blight (% diseased plants) in plasticulture tomato production experiment for 2000. Bars with the same letters were not significantly different at $P < 0.05$ according to LSD of arcsin transformed data.

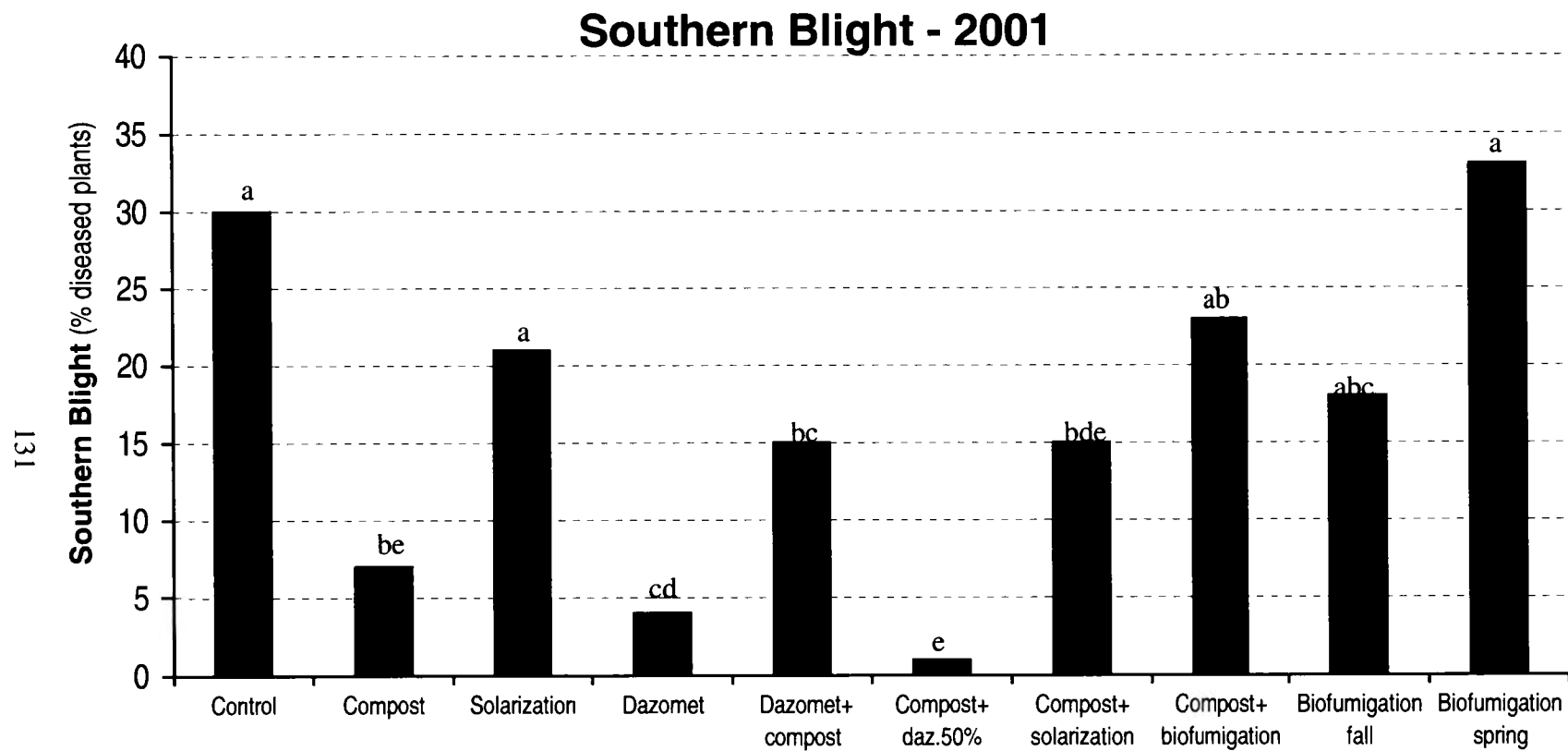


Figure 8. Incidence of Southern Blight (% diseased plants) in plasticulture tomato production experiment for 2001. Bars with the same letters were not significantly different at $P < 0.05$ according to LSD of arcsin transformed data.

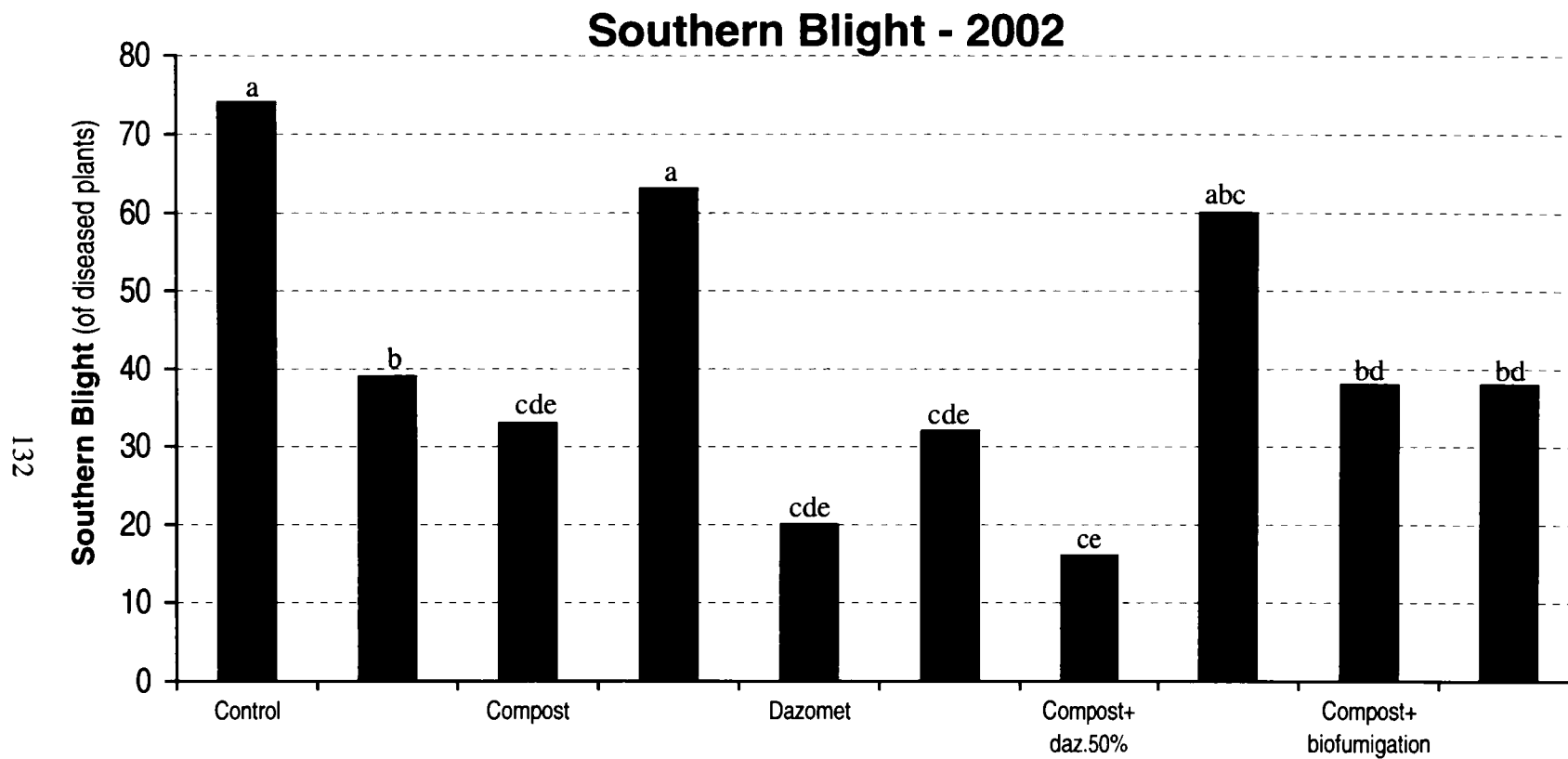


Figure 9. Incidence of Southern Blight (% diseased plants) in plasticulture tomato production experiment for 2002. Bars with the same letters were not significantly different at $P < 0.05$ according to LSD of arc-sine transformed data.

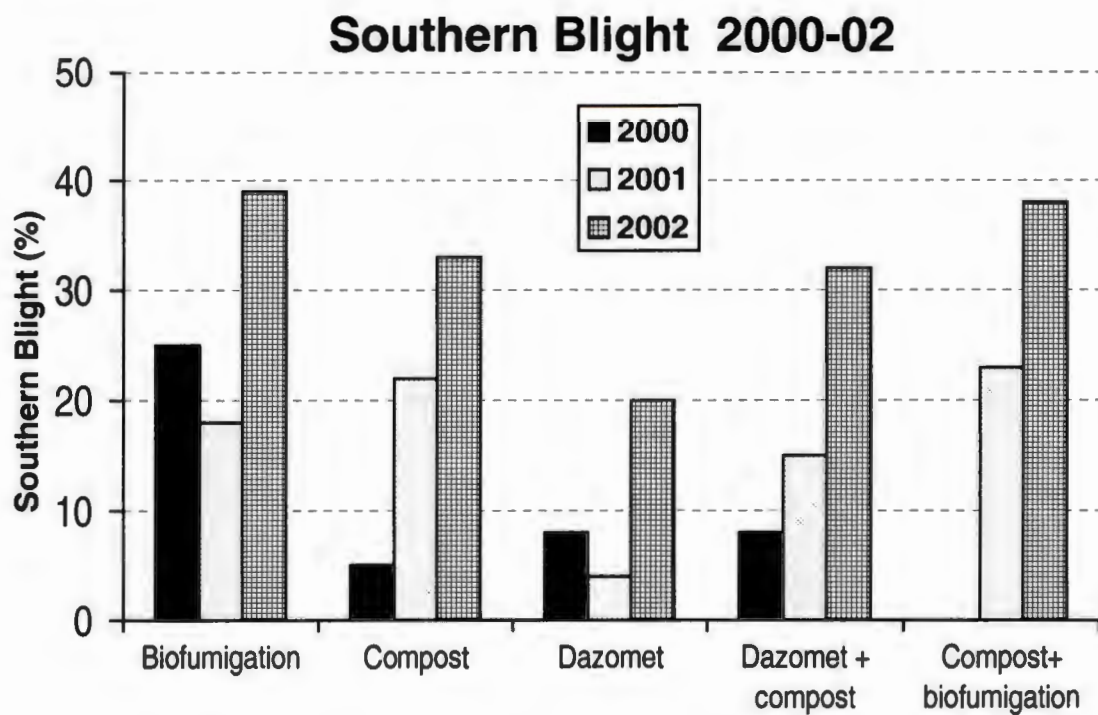


Figure 10. Southern Blight (%) comparison from 2000-02 for plasticulture tomatoes. Each bar represents percentage of disease for that year for selected treatments. Biofumigation 2000 = average of all three treatments, 2001 = fall treatment. Compost 2000 = average of both treatments.

Table 10. Stand of Brassica cover crop relative to full coverage prior to mowing and incorporation for 2000–02. All Brassica plots were assessed each year. The mean stand for the treatment type is expressed, as is the range of evaluations.

Brassica Plant Stand		
Brassica Treatment	% of full cover	Range
<u>2000</u>		
Indian mustard ^z	57	10-95
Fall raab ^z	20	5-100
Mix ^z	23	5-75
<u>2001</u>		
Fl. broadleaf - spring ^z	53	40-75
Fl. broadleaf - fall ^x	96	80-100
Fl. broadleaf + compost ^x	92	80-100
<u>2002</u>		
Fl. broadleaf ^x	87	75-100
Fl. broadleaf + 50% dazomet ^{xy}	88	70-100
Fl. broadleaf + compost ^x	89	75-100

^z spring incorporated

^x fall incorporated

^y dazomet applied after Brassica crop mowed (no effect on emergence)

Table 11. Dry weight and percentage of Brassica plant material in the soil of biofumigation treatments after mowing and incorporation of cover crop for 2000.

Brassica Plant Material in Soil						
2000	Plot ID	% of full cover	Soil wt. (g)	Soil dry wt. (g)	Plant dry wt. (g)	% plant material
Ind. mustard	A13	85	551	413.5	7.75	1.8
Ind. mustard	C10	75	750	607.3	6.05	1.0
Ind. mustard	C14	95	318	221.8	9.24	4.0
Ind. mustard	D3	80	604	501.3	3.35	0.7
Ind. mustard	D20	80	398	300.0	5.31	1.7
Fall raab	A12	100	489	383.7	3.40	0.9
Mix	B11	75	626	469.8	10.26	2.2

N.B. Soil cores taken after biofumigation beds were formed and plastic mulch laid.

Table 12. Dry weight and percentage of Brassica plant material in the soil of biofumigation treatments after mowing and incorporation of cover crop for 2001.

Brassica Plant Material in Soil						
2001	Plot ID	% of full cover	Soil wt. (g)	Soil dry wt. (g)	Plant dry wt. (g)	% plant material
Fl. broadleaf (fall)	B7	100	685	514.1	15.9	3.0
	B17	100	711	495.9	14.8	2.9
	B19	100	701	581.8	20.5	3.4
	B27	100	652	511.6	9.9	1.9
	B30	100	492	369.2	11.0	2.9
	C10	100	589	410.8	14.9	3.5
Fl. broadleaf (spring)	A1	55	522	409.6	4.6	1.1
	A13	75	753	565.1	15.1	2.6
	B1	60	606	490.7	14.2	2.8
	C14	60	648	452.0	9.2	2.0
	D1	45	625	518.7	7.4	1.4
	D20	70	698	526.2	15.7	2.9
	D27	60	722	566.5	11.0	1.9
Fl. b'leaf + compost	B5	100	507	397.8	14.9	3.6
	B11	100	580	469.6	20.6	4.2
	B25	100	523	394.3	13.0	3.2
	C2	100	456	369.2	15.4	4.0
	C20	100	702	582.6	19.9	3.3
	C26	100	641	483.2	24.4	4.8

N.B. Soil cores taken after biofumigation beds were formed and plastic mulch laid.

Table 13. Dry weight and percentage of Brassica plant material in the soil of biofumigation treatments after mowing and incorporation of cover crop for 2002.

Brassica Plant Material in Soil						
2002	Plot ID	% of full cover	Soil wt. (g)	Soil dry wt. (g)	Plant dry wt. (g)	% plant material
Fl. broadleaf	B17	100	664	537.7	22.4	4.0
	C10	100	712	558.7	23.9	4.1
	C13	100	688	516.3	19.3	3.6
	C24	100	702	582.6	17.4	2.9
Fl. b'leaf + daz.50%	B7	100	741	581.4	23.6	3.9
	B19	100	685	477.8	15.8	3.2
	B27	100	621	468.2	14.0	2.9
	C21	100	650	453.4	9.3	2.0
Fl. b'leaf + compost	B11	100	711	533.6	24.6	4.4
	B24	100	598	496.3	23.4	4.5
	C20	100	596	482.6	23.3	4.6
	C26	100	691	520.9	18.8	3.3

N.B. Soil cores taken after biofumigation beds were formed and plastic mulch laid.

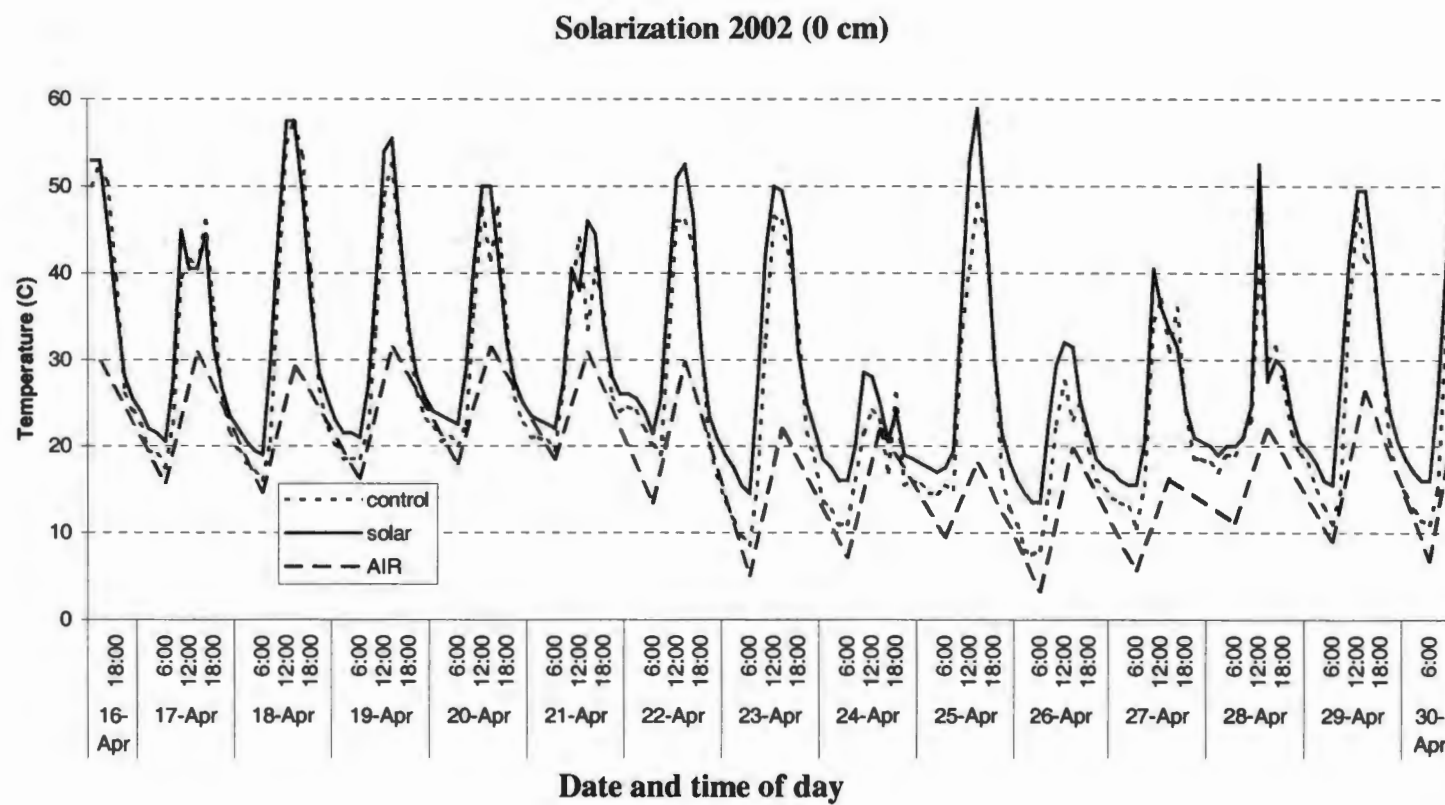


Figure 11. Temperatures at soil surface level under black plastic (control), under black plastic + clear plastic (solar), and air above bare ground.

Solarization 2002 (15 cm)

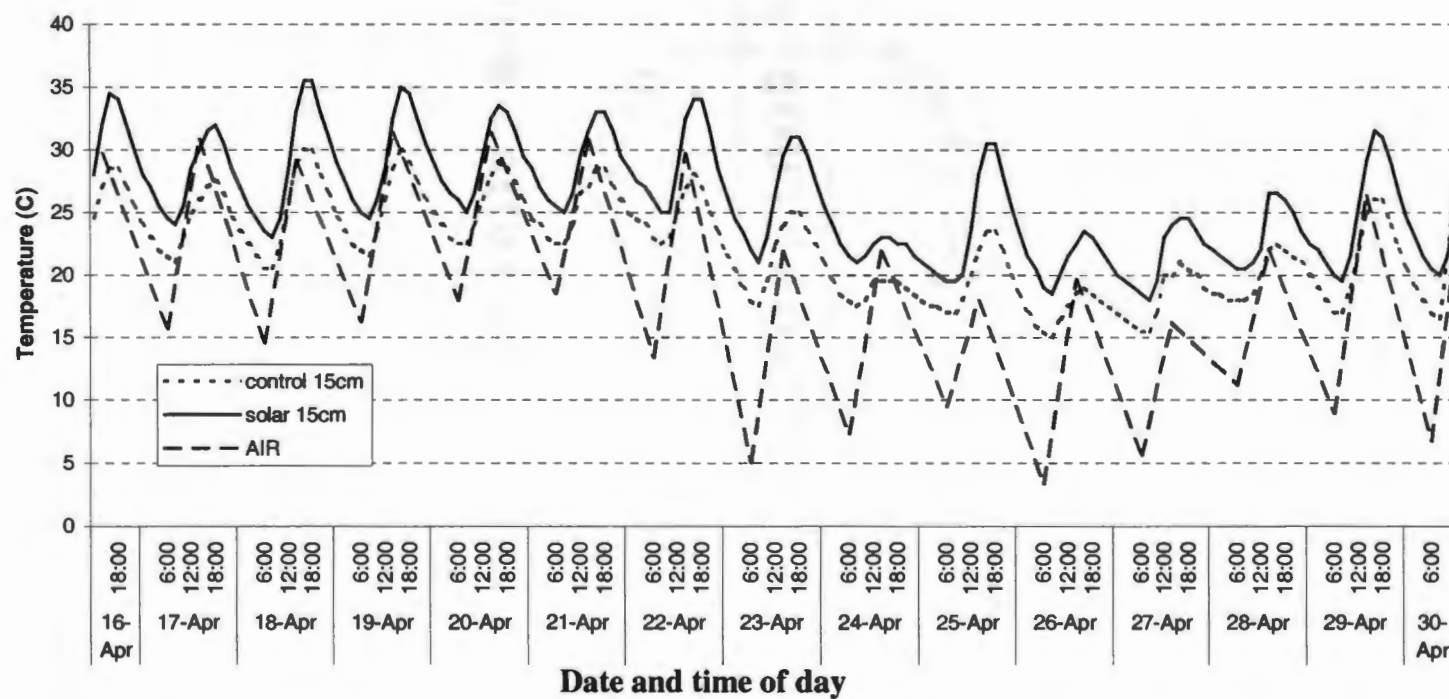


Figure 12. Soil temperatures at 15 cm depth under black plastic (control), under black plastic + clear plastic (solar), and air above bare ground.

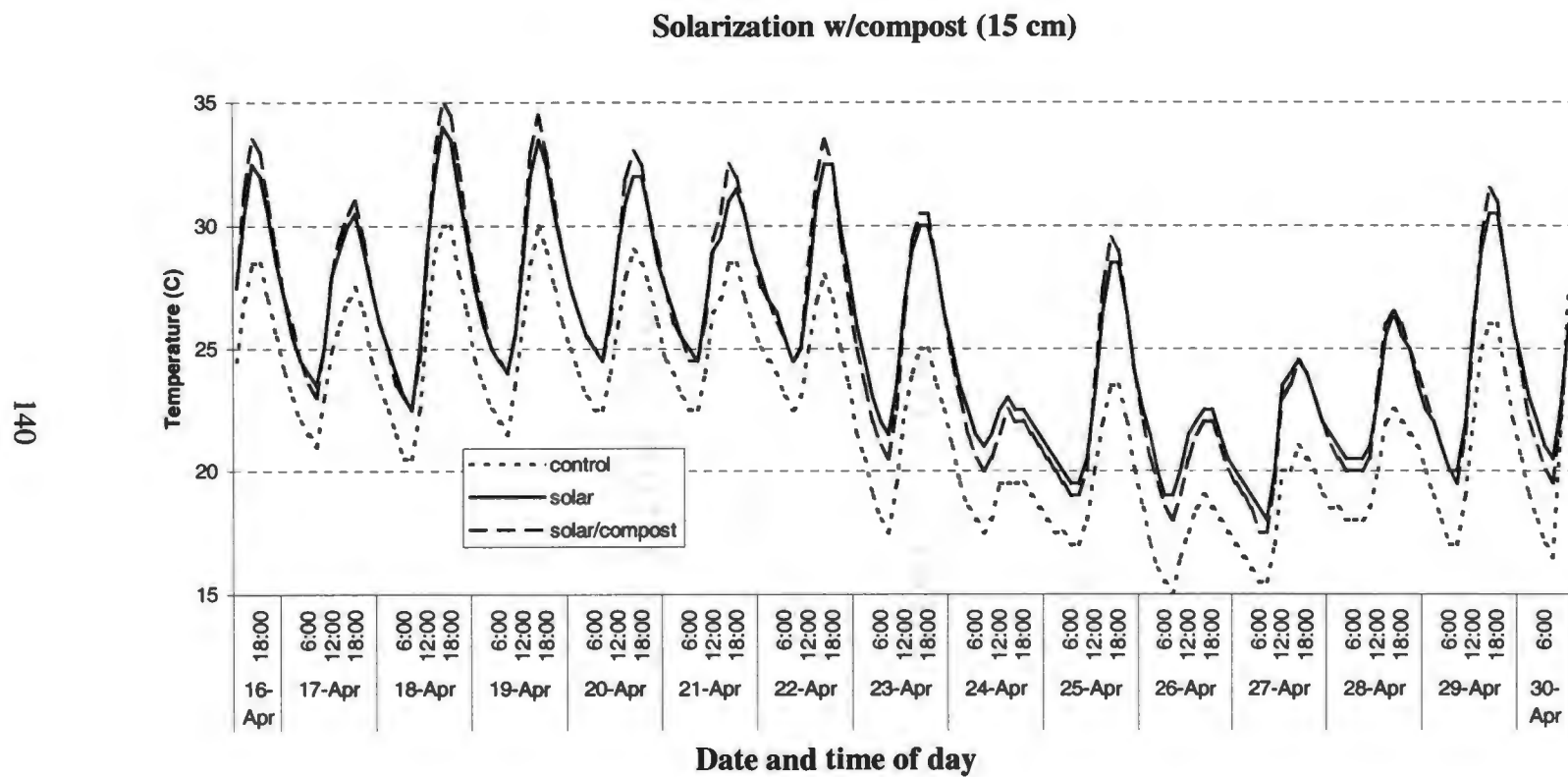


Figure 13. Soil temperatures at 15 cm depth below black plastic (control), below black plastic + clear plastic (solar), and below black plastic + clear plastic in compost amended soil (solar/compost).

Table 14. Viability of *S. rolfii* and *R. solani* sclerotia after retrieval from experimental plots in 2000-02.

Sclerotia Viability Assay				
	<i>S. rolfii</i>		<i>R. solani</i>	
	Sclerotia viable/buried	Viable (%)	Sclerotia viable/buried	Viable (%)
<u>2000</u>				
Control	79/80	99	67/80	84
Biofumigation (Ind.mstd)	50/80	63	37/80	46
Compost (SMS)	75/80	94	62/80	78
Dazomet	14/80	18	3/80	4
<u>2001</u>				
Control	58/60	96	54/60	90
Biofumigation (spring)	55/60	92	50/60	83
Biofumigation (fall)	33/60	55	27/60	45
Compost	53/60	88	48/60	80
Dazomet	9/60	15	1/60	2
Compost + daz.50%	28/60	47	18/60	30
Solarization	39/60	65	40/60	67
Solar. + compost	36/60	60	28/60	46
<u>2002</u>				
Control	59/60	98	52/60	86
Biofumigation	35/60	59	31/60	52
Biofum. + 50%daz.	28/60	40	21/60	28
Compost	54/60	90	50/60	83
Dazomet	12/60	20	5/60	8
Compost + 50%daz.	27/60	45	22/60	37
Solarization	46/60	77	44/60	73
Solar. + compost	38/60	64	35/60	58

Table 15. *Sclerotium rolsii* sclerotia germination using methanol assay from 100g soil samples taken from experimental plots from 2000 – 2002.

<i>S. rolsii</i> sclerotia germination 2000-02					
<u>2000</u>	Sclerotia/ 100g soil	<u>2001</u>	Sclerotia/ 100g soil	<u>2002</u>	Sclerotia/ 100g soil
Control	4.17	Control	5.48	Control	10.55
Biofumigation (Ind. Mstd.)	4.50	Biofumigation(spring)	4.87	Solarization	6.92
Biofumigation (Mix)	3.45	Biofumigation(fall)	6.02	Compost + solarization	6.08
Biofumigation (Fall Raab)	2.65	Compost + Biofumigation	3.58	Compost + biofumigation	6.57
Compost (PWC)	3.57	Solarization	5.28	Biofumigation	6.25
Compost (SMS)	3.68	Compost + solarization	2.87	Biofumigation + 50%dazomet	4.45
Dazomet	1.73	Dazomet + compost	4.70	Compost + 50% dazomet	3.40
Dazomet + compost	3.07	Compost (SMS+PWC)	5.03	Dazomet + compost	5.62
		Dazomet (Basamid)	2.55	Dazomet (Basamid)	3.18
		Compost + 50% dazomet	2.70	Compost (SMS+PWC)	6.30

Table 16. Fungal, bacterial, and *Trichoderma* spp. soil populations from 2000 – 2002.

Microbial Counts 2000-02						
	Total Fungi		Trichoderma		Bacteria	
	CFUs/gram soil(10^3)		CFUs/gram soil(10^3)		CFUs/gram soil(10^6)	
	pre-plant	post-harvest	pre-plant	post-harvest	pre-plant	post-harvest
<u>2000</u>						
Control	29.22	53.54	4.10	5.36	95.65	107.53
Biofumigation (Ind. Mstd.)	12.68	22.31	2.23	3.10	69.32	61.78
Biofumigation (Mix)	15.32	17.36	1.24	1.68	52.31	78.98
Biofumigation (Fall Raab)	9.58	13.87	0.96	2.25	58.64	62.20
Dazomet	0.11	5.10	0.22	1.12	26.63	121.85
Dazomet + compost	25.22	163.84	4.66	12.26	226.39	563.84
Compost (PWC)	159.36	201.12	19.36	21.54	560.21	891.54
Compost (SMS)	119.65	188.52	15.88	16.97	398.44	680.03
<u>2001</u>						
Control	36.36	59.10	2.33	3.67	73.06	95.36
Biofumigation(spring)	31.52	38.78	0.35	1.66	62.11	88.23
Biofumigation(fall)	25.89	39.14	2.35	3.12	45.18	66.31
Compost + Biofumigation	100.22	155.93	12.39	14.09	220.12	386.34
Solarization	12.96	28.20	2.56	4.01	108.14	79.68
Compost + solarization	156.13	145.95	18.36	21.59	488.21	510.22
Dazomet (Basamid)	0.08	2.85	0.35	0.89	15.32	98.97
Dazomet + compost(2nd year)	98.25	67.89	10.75	8.96	152.08	165.13
Compost (SMS+PWC)	188.10	197.51	22.16	16.35	323.28	1116.80
Compost + 50% dazomet	58.68	61.30	11.18	29.52	156.14	745.30
<u>2002</u>						
Control	38.37	47.61	1.75	2.22	80.59	139.44
Biofumigation	15.63	19.98	0.56	3.63	47.91	78.63
Biofumigation + 50%dazomet	11.58	22.61	0.93	1.55	72.93	198.51
Compost + biofumigation	112.06	147.29	6.38	6.93	336.92	300.12
Solarization	19.53	23.63	1.85	2.20	91.49	65.39
Compost + solarization	121.18	189.61	12.28	8.89	963.21	1405.53
Dazomet (Basamid)	1.13	10.42	0.45	1.90	37.50	228.11
Dazomet + compost	19.91	133.68	3.69	9.61	423.79	895.55
Compost (SMS+PWC)	175.26	238.63	28.11	31.47	782.65	1256.20
Compost + 50% dazomet	96.22	83.88	8.63	22.12	202.08	556.72

Part V

**Phospholipid Fatty Acid Analysis of the
Soil Microecology in Tomato Production Plots.**

Abstract

Over the past two decades, the science of phospholipid fatty acid (PLFA) analysis has developed into an effective, comprehensive, and quantitative assessment method for soil microbiota. It defines the viable biomass, community composition, and nutritional/physiological status of environmental microbial communities. The methods are based on the application of signature lipid biomarker (SLB) analysis. Using SLB enables the *in situ* quantification of the effect of synthetic chemical, biologically based, and integrated soil treatments on changes in total biomass and microbial diversity.

In 2001 and 2002, soil treatments investigated were: compost, biofumigation, dazomet fumigation, integrated dazomet plus compost, and untreated control. Total lipid extracts from the soil samples were fractionated into neutral, glycolipids, and polar lipids. Polar lipids were transesterified into fatty acid methyl esters (FAMES) and analyzed by capillary gas chromatography. When polar lipid fractions were examined for patterns of fatty acids, it proved possible to detect differences in soil microbiota as a result of various agricultural practices.

Total microbial biomass PLFA were higher in the compost treatments than the non-amended control in 2001 and 2002. The dazomet plus compost treatment had similar elevated biomass in 2001. Both polyunsaturated PLFA, an indicator of soil microeukaryotes, and 18:2 ω 6, a fungal specific lipid biomarker, were relatively lower in the compost and 'dazomet plus compost' treatments compared to control in 2001. Conversely, monounsaturated PLFA were lower relative to total biomass in the compost treatments in both years. Levels of monounsaturated PLFA are positively linked with populations of Gram-negative bacteria. The conclusion is that compost amendments

establish an elevated microbial biomass and favor a high bacteria:fungal ratio; specifically Gram-negative bacteria.

Based on hierarchical cluster analysis of all PLFA biomarkers, compost-based treatments share similar microecological profiles. A close link between improved tomato yields, high Gram-negative bacterial populations, and compost-based soil treatments was established with ordination modeling.

Introduction

The utilization of biologically based and integrated pre-plant soil treatments for commercial vegetable production results in alterations to the soil microecology. The re-establishment of a balanced soil microbial community may resist the endemic proliferation of pathogens, providing sustainable disease suppression. A better understanding of the biology of disease control in agricultural soils may be developed if the mode of action of disease suppression is described. That can be accomplished via analyzing the changes that occur in the soil microecology because of soil additions.

It has been well documented that soil management can significantly impact soil biological and biochemical properties. Doran (1980) observed that soils in no-till cropping systems have higher microbial populations than soils under tillage. He also observed a shift in microbial community structure. Alvarez (1998) observed that higher concentrations of soluble organic carbon compounds resulted in the enhancement of microbial properties. Products such as manures, composts, and cover crops are likely to serve this purpose.

Traditional methods of enumeration and isolation via culture plating have flaws and limitations. A problem with characterizing changes in soil microbial communities

has been the lack of sensitivity associated with many of the traditional methods. Standard plate count methods may recover only a few percent of the organisms present. Those isolated are the ones suited to growth on the medium of choice. Measurements of soil microbial biomass give an indication of the total existing microbial life in the soil, but give no indication of structure. Traditional methodologies lack the ability to quantitatively and comprehensively describe the diverse communities that may be found in agricultural soils. The application of molecular techniques to the study of microorganisms *in situ* overcomes a number of these deficiencies. This study utilizes one such method - the measurement of phospholipid fatty acids (PLFA) in the soil.

Phospholipid fatty acid (PLFA) analysis permits both more precise calculations of microbial biomass and identification of the microbial community members (White et al., 1995). By utilizing techniques that can quantify PLFAs and their related biomarkers, how a treatment alters microbial populations and the community structure can be described. Relating this information to yields and disease incidence will accrue valuable information regarding soil microbial community diversity and pathogen suppression.

The biomass of complex microbial consortia can be determined by measuring the concentrations of the components of cell membranes. The detailed analysis of universal components, such as the lipids found in all cell membranes, yields detailed patterns of components that have been established as biomarker “signatures” for specific groups of microorganisms. The patterns can be utilized as quantitative definitions of microbial community structure (Zelles, et al., 1992). Phospholipid fatty acids can be used to describe viable microbial communities in terms of total biomass (Balkwill et al., 1988) and community composition (Vestal and White, 1989). Since phospholipids are rapidly

degraded by endogenous and exogenous phospholipases upon cell death, they are reliable measures of viable cell biomass (White et al., 1979). The extraction and purification methods used are optimized for phospholipid molecules, so 'fossil' fatty acids, such as those attached to humic substances, are not detected. PLFA have been shown to be sufficiently distinct to allow for the identification of individual species of bacteria as well as provide insight into the composition of whole communities (Frostegard and Baath, 1996)

Differences in the end products of the fatty acid biosynthetic pathways provide insight into the microbial community. The branched-chain pathway used by Gram-positive bacteria results in the formation of terminally methyl-branched saturated fatty acids (iso15:0, anteiso15:0), whereas the anaerobic desaturase pathway used by Gram-negative bacteria results in the formation of monoenoic fatty acids (16:1 ω 7c, 18:1 ω 7c) (Guckert et al., 1985). Gram-positive bacteria tend to be slower growing, more resilient, and capable of degrading more complex compounds than Gram-negative organisms. Gram-negative bacteria generally are faster growing, able to utilize many carbon sources, and adapt quickly to a variety of environments. Bacteria characteristically contain odd-numbered chains, methyl-branched, and cyclopropane fatty acids. Fungi typically synthesize saturated, even-numbered chains and polyenoic fatty acids (Tunlid and White, 1992). Federle (1986) found that in 47 species of fungi the fatty acid 18:2 ω 6 constituted, on average, 43% of the total PLFA. The use of 18:2 ω 6 as a fungal biomarker was confirmed by Frostegard and Baath (1996) who reported that the soil content of 18:2 ω 6 was correlated with ergosterol content; another molecular marker of fungal species.

Detection and identification can be made independent of cell culture and growth. Automated systems utilizing successive enhanced extractions can be coupled to GC/MS or, providing even greater specificity/sensitivity, to HPLC/electrospray/MS/MS. With the electrospray ionization and in ideal conditions, sensitivity is near single microbe levels (Lytle et al., 2000). Shifts of signature component patterns can be followed as responses to environmental alterations, as might be expected from the various soil treatments that were implemented during this research project.

The objective of this study was to utilize the technique of PLFA analysis to assess the viable biomass and community composition of the soil microbial communities for selected pre-plant treatments implemented in plasticulture tomato production.

Materials and Methods

Soil Treatments and Sampling

As described in detail in chapters two and three, ten pre-plant soil treatments were implemented as part of the tomato plasticulture production study in 2001 and 2002. These treatments were: compost, biofumigation, dazomet, solarization, compost plus biofumigation, compost plus solarization, dazomet plus compost, compost plus 50% dazomet, biofumigation plus 50% dazomet, and controls. Yield and disease incidence were evaluated during the study. Microbial biomass and community composition data supplements these two major components of the study.

The treatments were incorporated into replicated 10 m plots. Soil samples were collected from the designated treatments. Cores were taken to a depth of 15 cm using a 2.5-cm soil auger, at random along the row within the tomato root zone i.e. within 15 cm of the center of the row. The soil was immediately placed into a cooler and frozen on dry

ice. Samples were returned to the lab and stored in an ultra-low freezer at -70C until analyzed.

Sample Preparation

While still frozen, the soil samples were lyophilized. Glassware for lipid analysis was cleansed of lipid contaminants by washing with non-phosphate detergent and rinsing with ten volumes of clean tap water, then ten volumes of deionized water, and heating in a muffle furnace at 450C for 4 hours. Solvents used were GC grade from Fisher Scientific, Fairlawn, NJ.

Approximately five grams of lyophilized soil was weighed and the exact quantity recorded. The initial extraction was completed using a single-phase chloroform-methanol-buffer system described by Bligh and Dyer (1954), as modified by White et al. (1979). Five ml of chloroform, 10 ml of methanol, and 5 ml of phosphate buffer (50 mM, pH = 7.4) were added to the soil, mixed, and allowed to equilibrate for 2.5 h. The single-phase extractant was separated from the solid material by centrifugation at 2000 rpm for 20 min and decanted. Five ml of chloroform was used to wash the pelleted solids, which were then re-centrifuged. The upper chloroform layer was decanted and added to the original extractant. An additional 5 ml of water was added to the extract to force the separation of the aqueous from the organic phases (ratio was 1:1:0.8 MeOH:CHCl₃:buffer, v:v:v). After separation for approximately 12 h, the organic phase was pipetted to a new test tube and the solvent removed with a stream of dry nitrogen at 37C.

The total lipid extract was fractionated into neutral lipids, glycolipids, and polar lipids by silicic acid column chromatography (Guckert et al., 1985). Pasteur pipettes (1-

cm-diameter) partially plugged with glass wool were prepared, and 0.5g silicic acid added as a slurry in 30 mM ammonium acetate in methanol. The columns were pre-eluted with 5 ml of acetone and 5 ml of chloroform, and the samples transferred to the column with three 100- μ l washes of chloroform. Neutral lipids were eluted with 5 ml chloroform, glycolipids with 5ml acetone, and polar lipids with 10 ml of methanol. The solvent was removed from the polar lipids under a stream of dry nitrogen at 37C. All results presented are for the polar lipid fraction.

The polar lipids were transesterified to the fatty acid methyl ester by a mild alkaline methanolysis (Guckert et al., 1985). The polar lipid extract was dissolved in 1 ml of chloroform/methanol 1:1(v/v), 1 ml of methanolic KOH was added, and the mixture heated at 60C for 30 min. Fatty acid methyl esters (FAMES) were recovered from the organic fraction of the sample after adding 2 ml of hexane and 2 ml of water to separate layers according to density.

FAME Analysis

A Hewlett-Packard 5890 series II gas chromatograph coupled to a flame ionized detector (FID) provided quantitative analysis of FAME components. Chromatographic separations were carried out on a HP-1 fused-silica capillary column (50 m by 0.2 mm, inner diameter) of 0.11 μ m film thickness. The injector and detector were maintained at 270C and 290C, respectively. The column was kept at an initial temperature of 60C for 2 min, and then programmed to ramp at 10C per min to 150C, then ramped to 312C at 3C per min. Splitless injections were performed with a Hewlett-Packard model 7673 autosampler. Hydrogen gas was the carrier.

Preliminary peak identification was by comparison of retention times with known standards. Detailed identification of peaks was by gas chromatography/mass spectroscopy of selected samples using a Hewlett-Packard 5890 series 2 gas chromatograph interfaced to a Hewlett-Packard 5971 mass-selective detector using the same column and temperature program as previously described. Mass spectra were determined by electron impact at 70 eV. Methyl nonadecanone (c21:0) was used as the internal standard, and the PLFA expressed as equivalent peak response to the internal standard. Peak areas were determined using a programmable laboratory data system, ChromPerfect v.3.5 (Chromatography Data Systems, Palo Alto).

Fatty acids are named according to the convention X:Y ω Z, where X represents the number of carbon atoms in the chain, Y represents the number of unsaturations, and Z the number of carbon atoms from the methyl end of the molecule to the first unsaturation encountered. The prefix “cy” represents the cyclopropyl isomer of a fatty acid, and “i” and “a” represent the *iso*-branched and *anteiso*-branched isomers, respectively. The number preceding the abbreviation “Me” indicates the position of a methyl group relative to the carbonyl carbon followed by the number of carbons in the fatty acid chain. Suffixes “c” and “t” represent *cis* and *trans* geometric isomers of the unsaturation, respectively. An ambiguity in this naming convention is that the number of carbons given for *iso*-, and *anteiso*-, unknown branched, and cyclopropyl fatty acids include all of the carbons in the molecule except the esterified methyl group. Therefore, 10Me18:0 indicates a methyl group attached to an 18-carbon side chain at the 10 position from the carbonyl (Kates, 1986).

FAMES are expressed in pmol/g or as mole percentages. If mole percent values were used for statistical analysis, arcsine transformation was applied to the data to remove the percentage bias. STATISTICA software (StatSoft, Tulsa, OK) was utilized for cluster analysis. Cluster analysis was performed using complete linkage rules and Euclidean distances. Linkage distances can measure similarity between samples. Models are generated utilizing unconstrained ordination (Redundancy analysis, RDA). The RDA was performed on arc-sin transformed relative proportion (mol %) data using the statistical package "Canoco 4" (Microcomputer power, Ithaca, NY).

Results and Discussion

Microbial Biomass

Total PLFA extracted from the soil samples obtained from the treatment plots are an indication of total viable biomass. The viable biomass measurements for 2001 from the four major treatments investigated during the study i.e. biofumigation, composted amendments, synthetic fumigation (dazomet), and the integrated dazomet/compost treatment are displayed in Figure 14 (All tables and figures are in appendix). The two compost-based treatments had the highest quantities of total PLFA. The compost alone treatment had a mean of 1.19×10^5 picamol PLFA per gram of dry soil (pmol/g). The compost/dazomet integrated treatment had a slightly lower mean PLFA content of 1.14×10^5 pmol/g. The data from these organically amended plots contrasts with the PLFA content mean from the control plots of 0.77×10^5 pmol/g. The other non-organically amended treatment, dazomet soil fumigation, had a similar mean to the control plots, 0.79×10^5 pmol/g. Brassica biofumigation also involves the incorporation of organic material into the soil of the production beds. The mean biomass reading for the

biofumigation plots, 0.90×10^5 pmol/g, was between the controls and the compost plots. The biofumigation plots have a higher biomass than the control and dazomet plots, but not as high as the heavily organically amended compost plots.

This data set supports the concept that soil microbial biomass correlates closely with the quantity of available organic matter present in the soil. The organic material acts as both a nutrient source for the microbial population present, adding essential carbon and nitrogen, and as a substrate for growth. The presence of a high microbial biomass in compost-amended soils is linked to the inoculant potential of the compost. Compost additions introduce numerous and varied species of microorganisms to the soil as well as providing a favorable environment for their growth and adding nutrients to the substrate (Hoitink and Fahy, 1986). Brassica amendments provide organic matter to the soil (although not in the same humic state as composts) and, so, are likely to improve physiochemical properties and provide an improved substrate for microbial growth. The rapid introduction of large numbers of microorganisms is less likely to occur with Brassica incorporations than with compost additions to the soil. The PLFA microbial biomass data tends to support these conclusions.

In general, the total PLFA biomass data for 2002 corresponds with the 2001 data (Figure 15). The compost treatment again had the highest reading. The primary differences were that the samples from the control plots had higher biomass readings than the biofumigation and compost/dazomet treatments. The latter had the lowest biomass reading. The data as displayed is a summation of all PLFAs extracted. Total biomass, however, does not reveal any changes that may be occurring in relation to relative populations of major groups of microorganisms, such as fungi versus bacteria, or specific

genera and species. The disease data for 2002 indicated an increase in Southern Blight incidence throughout the field. The control plots had the highest incidence of disease and significant populations of *Sclerotium rolfii*. The elevated biomass reading from the compost treatment could indicate a relative proliferation of one species e.g. the pathogen, compared with 2001. The information gained from PLFA analysis is not as accurate, at present, at a species level. However, further division of the microbial phylogeny is possible with the analysis of specific signature lipid biomarkers.

Community Structure

Measuring the viable biomass of a community gives an indication of that community's capacity for transformations. PLFA are discriminatory enough to allow various subsets of the microbial community to be identified (White et al., 1979). They can give evidence of the phenotypic properties of the community *in situ*. Polyunsaturated PLFA have been linked with the presence of soil micro-eukaryotes (White and Ringelberg, 1995). The data shown in Figure 15 is expressed in transformed mole percentages. It permits the comparison of the relative proportions of PLFA subsets to total PLFA for that treatment.

In 2001, the compost treatment (0.67%) and the compost/dazomet treatment (0.65%) both had lower means for relative polyunsaturates than the control (0.72%) (Figure 16). Fungi are predominant soil microeukaryotes. The data indicates that the compost-amended plots have larger relative proportions of prokaryotes to eukaryotes. This signifies that bacterial populations are more dominant, in terms of biomass, than eukaryotes such as fungi, although expanded analysis of the signature lipid biomarkers directly associated with fungi would be required to confirm this shift. The dazomet

treatment (0.67%) had a similar relative proportion of eukaryotes to prokaryotes as the composts, however, the information already examined pertaining to total biomass would still suggest that the absolute quantity of eukaryotes in the compost amended plots is higher than the dazomet plots.

The signature lipid biomarker 18:2 ω 6 is associated with fungi (Fedrle, 1986). The two compost treatments had the lowest relative proportions of this PLFA subset (Figure 17). The compost-amended soils had a mean of 0.45 transformed mol% compared to 0.52% for the controls. This substantiated the observation of higher bacteria to fungi ratios in the composted soils.

The relative biomass of all monounsaturated signature lipid biomarkers was highest in the compost treatment (Figure 18). The transformed mole percent was 1.11% in the compost treatment, 1.10% in the compost/dazomet, and 1.10% in the biofumigation treatment. The non-organically amended plots had the lowest proportions of monounsaturates i.e. control (1.08%) and dazomet (1.08%). In 2002, the relationship of control to compost plots pertaining to monounsaturated PLFA was similar to that observed in 2001, i.e. 1.32% in the compost samples compared to 1.26% in the control (Figure 19). The dazomet/compost treatment had the highest mol percent of 1.36%. Monoenoic fatty acids are frequently found in Gram-negative bacteria (Guckert et al., 1985). The data, therefore, indicates that Gram-negative bacteria are relatively more abundant than other classes of microorganisms in compost amended soils than non-treated soil. There was a distinct increase in mol% of monounsaturates in all treatments from 2001 to 2002. The increase in relative abundance in the compost, as well as the dazomet/compost treatment, was higher than the increase recorded in controls.

Cluster Analysis

The hierarchical tree diagram depicts a cluster analysis for thirty samples from four treatments and controls in 2001 (Figure 20). Using linkage rules and Euclidean distances, any relationship between individual samples, based on all of the PLFA biomarkers analyzed, can be identified. The controls seem to be dispersed non-uniformly among the samples. Five of the six compost samples were grouped together on the tree, indicating that the total set of PLFA for these samples are more similar to each other than to any other group of samples. Adjacent to the compost treatments, the dazomet/compost samples are also clustered. Using PLFAs as an accepted indicator of microbial community structure, this summation of biomarkers identifies closely related soil microecologies. The hierarchical analysis indicates that a relationship between treatment type and soil microecology exists.

Ordinations

Identifying relationships between the soil microecology and the major agricultural factors of yield and disease requires statistical modeling that includes all available parameters. Ordinations that included PLFA biomarkers grouped into signature subsets, full yield and disease data from each year (2000 and 2001, respectively), and pre-plant treatment type are shown in Figures 21 and 22. The 2001 model was significant at $P = 0.026$ and the 2002 model at $P = 0.10$. In the 2001 model, the arrow representing yield points towards the “compost” centroid. This indicates a relationship between this treatment type and increased yields. If the arrow is continued, a line drawn at right angles towards the “compost” centroid would be shorter than that required to link the centroid for any other treatment with the yield line (forming a right angle). Hence, the

strongest correlation exists between compost treatments and yield. The line for disease indicates a similar trend by pointing toward the control centroid. When considering the “disease” line, the biofumigation point would be the next closest. This correlates with the disease incidence data that indicates that biofumigation was more disease suppressive than the controls but less than the other three treatments listed.

The model indicates a very strong correlation between yield and the PLFA biomarkers that indicate the presence of monounsaturated and branched monounsaturated membrane lipid- containing organisms. As discussed previously, monounsaturated PLFAs correlate very well with Gram-negative bacteria. Branched monounsaturates have been linked with actinomycetes species (White et al., 1997). Their abundance in this soil type may indicate general soil health. The line indicating polyunsaturates correlates with disease incidence in this model. Since it is recognized that this biomarker is an indicator for microeukaryotes, including fungi, we hypothesize that there was a greater abundance of the pathogen, *S. rolfisii*, in the soils of plots containing higher levels of this biomarker, hence, the correlation with disease. When linked with established knowledge of PLFA biomarkers, the model indicates that higher yields and decreased disease are related to a high ratio of bacteria to fungi in the soil.

The 2002 model suggests some of the same tendencies as the 2001 model. In particular, the very close relationship between high yields and low disease incidence with Gram-negative bacteria-dominated soil microbial communities. The PLFA data examined previously (Figure 18), indicated that the populations of Gram-negative bacteria increased more in the two compost treatments from 2001 to 2002 than the

controls. The dazomet/compost treatment had the largest increase, 0.26 %. This treatment also had the highest yields in 2002 (Table 6).

Conclusion

PLFA analysis enabled the quantification of microecological differences in the soil of various pre-plant treatments. Microbial biomass was elevated in compost-amended soils. When examined relative to total biomass for each treatment, differences were also measurable in fungal and bacterial populations. The addition of compost to the soil resulted in high Gram-negative bacteria populations. In these treatments, high bacteria:fungi ratios were evident. The examination of all PLFA biomarkers using hierarchical clustering indicated relationships in the microbial community composition of compost-amended treatments. Ordination modeling established relationships between high populations of Gram-negative bacteria, improved tomato yields, and compost-based soil treatments.

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Appendix

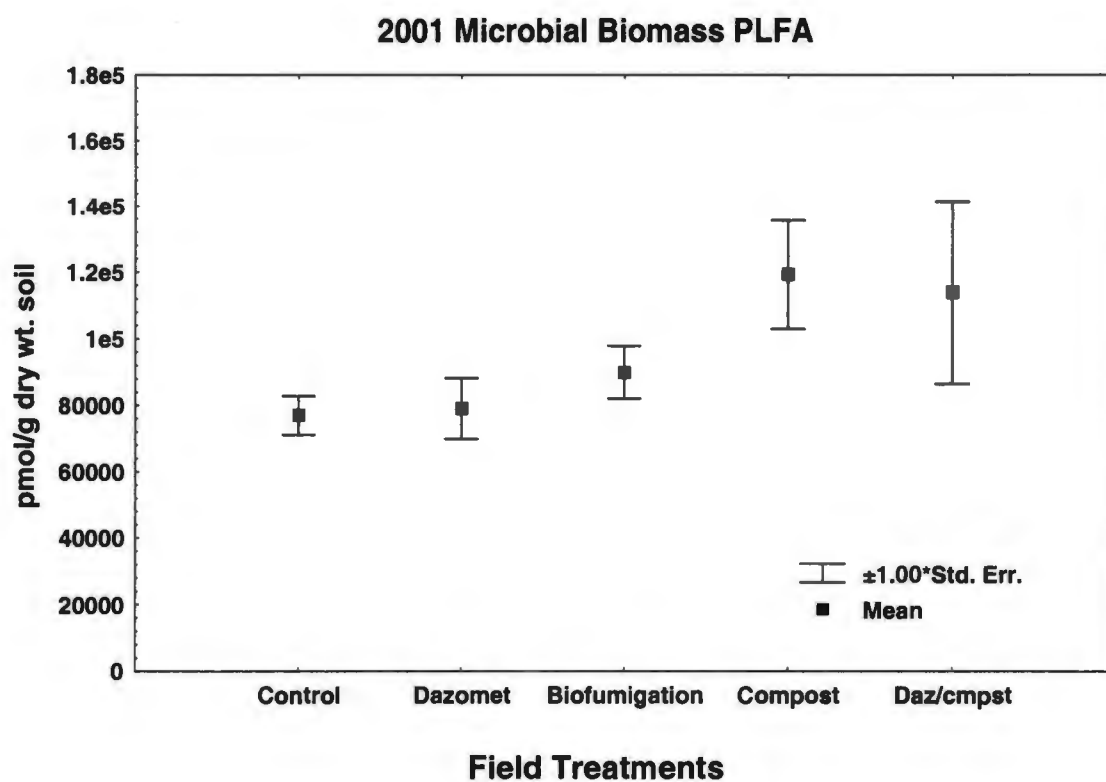


Figure 14. Microbial biomass of total PLFA for four treatments and control in tomato plasticulture production experiment in 2001.

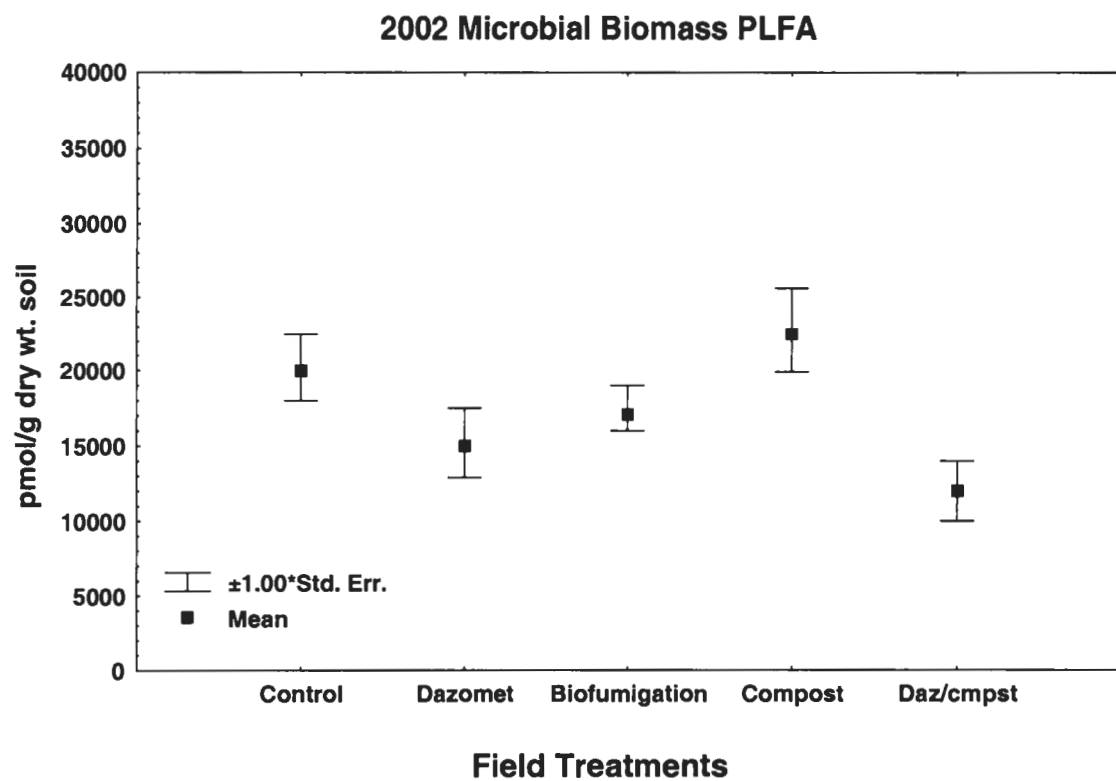


Figure 15. Microbial biomass of total PLFA for four treatments and control in tomato plasticulture production experiment in 2002.

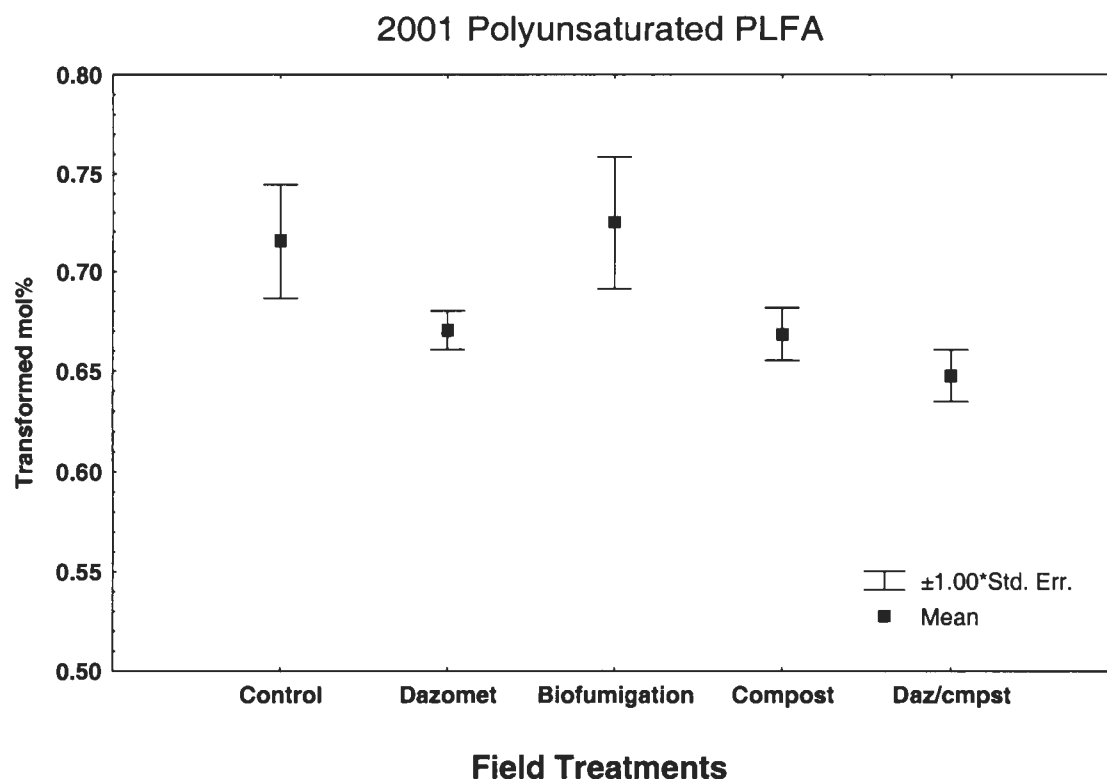


Figure 16. Polyunsaturated PLFA, an indicator of soil microeukaryotes for four treatments and control in tomato plasticulture production experiment in 2001.

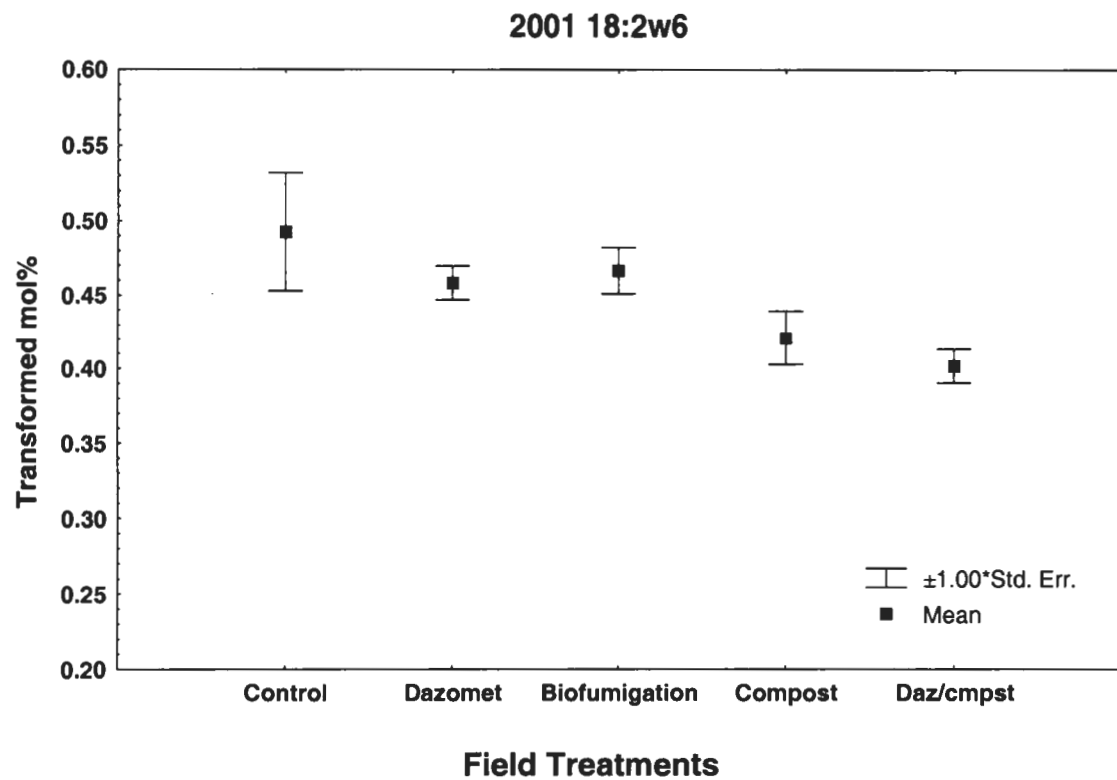


Figure 17. 18:2w6 PLFA, an indicator of soil fungi, for four treatments and control in tomato plasticulture production experiment in 2001.

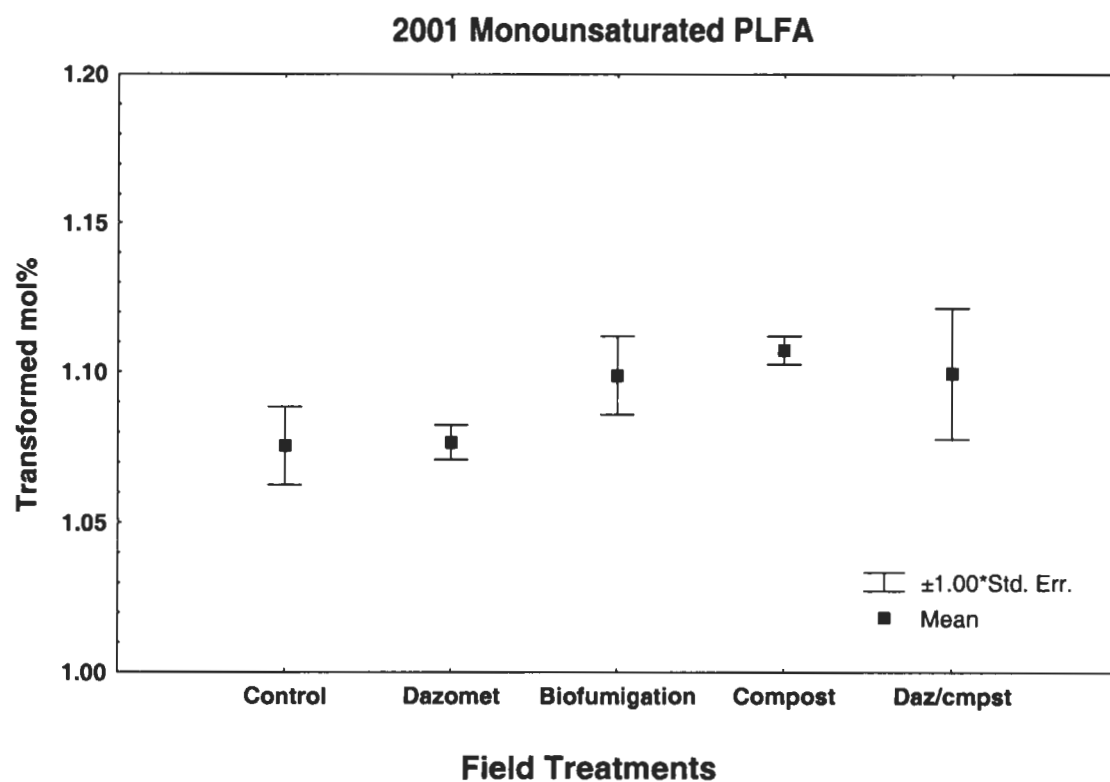


Figure 18. Monounsaturated PLFA, an indicator of Gram-negative bacteria, for four treatments and control in tomato plasticulture production experiment in 2001.

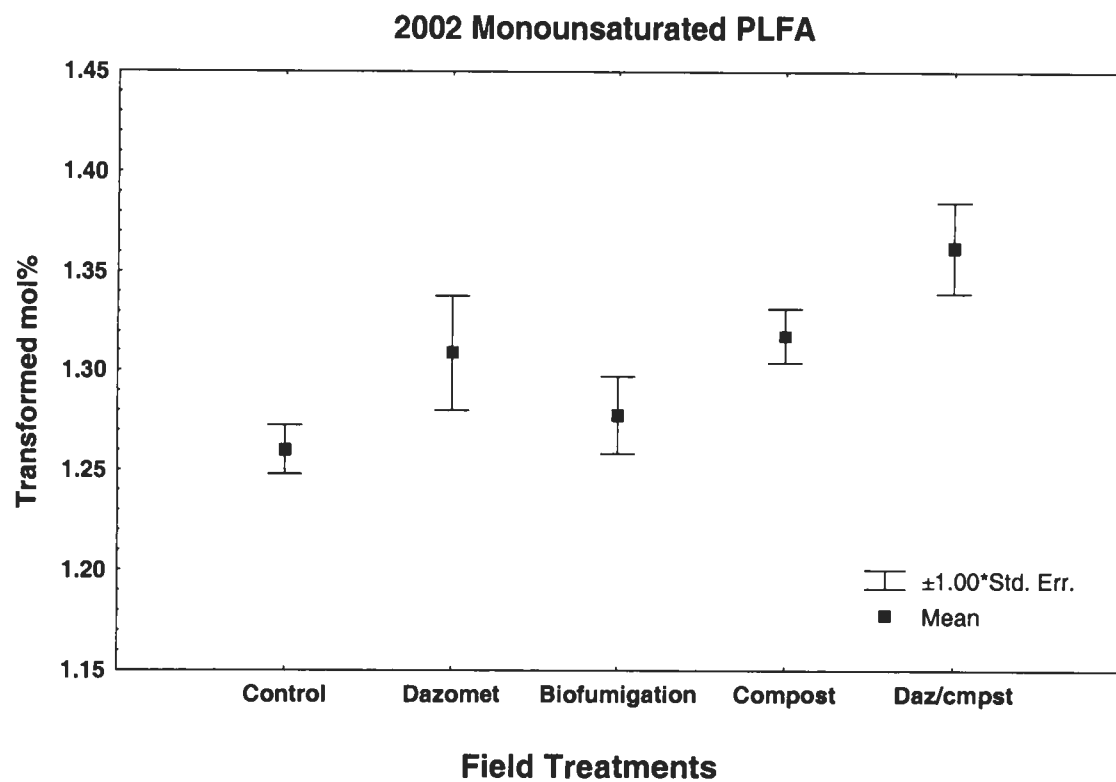


Figure 19. Monounsaturated PLFA, an indicator of Gram-negative bacteria, for four treatments and control in tomato plasticulture production experiment in 2002.

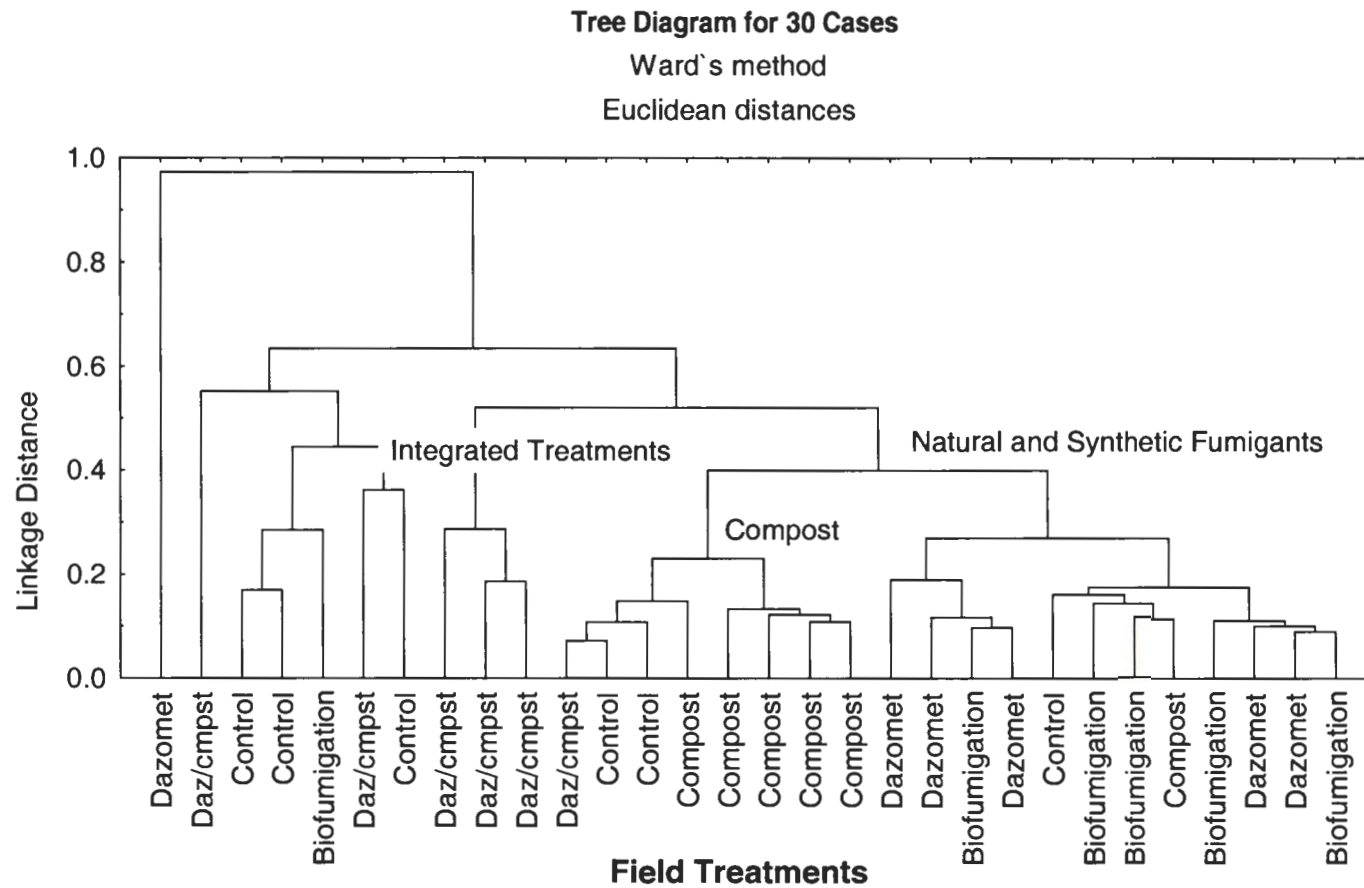


Figure 20. Hierarchical cluster tree diagram of thirty samples for four treatments and control in tomato plasticulture production experiment in 2001.

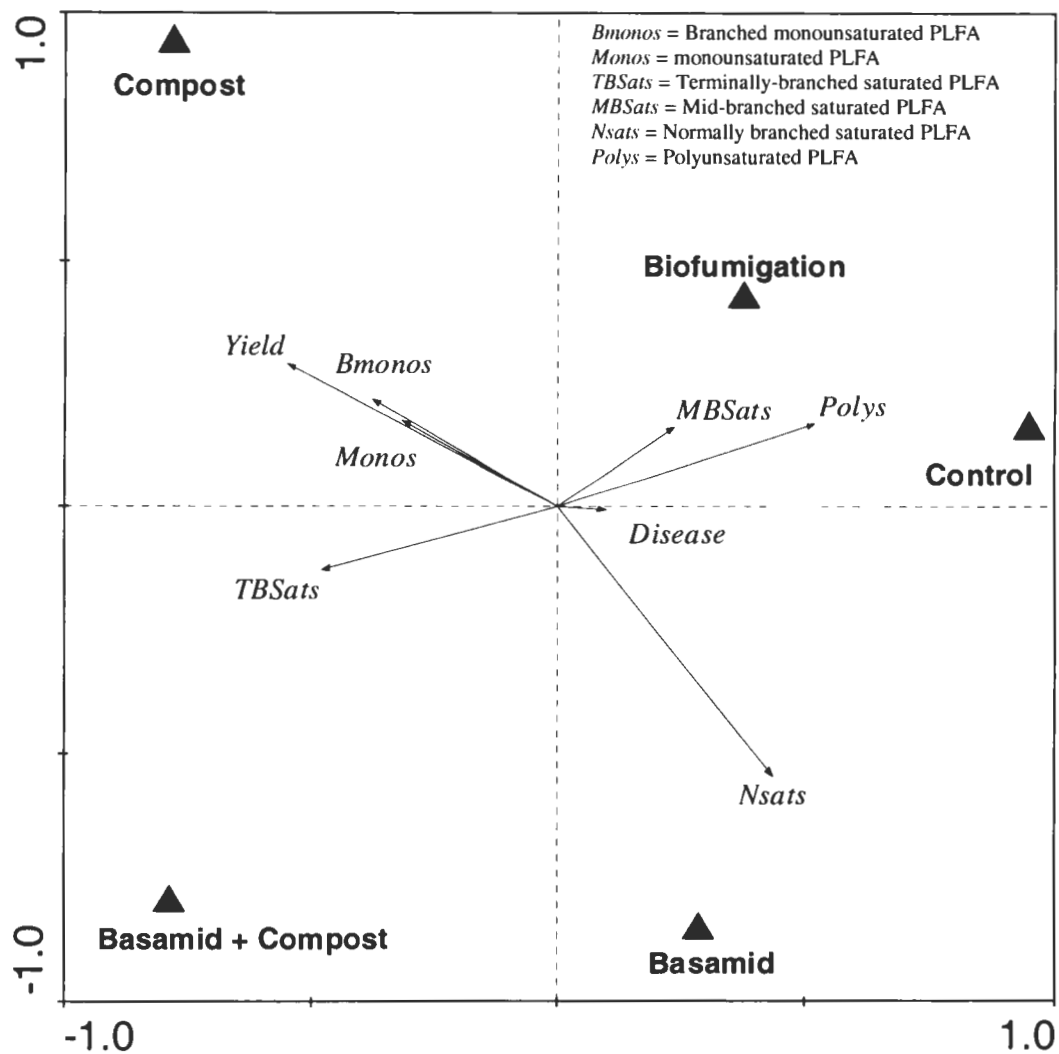


Figure 21. Ordination model of relationships between treatment type, yields, disease incidence, and PLFA biomarker sets for four treatments and control in tomato plasticulture production experiment in 2001.

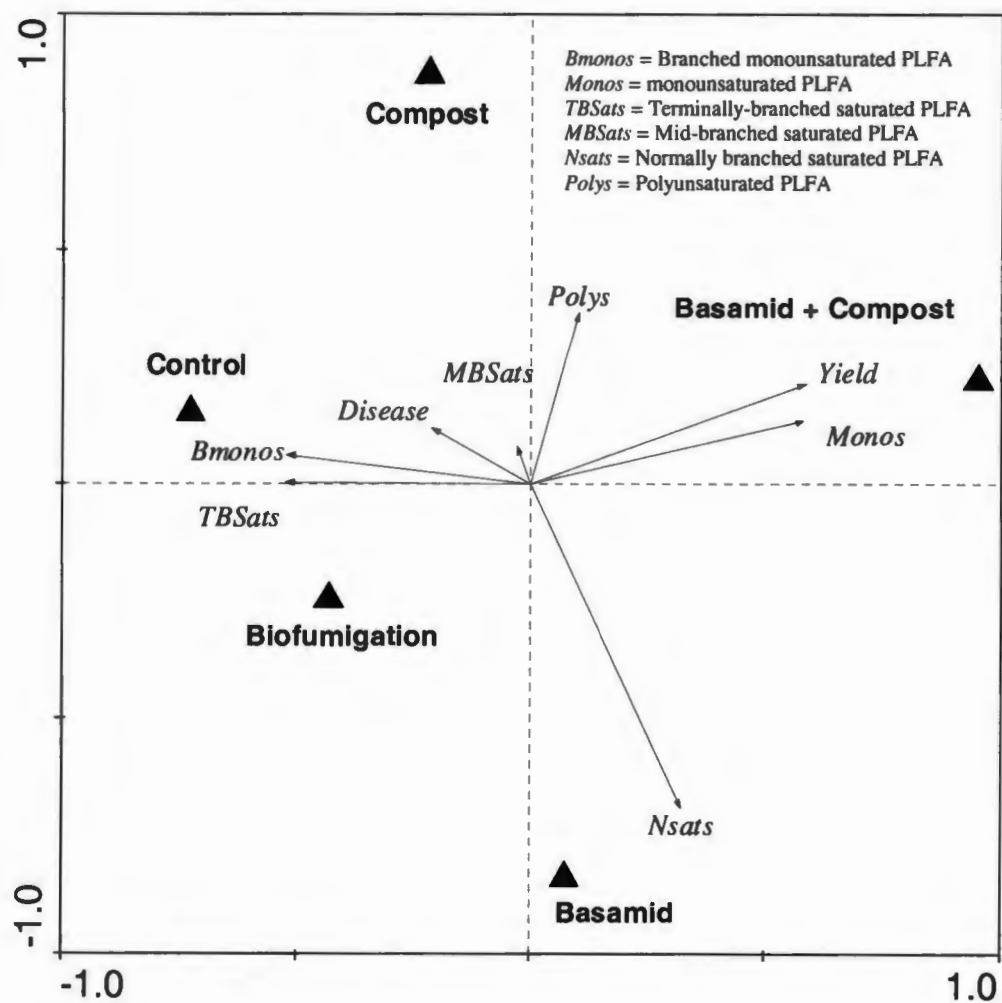


Figure 22. Ordination model of relationships between treatment type, yields, disease incidence, and PLFA biomarker sets for four treatments and control in tomato plasticulture production experiment in 2002.

Vita

Martin Terence Lyons born in Melbourne, Australia in 1962. He attended Huntingdale High School until graduation in 1980. Mr. Lyons attended Edinboro University of Pennsylvania where he obtained a Bachelor of Science in Biology in 1994. Awarded a scientific internship with the Department of Conservation and Natural Resources, Mr. Lyons worked on a M. S. degree in Biological Sciences with Dr. Barry Hunter at California University of Pennsylvania. He completed that work in 1998. In the Department of Plant and Soil Sciences at The University of Tennessee, Mr. Lyons worked, firstly, as a Graduate Teaching Assistant and then as a Graduate Research Assistant for Dr. Carl Sams. Immediately following the completion of his Doctor of Philosophy in Plant and Soil Sciences in August of 2003, he accepted a post-doctoral fellowship within the Department of Plant Sciences at The University of Tennessee.

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