EVALUATION OF SOIL SOLARIZATION TO CONTROL SOILBORNE FUNGI AND DISTRIBUTION STUDIES OF SELECTED SOILBORNE FUNGI IN OKLAHOMA

By

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CHAPTER I

INTRODUCTION

Agricultural crop systems can sustain heavy losses annually due to diseases caused by soilborne pathogens. High population levels of these pathogens may eventually occur when the same crop is repeatedly planted in the same location, resulting in severe disease and often economic losses. Effective control measures however, can decrease pathogen populations and increase crop yield and quality.

A variety of control measures exist, however none can be used in all situations. Fumigation and heat treatment of soil using steam are expensive and thus restricted to high value crops. Also, phytotoxic substances may be released into the soil due to the effects of these soil treatments.

Pesticides vary significantly in their effectiveness depending on soil types, microflora present and environmental conditions. In addition, use of pesticides may be detrimental to the beneficial soil microflora.

Soil solarization or solar heating of soil is a method of soilborne pathogen control that involves spreading of thin, transparent polyethylene mulch over the surface of the soil. This method utilizes solar energy to increase soil temperatures near the soil surface, usually for a period of four weeks or longer. Many soilborne pathogens are sensitive to temperatures above the normal range in the soil and their populations can be significantly reduced upon repeated exposure to

the daily elevated temperatures that occur during soil solarization. Since 1976 when the technique of soil solarization was first employed, excellent control has been obtained in numerous experiments in tropical and subtropical regions of the world. Unfortunately, little success has been achieved in the control of thermophilic organisms using soil solarization. In addition, only limited control may be obtained in areas or during seasons lacking in high temperatures and/or prolonged daily intervals of direct sunlight.

The Oklahoma State Department of Agriculture Central and Western Forest Regeneration Center (OSDACWFRC) in Washington, Oklahoma relies primarily on fumigation with methyl bromide every third year to control populations of soilborne pathogens that cause root and crown rot diseases of various conifer and deciduous tree seedlings. Recently, the control of soilborne pathogens by fumigation at the OSDACWFRC has been increasingly short-lived. Due to this problem and economic considerations, the OSDACWFRC is seeking alternative methods of soilborne pathogen control. Several soil solarization experiments and thermal death studies were conducted during this study to determine the feasibility of soil solarization as a method of soilborne pathogen control in Oklahoma.

Prior to this study, isolation of fungi from diseased conifer and deciduous tree seedling tissue indicated that Pythium spp. and Fusarium spp. were the primary soilborne fungal pathogens at the OSDACWFRC. In addition to these fungi, isolations conducted during this study also revealed that Macrophomina phaseolina (Tassi) Goid. was present and possibly a major soilborne pathogen at the OSDACWFRC. The pathogenic potential of isolates of Pythium irregulare Buisman and M. phaseolina obtained from diseased seedlings was investigated in this study.

The thermal death rate of an organism is the rate at which the viable population of an organism decreases over time upon exposure to temperatures above the maximum for growth. The thermal death rate is greatly influenced by the presence of moisture. Determination of the thermal death rate aids in the characterization of the population of an organism and provides an effective tool for predicting the effectiveness of soil solarization.

Populations of biological organisms are associated in a spatial arrangement or pattern. This spatial pattern may be affected by cultural, biological and environmental factors and may in turn affect spatial patterns of other biological populations. Analyses of spatial patterns provide a method to better characterize these populations and understand relationships between individual components within an agronomic system. In this study, spatial patterns of selected soilborne fungi were analyzed to correlate relationships between several biotic and abiotic components at OSDACWFRC. The accuracy of using radiospectrophotometry as a quick method of disease assessment at the nursery was also investigated.

CHAPTER II

LITERATURE REVIEW

Solarization

By the end of the nineteenth century, artificial heating of soil, usually by steam, was being used to control diseases caused by soilborne pathogens (Baker and Cook, 1974). Although some improvements have been introduced, such as use of aerated steam for better penetration into the soil profile, this method is still practiced only in small areas.

In 1939, Grooshevoy first employed solar energy in Russia to control soilborne pathogens (1939). He heated the top layer of soil in cold frames with direct sunlight raising the soil temperature to between 40 C and 60 C. This reduced the incidence of Black Rot of tobacco, caused by *Thielaviopsis basicola* to less than 4% compared to 65% in unheated controls. Grooshevoy further showed reductions in the population of *T. basicola* exposed to temperatures from 40 C to 60 C over several time intervals.

Though polyethylene was first introduced on a commercial scale in 1939 (Baker and Cook, 1974), it was not until 1976 that the combination of polyethylene and solar energy was considered for the control of plant disease (Katan et al.,1976). In solarization, an area targeted for soilborne pest control is mulched with thin polyethylene sheets for a period of several weeks or more primarily in fallow periods during times of maximum insolation and temperature. When transparent polyethylene is

used most of the visible and ultraviolet components of the solar energy striking the surface of the mulch passes through and is adsorbed into the soil surface where it is converted to infrared radiation which heats the soil surface and radiates back upward. However, polyethylene is not highly transparent to infrared radiation and neither are the droplets of moisture that evaporate from the heated soil and condense on the underside of the polyethylene. Therefore, much of the infrared radiation is contained under the mulch increasing the heating effect (Waggoner et al., 1960, Katan, 1981).

Katan et al. (1976) achieved temperatures as high as 52 C and eliminated the population of _Verticillium_ _dahliae_ to a depth of 25 cm in mulched plots. Verticillium wilt of tomato and eggplant was reduced 25 to 95% and weed populations were reduced. In addition, plant growth and yield increased in mulched plots.

Research involving solarization has received much attention in the past twelve years. Differences in the effectiveness of various types of polyethylene have been studied. Horowitz (1980) used solarization to control Egyptian broomrape (_Orobanche_ _aegyptiaca_), a plant parasitic weed and determined that transparent polyethylene mulch was superior to black polyethylene mulch in increasing soil temperatures and reducing weed populations. Rubin and Benjamin (1983) found perforated mulch to be less effective in controlling annual weed populations than nonperforated polyethylene of a similar type. Ultraviolet-stabilized polyethylene was found less efficient than transparent polyethylene in raising soil temperatures and decreasing populations of _Phytophthora cinnamomi_ and _Pythium_ spp. (Kassaby, 1985).

The thickness of polyethylene used for solarization is an important consideration. Thinner polyethylene (25 micron) permits more solar

energy to pass through and slightly increases the heating efficiency resulting in marginal increases in pest control compared to thick (100 micron) polyethylene (Pullman et al., 1981b; Horowitz et al., 1983). However, thicker polyethylene possesses greater resistance to breakdown caused by exposure to environmental factors such as temperature and sunlight (Waggoner et al., 1960). Therefore, thick polyethylene would be useful for longer periods and may be used several times.

Soil moisture increases the thermal sensitivity of microorganisms (Farrell and Rose, 1967) and improves heat conduction (Katan et al., 1976). LaMondia and Brodie (1984) obtained fewer surviving cysts of Globodera rostochiensis in irrigated mulched plots than in nonirrigated mulched plots. However, Ashworth and Gaona (1982) found no difference in the amount of infection of pistachio trees by Verticillium dahliae between irrigated and nonirrigated transparent polyethylene mulched plots. Both provided good control compared to nonmulched plots. Drip irrigation under the mulch during the solarization period provides slightly enhanced control of pathogenic fungi and weeds under optimal conditions compared to a thorough wetting prior to mulching (Grinstein et al., 1979; Jacobsohn et al., 1980; Katan et al., 1980; Horowitz et al., 1983; Katan et al., 1983; Stapleton and DeVay, 1984).

The effect of solarization is greatest near the soil surface and diminishes with increasing depth. The population of Fusarium oxysporum f. sp. lycopersici was reduced 94 to 100% at a depth of 5 cm, 68 to 100% at 15 cm and 54 to 63% at 25 cm in solarized plots compared to control plots (Katan et al., 1976). Porter and Merriman (1983) found that the population of V. dahliae was reduced 100% at 0 to 11 cm depth, but only 64% at 14 to 26 cm depth in mulched plots compared to nonmulched controls. Though solarization is less effective as depth increases, it

is important to note that pathogen populations are generally greater near the soil surface where effective control is most important. Often, a plant is most vulnerable to pathogenic infection in the seedling stage. If the plant escapes infection beyond the seedling stage, it will usually develop faster and produce greater yield. That is why most methods of soilborne disease control are targeted at the seedling stage (Baker and Cook, 1974).

Sufficient exposure time is critical for effective control of soilborne pests. Horowitz et al. (1983) found that the total population of field bindweed germinating within two months after solarization in plots mulched two, four and six weeks was reduced 35%, 94% and 100%, respectively, compared to the nonmulched control. Working with Verticillium wilt of safflower, Pullman et al. (1981b) found disease incidence in plots mulched for two weeks was reduced by 39% of the nonmulched control and no disease occurred in plots mulched for four weeks. As the length of the solarization period was extended, the depth at which some control was achieved increased and the extent of control at any specific depth was enhanced (Katan, 1981). Two weeks of solarization reduced populations of _Pythium_ spp. by 97%, 83% and 63% at 0 to 15 cm, 15 to 30 cm and 46 cm depths, respectively. The reductions were 99%, 99% and 96% at 0 to 15 cm, 15 to 30 cm and 46 cm depth, respectively after a solarization period of four weeks (Pullman et al., 1981b).

An increased growth response due to solarization beyond what could be explained by only pathogen control, has also been observed (Katan, 1981). Tomato seedlings in water extracted from solar heated soil grew 82% taller than those grown in tap water, while those grown in extracts from unheated soil grew only 36% taller than those grown in tap water

(Chen and Katan, 1980). Several factors have been implicated to account for this increased growth response. Nitrate concentrations in field soils solarized for six to seven weeks were as much as eight times greater than nonmulched soils (Chen and Katan, 1980). Even in soil mulched with black polyethylene for three weeks, Black and Greb (1962) found more than 80% higher accumulation of nitrate than in nonmulched soils. Increased concentrations of available calcium, magnesium, sodium, potassium, ammonium, phosphorus and organic matter, all of which would contribute to plant growth, have been found in solarized soils (Stapleton et al, 1983; Chen and Katan, 1980). Stapleton and DeVay (1982a) found that population densities of plant growth promoting rhizobacteria on sugarbeet roots in solarized soil were 469% higher than on roots in nonmulched soil.

Though several similarities existed between soil solarization and soil fumigation or steam sterilization of soil such as reduced disease, increased growth and greater yields (Katan, 1981), the differences were quite important. Phytotoxic substances may be released into the soil by fumigation or steam sterilization, but this was not found with solarization (Katan et al., 1976). With fumigation or steam sterilization, pests were destroyed as well as nearly all antagonists and saprophytic competitors of the pests. This created an area in the soil virtually devoid of life and available for rapid recolonization, which was termed a biological vacuum (Harper, 1974). Pathogens invaded these areas readily and pathogen infestations more severe than prior to treatment have resulted (Bollen, 1974; Olsen and Baker, 1968).

Thermophiles are organisms that grow above the temperatures considered to be the maximum limits for growth of most forms of life. (Crisan, 1973). Thermophilic organisms such as most bacteria,

actinomycetes and certain fungi were not harmed by the temperatures normally achieved by solarization (Baker and Cook, 1974; Katan, 1981). In addition, the flush of nutrients that occurs with solarization (Stapleton et al, 1983; Chen and Katan, 1980) may have enabled the populations of these thermophilic saprophytes and antagonists to increase dramatically during and immediately after solarization. These populations then inhibited the reentry of the nonthermophilic, less saprophytically competitive pathogens into the solarized area (Elad et al., 1980b; Stapleton and DeVay, 1982b). Heat sensitive pests not killed by solarization may be weakened by the heating effect and become more vulnerable to attack by biocontrol agents such as Trichoderma spp., Penicillium spp. and bacteria (Baker and Cook, 1974; Katan et al., 1976; Olsen and Baker, 1968; Elad et al., 1980a).

As a result of solarization and the enhanced action of biocontrol agents, long term control of pests may occur. Katan et al. (1983) found that in the third year after solarization incidence of Fusarium wilt of cotton was 60% to 90% less in previously solarized plots than in control plots and weed populations were still lower in solarized plots. Stapleton and DeVay (1983) found that the population of all free-living phytoparasitic nematodes was still 58 to 67% lower in solarized plots than control plots nine months after a six week period of solarization.

Solarization has been used for the control of disease and weeds in many studies. Populations of Sclerotium rolfsii Sacc. have been eliminated using solarization (Elad et al., 1980b; Grinstein et al., 1979; Mihail and Alcorn, 1984). Diseases caused by Verticillium dahliae have been drastically reduced by solarization (Katan et al., 1976; Elad et al., 1980b; Pullman et al., 1981a and 1981b; Ashworth and Gaona, 1982; Conway et al., 1983). Pythium spp. populations were reduced

(Pullman et al., 1981a; Stapleton and DeVay, 1984; and Kassaby, 1985). Reductions in weed and nematode populations were also documented (Grinstein et al., 1979; Horowitz, 1980; Jacobsohn et al., 1980; Horowitz et al., 1983; Rubin and Benjamin, 1983 and 1984; Stapleton and DeVay, 1983; Porter and Merriman, 1983 and 1985; LaMondia and Brodie, 1984; Standifer et al., 1984; Kassaby, 1985; Heald and Robinson, 1987). However, studies of solarization for the control of Macrophomina phaseolina have been unsuccessful (McCain et al., 1982; Mihail and Alcorn, 1984).

Thermal Death Studies

Thermal energy and water are two of the most important factors necessary for the survival and growth of any organism (Farrell and Rose, 1967). The population of each species is maintained only within a narrow range of heat and moisture. At any temperature above the maximum for growth the population begins to decline. Genetic variation, physiological condition and minute environmental factors contribute to differences in the sensitivity of individuals to heat above the maximum for growth.

The heat tolerance of microorganisms is greatly affected by the amount of water present and is generally lowest in the presence of abundant moisture. As the amount of available water decreases, tolerance to the effects of heat increases (Farrell and Rose, 1967; Precht et al., 1973).

Bigelow (1921) determined that at any continuous temperature above the maximum for growth of certain bacteria would reduce populations logarithmically as exposure time increased. In other words, a constant fraction of the remaining population would die per unit time. At higher

temperature the decline was more rapid, but the logarithmic nature of the rate of death was maintained. The thermal death time was defined as the time necessary to completely inactivate a population at a specific temperature. The change in density of the population at a specific temperature plotted over time produces the thermal death curve.

Smith (1923) showed that a fungal population exposed to some temperature above the maximum for growth declined in a manner similar to bacteria. She characterized the thermal death curve of Botrytis cinerea by determining the time required to reduce the viable population by 50% (i.e. LD_{50}) at different temperatures in sterile water. At 31 C, the LD_{50} was 202 hours; at 37 C, 4 hours; at 47 C, 2 minutes and at 50.3 C, 55 sec.

Other expressions have been used for the description of thermal death rates (Katan, 1981). The thermal death point was defined as the lowest temperature at which a population of microorganisms was killed completely in a specified length of time, usually ten minutes. The LD_{90} or decimal reduction time was the time necessary at a specified temperature to reduce the population by 90%. Unfortunately, no expression of thermal death rate was superior in all situations, so none has emerged as the standard, though work continues in this area.

The thermal death rates of several soilborne plant pathogens have been described using several methods under various environmental conditions. In sterile water, less than one minute was required to inactivate mycelium of Verticillium albo-atrum on two-day-old agar slants at 65 C and four minutes was required at 53 C (Miller and Stoddard, 1956). A minimum of five minutes was required to inactivate seven-day-old mycelium and conidia at 47 C (Nelson and Wilhelm, 1958). A minimum of ten minutes was required to nullify microsclerotia at 50 C

(Nelson and Wilhelm, 1958) and a minimum of four minutes was required at 55 C (Miller and Stoddard, 1956). Mycelium of Rhizoctonia solani on two-day-old agar slants was inactivated in one minute at 46 C in sterile water and fourteen-day-old mycelia and sclerotia on agar slants were inactivated within four minutes at 53 C in sterile water (Miller and Stoddard, 1956). In soil, with moisture at field capacity, the LD_{90} of R. solani was ten minutes at 50 C and fourteen days at 39.1 C (Nelson and Wilhelm, 1958).

The thermal death time of Fusarium spp. on two-day-old agar slants in sterile water at 65 C was seven minutes (Miller and Stoddard, 1956). Larvae of the nematode Globodera rostochiensis contained in cysts were killed immediately in sterile water at 55 C, but remained unharmed after 47 hours in soil at 0% moisture at 55 C (LaMondia and Brodie, 1982). The LD_{90} of Thievaliopsis basicola in soil at field moisture capacity was 67 minutes at 50 C and 33 days at 37 C (Pullman et al., 1981a). Verticillium dahliae had an LD_{90} of 9 minutes at 50 C and 14 days at 37 C (Pullman et al., 1981a). The LD_{90} of Pythium ultimum Trow. was 31 minutes at 50 C and 18 days at 37 C (Pullman et al., 1981a).

The LD_{50} of Sclerotium cepivorum in field soil at -0.15 bars moisture was 129.6 hours at 35 C and 0.8 hours at 50 C (Adams, 1987). The LD_{50} of mycelium of Phytophthora cinnamomi on agar in sterile water varied slightly among two isolates studied. The LD_{50}'s were 26 and 52 minutes at 39 C and 2.7 and 3.3 minutes at 46 C, respectively, for the two isolates (Benson, 1978).

Porter and Merriman (1983) treated several plant pathogens in soil with moisture at field capacity with daily six hour intervals of heating for two weeks. At 45 C, the population of Sclerotium rolfsii was unaffected, but was reduced 99.4% at 50 C. Reductions obtained were

75%, 96% and 100% in populations of *Pythium irregulare*, *Fusarium oxysporum* and *Verticillium dahliae*, respectively. The nematodes *Pratylenchus penetrans* and *Tylenculus semipenetrans* were completely eliminated from the soil at 50 C.

Usmani and Ghaffar (1986) conducted studies of continuous heating on *Sclerotium oryzae*. In soil at 96 to 100% soil moisture, less than one day was required for the complete killing of sclerotia at 45 C and above. At 40 C, *S. oryzae* survived seven days and no effect was apparent at 35 C or below. In dry soil (2 to 3% soil moisture), sclerotia survived three days at 60 C, but only minor declines in populations occurred at less than 55 C with ten days of heat treatment.

In an attempt to simulate the effects of solarization, Usmani and Ghaffar (1986) also studied reductions in populations of *S. oryzae* in soil treated with cycles of two hour periods of high temperature per day. Sclerotia in wet soil were completely eliminated in one cycle with periods at 55 C and 60 C, but survived three cycles at 50 C. Sclerotia survived twelve cycles at 60 C in dry soil, but with cycles of 55 C or below little effect was apparent even after fifteen days.

Distribution Studies

Population densities of biological organisms vary both spatially and temporally. Analysis of the spatial arrangement at a specific time helps to characterize the attributes of a population of organisms by summarizing data in such a way that features which are not readily apparent from direct observation can be appreciated (Hill, 1973). This information also provides a method of predicting probable cause-effect relationships between the spatial pattern of a specific organism and those of other organisms or environmental factors (Southwood, 1987).

The pattern of individuals over a defined area may be random, uniform (regular) or contagious (aggregated or clumped) (Cliff and Ord, 1973). Though many techniques have been developed to quantify spatial pattern, two distinct categories of analysis are available, depending on the type of data. One category, plotless sampling, involves measurement of distances between distinct individuals in a population (Pielou ,1977). Though this may be useful in working with natural plant communities, direct observation and counting of individual propagules of inoculum in soil is seldom practical (Menzies, 1963).

The second category of analysis techniques is based on quadrat sampling, which, in the case of soilborne pests, involves determination of mean inoculum density within a specified area by dilution plating of subsamples or other suitable techniques. This method is relatively simple and a more suitable alternative, though some prior knowledge of the distribution may be necessary to determine the appropriate quadrat size (Menzies, 1963).

There are four basic approaches to spatial pattern analysis using quadrat sampling. The first approach involves mapping of scaled inoculum densities within each quadrat. To illustrate the clumped pattern of _Verticillium_ _dahliae_ in a potato field, Smith and Rowe (1984) used mapping. Dillard and Grogan (1984) mapped the spatial pattern of lettuce plants infected with _Sclerotinia_ _minor_ to illustrate the temporal changes in the distribution of lettuce drop. Mapping simplifies visual assessment and interpretation of disease, but its use is limited, because there is no basis for statistical analysis (Campbell and Noe, 1985).

The fit of different probability distributions to frequency distributions of field data is the second approach to spatial pattern

analysis. The Poisson series, a form of the positive binomial, describes a random arrangement (Elliot, 1977). The value of the cumulative Poisson distribution (equal to one) is compared with the value of the cumulative frequency distribution and the chi-squared goodness-of-fit test is applied to the observed and expected (Poisson) frequency distributions. Frequency distributions with Poisson parameters significantly greater than one are considered clumped and a parameter significantly less than one indicates uniform distribution.

Campbell and Pennypacker (1980) used the Poisson series to determine the distribution of snapbean hypocotyl rot caused by *Rhizotonia solani* and randomness was indicated. The distribution of plants infected with cabbage black rot caused by *Xanthomonas campestris* was tested against the Poisson distribution by Strandberg (1973) and the pattern was found to be clumped. Tomasino (1986) compared the frequency distribution of sclerotia of *Sclerotium rolfsii* with Poisson's distribution and found a random pattern.

Data fitting the negative binomial distribution is considered nonrandom (Elliot, 1977). Martin et al. (1983) tested the fit of the horizontal distribution of propagules of *R. solani* in soil to the negative binomial distribution and clumping was found. The frequency distribution of propagules of *Cylindrocladium crotalariae* in a peanut field was not significantly different from the negative binomial distribution, which indicated nonrandomness (Hau et al., 1982).

Caution must be exercised in making assumptions from frequency distribution testing. Due to the power of the chi-squared test, the frequency distribution of data may fit both the negative binomial and Poisson distributions (Cliff and Ord, 1973; and Pielou, 1977). Also, these tests have a significant limitation. Though aggregation may be

indicated, there is no means of determining grain (clump size) or intensity (the extent to which density varies), so comparison with patterns of other components of the field is limited (Nicot et al., 1984).

The third approach to spatial pattern analysis is the use of indices of dispersion, which provide a measure of spatial aggregation that may be tested for statistical significance. The variance to mean ratio is the most simple index. In theory, a totally randomly distributed population has a variance equal to the mean and thus a variance to mean ratio equal to one. An aggregated (contagiously distributed) population has a variance greater than the mean with a variance to mean ratio significantly greater than one. Lastly, a uniformly distributed population has a variance less than the mean with a variance to mean ratio significantly less than one (Elliot, 1977). Using the variance to mean ratio, Sclerotium rolfsii (Punja et al., 1985) and Verticillium dahliae (Smith and Rowe, 1984) were found to be contagiously distributed.

Unfortunately, the variance to mean ratio is somewhat sensitive to the size of the mean population density (Grieg-Smith, 1983). A simple data transformation or change in the efficiency of propagule recovery may alter the ratio significantly and indicated a different pattern type (Cliff and Ord, 1973).

Morisita's index of dispersion (Morisita, 1959) is relatively insensitive to the size of the mean population density and thus, provides a more reliable method of spatial pattern analysis (Patil, 1974 and Grieg-Smith, 1983). In addition, Morisita's index of clump size, a simple manipulation of Morisita's index of dispersion, provides a method for describing the grain of the pattern of a population (Morisita,

1959). Schuh et al. (1986) found that the distribution of plants infected with sorghum downy mildew was clumped and determined the size of these clumps in the field.

Quadrat variance techniques, the fourth approach to spatial pattern analysis, provide descriptions of both grain and intensity, the two major components of dispersion (Pielou, 1977). Several methods of quadrat variance analysis are available and have been utilized extensively in ecology, but use of these methods has been limited in phytopathology (Campbell and Noe, 1985).

Grieg-Smith (1952) developed the Blocked Quadrat Variance Analysis method (BQV). A beginning quadrat is chosen from a straight transect of no particular length consisting of quadrats of a uniform specified size. The population variance among one-quadrat-sized blocks is calculated from the mean population density differences between contiguous quadrats one and two, then three and four, etc. Larger blocks consist of some integer power of two contiguous quadrats, up to one-half the maximum power of two contiguous quadrats in the transect. The variance of each larger block size, is calculated by first computing the differences between sum populations in consecutive pairs of contiguous blocks (i.e. block one and two, three and four, etc.). The differences are then divided by the number of quadrats per block, providing the values for the variance calculation at that block size. The grain of the distribution is indicated by the peak block size variance. The intensity of the distribution is indicated by the sharpness of the peak.

An important drawback with the BQV method is that the choice of the beginning quadrat may shift the calculations of the variances considerably (Ludwig, 1979). Usher (1969) corrected this problem when he developed the Stepped Blocked Quadrat Variance method (SBQV). For

the SBQV, the block size variance for each power of two quadrats is calculated in a similar manner as the BQV, but this procedure is repeated for each possible beginning quadrat, which is dependent upon the number of quadrats greater than one-half the maximum power of two quadrats within the transect studied and an average variance for each block size is determined. This method utilizes all available quadrats and results in an estimate of each block size variance that more accurately estimates the actual distribution. The main drawback of both the BQV and the SBQV is that these methods only determine the variances at block sizes equal to some integer power of two. If the actual variance peak lies between block sizes of two consecutive powers of two, it is possible that no peak will be indicated (Ludwig, 1979).

The two-term local quadrat variance method (TTLQV) developed by Hill (1973) calculates the variance of block sizes in a manner similar to the SBQV, but avoids the restriction of block sizes only being equal to powers of two by calculating variances for all block sizes up to the maximum possible at one-half the total number of quadrats. Ludwig (1979) compared the TTLQV with several other methods of quadrat variance analysis and found that the TTLQV most accurately detected both primary and secondary known scales of patterning. Grain and intensity are determined in the same manner as in the BQV and the SBQV. When two scales of pattern exist, TTLQV tends to emphasize the larger scale of pattern by producing a higher peak. Noe and Campbell (1985) used the TTLQV to determine the two dimensional horizontal spatial distribution of each of the plant parasitic nematode species *Meloidogyne incognita*, *Tylenchorhynchus claytoni*, *Helicotylenchus dihystera* and *Criconemella ornata*. They found that population clusters were ellipsoidal with their long axis orienting along plant rows.

CHAPTER III

MATERIALS AND METHODS

Solarization

Solarization Field and Greenhouse Studies

Field studies were conducted in Washington and Stillwater, Oklahoma. The studies at Washington were performed at two sites at the Oklahoma State Department of Agriculture Central and Western Forest Regeneration Center. The soil type was a silty loam (35% sand, 7% clay and 58% silt). Studies at Stillwater were at two sites at the Oklahoma State University Plant Pathology Farm (PPF). The soil type was a sandy loam (56% sand, 17% clay and 27% silt). Greenhouse studies were conducted at Stillwater, Oklahoma.

1986 Washington Field Study

Soil in the 1.2 by 2.0 m plots was disked and irrigated to field capacity prior to the study. Soil thermistors were placed at 4 and 24 cm depths in the soil. Temperatures were recorded at one hour intervals over the duration of the study.

Two treatments were compared using a randomized complete block design replicated three times. One treatment consisted of mulching the soil with 4 mil, transparent polyethylene sheets for the duration of the study, from April 29 to May 27. In the control treatment, no mulch was applied.

Soil samples were obtained just prior to mulching and at the conclusion of the study. Samples were extracted using a 2 cm diameter soil probe to a depth of 24 cm. Five samples from each plot were extracted in an X pattern, bulked in sealed polyethylene bags and stored at 4 C. Bulked soil samples from individual plots were assayed for populations of _Fusarium oxysporum_, _Pythium_ spp., _Trichoderma_ spp. and total fungi.

For the assay of populations of _Pythium_ spp., each soil sample was prepared following the general procedure of Conway (1985) using Pythium selective agar (PSA) (Conway, 1985) in 20 petri dishes. The average number of colonies of _Pythium_ spp. per gram soil for each sample was determined.

Populations of _Fusarium oxysporum_ were assayed by mixing each one gram soil sample in 9 ml distilled water. The resulting suspension was spread on Komada medium (1975) in each of twenty petri dishes. Petri dishes containing the soil suspension were incubated in the dark at 25 C for two days and average number of colonies of _F. oxysporum_ per soil sample were determined.

Populations of _Trichoderma_ spp. and total fungi were determined in the same manner as _Fusarium oxysporum_ populations with exception. Trichoderma selective media (TME) (Papavizas 1982) was used for the assay of _Trichoderma_ spp. at dilutions of 1:100. Populations of total fungi at dilutions of 1:10,000 were determined using oxgall agar, a semiselective medium that restricts radial growth of most fungi. It consisted of 2% agar in deionized H_2O, 15 g Difco Oxgall/L H_2O, 100 mg streptomycin sulfate/L H_2O and 200 mg sodium ampicillin/L H_2O.

1986-87 Stillwater Field Study

Sclerotia of *Sclerotium rolfsii* were obtained from the soil surface adjacent to carrots grown in artificially-infested field soil and placed in sacs of fifty sclerotia per sac for burial in the soil. Vispore[R] (porous polyethylene, Ethyl Corporation) was used to construct the individual sacs which were closed by a wirestring twist-tie. Pores in the Vispore were small enough to prevent loss of sclerotia.

Soil in 0.5 by 7.5 m plots was disked prior to the study and soil temperature probes were placed at 4 cm depths in the soil. Drip irrigation lines were placed in all plots. Treatments were compared using a split-split plot within a randomized complete block design replicated eight times. Main plots consisted of two time treatments. The first treatment consisted of leaving the sacs in the soil from 20 August to 20 September, 1986 for a four week solarization study. In the second treatment, sacs were placed in the soil 20 August, 1986 and removed 3 March, 1987, to determine the effect on sclerotia of mulching continued through the winter.

Sub plots consisted of four location treatments. For three treatments, four mil transparent polyethylene mulch was installed over drip irrigation lines. Two sacs containing sclerotia were placed under the mulch at the center, margin or intermediate (0.25 m from the center) in the mulched area. The fourth treatment consisted of placing two sacs containing sclerotia only at the center of nonmulched plots. Drip irrigation was applied in all plots as needed to maintain soil moisture at or near field capacity under the mulch from 20 August to 20 September, 1986.

Sub-sub plots consisted of four depth observations. Sacs

containing sclerotia were placed within the soil profile at either 0, 8, 16, or 24 cm depths.

At the conclusion of each time period for the main plots, one randomly selected sac was removed from the soil from each location in each plot. The number of sclerotia remaining in each individual sac was recorded. Sclerotia from individual sacs were placed in petri dishes on moistened filter paper and incubated at room temperature (approximately 25 C) on a lab bench for five days. Moisture content of the filter paper was maintained at the level at which little free water was present and desiccation of sclerotia would not occur. Sclerotia from the inoculum source were stored in a sealed polyethylene bag on a laboratory bench at room temperature.

Statistical analyses were performed on the number of sclerotia missing from sacs, the number of sclerotia germinated, the percent recovered sclerotia that germinated and the number of nongerminated sclerotia. Analyses were also performed on the percent incidence of epiphytic *Trichoderma* spp. on germinated sclerotia, and the percent incidence of epiphytic *Trichoderma* spp. on nongerminated sclerotia. These analyses were performed for each individual depth and on the total sclerotia (mean of those at all depths).

1987 Washington Field Study

Disked 1.2 by 2.4 m plots were irrigated to field capacity prior to the study. Five soil samples, taken in an X pattern to a depth of 15 cm, were removed from each plot using a 5 cm diameter soil probe, both prior to and at the conclusion of the study. Soil samples from the same plot and time of sampling were bulked, placed in sealed polyethylene bags and stored at 4 C. Bulked soil samples were then assayed for

populations of Fusarium oxysporum, Pythium spp., Macrophomina phaseolina and total fungi.

Treatments were compared using a split plot within a randomized complete block design with four replicates. Main plots consisted of two treatments. One treatment consisted of mulching plots with four mil, transparent polyethylene sheets from 7 July to 3 September. In the control, no mulch was applied.

The subplot treatments were the effects of solarization at three soil depths, 0-5 cm, 5-10 cm and 10-15 cm. Soil samples taken from plots at the conclusion of the study were separated by depth prior to bulking. Soil temperatures were not recorded during the solarization period, but air temperature data was obtained from the National Weather Service (NWS) at Will Rogers World Airport at Oklahoma City, Oklahoma.

Populations of F. oxysporum, Pythium spp. and total fungal populations were assayed according to the procedure in the 1986 Washington Field Study, except only ten petri dishes were used to compute average numbers of colonies. Populations of F. oxysporum Pythium spp. and total fungi were determined at dilutions of 1:1,000 1:20 and 1:10,000, respectively.

Populations of M. phaseolina at dilutions of 1:100 were assayed according to the procedure of Papavizas and Klag (1975) using Dexon Oxgall PCNB Captan agar (DOPC) (DOP agar (Mihail and Alcorn, 1982) modified by the addition of 50 mg Captan / L H_2O to reduce growth of colonies of Fusarium spp. found on DOP agar alone (Conway, unpublished)). The population of M. phaseolina per gram soil in each sample was determined from the average number of colonies of M. phaseolina on the surface of DOPC in ten petri dishes.

1987 Parsley Greenhouse Study

The effect of solarization on seed survival and seedling growth of parsley (Petroselinum crispum (Mill.) Nym.) was determined in soil samples collected from the top 15 cm of solarized and control plots one week after the conclusion (removal of the polyethylene mulch) of the 1987 Washington Field Study. Treatments consisted of 15.2 cm pots filled with subsamples of bulked, lightly-packed, solarized or control soil. Seed of parsley was germinated in a column of oxygenated tap water for five days prior to this study. Ten parsley seed were planted evenly distributed at approximately 1 cm depth in each pot. Ten pots of each soil treatment were placed in a completely randomized design on a greenhouse bench and were misted for 24 seconds every 12 minutes daily between 10 AM and 4 PM to provide moisture conditions suitable for seedling growth. Plants were counted twice weekly for seven weeks. Analyses were performed on the number of seedlings per pot, mean root weight per plant per pot, as well as fresh shoot weight and dry shoot weight of parsley plants. This study was not repeated.

1987 Weed Greenhouse Study

The effect of solarization on weed seed survival was determined in soil samples collected, placed in 20 pots per soil treatment and maintained as described in the 1987 Parsley Greenhouse Study without the addition of Parsley seed. Populations of all emergent weeds species were counted twice weekly over an eight week growth period. This study was not repeated.

1987 Nematode Greenhouse Study

Solarization effects on the size of nematode populations were compared by assaying populations of nematodes remaining in the soil in one-half of the pots used in the 1987 Weed Greenhouse Study at the conclusion of the study. Populations of microphages, predators, myceliophages, seinurids and phytoparasitic nematodes were assayed using the "Okie Tub Method" (Alby, 1975). Nematodes populations were observed and enumerated under a dissecting scope. This study was not repeated.

1987 Stillwater Field Study

Cultures of *Macrophomina phaseolina* were grown in two liter flasks on potato dextrose broth for two weeks. The cultures were then placed in a Waring commercial blender and blended for thirty seconds on low speed, poured onto a 325 mesh sieve and rinsed thoroughly with tapwater. The remaining sclerotia were spread on paper towels and dried overnight. Dried sclerotia were mixed with field soil to a concentration of approximately twenty-eight sclerotia per gram soil. Two gram samples of the sclerotia/soil mixture were placed in sacs as described in the 1986-87 Stillwater Field Study, except a tight weave polyester cloth was used in this case.

Vispore[R] sacs, constructed as in the 1986-87 Stillwater Field Study, contained thirty sclerotia of *S. rolfsii* obtained from the same source and stored in the same manner as above.

Drip irrigation was installed in disked 1.5 by 2.1 m plots prior to the study. Treatments for each fungal species were separately compared using split plot designs within randomized complete blocks. Main plots consisted of two solarization treatments. One treatment included

mulching plots with 4-mil transparent polyethylene sheets for the period from 23 July to 3 September. In the control, no mulch was applied. During the study, irrigation was uniformly applied as needed to maintain soil moisture at or near field capacity under the mulch.

Sub plots consisted of two depth treatments. Sacs of sclerotia of M. phaseolina and of S. rolfsii were placed in the soil at 5 and 15 cm depths at random locations within the plots prior to mulching.

Sacs were removed at the conclusion of the study. The number of germinable sclerotia of M. phaseolina per gram soil per sac was determined by distributing 0.25 grams of soil from sacs on DOPC agar contained in 10 petri dishes and incubated at 32 C for seven days in darkness. The average number of colonies of M. phaseolina per gram of soil was calculated.

The number of sclerotia of S. rolfsii missing from each sac was recorded and sclerotia from each sac were placed on moistened filter paper in petri dishes for seven days at 25 C. The various components of the germination of S. rolfsii were determined and analyzed as in the 1986-87 Stillwater study. In addition, the percent incidence of epiphytic fungi including Trichoderma spp., Penicillium spp., Rhizopus spp., Chaetomium spp., or Aspergillus spp. on germinated or nongerminated sclerotia were recorded.

Thermal Death Studies

Inoculum of field isolate ESp3 of P. irregulare was prepared by growing the isolate on V-8 broth contained in autoclaved biohazard bags. Cultures were removed from the sealed bags and rinsed thoroughly with tap water after two weeks growth in darkness at 25 C. The cultures were blended twice at low speed for 30 second intervals in a Waring

commercial blender to separate oospores and break hyphae into minute fragments. The slurry was mixed thoroughly into moistened autoclaved soil (81% sand, 2% clay and 17% silt). Infested soil was spread 2 cm deep in 23 by 33 cm pans and air dried by mixing periodically in a laminar flow hood for 2 days.

Germinable oospore populations were assayed weekly by spreading 0.1 grams inoculated soil on PSA agar. After six weeks, when the germinable population had stabilized in dry soil, additional soil was added adjusting the concentration to approximately 26 germinable oospores per 0.1 grams soil.

Sclerotia of _M_. _phaseolina_ were produced and mixed into pasteurized OSDACWFRC field soil as previously described to obtain a concentration of 28 sclerotia/ 0.1 g soil.

Soil infested with either _P_. _irregulare_ or _M_. _phaseolina_ was either moistened with distilled water to 15% soil moisture or left dry. Two gram infested soil samples were placed in 10 ml glass vials. Parafilm was stretched over the mouth of each vial before the lid was screwed on to prevent moisture exchange with the surrounding environment.

The vials were placed in incubators, heated to either 40, 45, 50 55 or 60 C or left at 25 C. Incubators either maintained constant temperature or were set on a daily cycle to increase temperature from approximately 25 C (room temperature) to the desired maximum temperature for two hours and then returned to room temperature.

At specified times five randomly-chosen vials were removed from incubators at each temperature to assay fungal populations. For constant temperature, vials were removed at 1.5, 3, 6 and 12 hours, as well as 1, 2, 4, 8, 16, and 32 days. From incubators set for two hour maximum temperature daily cycles, vials were removed at 1, 2, 4, 8, 16

and 32 days.

Fungal populations were determined by spreading 0.1 g soil from each vial on DOPC agar and PSA agar in petri dishes for M. phaseolina and P. irregulare, respectively. Colony counts on DOPC agar were determined after the petri dishes were placed at 30 C for seven days. After 2-3 days, colony counts from PSA agar in petri dishes placed at 25 C in darkness were determined. The portion of this study involving continuous exposure of fungi to 25, 50, 55 and 60 C was replicated twice and other portions of this study were conducted only once.

Pathogenicity Studies

Isolations

Roots of stunted or chlorotic seedlings of silver maple (Acer saccharinum L.), sycamore (Platanus occidentalis L.), scots pine (Pinus sylvestris L.)and virginia pine (Pinus virginiana Mill.) were obtained from the OSDACWFRC in Washington, OK. Two cm sections were vertically sectioned, soaked two minutes in 0.525% NaClO, rinsed in deionized water and placed on either potato-dextrose agar amended with 300 mg / liter of sodium ampicillin agar (PDAA) or Pythium Selective agar (PSA) in petri dishes. Cultures on PSA were rinsed after 1 day as previously described. All cultures were incubated two to three days in darkness at 25 C. Hyphal tips of fungal colonies were removed and grown on PDA to obtain clean cultures. Colonies of Macrophomina phaseolina were identified by comparison with the description of the species by Holliday and Punithalingham (1970).

Colonies of Fusarium spp. were transferred to the surface of Carnation-leaf agar (CLA) (Nelson et al., 1983). Species of Fusarium

were identified by comparison of morphology of macroconidia, microconidia and chlamydospores on CLA, as well as colony morphology on PDA with the descriptions of various species by Nelson et al. (1983).

Green wheat leaves, cut into 0.5 cm square sections were placed in deionized H_2O and boiled for approximately five minutes to sterilize the leaves. The mixture was placed in petri dishes and cooled. Oospores of Pythium spp. were produced by transferring hyphae of actively-growing cultures to the wheat leaf mixture and incubating in darkness at 25 C on a lab bench for 3 to 5 days. Identification of Pythium spp. was performed by comparison of oogonial and antheridial characteristics, in addition to oospore morphology and optimum growth temperature, with descriptions of the species by Plaats-Niterink (1981).

Preliminary Pathogenicity Greenhouse Study

Scots pine seedlings were grown individually in Ready Mix (a vermiculite/sphagnum moss commercial potting medium) in 10 cm plastic pots. One-half cm disks of three field isolates of Pythium irregulare (ESp3, ESp4 and ESp5) from scots pine grown on PDA were placed 2 cm below the seedling crowns within the potting media. Six pots for each tree species were placed randomly on a greenhouse bench and misted as above. Noninoculated seedlings in six other pots constituted the control. Trees were observed and disease symptoms recorded twice weekly for four weeks. Three isolations were made from roots of each seedling as described above. This study was not repeated.

Pathogenicity Greenhouse Study I

Seedlings of sycamore, scots pine, virginia pine and arborvitae (Thuga occidentalis L.) grown in Ready Mix in trays were transferred

individually into individual 4 by 4 by 8 cm deep plastic pots. One-half cm disks of field isolates of fungi grown on PDA were placed 2 cm below the potting media surface adjacent to roots of seedlings with one isolate per seedling. The isolates used were *Pythium irregulare* (ESp3) isolated from scots pine, *M. phaseolina* isolated during this study (SyV1 and SyV2 obtained from sycamore and VpV4 obtained from virginia pine) and isolates of *M. phaseolina* (designated 38, 40, 61, 62, 98, 155, 169 and 244) obtained prior to this study (Conway, unpublished). Isolate 38 was obtained from arborvitae. Isolate 40 was obtained from silver maple. Isolate 61 was from sycamore. Isolates 62, 155 and 169 were from apple (*Malus sylvestris*). Isolate 98 was from asparagus (*Asparagus officinalis*) and isolate 244 was from cantaloupe (*Cucumis melo* var *reticulatus*).

Three seedlings of each tree species were inoculated with *P. irregulare* and each isolate of *M. phaseolina*, except due to germination problems, sycamore seedlings were not inoculated with the isolates of *M. phaseolina* obtained prior to this study. Pots were arranged randomly on a greenhouse bench and misted as above.

Trees were observed and symptoms recorded twice weekly. After four weeks, isolations and identifications were made from roots of each seedling as described above. This study was repeated once.

Pathogenicity Greenhouse Study II

Virginia pine and sycamore seedlings grown six weeks in Ready Mix were transferred to six inch clay pots containing lightly packed, pasteurized soil obtained from OSDACWFRC. Three seedlings were placed in each pot.

Sorghum seed was autoclaved twice (1.55 kg/cm^2, 30 minutes) in 250 ml flasks was inoculated with an isolate of *M. phaseolina* (SyV2) from sycamore grown on PDA. After three weeks, the infested seed was removed from the flasks and dried in a laminar flow hood for two days.

The effect of different moisture conditions on the pathogenicity of *M. phaseolina* was studied. Four treatments were employed and were replicated eight times for each tree species. One treatment consisted of infesting all seedlings within a pot by placing infested sorghum seed at 3 cm depth within the soil adjacent to the plant roots. Seedlings were placed randomly on a greenhouse bench and misted as above. In the second treatment, the soil seedlings were infested in the same manner, but two weeks after infestation and again four weeks after infestation, pots were removed from misting for three days to create a moisture stress condition for the seedlings. In the third treatment, seedlings were not infested, but were moisture stressed as in the second treatment. Controls were neither infested nor removed from the mister during the study. Symptoms were recorded and isolations from roots of each tree were made as above, except DOPC was used.

Distribution Study

The field study was conducted at one location at OSDACWFRC in Washington, Oklahoma on an established 1.2 m wide bed of 1 year old Scot's pine seedlings. A transect of 128 contiguous 1.2 by 1.2 m quadrants was established on the bed.

Five soil samples, taken in an X pattern from between seedling rows, were obtained using 2 cm soil probes to a depth of 15 cm. Samples from each quadrant were bulked, sealed in polyethylene bags and stored at 4 C until analysis.

Observations of the approximate number of trees in each quadrat were recorded on a scale (0 - 10) with a value of 0 representing 0-10 trees and a value of 1 representing 11-20 trees, etc. The approximate percent coverage of each quadrat by woodchip mulch was also recorded with a value of 1 representing 0-33% coverage, a value of 2 representing 34-66% coverage and a value of 3 representing 67-100% coverage. Three recordings each of eight wavelengths of light (500 nm - 850 nm wavelengths) were taken of each quadrant. Readings were obtained using a hand held multispectral radiometer (Nutter et al., 1985). Readings of each of the eight wavelengths from individual quadrants were adjusted according to incoming light at the time of reading, averaged and recorded.

Percent soil moisture of each sample was determined by removing and weighing ten grams soil from the polyethylene bag and drying it in a microwave oven for six minutes. Samples were then reweighed and the percent soil moisture of the original sample calculated. Assays for mean populations of _M. phaseolina_ and _P. irregulare_ per quadrat were performed as previously described for the 1987 Solarization Field Study.

Field patterns of _M. phaseolina_, _P. irregulare_, numbers of seedlings, mulch cover, percent soil moisture and radiospectrophotometer readings were statistically correlated and analyzed by Hill's Two-Term Local Quadrat Variance method (TTLQV)(Hill 1973). TTLQV was employed to determine the grain and intensity of spatial patterns over the entire transect. Variances for each block size up to 64 (one-half of the entire transect), calculated by TTLQV for each parameter, were also statistically correlated.

CHAPTER IV

RESULTS

Solarization

1986 Washington Field Study

Prior to mulching, population densities of all fungi assayed were similar in solarized plots compared to control plots (Table I). Population densities of selected soilborne fungi recorded after solarization were not significantly lower (P = 0.05) in solarized plots compared to control plots.

The mean maximum daily soil temperature at 4 cm depth was 35.8 and 29.2 C in solarized and control plots, respectively. At 24 cm depth, the mean maximum daily soil temperature was 27.7 and 24.4 C in solarized and control plots, respectively. The average maximum and minimum daily air temperatures were computed from readings provided by the National Weather Service (NWS) at Will Rogers World Airport southwest of Oklahoma City approximately 35 miles from OSDACWFRC. The average minimum daily air temperature from the NWS was less than 1 C from the average minimum daily air temperature taken at the site of this study. The average maximum and minimum daily air temperatures were 25.9 C and 15.1 C, respectively. Daily skycover ratings, which include the percent cloud cover and opacity from sunrise to sunset, were also obtained from the NWS. A rating of 10 indicates thick, continuous cloud cover throughout the day and a rating of 0 indicates clear, blue skies throughout the

TABLE I

EFFECT OF SOLARIZATION[1] AT WASHINGTON, OKLAHOMA DURING 29 APRIL TO 27 MAY, 1986 ON POPULATIONS OF SELECTED SOILBORNE FUNGI[2]

	Fusarium oxysporum[3] (X10)	*Pythium* spp.[4] (X1)	*Trichoderma* spp.[5] ($X10^2$)	Total Fungi[6] ($X10^4$)
Prior to solarization				
Solarized	6.20[7]a[8]	6.40 a	1.33 a	5.80 a
Control	7.50 a	6.60 a	2.48 a	4.28 a
Post Solarization				
Solarized	0.30 a	3.30 a	1.72 a	7.58 a
Control	2.17 a	6.60 a	1.45 a	7.21 a

[1]1.2 X 2.0 m plots were mulched with 4-mil, transparent polyethylene.
[2]Using a 2 cm diameter soil probe to a depth of 24 cm, five soil samples were taken in an X pattern from each plot and bulked.
[3]*Fusarium oxysporum* isolated on Komada media.
[4]*Pythium* spp. isolated on Pythium selective agar.
[5]*Trichoderma* spp. isolated on Trichoderma selective media.
[6]Total fungi isolated on oxgall agar.
[7]Values are means of colony forming units per gram soil in 20 subsamples from each of three plots.
[8]Letters represent mean separation in columns and sampling times by Student-Newman-Keuls at P = 0.05.

day. The average skycover rating throughout the period of this study was 7.27.

1986-87 Stillwater Field Study

The number of total sclerotia (mean of those at all depths) germinated from sacs recovered from mulched plots after four weeks of solarization was not lower (P = 0.05) than the number in control plots (Table II). However, of the number recovered from the margin of solarized plots, the percent germinated of the total sclerotia recovered at that location was lower (P = 0.05) than those from control and from intermediate locations in the solarized treatment. Fewer total sclerotia recovered from control plots did not germinate than sclerotia from the margin of solarized plots (P = 0.05), but there was no significant difference at individual depths.

The number of total sclerotia missing from sacs in control plots was not greater (P = 0.05) than from solarized plots, but there was a significant difference (P = 0.05) at the 24 cm depth between the number of sclerotia missing from sacs in control plots and those at the margin of solarized plots (11.1 vs. 4.5 sclerotia, respectively). The percent incidence of epiphytic *Trichoderma* spp. on nongerminating total sclerotia, was greater (P = 0.05) in control plots than in solarized plots at the intermediate location, but at individual depths, no significant difference was found.

At the 4 cm depth, the mean maximum daily soil temperature in solarized plots was 40.9 C, which was 6.0 C greater than the temperature in control plots. The average minimum daily air temperature from Stillwater was less than 1 C from the average minimum daily air temperature at the National Weather Service. The average maximum and

TABLE II

EFFECT OF SOLARIZATION[1] AT STILLWATER, OKLAHOMA DURING 20 AUGUST TO 20 SEPTEMBER,1986 ON SCLEROTIA[2] OF SCLEROTIUM ROLFSII

Treatment	Germinated	% Germinating of # Recovered	Nongerminated	% Incidence of Epiphytic *Trichoderma* spp. on Nongerminated	Missing
Total Sclerotia					
Solarized					
Center[3]	20.5[6]a[7]	49.0 ab	21.7[6]ab	21.2 ab	7.9[6]a
Intermediate[4]	21.1 a	52.2 a	19.5 a	14.9 a	9.4 a
Margin[5]	17.6 a	41.7 b	25.1 b	17.4 ab	7.3 a
Control	21.1 a	53.6 a	18.9 a	32.2 b	10.0 a
0 cm Depth					
Solarized					
Center	26.3 a	64.0 a	15.3 a	24.4 a	8.5 a
Intermediate	24.0 a	59.1 a	16.6 a	9.6 a	9.4 a
Margin	19.5 a	43.7 a	25.8 a	22.3 a	4.8 a
Control	26.6 a	63.3 a	15.3 a	30.8 a	7.9 a
8 cm Depth					
Solarized					
Center	18.4 a	43.2 a	24.4 a	18.5 a	7.3 a
Intermediate	18.3 a	44.2 a	23.1 a	13.1 a	8.6 a
Margin	15.4 a	40.3 a	24.0 a	13.6 a	10.6 a
Control	20.9 a	54.6 a	17.8 a	30.4 a	11.4 a
16 cm Depth					
Solarized					
Center	16.9 a	39.2 a	26.8 a	17.0 a	6.3 a
Intermediate	21.8 a	48.5 a	18.3 a	12.6 a	10.0 a
Margin	17.4 a	42.8 a	23.4 a	16.1 a	9.3 a
Control	20.9 a	53.7 a	19.5 a	29.4 a	9.6 a

TABLE II (Continued)

Treatment	Germinated	% Germinating of # Recovered	Nongerminated	% Incidence of Epiphytic *Trichoderma* spp. on Nongerminated	Missing
<u>24 cm Depth</u>					
Solarized					
Center	20.5 a	49.6 a	20.1 a	25.0 a	9.3 ab
Intermediate	20.3 a	50.6 a	20.1 a	24.4 a	9.6 ab
Margin	18.3 a	39.8 a	27.3 a	17.5 a	4.5 a
Control	16.3 a	42.8 a	22.7 a	38.1 a	11.1 b

[1]Solarized plots were mulched with 4-mil,transparent polyethylene from 20 August,1986 to 3 March, 1987 with drip irrigation applied to maintain soil moisture at or near field capacity.
[2]Sclerotia, obtained from artificially-infested field-grown carrots were placed in sacs at 50 sclerotia/sac, buried in 0.5 X 7.5 m plots prior to mulching and removed 20 September, 1986.
[3]Sclerotia placed under the mulch at the center of the plot.
[4]Sclerotia placed under the mulch halfway between the center and margin.
[5]Sclerotia placed under the mulch at the margin of the plot.
[6]Values represent means of sclerotia in individual sacs in 8 plots.
[7]Letters represent mean separation in columns and depths by Student-Newman-Keuls at p = 0.05.

minimum daily air temperature computed from readings provided by the NWS were 28.5 C and 19.1 C, respectively. Based on readings of skycover obtained at NWS, the average skycover rating throughout this study was 6.75.

After mulching through the winter (Table III), the number of total sclerotia germinating from sacs at the center of mulched plots was greater (P = 0.05) than those germinating from sacs in control plots. At 16 cm depth, the number of sclerotia germinating at the margin of mulched plots was also greater (P = 0.05) than sclerotia germinated in control plots. The percent germination of sclerotia recovered at the 16 cm depth in the center of mulched plots was greater (P = 0.05) than in nonmulched plots at the same depth. The number of sclerotia remaining nongerminated from sacs at 16 cm depth at the center of mulched plots was lower (P = 0.05) than in nonmulched plots at the same depth.

The percent incidence of epiphytic *Trichoderma* spp. on nongerminating total sclerotia in all locations in mulched plots was lower (P = 0.01) than that in control plots. In addition, the percent incidence of epiphytic *Trichoderma* spp. on nongerminating sclerotia in mulched plots at all locations was lower (P = 0.05) at the soil surface and lower (P = 0.01) at 16 cm depth than that at the same depths in control plots.

Significant differences in mean values over all treatments were found between sclerotia at the conclusion of the 4-week solarization period compared to those at the conclusion of the mulching through the winter (Table IV). The number and percent germination of sclerotia recovered was greater (P = 0.001) for total sclerotia and at depths 8, 16 and 24 cm at the conclusion of mulching through the winter than after the 4-week solarization period. In addition, the percent germination of

TABLE III

EFFECT OF SOLARIZATION[1] AND MULCHING CONTINUED THROUGH THE WINTER AT STILLWATER, OKLAHOMA DURING 20 AUGUST, 1986 TO 3 MARCH, 1987 ON SCLEROTIA[2] OF SCLEROTIUM ROLFSII

Treatment	Germinated	% Germinating of # Recovered	Nongerminated	% Incidence of Epiphytic *Trichoderma* spp. on Nongerminated	Missing
Total Sclerotia					
Solarized					
Center[3]	32.3[6]a[7]	79.2 a	8.3[6]a	3.2 a	9.3[6]a
Intermediate[4]	30.3 ab	75.2 a	10.1 a	9.6 a	9.6 a
Margin[5]	30.9 ab	79.4 a	7.7 a	9.1 a	11.6 a
Control	27.3 b	73.8 a	9.8 a	19.8 b	12.9 a
0 cm Depth					
Solarized					
Center	28.8 a	75.1 a	8.5 a	5.1 a	12.8 a
Intermediate	27.8 a	72.1 a	10.5 a	6.5 a	11.8 a
Margin	24.3 a	71.2 a	9.5 a	13.3 a	16.3 a
Control	31.6 a	85.4 a	5.3 a	30.5 b	13.1 a
8 cm Depth					
Solarized					
Center	35.9 a	80.4 a	8.6 a	0.0 a	5.5 a
Intermediate	31.8 a	77.2 a	9.9 a	13.4 a	8.4 a
Margin	34.0 a	80.7 a	7.6 a	5.2 a	8.4 a
Control	27.9 a	71.6 a	10.8 a	17.1 a	11.4 a
16 cm Depth					
Solarized					
Center	30.9 ab	83.7 a	6.3 a	5.3 a	12.9 a
Intermediate	30.5 ab	77.8 ab	8.8 ab	0.0 a	10.8 a
Margin	32.4 a	79.6 ab	7.6 ab	5.4 a	10.0 a
Control	24.0 b	65.5 b	13.1 b	18.5 b	12.9 a

TABLE III (Continued)

Treatment	Germinated	% Germinating of # Recovered	Nongerminated	% Incidence of Epiphytic *Trichoderma* spp. on Nongerminated	Missing
24 cm Depth					
Solarized					
Center	33.9 a	77.5 a	9.9 a	2.4 a	6.3 a
Intermediate	31.3 a	73.5 a	11.1 a	18.4 a	7.6 a
Margin	33.1 a	86.3 a	5.1 a	12.5 a	11.8 a
Control	25.6 a	72.8 a	10.1 a	12.9 a	14.3 a

[1]Solarized plots were mulched with 4-mil,transparent polyethylene and drip irrigation was applied during 20 August to 20 September, 1986 to maintain soil moisture under the mulch at or near field capacity.
[2]Sclerotia, obtained from artificially-infested field-grown carrots were placed in sacs at 50 sclerotia/sac, buried in 0.5 X 7.5 m plots prior to mulching and removed at the conclusion of mulching.
[3]Sclerotia placed under the mulch at the center of the plot.
[4]Sclerotia placed under the mulch halfway between the center and margin.
[5]Sclerotia placed under the mulch at the margin of the plot.
[6]Values represent means of sclerotia in individual sacs in 8 plots.
[7]Letters represent mean separation in columns and depths by Student-Newman-Keuls at P = 0.05.

TABLE IV

COMPARISON OF THE EFFECT OF DATE OF RECOVERY ON SCLEROTIA OF *SCLEROTIUM ROLFSII* BURIED IN THE SOIL BEGINNING 20 AUGUST, 1986 IN STILLWATER, OKLAHOMA

Recovery Date	Germinated	% Germinating of # Recovered	Nongerminated	% Incidence of Epiphytic *Trichoderma* spp. on Nongerminated	Missing
Total *Sclerotia*					
September[2]	20.10[4]	49.13	21.28[4]	21.43	8.64[4]
March[3]	30.20***[5]	76.90***	8.91***	10.41**	10.87
0 cm Depth					
September	24.08	57.52	18.28	21.77	7.64
March	28.09	75.99**	8.84***	13.84	13.47*
8 cm Depth					
September	18.22	45.60	22.31	18.91	9.46
March	32.38***	77.46***	9.22***	8.94*	8.41
16 cm depth					
September	19.22	47.69	21.97	18.78	8.81
March	29.44***	76.62***	8.94***	7.32*	11.63
24 cm Depth					
September	18.81	45.72	22.55	26.27	8.64
March	30.97***	77.53***	9.06***	11.55	9.97

[1]Sclerotia, obtained from artificially-infested field-grown carrots were placed in sacs at 50 sclerotia/sac buried in 0.5 X 7.5 m plots with drip irrigation applied during 20 August to 20 September, 1986 to maintain soil moisture at or near field capacity.
[2]September = Sclerotia recovered 20 September, 1986.
[3]March = Sclerotia recovered 3 March, 1987.
[4]Values represent means of sclerotia in individual sacs in 16 plots at the center, margin and intermediate locations under mulch and in the nonmulched location.
[5]* represent mean separation in columns and depths by Student-Newman-Keuls at: * P = 0.05, ** P = 0.01, *** P = 0.001.

sclerotia recovered at the soil surface after mulching through the winter was greater (P = 0.05) than after the 4-week solarization period. The number of nongerminating total sclerotia and the number of nongerminating sclerotia at each depth at the conclusion of mulching through the winter was lower (P = 0.001) than after the 4-week solarization period. The number of total sclerotia missing and the number of sclerotia missing at depths 8, 16 and 24 cm was not greater (P = 0.05) after mulching through the winter than after the 4-week solarization. However, the number of sclerotia missing from sacs at the soil surface after mulching through the winter was greater (P = 0.05) than after the 4-week solarization period.

The percent incidence of epiphytic *Trichoderma* spp. on nongerminating total sclerotia after the winter mulching was lower (P = 0.01) than at the conclusion of the 4-week solarization period. At 8 and 16 cm depth, the percent incidence of epiphytic *Trichoderma* spp. on nongerminating sclerotia after mulching through the winter was lower (P = 0.05) than after 4-week solarization.

1987 Washington Field Study

Prior to mulching, population densities of all fungi assayed were not significantly different between solarized and control plots (Table V). After solarization, *Fusarium oxysporum* and *Pythium* spp. were not recovered from solarized plots at 0 - 5 cm depth. At 5 - 15 cm depth, the population densities of *F. oxysporum* and *Pythium* spp. were lower (P = 0.05) in solarized plots compared to control plots. At each depth, the population of total fungi was also lower (P = 0.05) in solarized plots compared to control plots. The population density of *Macrophomina phaseolina* was not less (P = 0.05) in solarized plots at any individual

TABLE V
EFFECT OF SOLARIZATION IN WASHINGTON, OKLAHOMA DURING 7 JULY TO 4 SEPTEMBER, 1987 ON POPULATIONS OF SELECTED SOILBORNE FUNGI

	After Mulching				Prior to Mulching
	0-5 cm	5-10 cm	10-15 cm	All Depths	All Depths
Fusarium oxysporum (X10^{3})[4]					
Solarized	0.0[1]a[2]	0.6 a	1.65a	0.73 a	4.10 a
Control	5.6 b	6.35 b	6.55 b	6.17 b	3.00 a
Pythium spp. (X10)[5]					
Solarized	0.0 a	0.95 a	6.2 a	2.38 a	3.50 a
Control	3.55 b	5.5 b	13.05 b	7.37 b	2.70 a
Macrophomina phaseolina (X1)[6]					
Solarized	9.00 a	1.80 a	0.30 a	3.60 a	7.80 a
Control	16.50 a	5.50 a	0.30 a	7.40 b	4.30 a
Total Fungi (X10^{4})[7]					
Solarized	1.65 a	9.25 a	5.45 a	5.45 a	11.15 a
Control	15.01 b	14.9 b	20.55 b	16.85 b	10.25 a

[1]1.2 X 2.4 m plots were mulched with 4-mil, transparent polyethylene after irrigation.
[2]Values are means of colony forming units per gram soil in 10 subsamples in each of 4 plots.
[3]Letters represent mean separation in column within fungi by Student-Newman-Keuls at $p = 0.05$.
[4]*Fusarium oxysporum* isolated on Komada media.
[5]*Pythium* spp. isolated on Pythium selective agar.
[6]*Macrophomina phaseolina* isolated on Dexon Oxgall PCNB Captan agar.
[7]Total Fungi isolated on oxgall agar.

depth. However, the population in solarized plots was lower (P = 0.05) than that in control plots when the mean densities for all depths were compared.

The average maximum daily air temperature as provided by the NWS was 32.9 C and the minimum was 21.6 C during the solarization period in this study. The average skycover rating was 3.6.

1987 Parsley Greenhouse Study

The mean fresh root weight per plant in solarized soil was 119% greater (P = 0.05) than in control soil (Table VI). The mean number of parsley plants that germinated in soil from solarized plots was 76% greater than the number germinating in soil from control plots, however this difference was not significant (P = 0.05). Though also not significant (P = 0.05), the mean fresh weight of parsley shoots was 73% greater in solarized soil than in control soil and the mean dry weight of parsley shoots grown in solarized soil was 79% greater than in control soil.

TABLE VI

EFFECT OF SOLARIZATION[1] AT WASHINGTON, OKLAHOMA DURING 7 JULY TO 4 SEPTEMBER, 1987 ON SUBSEQUENT GERMINATION AND GROWTH OF PARSLEY[2] IN SOIL IN THE GREENHOUSE IN STILLWATER, OKLAHOMA

	Plants[3]	Fresh Shoot Weight[4]	Dry Shoot Weight	Root Weight
Solarized	4.4	2.6	0.34	16.0
Control	2.5	1.5	0.19	7.3
Significance[5] at P =	.0661	.1037	.1052	.0496

[1]Samples of soil from the top 15 cm of plots taken 1 week after the conclusion of mulching were bulked. Subsamples were placed in pots on a greenhouse bench.
[2]Parsley seed was pregerminated in oxygenated water for 1 week and placed 10/pot at 1 cm below the soil surface. Misting was applied 6 hours/day. Parsley plants were counted twice weekly until 7 weeks when the study was concluded and weights determined.
[3]Values represent the mean number of plants/pot in 10 pots.
[4]Values of all weights represent the mean weight/plant/pot.
[5]Significance values obtained from mean separation by Student-Newman-Keuls.

1987 Weed Greenhouse Study

The total population of weeds in solarized soil was 23% (significant at P = 0.001) of that in control soil (Table VII). In solarized soil populations of *Ephorbia suprina* L. (prostrate spurge) and *Chenopodium pumillo* L. were reduced (P = 0.05) compared to controls. *Solanum nigrum* L. (black nightshade) and *Digitaria sanguinalis* (L.) Scop. (large crabgrass) populations were eliminated or absent in solarized soil. There were no significant differences in populations of *Cyperus esculentus* L. (yellow nutsedge) or populations of unidentified weeds (too small to identify when premature death occurred) between solarized soil and the control.

TABLE VII

EFFECT OF SOLARIZATION[1] IN WASHINGTON, OKLAHOMA DURING 7 JULY TO 4 SEPTEMBER, 1987 ON SUBSEQUENT NATURAL POPULATIONS OF WEEDS[2] IN SOIL IN THE GREENHOUSE AT STILLWATER, OKLAHOMA

	Total Weeds	*Euphorbia suprina*	*Chenopodium pumillo*
Solarized	1.15[3] a[4]	0.4 a	0.35 a
Control	5.1 b	2.1 b	1.80 b

	Cyperus esculentus	*Solanum nigrum*	*Digitaria sanguinalis*	Unidentified Weeds[5]
Solarized	0.30 a	0.00 a	0.0 a	0.1 a
Control	0.15 a	0.25 a	0.4 b	0.4 a

[1]Samples from the top 15 cm of soil in plots was taken 1 week after the conclusion of mulching and bulked. Subsamples were placed in pots on a greenhouse bench. Misting was applied 6 hours/day.
[2]Weeds were identified and enumerated twice weekly for 8 weeks.
[3]Values represent mean numbers of weeds per pot in 20 pots.
[4]Letters represent mean separation in columns and weed types by Student-Newman-Keuls at $p = 0.05$.
[5]Unidentified weeds = weeds that died too immature to identify.

1987 Nematode Greenhouse Study

Plant parasite nematodes, consisting of *Tylenchus* spp., *Miculenchus* spp. and *Pratylenchus* spp., were found only in control soil (significant at P = 0.05). Seinurids (*Seinura* spp.), beneficial to plants because they are especially aggressive predators on other nematodes, were found only in solarized soil (Table VIII). The population of predator/omnivore nematodes, consisting of *Leptonchus* sp. and various Dorylaimidae, that may alternatively attack plants, was lower (P = 0.05) in solarized soil than in control soil. Populations of beneficial myceliophagous (*Aphelenchus* spp.) and microphagous nematodes (Cephalobinae, Rhabditinae and Acrobelinae) were not significantly different (P = 0.1) between solarized and control soils.

TABLE VIII

EFFECT OF SOLARIZATION[1] AT WASHINGTON, OKLAHOMA 7 JULY TO 4 SEPTEMBER, 1987 ON SUBSEQUENT SOIL NEMATODE POPULATIONS IN THE GREENHOUSE AT STILLWATER, OKLAHOMA

	Nematodes Recovered[2]		
	Microphagous[3]	Omnivors[4]	Plant Parasitic[5]
Solarized	99.5[6]a[7]	3.2 a	0.0 a
Control	89.1 a	8.3 b	5.8 b
	Aphelenchus spp.	*Seinura* spp.	
Solarized	0.9 a	0.4 a	
Control	1.9 a	0.0 a	

[1]Samples of soil from the top 15 cm of plots taken 1 week after the conclusion of mulching were bulked. Subsamples were placed in pots on a greenhouse bench and misting was applied 6 hours/day.
[2]Nematodes/100 ml aliquants after 8 weeks in greenhouse.
[3]Cephalobinae, Rhabditinae and Acrobelinae.
[4]*Leptonchus* sp. and Dorylaimidae.
[5]*Tylenchus* spp., *Miculenchus* spp. and *Pratylenchus* spp.
[6]Values represent mean numbers of nematodes in 10 pots.
[7]Letters represent mean separation in columns and nematode types by Student-Newman-Keuls at P = 0.05.

1987 Stillwater Field Study

No significant difference (P = 0.05) in the population of M. phaseolina was found between solarized and control plots (Table IX). The population of M. phaseolina in all plots at 15 cm depth was lower (P = 0.001) than the population at 5 cm depth.

No sclerotia of Sclerotium rolfsii in solarized plots germinated after the solarization period (Table X). The mean number of sclerotia that did not germinate from solarized plots was 240% (significant at P = 0.001) of those from control plots. The number of sclerotia lost from sacs at 5 cm depth was greater (P = 0.05) in solarized plots than in control plots.

The percent incidence of fungal epiphytes on nongerminating total sclerotia and nongerminating sclerotia at 5 cm depth in solarized plots was not significantly different (P = 0.05) from those in control plots at the same depth. However at the 15 cm depth, the percent incidence of fungal epiphytes in solarized plots was lower (P = 0.05) than those in control plots. Fungal epiphytes were identified as Trichoderma spp., Penicillium spp., Rhizopus spp. and Chaetomium spp. and Aspergillus spp.

The average daily maximum and minimum air temperatures as provided by the NWS were 33.7 and 21.7 C, respectively and the average skycover rating during this study was 3.5.

TABLE IX

EFFECT OF SOLARIZATION[1] AT STILLWATER, OKLAHOMA DURING 23 JULY TO 4 SEPTEMBER, 1987 ON SCLEROTIA OF *MACROPHOMINA PHASEOLINA*[2]

	5 cm	15 cm	All Depths
Solarized	26.4[3]a[4]	8.0 a	17.2 a
Control	17.2 a	10.4 a	13.8 a

[1]1.5 X 2.1 m plots were mulched with 4-mil transparent polyethylene.
[2]After 2 weeks growth on PDA broth, sclerotia were sieved, rinsed in tapwater and dried. Sclerotia were mixed into pasteurized field soil at 28 sclerotia/ gram soil and placed in sacs.
[3]Values represent means of sclerotia/ gram soil in six sacs/ plot in 4 plots assayed on DOPC agar.
[4]Letters represent mean separation in columns by Student-Newman-Keuls at $P = 0.05$.

TABLE X

EFFECT OF SOLARIZATION[1] AT STILLWATER, OKLAHOMA DURING 23 JULY TO 4 SEPTEMBER, 1987 ON SCLEROTIA OF SCLEROTIUM ROLFSII[2]

Treatment	Germinated	% Germinating of # Recovered	Nongerminated	Missing	% Incidence of Epiphytic Fungi on Nongerminated
Total Sclerotia					
Solarized	0.0[3]	0.0	131.0	49.0	13.7
Control	78.9***[4]	59.4***	54.0***	47.1	23.5
5 cm Depth					
Solarized	0.0	0.0	124.8	55.3	20.6
Control	62.0**	45.3**	74.5**	43.5*	22.4
15 cm Depth					
Solarized	0.0	0.0	137.3	42.8	6.9
Control	95.8**	73.5***	33.5***	50.8	24.6*

[1]1.5 X 2.1 m plots were mulched with 4-mil, transparent polyethylene.
[2]Sclerotia, obtained from artificially infested field-grown carrots were placed at 30 sclerotia/sac and buried.
[3]Values represent means of sums of sclerotia in six sacs/plot in 4 plots.
[4]* represent mean separation in columns and depths by Student-Newman-Keuls at: * P = 0.05, ** P = 0.01, *** P = 0.001.

Thermal Death Studies

Continuous Temperature Effects

Pythium irregulare oospores were completely inactivated in wet or dry soil in less than 1.5 hours when exposed to 55 or 60 C (Table XI). Oospores were completely inactivated in wet soil in less than 6 hours, 2 days and 8 days at 50, 45 and 40 C, respectively and in dry soil in less than 12 hours, 4 days and 16 days at 50, 45 and 40 C, respectively.

The LD_{50} of *P. irregulare* in wet soil with continuous heating was 1.9 days, 12 hours and 2 hours and in dry soil was 4.3 days, 16 hours and 3.5 hours at 40, 45 and 50 C, respectively. The LD_{90} of *P. irregulare* was 4.3 days, 22.2 hours and 2.7 hours in wet soil at 40, 45 and 50 C, respectively and in dry soil, 9.3 days, 1.7 days and 5.6 hours at 40, 45 and 50 C respectively. LD_{50}'s and LD_{90}'s at 55 and 60 C were less than 1.5 hours in wet or dry soil.

All sclerotia of *M. phaseolina* in wet soil were inactivated in less than 3 hours, 6 hours and 4 days at 60, 55 and 50 C, respectively. After 32 days in wet soil at 45 C, *M. phaseolina* was reduced by greater than 99%, but at 40 C only 19% of sclerotia were inactivated. In dry soil, *M. phaseolina* was eliminated at 60, 55, and 50 C within 6 hours, 8 days and 16 days, respectively. Only 40% of the sclerotia in dry soil were eliminated within 32 days at 45 C and no reduction of *M. phaseolina* in dry soil at 40 C was apparent even after 32 days.

In wet soil, the LD_{50} of *M. phaseolina* was 11.3 days,20.4 hours, 2.6 hours and 0.9 hours at 45,50,55 and 60 C, respectively. In dry soil, the LD_{50} was 30.4 days, 2.8 days, 23.5 hours and 2.3 hours at 45, 50, 55 and 60 C, respectively. The LD_{90} in wet soil was 21.5 days, 1.6 days, 4.9 hours and 1.2 hours at 45, 50, 55 and 60 C, respectively. In dry soil,

TABLE XI

EFFECT OF CONTINUOUS HEATING IN LABORATORY INCUBATORS ON POPULATIONS OF *PYTHIUM IRREGULARE*[1] OR *MACROPHOMINA PHASEOLINA*[2] IN ARTIFICIALLY-INFESTED STERILIZED FIELD SOIL IN VIAL[3]

	Macrophomina phaseolina		*Pythium irregulare*	
Temperature	Dry Soil	Wet Soil	Dry Soil	Wet Soil
<u>40 C</u>				
LD_{50}[4]	NR[5]	>32 days	4.3 days	1.9 days
LD_{90}	NR	>32 days	9.3 days	4.3 days
Complete Inactivation	>32 days	>32 days	<16 days	< 8 days
<u>45 C</u>				
LD_{50}	30.4 days	11.3 days	16.5 hours	12.0 hours
LD_{90}	>32 days	21.5 days	1.7 days	22.2 hours
Complete Inactivation	>32 days	>32 days	< 4 days	< 2 days
<u>50 C</u>				
LD_{50}	2.8 days	20.4 hours	3.5 hours	2.0 hours
LD_{90}	6.1 days	1.6 days	5.6 hours	2.7 hours
Complete Inactivation	<16 days	< 4 days	<12 hours	< 6 hours
<u>55 C</u>				
LD_{50}	23.5 hours	2.6 hours	< 1.5 hours	<1.5 hours
LD_{90}	2.4 days	4.9 hours	< 1.5 hours	<1.5 hours
Complete Inactivation	< 8 days	< 6 hours	< 1.5 hours	<1.5 hours

TABLE XI (Continued)

Temperature	Macrophomina phaseolina Dry Soil	Macrophomina phaseolina Wet Soil	Pythium irregulare Dry Soil	Pythium irregulare Wet Soil
60 C				
LD_{50}	2.3 hours	0.9 hours	< 1.5 hours	<1.5 hours
LD_{90}	3.2 hours	1.2 hours	< 1.5 hours	<1.5 hours
Complete Inactivation	< 6 hours	< 3 hours	< 1.5 hours	<1.5 hours

[1]P. irregulare grown on V-8 broth was rinsed, blended and mixed into autoclaved soil at 20 oospores/ g soil. Populations were assayed on PSA agar.
[2]M. phaseolina grown on PDA broth was sieved, rinsed and mixed into pasteurized field soil at sclerotia/ g soil. Populations were assayed on DOPC agar.
[3]2 g infested soil samples were placed into 10 ml vials and sealed. Vials were placed in incubators set at specified temperatures and five vials were withdrawn at 1.5, 3, 6 and 12 hours and at 1, 2, 4, 8, 16 and 32 days. Mean populations at each withdrawal time for each temperature were determined.
[4]All calculations were made using probit analysis.
[5]NR = No reduction in population was found.

the LD_{90} was 6.1 days, 2.5 days, 3.2 hours at 50, 55 and 60 C, respectively. The LD_{90} at 45 C in dry soil and LD_{50}'s and LD_{90}'s at 40 C were beyond the time interval of this study or no reduction in the population of M. phaseolina had occurred.

Daily Two-Hour Interval High Temperature Effects

Pythium irregulare oospores were completely inactivated at 60 C in wet or dry soil and at 55 C in dry soil prior to the end of the first daily high temperature interval (Table XII). At 55 C in wet soil, more than 97% of oospores were inactivated within the first daily high temperature interval. Oospores in wet soil were completely inactivated in less than 8 days and 32 days at 50 and 45 C, respectively and at 40 C, 58% of the oospores remained viable after 32 days. In dry soil at 50 C, oospores were eliminated in less than 16 days. Pythium irregulare in dry soil was reduced by 99% and 24% after 32 days at 45 and 40 C, respectively.

The LD_{50} of P. irregulare in wet soil was 26.4, 5.3 and 1.5 days at 40, 45 and 50 C, respectively (Table XII). The LD_{90} of P. irregulare at 45 and 50 C was 12.3 and 3.7 days, respectively, in wet soil and 20.4 and 9.7 days, respectively, in dry soil. LD_{50} and LD_{90} at 40 C in dry soil and LD_{90} in wet soil at 40 C were beyond the time interval of this study. LD_{50}'s and LD_{90}'s at 55 and 60 C in wet or dry soil were less than one day.

Sclerotia of M. phaseolina were eliminated in less than 4 and 16 days in dry soil and in less than 1 and 4 days in wet soil at 60 and 50 C, respectively. In dry soil, 97%, 88% and 63% of sclerotia remained viable after 32 days at 40, 45 and 50 C, respectively. In wet soil, 93%, 70% and 6% of sclerotia were still viable after 32 days at 40, 45

TABLE XII

EFFECT OF DAILY TWO-HOUR INTERVALS OF HEATING IN LABORATORY INCUBATORS ON POPULATIONS OF PYTHIUM IRREGULARE[1] OR MACROPHOMINA PHASEOLINA[2] IN ARTIFICIALLY-INFESTED STERILIZED FIELD SOIL IN VIALS[3]

	Macrophomina phaseolina		*Pythium irregulare*	
Temperature	Dry Soil	Wet Soil	Dry Soil	Wet Soil
40 C				
LD50[4]	NR[5]	NR	>32 days	26.4 days
LD90	NR	NR	>32 days	>32 days
Complete Inactivation	NR	NR	>32 days	>32 days
45 C				
LD50	NR	>32 days	9.4 days	5.3 days
LD90	NR	>32 days	20.4 days	12.3 days
Complete Inactivation	NR	>32 days	>32 days	<32 days
50 C				
LD50	>32 days	14.7 days	4.2 days	1.5 days
LD90	>32 days	29.3 days	9.7 days	3.7 days
Complete Inactivation	>32 days	>32 days	<16 days	< 8 days
55 C				
LD50	4.2 days	1.4 days	<1.5 hours	<1.5 hours
LD90	7.2 days	2.0 days	<1.5 hours	<1.5 hours
Complete Inactivation	<16 days	< 4 days	<1.5 hours	<3.0 hours

TABLE XII (Continued)

	Macrophomina phaseolina		*Pythium irregulare*	
Temperature	Dry Soil	Wet Soil	Dry Soil	Wet Soil
60 C				
LD50	1.5 days	< 1 day	<1.5 hours	<1.5 hours
LD90	2.1 days	< 1 day	<1.5 hours	<1.5 hours
Complete Inactivation	< 4 days	< 1 day	<1.5 hours	<1.5 hours

[1]*P. irregulare* grown on V-8 broth was rinsed, blended and mixed into autoclaved soil at 20 oospores/ g soil. Populations were assayed on PSA agar.
[2]*M. phaseolina* grown on PDA broth was sieved, rinsed and mixed into pasteurized field soil at sclerotia/ g soil. Populations were assayed on DOPC agar.
[3]2 g infested soil samples were placed into 10 ml vials and sealed. Vials were placed in incubators set to increase to specified temperatures, maintain that temperature for 2 hours each day and cool to room temperature. Five vials were withdrawn at 1, 2, 4, 8, 16 and 32 days. Mean populations at each withdrawal time for each temperature were determined.
[4]All calculations were made using probit analysis.
[5]NR = No reduction in population was found.

and 50 C, respectively.

The LD_{50} of *M. phaseolina* in wet soil at 50 and 55 C was 14.7 and 1.4 days, respectively. In dry soil, the LD_{50} was 4.2 days and 1.5 day at 55 and 60 C, respectively. The LD_{90} of *M. phaseolina* was 29.3 and 2.0 days in wet soil at 50 and 55 C, respectively and 7.2 and 2.1 days at 55 and 60 C, respectively in dry soil. The LD_{50} and LD_{90} in wet soil was less than one day at 60 C. At 40 and 45 C in dry soil and 40 C in wet soil, no population reductions occurred. The LD_{50} and LD_{90} at 45 C in wet soil and 50 C in dry soil were greater than 32 days.

Pathogenicity Studies

Isolations

Pythium irregulare was isolated from all species of trees used in this study. Isolates designated ESp3, ESp4 and ESp5 were used as inoculum sources in other portions of this study. *P. debaryanum* was isolated from scots pine and sycamore. Several other species of *Pythium*, including an isolate from maple with brown oospores, were isolated from various tree species, but identification was not possible, because either oospores were not formed or insufficient similarity was found between the isolate and descriptions of known species.

Fusarium oxysporum was isolated from all tree species used in this study. *F. solani* was isolated from sycamore and virginia pine. Two isolates of *Macrophomina phaseolina*, designated SyV1 and SyV2 were found on sycamore and one isolate designated VpV4 was found on virginia pine. These isolates of *M. phaseolina* were used as inoculum sources in other portions of this study.

Preliminary Pathogenicity Greenhouse Study

All scots pine seedlings grown in soil infested with *P. irregulare* isolate ESp3 were dead at the conclusion of this study. ESp3 was recovered from each seedling. Three of the six seedlings grown in soil infested with *P. irregulare* isolate ESp4 died and the tips of needles on the fourth seedling were browning at the conclusion of the study. ESp4 was only recovered from these four isolates. None of the seedlings in soil infested with *P. irregulare* isolate ESp5 showed any disease symptoms at the conclusion of this study and the isolate could not be recovered. Control seedlings also showed no symptoms and no *Pythium* spp. were isolated from them.

Pathogenicity Greenhouse Study I

Isolates 38 and 98 of *M. phaseolina* could not be recovered from any of the host plants (Table XIII). Isolate 62 was only recovered from one arbivatae seedling. Isolate 244 was recovered from only one seedling each of arborvitae and virginia pine. Isolate 61 was limited to *Pinus* spp., but was recovered from 83% of the pine seedlings. Isolates 40 and 169 were recovered from all host species, but only 67% of the individual seedlings.

Of the isolates from the OSDACWFRC, all were recovered from arborvitae and sycamore. Isolate SyV1 was only recovered from one seedling each of arborvitae and sycamore. Isolate VpV4 was recovered from only one seedling each of scots pine, arborvitae and sycamore. Isolate SyV2 was recovered from all host species, including one seedling each of arborvitae and scots pine, two seedlings of virginia pine and all seedlings of sycamore.

TABLE XIII

INFECTION OF TREE SEEDLINGS[1] IN PASTEURIZED FIELD SOIL ARTIFICIALLY INFESTED[2] WITH MACROPHOMINA PHASEOLINA OR PYTHIUM IRREGULARE

Macrophomina phaseolina isolate	Arborvitae sp.	Pinus sylvestris	Pinus virginiana	Platanus occidentalis
38[3]	0[5]	0	0	-[6]
40	1	2	1	-
61	0	2	3	-
62	1	0	0	-
98	0	0	0	-
155	2	2	2	-
169	2	1	1	-
244	1	0	1	-
SYV1[4]	1	0	0	1
SYV2	1	1	2	3
VPV4	1	1	0	1
Macrophomina phaseolina control	0	0	0	0
Pythium irregulare isolate ESP3	3	3	3	3
Pythium irregulare control	0	0	0	0

[1]Tree seeds were germinated in 5 cm deep flats of Ready Mix. Seedlings were transferred to individual 2 X 2 X 7.5 cm pots.
[2]Three seedlings of each species were grown in soil infested with 0.5 cm plugs of M. phaseolina or P. irregulare grown on PDA.
[3]Isolates beginning with numbers were obtained prior to this study (Conway, unpublished).
[4]Isolates beginning with letters were obtained during this study from field-grown seedlings.
[5]Values represent the number of trees from which the isolate was recovered after six weeks on a greenhouse bench with 6 hours misting daily.
[6]-Fungi not tested.

All seedlings including controls had some necrotic or dead needles and several were killed by the conclusion of this study, probably due to the extreme temperatures in the greenhouse. Seedlings from which _M. phaseolina_ was recovered had dead needles or were killed.

Isolate ESp3 of _Pythium irregulare_ was recovered from all individual plants of each host species. All seedlings in soil infested with ESp3 were dead at the conclusion of this study. No _M. phaseolina_ or _Pythium_ spp. was recovered from any control seedling.

Pathogenicity Greenhouse Study II

No colonies of _M. phaseolina_ were recovered from pasteurized field soil spread on DOPC. Though _M. phaseolina_ isolate SyV2 was recovered from the majority of sycamore and scots pine seedlings grown in infested Ready Mix above, a much lower recovery was obtained from seedlings grown in infested pasteurized field soil. _Macrophomina phaseolina_ was recovered from one sycamore and one virginia pine seedling with no moisture stress. With plants exposed to moisture stress conditions, the isolate was recovered from 25% of sycamore seedlings, but only 8.3% of virginia pine seedlings. _Macrophomina phaseolina_ was not recovered from any control seedlings.

All sycamores from which _M. phaseolina_ was isolated were killed by the conclusion of this study. However, some of the other sycamore seedlings including controls were dead or possessed necrotic lower leaves or leaf tips probably due to the extreme temperatures (as great as 45 C) in the greenhouse. Most virginia pines grown in noninfested soil remained completely green, though some needle necrosis occurred. Virginia pines grown in infested soil showed no symptoms other than those in common with control plants.

Distribution Study

The patterns of sclerotia of *M. phaseolina* and propagules *P. irregulare* in this study were clumped, both with the most distinct clump size greater than 64 blocks within the transect analyzed. *Macrophomina phaseolina* was distributed within a less distinct clump size of approximately 25 blocks, while no less distinct cluster occurred in the pattern of *P. irregulare*.

The patterns of percent soil moisture, mulch coverage, number of pines and reflectance readings were also clumped. The most distinct clump size of the pattern of pines was 36 quadrats and the less distinct clump size was 46 quadrats large. Mulch coverage was distributed with the most distinct clump of 46 quadrats and a less distinct clump of 36 quadrats. Analysis of 500 nm reflectance revealed a very distinct clump size between 44 and 50 quadrats and a less distinct clump size of 36 quadrats. The most distinct clump size of percent soil moisture was larger than one-half of the transect with an indistinct clump size between 44 and 51 quadrats. Analysis of 850 nm wavelength reflectance revealed a distinct clump size of 62 quadrats and indistinct clump sizes of between 38 and 43 quadrats and near 16 quadrats.

The pattern of the number of pine seedlings across the transect was negatively correlated ($P = 0.001$) with the pattern of the wood chip mulch covering, percent soil moisture and the 500 nm wavelength reflectance (Table XIV) (Patterns of field parameters evaluated are displayed in Figures 1 - 7. Figures were constructed primarily for visual comparison of pairs of figures). The pattern of the mean density of *Pythium* spp. propagules also correlated negatively ($P = 0.05$) with the pattern of pine density.

TABLE XIV

REGRESSION CORRELATIONS OF PATTERNS OF FIELD ARRANGEMENT OF PARAMETERS EVALUATED IN A TRANSECT[1] SUPERIMPOSED OVER AN ESTABLISHED BED OF SCOTS PINE SEEDLINGS IN WASHINGTON, OKLAHOMA IN 1987

	Pine	*Macrophomina phaseolina*	*Pythium* spp.	Mulch	Moisture	500nm
Macrophomina phaseolina	-0.11					
Pythium spp.	-0.22 *[2]	0.29 ***				
Mulch	-0.48 ***	-0.13	-0.15			
Moisture	-0.67 ***	0.09	0.34 ***	0.40 ***		
500nm	-0.77 ***	0.06	0.28 **	0.49 ***	0.87 ***	
850nm	0.14	-0.09	-0.39 ***	-0.13	-0.22 *	-0.06

[1]Transect consisted of 128 1.2 X 1.2 m contiguous quadrats.
[2]* represent significant correlation at: * P = 0.05, ** P = 0.01, *** P = 0.001.

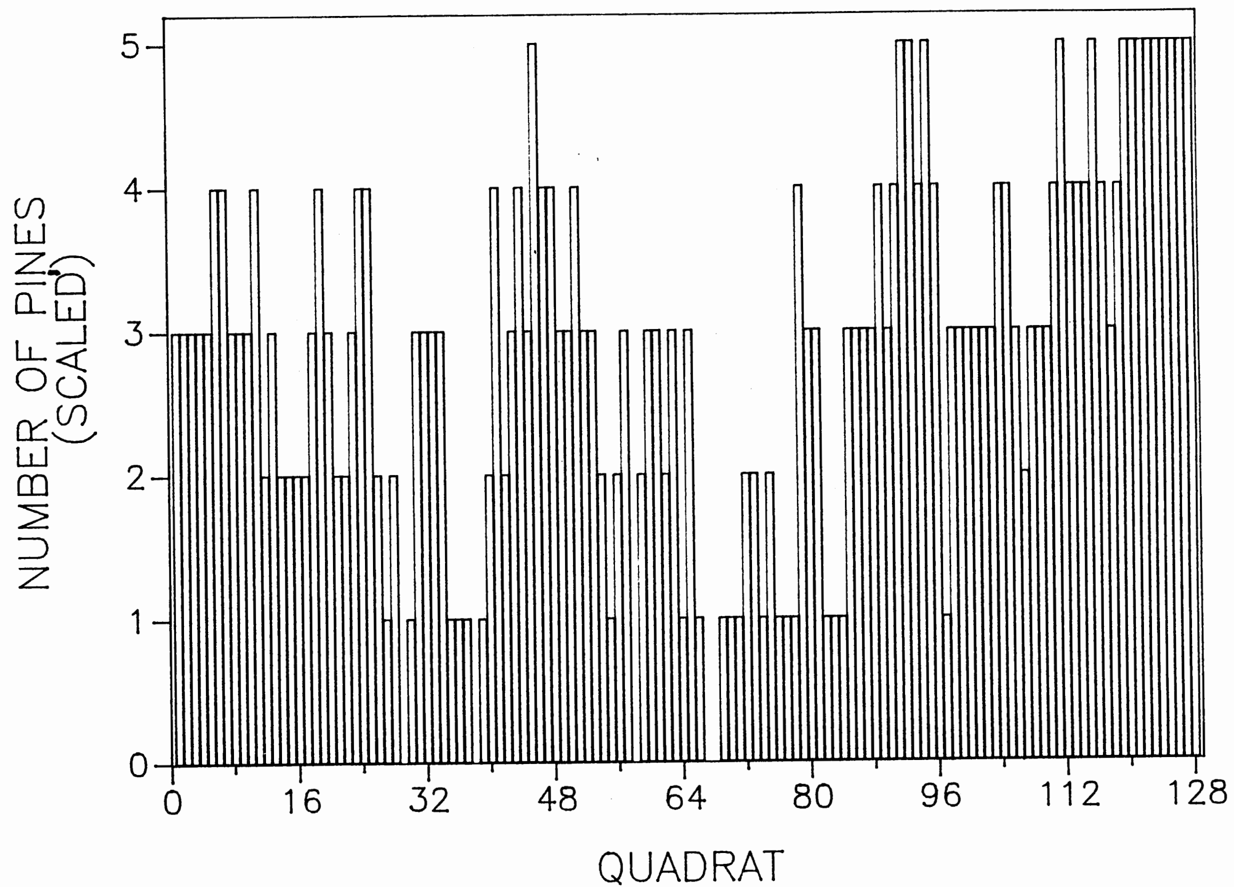

Figure 1. Pattern of pine seedling population across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots pine seedling bed in Washington, Oklahoma in 1987

[1]Scaled numbers were determined by dividing estimated counts by 10 and rounding down to the nearest integer.

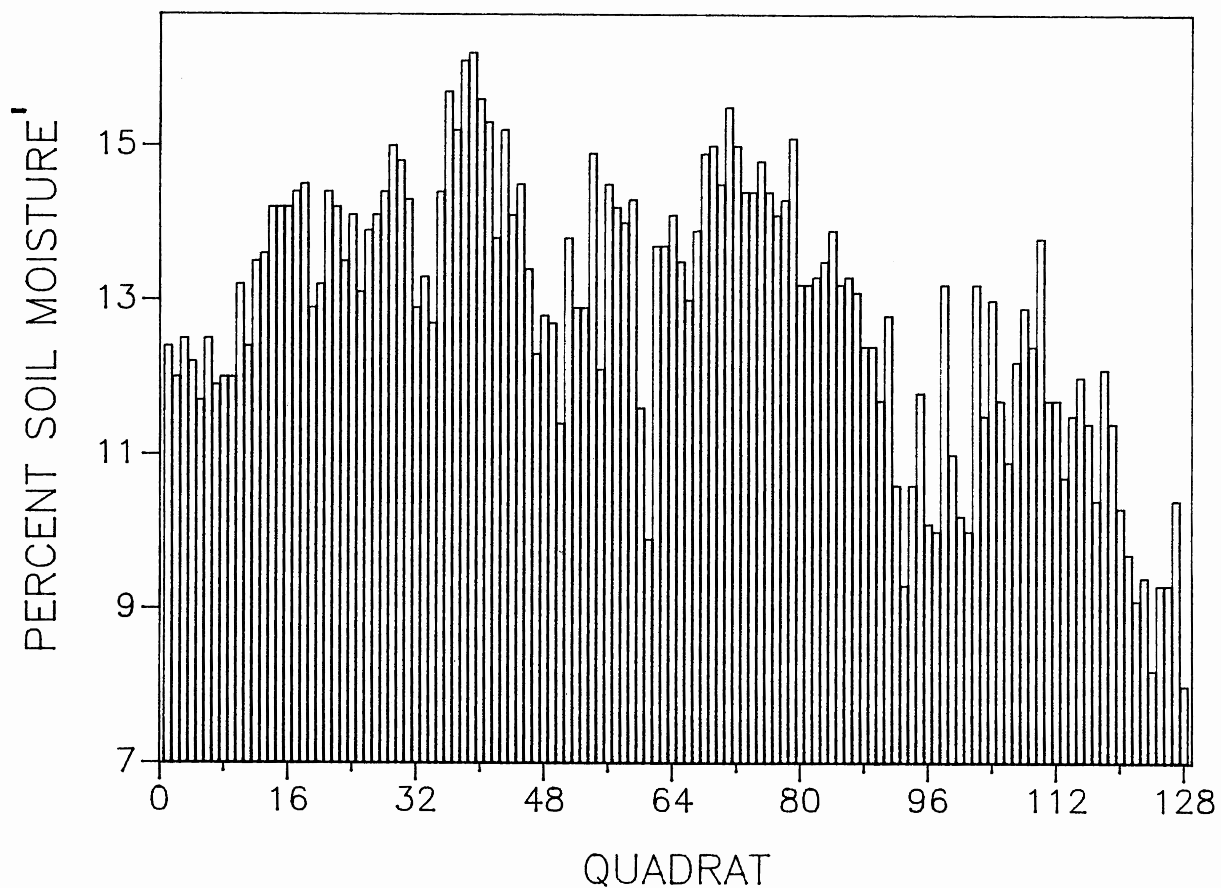

Figure 2. Pattern of percent soil moisture across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots pine seedling bed in Washington, Oklahoma in 1987

[1]Percent soil moisture determined from subsamples of 5 bulked soil samples taken to a depth of 15 cm using 2 cm diameter soil probes.

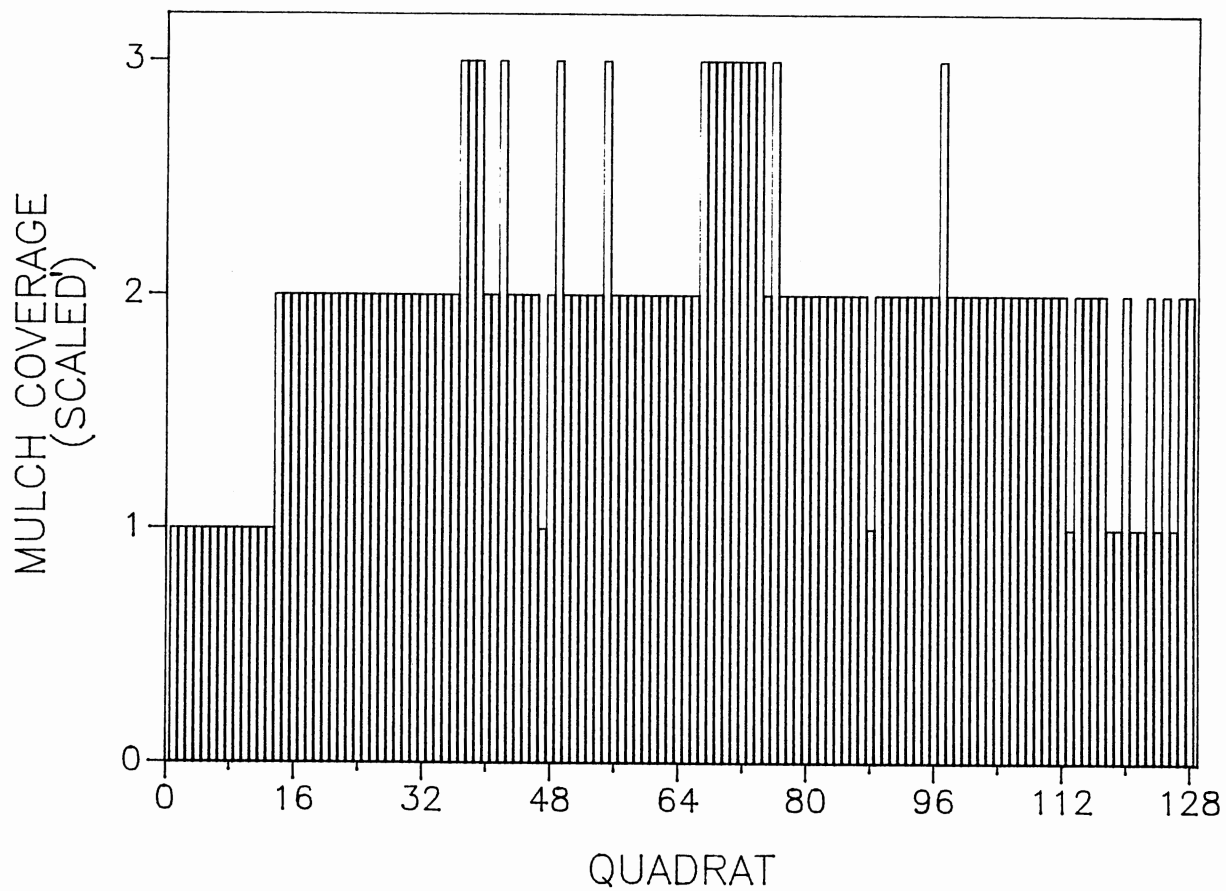

Figure 3. Pattern of mulch coverage across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots pine seedling bed in Washington, Oklahoma in 1987.

[1]Scaled numbers were determined by dividing estimated percent mulch coverage in each quadrat by 33 1/3 % and rounding up to the nearest integer.

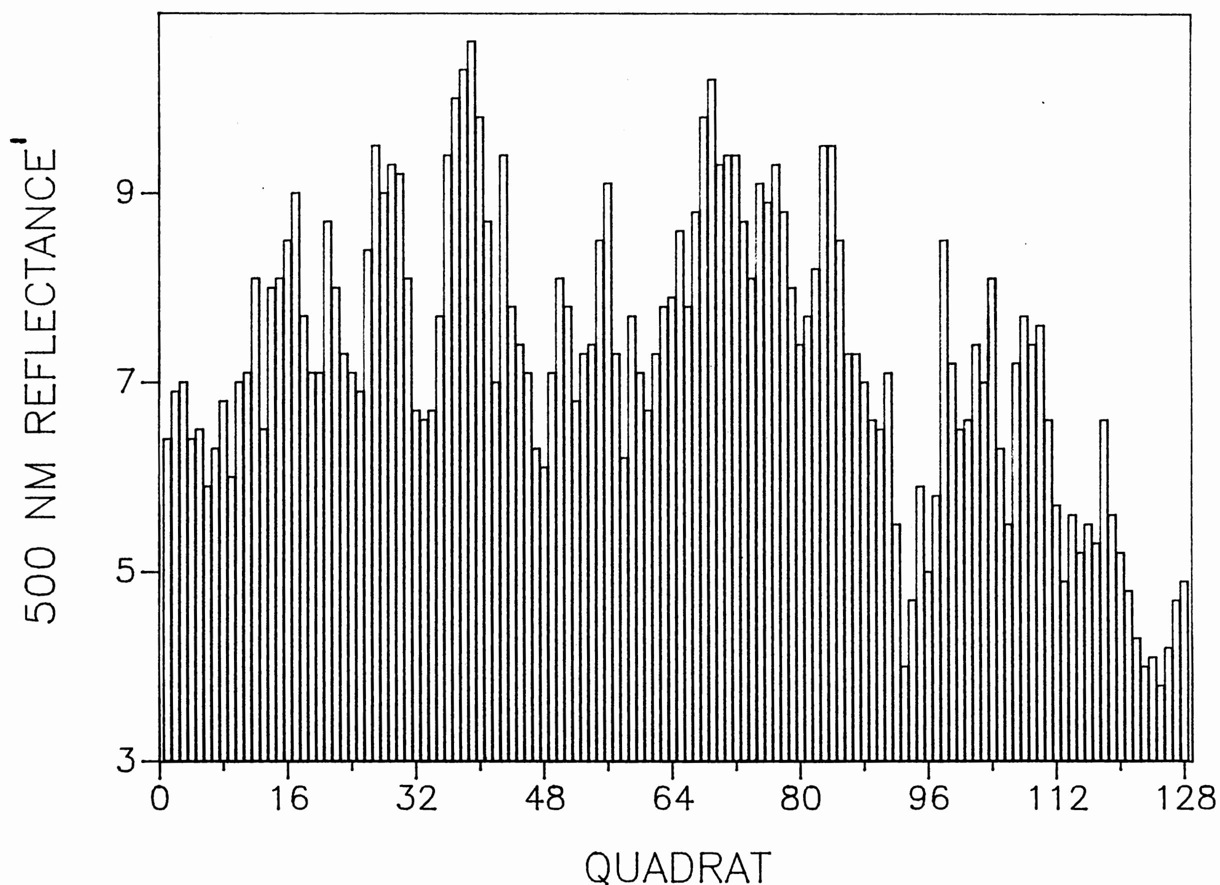

Figure 4. Pattern of 500 nmreflectance across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots pine seedling bed in Washington, Oklahoma in 1987

[1]3 reflectance readings were taken over each quadrat using a multispectral radiometer, adjusted according to incoming solar radiation and averaged.

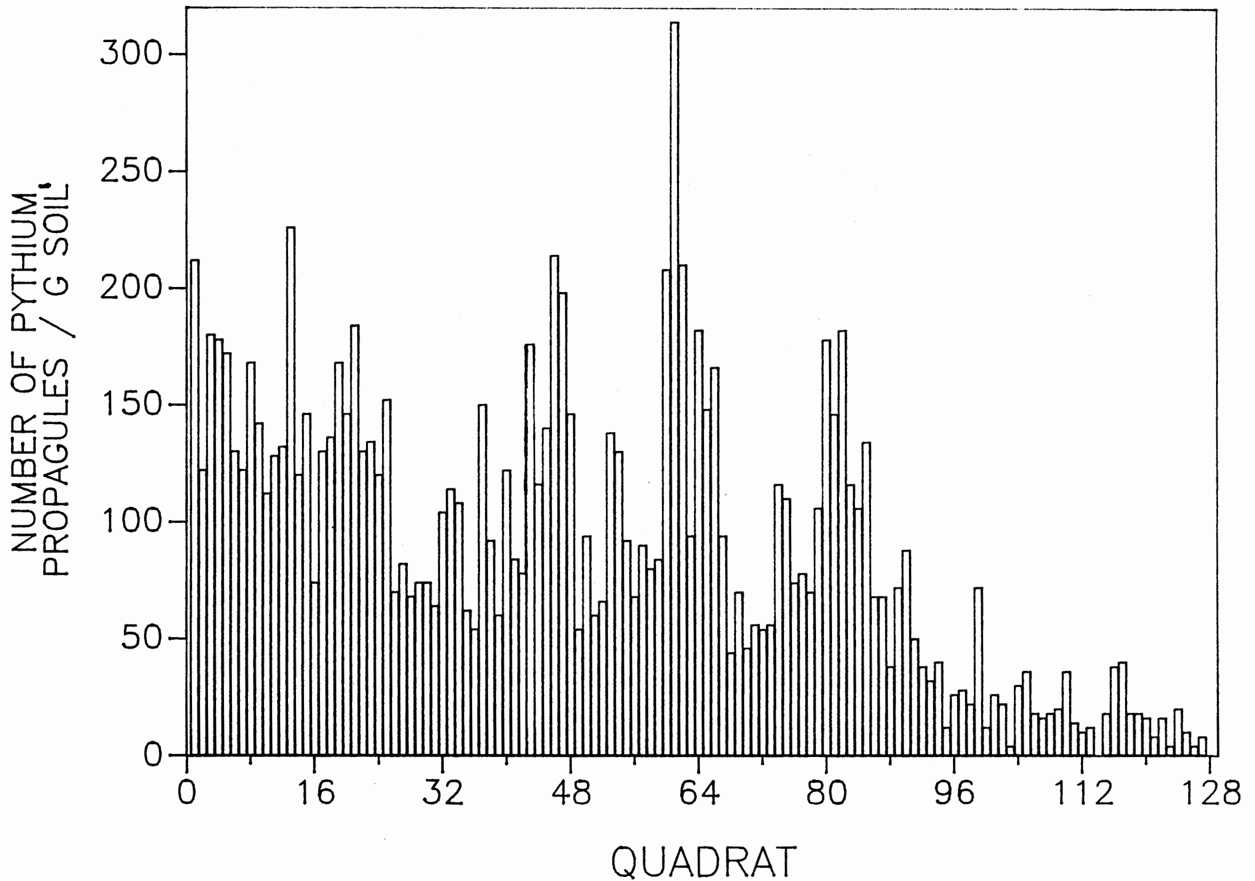

Figure 5. Pattern of Pythium spp. density across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots pine seedling bed in Washington, Oklahoma in 1987

[1]Number of Pythium spp. propagules determined by plating subsamples of 5 bulked soil samples taken to a depth of 15 cm using 2 cm diameter soil probes on Pythium Selective agar in 10 petri dishes and determining the mean number of colonies.

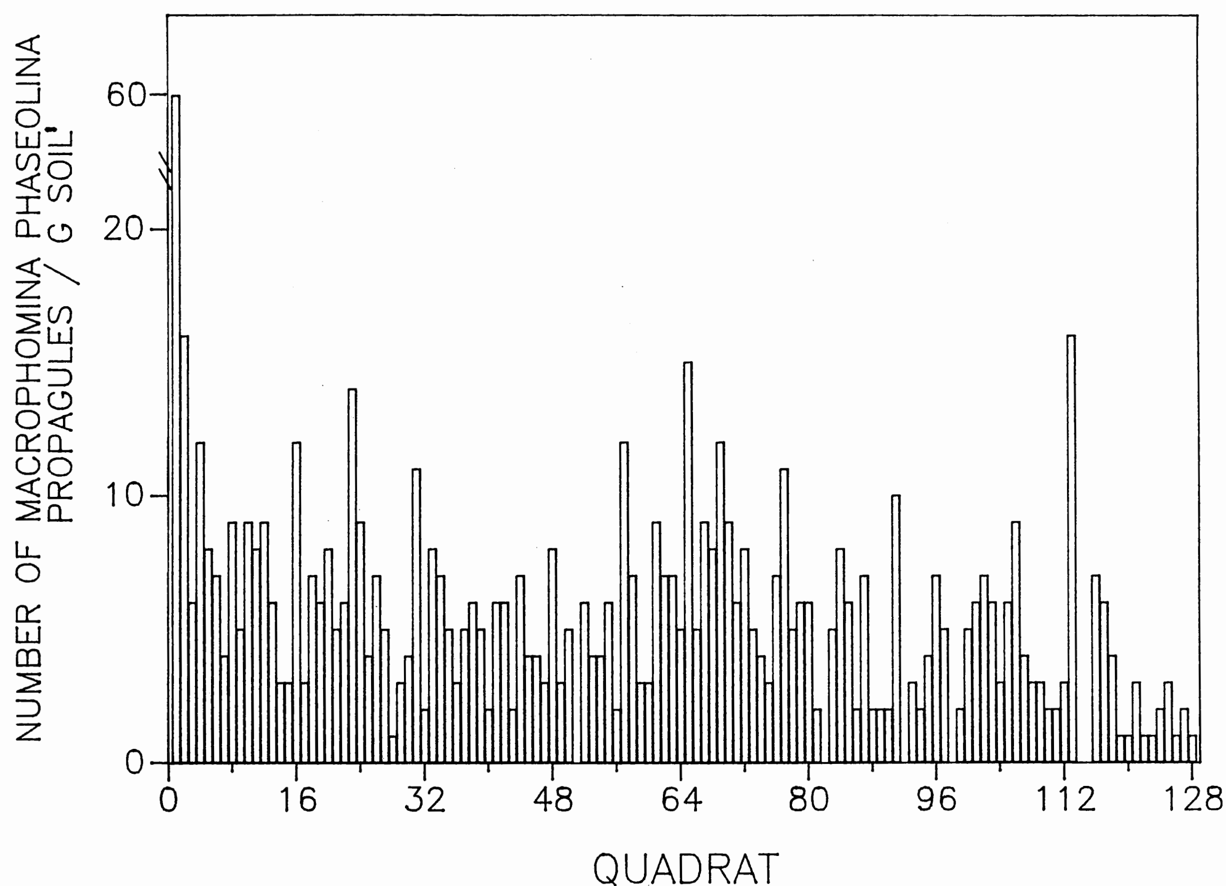

Figure 6. Pattern of Macrophomina phaseolina density across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots pine seedling bed in Washington, Oklahoma in 1987

[1]Number of Macrophomina phaseolina propagules determined by plating subsamples of 5 bulked soil samples taken to a depth of 15 cm using 2 cm diameter soil probes on Dexon- Oxgall-PCNB-Captan agar in 10 petri dishes and determining the mean number of colonies.

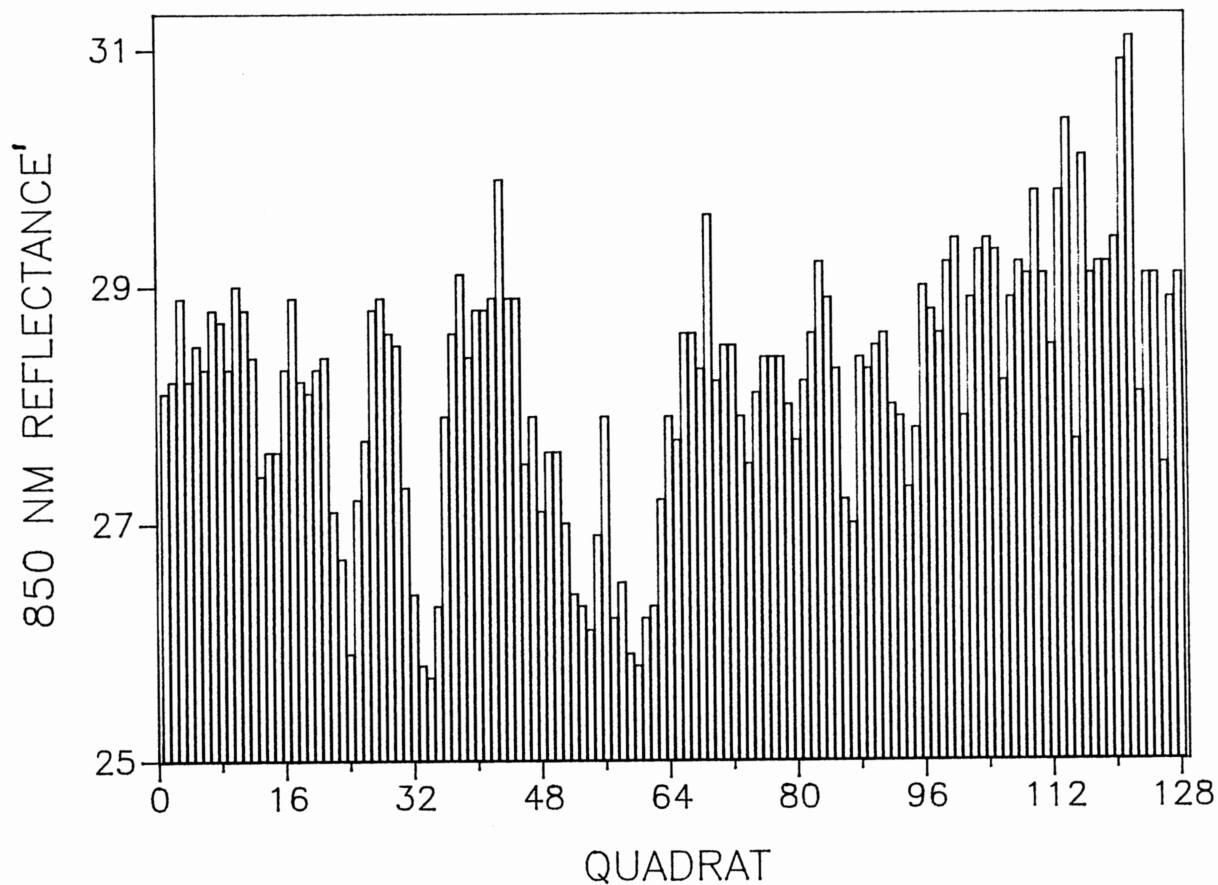

Figure 7. Pattern of 850 nm reflectance across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots pine seedling bed in Washington, Oklahoma in 1987

[1]3 reflectance readings were taken over each quadrat using a multispectral radiometer, adjusted according to incoming solar radiation and averaged.

The pattern of mean density of M. phaseolina propagules correlated positively (P = 0.001) with the Pythium spp. pattern, but with no other pattern evaluated. Density of Pythium spp. correlated positively with the pattern of percent soil moisture (P = 0.001) and the reflectance at the 500 nm wavelength (P = 0.01) and was negatively correlated (P = 0.001) with the pattern of 850 reflectance.

The mulch coverage pattern was positively correlated (P = 0.001) with the pattern of both percent soil moisture and 500 nm wavelength reflectance. The pattern of percent soil moisture was positively correlated (P = 0.001) with the pattern of 500 nm wavelength reflectance, but was negatively correlated (P = 0.05) with the pattern of 850 nm wavelength reflectance.

The variance distribution of each parameter across all block sizes, calculated by Hill's Two-term local quadrat variance method, were also correlated (Table XV) (Distributions of variance of field parameters are displayed in Figures 8 - 14. Figures were constructed primarily for visual comparison of pairs of figures). Distribution of pine density positively correlated (P = 0.001) with the distributions of mulch coverage, percent soil moisture and 500 nm wavelength reflectance and with the distribution of 850 nm wavelength reflectance (P = 0.05).

Macrophomina phaseolina distribution was positively correlated (P = 0.001) with the distribution of Pythium spp., percent soil moisture, reflectance at the 500 nm and 850 nm wavelengths. The Pythium spp. distribution also correlated positively (P = 0.001) with the distribution of percent soil moisture, 500 nm and 850 nm wavelengths of reflectance. The distribution of mulch coverage was positively correlated (P = 0.001) with the distribution of 500 nm wavelength reflectance and also with 850 nm wavelength reflectance (P = 0.01).

TABLE XV

REGRESSION CORRELATIONS OF VARIANCE[1] DISTRIBUTION ACROSS QUADRAT BLOCK SIZES OF PATTERNS OF FIELD ARRANGEMENT OF PARAMETERS EVALUATED IN A TRANSECT[2] SUPERIMPOSED OVER AN ESTABLISHED BED OF SCOTS PINE SEEDLINGS IN WASHINGTON, OKLAHOMA IN 1987

	Pine	*Macrophomina phaseolina*	*Pythium* spp.	Mulch	Moisture	500nm
Macrophomina phaseolina	0.19					
Pythium spp.	0.21	0.72 ***[3]				
Mulch	0.92 ***	-0.03	0.02			
Moisture	0.52 ***	0.56 ***	0.91 ***	0.40 **		
500nm	0.87 ***	0.42 ***	0.64 ***	0.76 ***	0.87 ***	
850nm	0.30 *	0.61 ***	0.98 ***	0.16	0.96 ***	0.73 ***

[1]Variances of quadrat block sizes were calculated using Hill's Two-Term Local Quadrat Variance method.
[2]Transect consisted of 128 1.2 X 1.2 m contiguous quadrats.
[3]* represent significant correlation at: * P = 0.05, ** P = 0.01, *** P = 0.001.

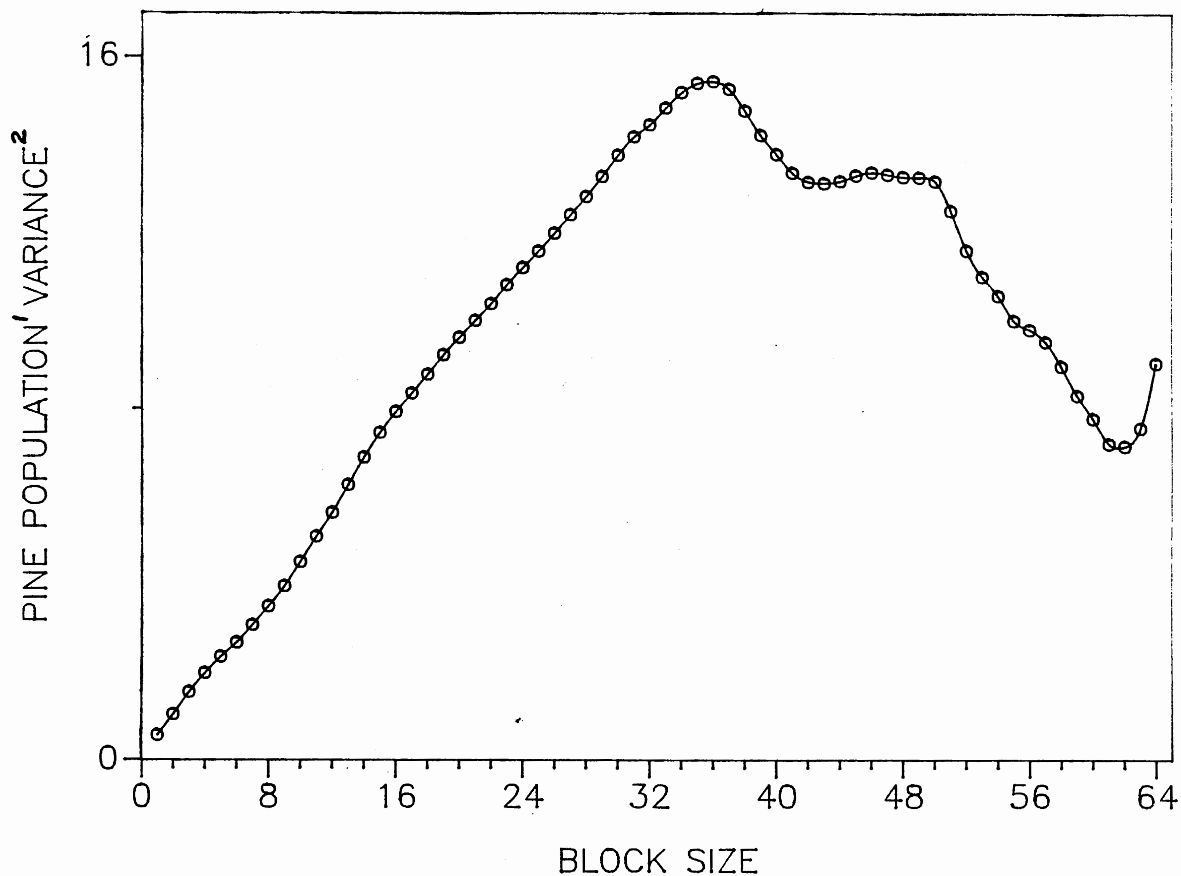

Figure 8. Distribution of block-size variance of pine seedling population[1] across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots pine seedling bed in Washington, OK in 1987

[1]Pine seedling populations recorded as scaled numbers which were determined by dividing estimated counts by 10 and rounding down to the nearest integer.
[2]Variances of individual quadrat block sizes were determined using Hill's Two-Term Local Quadrat Variance method.

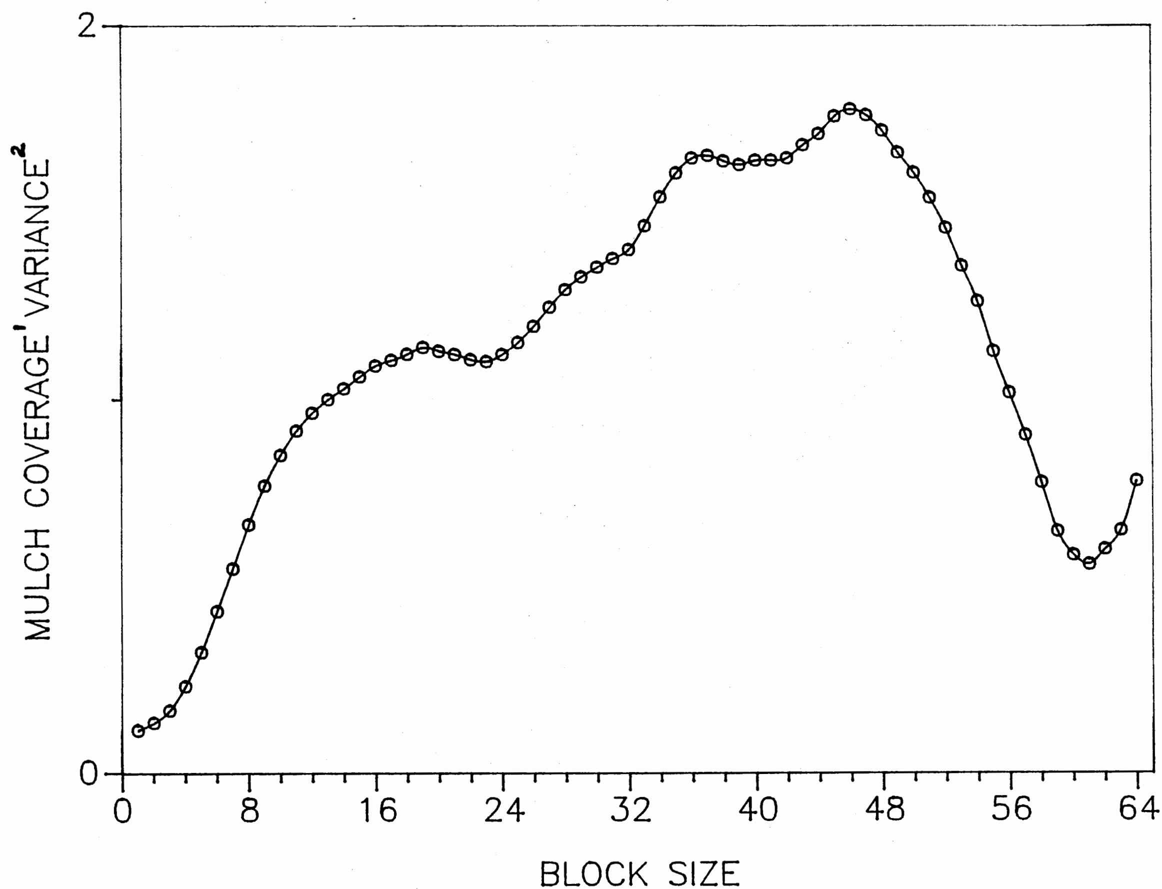

Figure 9. Distribution of block-size variance of mulch coverage[1] across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots pine seedling bed in Washington, Oklahoma in 1987

[1]Mulch coverage recorded as scaled numbers which were determined by dividing estimated percent mulch coverage in each quadrat by 33 1/3% and rounding up to the nearest integer.
[2]Variances of individual quadrat block sizes were determined using Hill's Two-Term Local Quadrat Variance method.

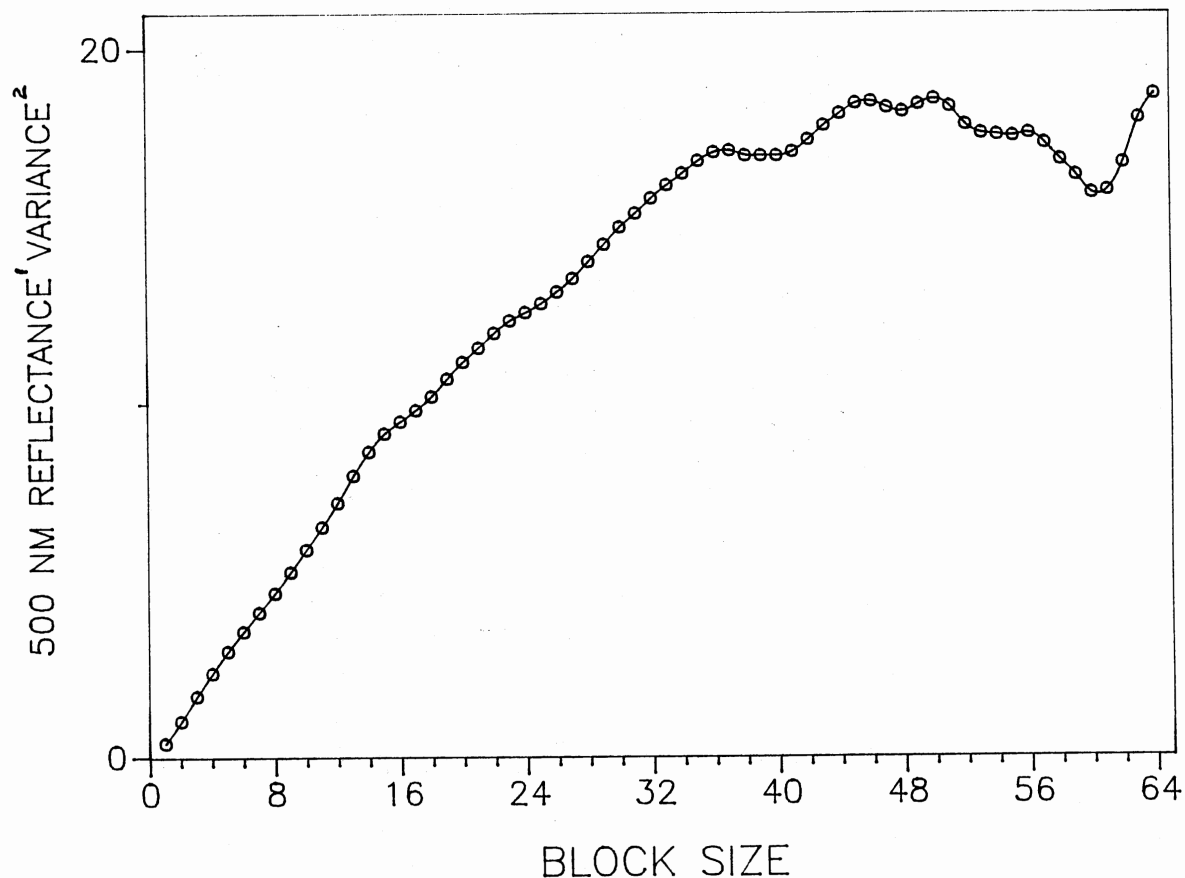

Figure 10. Distribution of block-size variance of 500 nm reflectance[1] across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots pine seedling bed in Washington, Oklahoma in 1987

[1] 3 reflectance readings were taken over each quadrat using a multispectral radiometer, adjusted according to incoming solar radiation and averaged.

[2] Variances of individual quadrat block sizes were determined using Hill's Two-Term Local Quadrat Variance method.

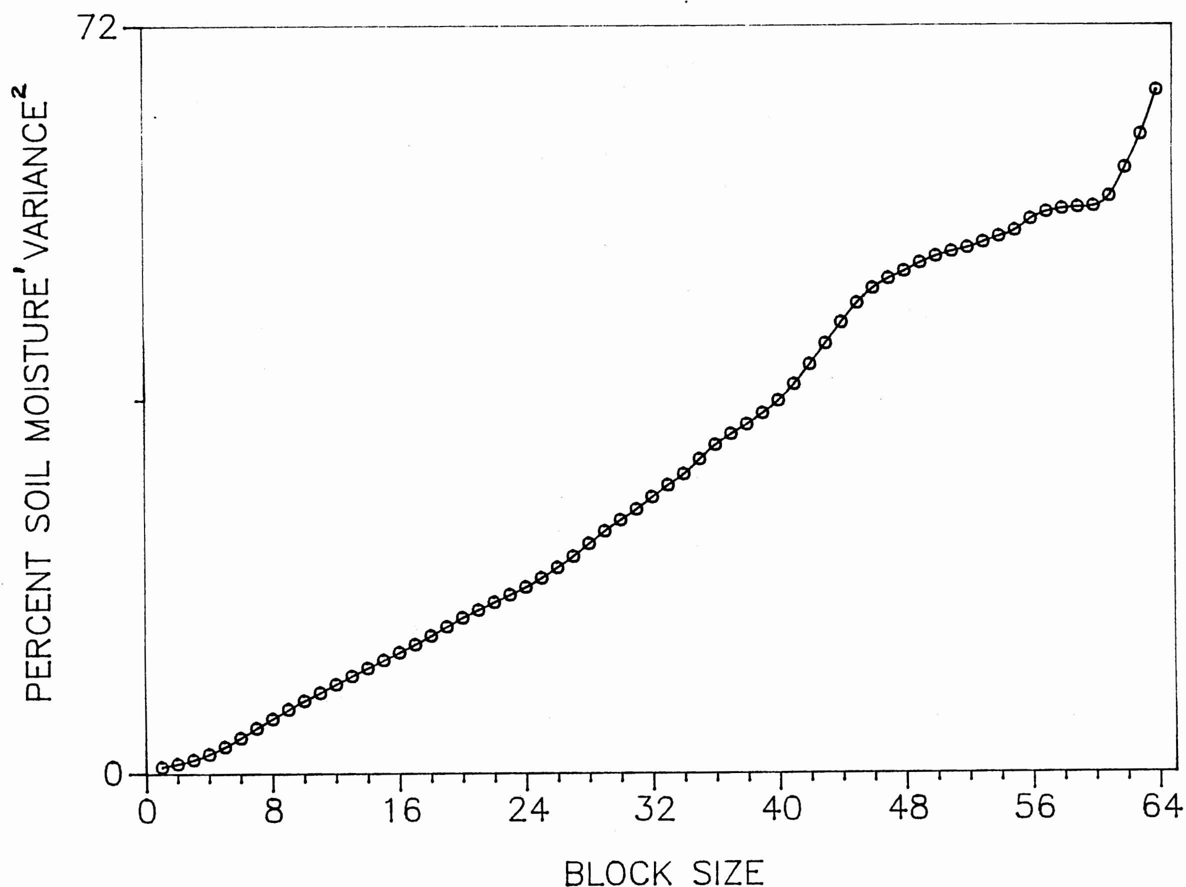

Figure 11. Distribution of block-size variance of percent soil moisture[1] across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots seedling bed in Washington, Oklahoma in 1987

[1]Percent soil moisture determined from subsamples of 5 bulked soil samples taken to a depth of 15 cm using 2 cm diameter soil probes.
[2]Variances of individual quadrat block sizes were determined using Hill's Two-Term Local Quadrat Variance method.

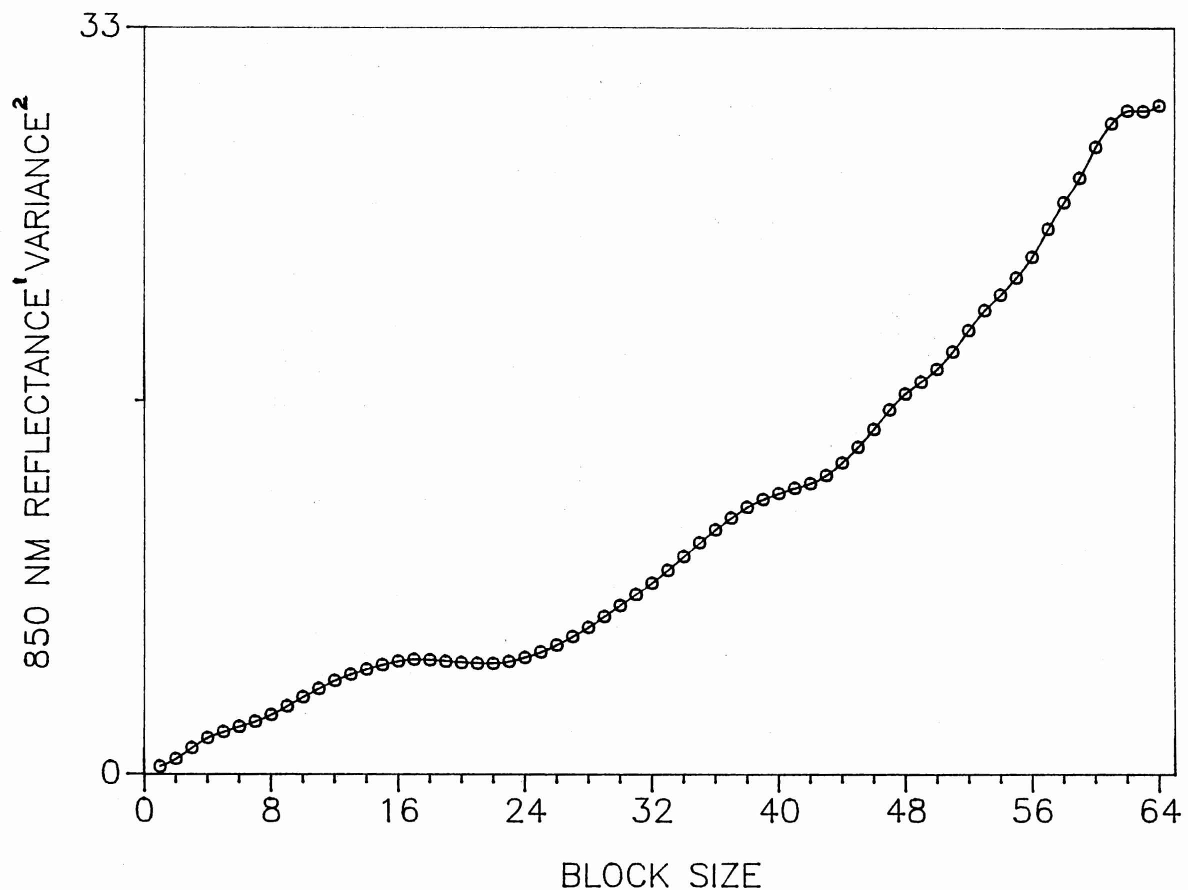

Figure 12. Distribution of block-size variance of 850 nm reflectance[1] across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots pine seedling bed in Washington, Oklahoma in 1987

[1]3 reflectance readings were taken over each quadrat using a multispectral radiometer, adjusted according to incoming solar radiation and averaged.
[2]Variances of individual quadrat block sizes were determined using Hill's Two-Term Local Quadrat Variance method.

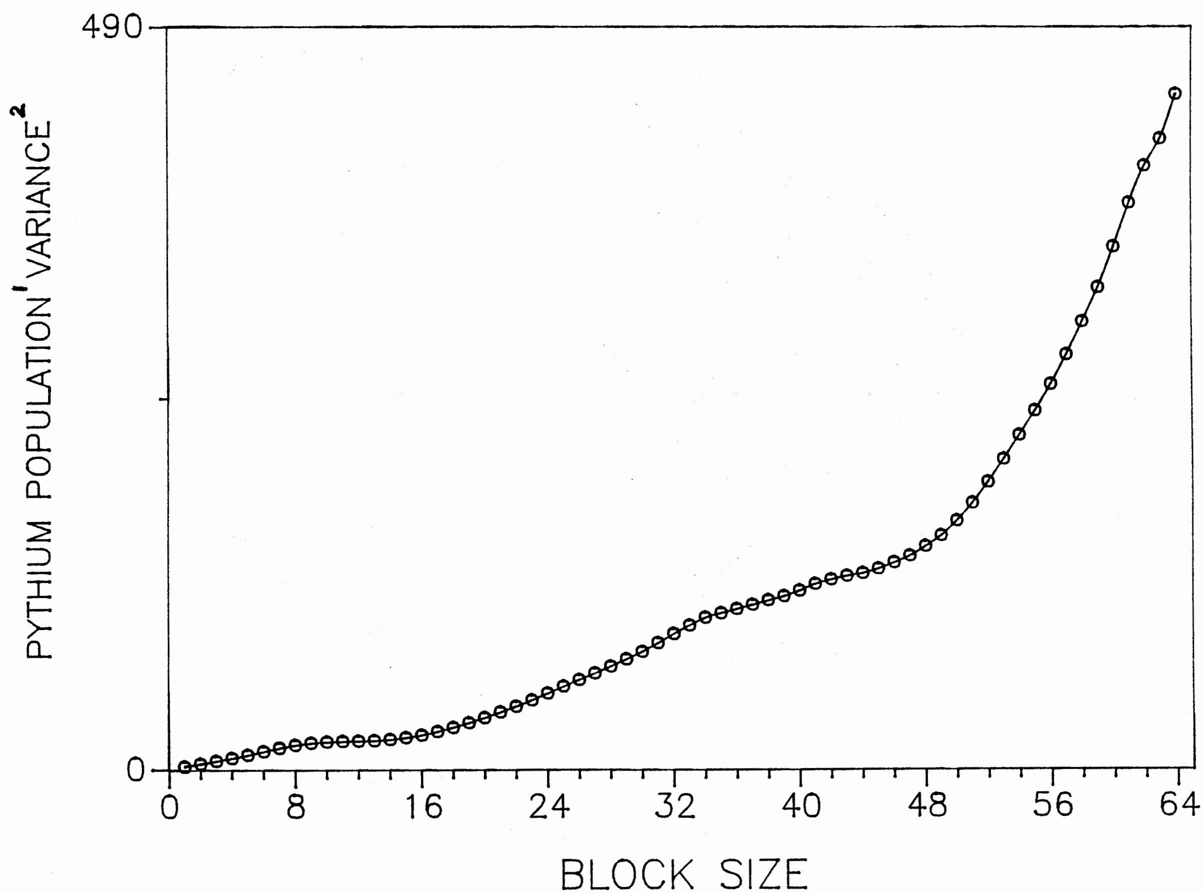

Figure 13. Distribution of block-size variance of <u>Pythium</u> spp. population[1] density across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots pine seedling bed in Washington, Oklahoma Oklahoma in 1987

[1]Number of <u>Pythium</u> spp. propagules determined by plating subsamples of 5 bulked soil samples taken to a depth of 15 cm using 2 cm diameter soil probes on Pythium Selective agar in 10 petri dishes and determining the mean number of colonies.
[2]Variances of individual quadrat block sizes were determined using Hill's Two-Term Local Quadrat Variance method.

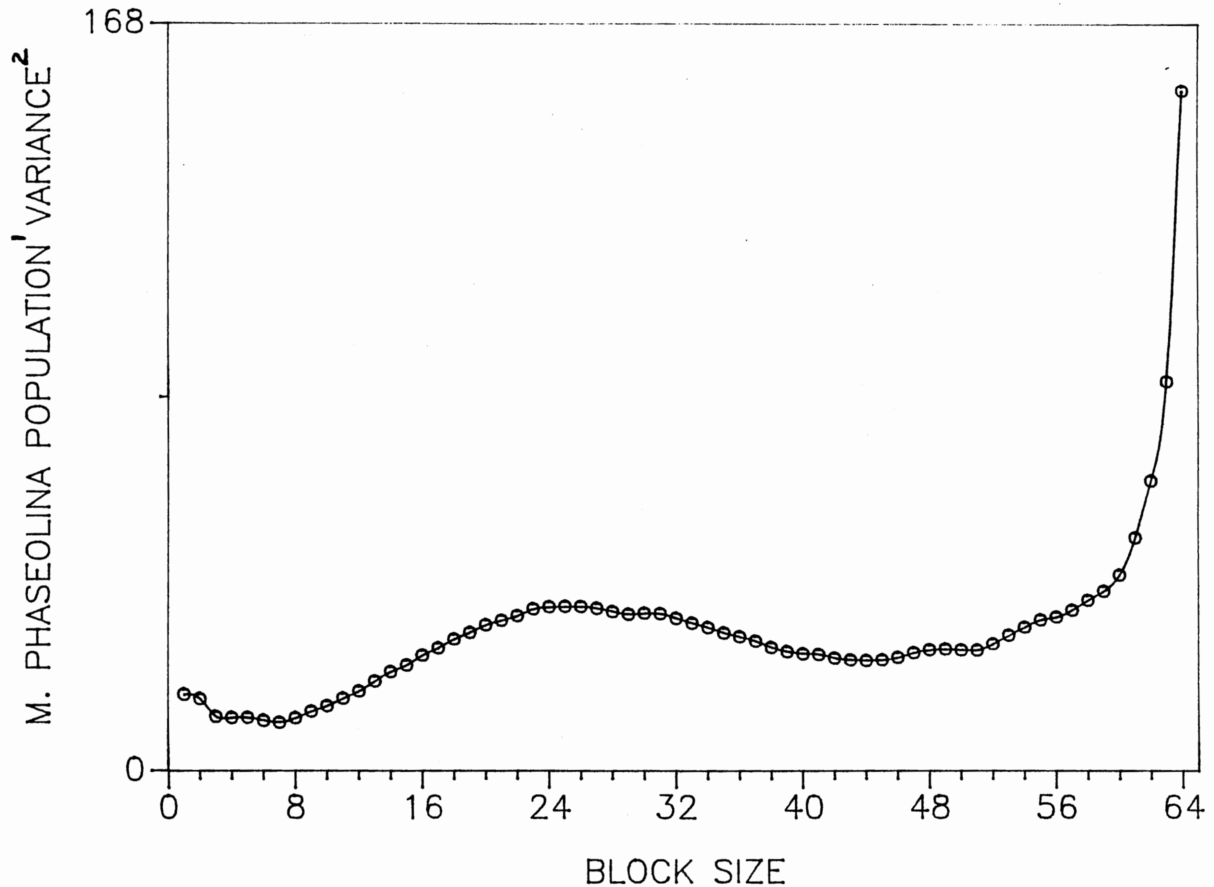

Figure 14. Distribution of block-size variance of Macrophomina phaseolina population[1] density across 1.2 X 1.2 m contiguous quadrats in a transect superimposed over an established scots pine seedling bed in Washington, OK in 1987

[1]Number of Macrophomina phaseolina propagules determined by plating subsamples of 5 bulked soil samples taken to a depth of 15 cm using 2 cm diameter soil probes on Dexon-Oxgall-PCNB-Captan agar in 10 petri dishes and determining the mean number of colonies.
[2]Variances of individual quadrat block sizes were determined using Hill's Two-Term Local Quadrat Variance method.

CHAPTER V

DISCUSSION

Solarization

Extended mid summer solarization treatment was most effective in reducing soilborne plant pest populations. No sclerotia of Sclerotium rolfsii from solarized soils germinated after the 1987 solarization period at Stillwater. Natural populations of Fusarium oxysporum and Pythium spp. were eradicated to a depth of 5 cm and significantly reduced to a depth of 15 cm. Similar results occurred in previous studies (Mihail and Alcorn, 1984; Porter and Merriman, 1983; Stapleton and DeVay, 1984; Katan, et al , 1976; Katan et al, 1983; Porter and Merriman, 1985; Kassaby, 1985; Martyn and Hartz, 1986; Pullman et al, 1981)

Preliminary studies indicated that most annual weed and plant parasitic nematode populations in solarized soils were either eradicated or reduced. Hershman et al. (1986) found parsley to be susceptible to attack by F. oxysporum, F. solani and P. irregulare among other soilborne fungi. All of these fungi were isolated from tree seedling roots from the Oklahoma State Department of Agriculture Central and Western Forest Regeneration Center at Washington. In this study, parsley was used as a trap plant for soilborne fungi from solarized and control soils. Root weight more than doubled and though not significant, an increased number of parsley seed germinated and the

plants grew larger in solarized soil than in control soil. The rapid growth of parsley plants and its' susceptibility to several common soilborne pathogens will provide a valuable assay of soilborne disease potential in the field and should be considered as a useful tool in future solarization and other soilborne disease control studies.

The natural population of Macrophomina phaseolina analyzed over all depths in the 1987 Washington Study was also reduced in solarized soils. However, reductions were not significant at depths assayed individually. In earlier published reports, 3 weeks of solarization and 6 weeks of solarization (McCain et al., 1982; Mihail and Alcorn, 1984), failed to control natural populations of M. phaseolina with solarization. The extended duration of the 1987 Washington Study (8 weeks) may account for the slight reduction found. Artificial populations of M. phaseolina in the concurrent 1987 Stillwater Study, concluded after 6 weeks, were not reduced by solarization. Unfortunately, even the residual populations in solarized soils after the 1987 Washington study were within the range reported for agricultural soils in which susceptible plants become infected. (Papavizas and Klag, 1975; Short et al, 1980; Young and Alcorn, 1982) If the agricultural system permits, projected research should include a solarization period of ten weeks or longer to determine the efficacy of longer periods of solarization for the control of M. phaseolina.

The addition of moisture to soil in the laboratory resulted in greater reductions in sclerotial populations of M. phaseolina at a specified temperature above the maximum for growth, indicating that abundant moisture should enhance population reduction in the field during solarization. However, results found in the current field studies suggested that moisture impaired the reduction of M. phaseolina

by solarization. Irrigation prior to solarization, which provided only a short term supply of moisture, was used in the 1987 Washington Study with some limited control of _M. phaseolina_, but drip irrigation, which maintained high soil moisture, was employed in the concurrent 1987 Stillwater Study with no control. Future solarization studies with drip irrigation and preirrigation employed in the same field for the control _M. phaseolina_ should be conducted.

Late spring solarization in the 1986 Washington Study resulted in limited control of _F. oxysporum_ and _Pythium_ spp. and late summer solarization in the 1986-87 Stillwater Study provided poor control of _S. rolfsii_. The limited control obtained may be due to the short four week duration solarization period. An extended period of solarization to control these fungi should be undertaken in future studies.

Another explanation for the variation in control between mid summer solarization and late spring or late summer solarization is the differences in ambient temperature and skycover. Average maximum and minimum air temperatures during the mid summer solarization periods were much higher than those during late spring and late summer solarization periods. In addition, the average amount of skycover from sunrise to sunset was much lower during mid summer solarization periods than late spring or late summer solarization in this study, greatly affecting the amount of sunlight reaching and heating the soil.

Obviously, ambient temperature and skycover during solarization can not be controlled at this time, but conducting solarization when the conditions are most likely to be optimal and solarizing for longer time periods will provide the best control possible within the limits of a specified system. Future research should include extended solarization periods at times and locations in which ambient temperature and skycover

are not ideal to determine if solarization is feasible under these marginal conditions.

Trichoderma harzianum Rifai added to field soil in previous studies controlled S. rolfsii (Wells et al, 1972) decreased infection of peanuts by S. rolfsii and increased yields (Backman and Rodriquez-Kabana, 1975). It was suggested that biocontrol techniques combined with heating or solarization might be used in more effective control of soilborne pathogens (Baker and Cook, 1974; Katan et al, 1976; Katan,. 1980). This synergistic control method was first demonstrated by Munnecke et al (1976) in the control of Armillaria mellea (Fr.) Quel.

Sclerotia of S. rolfsii exposed to sublethal heating are severely stressed and vulnerable to population reduction by microbial colonization (Lifshitz et al, 1983). Elad et al (1980) found that artificial infestation of T. harzianum immediately after solarization resulted in significant control of S. rolfsii beyond that provided by solarization.

The thermophilic nature of Trichoderma spp. (Crisan, 1973) would indicate that addition of this fungus to soil prior to solarization might further enhance the synergistic effect. However, though the effects of solarization on populations of Trichoderma spp. have not been directly investigated, studies indicate that the fungus would be detrimentally affected by solarization. The population of T. harzianum was negatively correlated to electrical conductivity of soil (Eastburn et al, 1985) which is greatly increased during solarization (Chen and Katan, 1980). Populations of most microorganisms decreased during solarization (Stapleton and DeVay, 1982, 1984), but thermophiles and highly competitive saprophytes decrease less during solarization than others. However, the cause of the reduction of populations of soilborne

thermophiles during solarization has not been thoroughly investigated and should be investigated in future studies. Due to increased nutrient levels within the solarized soil, (Chen and Katan, 1980), their populations increase rapidly after solarization. Elad et al. (1980) found that the low natural population of Trichoderma spp. at the conclusion of solarization increased dramatically after removal of the mulch.

Therefore, it is not surprising that at the conclusion of solarization in the current study, the population of Trichoderma spp. and most other epiphytes of S. rolfsii sclerotia were lower in solarized soils than control soils. A rapid increase in Trichoderma spp. may have been found in solarized soils, if the populations were assayed at a later date following removal of the mulch.

Another phenomena occurred in the 1986-1987 Stillwater Study. The average percent germination of S. rolfsii sclerotia was greater upon removal from soil in the spring of 1987 than those removed at the end of summer 1986, though those removed in the spring were first treated to the same effects as those from the summer of 1986. This suggested that a significant portion of those removed in 1986 may have been only dormant and not killed. The condition of inhibited germination of sclerotia in field soil has occurred in past research. Leach and Davey (1938) removed S. rolfsii sclerotia from soil in several fields at different times of the year by sieving. Their data showed that percent germinability, which he termed percent viability, was quite variable, and seemed independent of the site, time of the year or crop. Though fewer sclerotia were recovered in the spring, percent germination of recovered sclerotia was greater in several cases after the winter compared to late summer.

Javed and Coley-Smith (1973) recovered sclerotia of S. delphinii, (= S. rolfsii) buried at various depths in a field and illustrated the general decline in percent germination over a two year period. However, their data revealed that percent germination of sclerotia recovered from the soil occasionally dropped dramatically and recovered within short intervals such as a few months time. Germination was generally reduced or inhibited in September, as is indicated in the current study.

Much research, especially in recent years, has been devoted to the germination of sclerotia of Sclerotium rolfsii (Punja, 1985), but many inconsistencies have been found. Punja and Jenkins (1984) investigated the influence of temperature, moisture, modified gaseous atmosphere and depth of sclerotia within the soil on the germinability of sclerotia, but found that relationships were variable with soil type, available nutrients and with other microorganisms present in the soil. Beute and Rodriquez-Kabana (1979) concluded that wetting of dried, field-produced sclerotia was ineffective in stimulation of germination. A maximum of 18% germination was achieved with this method in their study. However in this current study, 100% germination of wetted, previously dried, field-produced sclerotia always occurred, if no treatment other than storage was applied. Linderman and Gilbert (1973) concluded that soil fungistasis of S. rolfsii is controlled by a complex balance of inhibitors and stimulators within the soil environment and further that both exogenous and endogenous factors, acting concurrently, consecutively or both may be involved in soil fungistasis.

Inhibition is an undeniable aspect of germinability of this fungus, especially with field-produced sclerotia. Different methods of assaying populations within the soil vary in their effectiveness to germinate sclerotia depending on many factors, but they may be sufficiently

reliable to determine population differences in an experiment. However, these are only germinable population differences. Assay of a germinable population at a single point in time may be misleading and/or not representative of the potential for viable inoculum over time. Researchers working with the control of S. rolfsii by solarization (Mihail and Alcorn, 1984; Porter and Merriman, 1983) or other means have referred to "the viable population," but the use of the phrase "germinable population at the time of the assay" may be more appropriate. Of primary importance is the crop yield increase obtained on current or subsequent crops by application of a disease control method. Whether this is gained through death of the fungus or by induced inhibition of germination, yield increase will still be realized on an annual crop planted immediately after control measures are applied. However, under these circumstances, long term disease control still remains a question. The effect which resulted in inhibited germination of a portion of the sclerotia, may have injured those sclerotia sufficiently to reduce pathogenicity when germination does occur or may result in a larger germinable population of the fungus present in the field to infect a future annual crop or a maturing perennial. A thorough investigation of the temporal fluxuations of the germinable population of S. rolfsii should be undertaken. In addition, more studies should be undertaken to determine the mechanisms involved in germination of sclerotia of S. rolfsii and to develop a reliable method for stimulating germination of any viable sclerotia.

Apart from cost factors, the efficacy of employing solarization for the control of soilborne pests is dependent upon the heat tolerance of the target pathogen and whether or not the remaining viable population is adequate to cause sufficient disease to result in economic loss. In

Oklahoma, climate conditions in mid summer are suitable for effective reduction of populations of nonthermophilic phytopathogenic soilborne fungi and reducing or eliminating disease, as well as drastically reducing the weed population. Unfortunately, many competitors and antagonists within the soil are reduced during solarization (Stapleton and DeVay, 1982). This may set the conditions for increased levels of disease caused by thermophilic organisms such as _M_. _phaseolina_, a possibility which should be thoroughly investigated prior to employing solarization in areas where _M_. _phaseolina_ and other thermophiles are important pathogens.

The long-term disease control aspects of solarization in Oklahoma should be investigated. Previous studies (Katan et al. 1983; Stapleton and DeVay, 1983) found that pathogen populations and disease were still lower in solarized plots after three years. The effects of solarization are not detrimental to either the consumer, environment or the individual applying the treatment (Katan, 1981). The agriculturalist may find that long term control of soilborne plant pests using solarization may be cost efficient in agriculture. Annual application of solarization is not economically feasible for most crops, but annual solarization may not be necessary.

The application of biocontrol technologies or specific pesticides, that provide some control alone, in combination with solarization may enhance the duration of control and may eliminate or effectively reduce populations of thermotolerant, soilborne plant pathogens, such as _M_. _phaseolina_. Investigation of possible synergistic control methods should be quite promising.

Thermal Death

The results in this study indicate that of the two fungal species examined, Pythium irregulare is much less tolerant to exposure to high temperature. At temperatures above 50 C, P. irregulare was nearly always completely inactivated within two hours in wet or dry soil. Sensitivity to 40-50 C was also apparent, though as expected, tolerance increased as the temperature decreased.

The presence of moisture greatly increases the sensitivity of microorganisms to the effects of temperature extremes (Farrell and Rose, 1967). In this study, the presence of soil moisture generally enhanced the reduction of viable oospores at each temperature. Continuous exposure to high temperatures more drastically affected oospores. Oospores exposed to two hour intervals of extreme temperature daily, which approximate the effects of solarization, withstood high temperature exposures for a longer total duration. With two hour intervals at 50 C daily in wet soil for example, the LD_{90} was 3.7 days or 7.4 hours of actual exposure to 50 C. However, with continuous heating the LD_{90} was only 2.7 hours.

Macrophomina phaseolina was tolerant to high temperature. The fungus survived nearly 6 hours of continuous exposure to 60 C in dry soil. Below 60 C, the sensitivity of M. phaseolina was greatly reduced and below 50 C, little or no reduction was found in dry soil with less than 32 days of continuous heating. Moisture had a profound effect on the susceptibility of M. phaseolina to temperature, especially at 50 C or higher. At 55 C in dry soil, 8 days of continuous heating was required to eliminate the viability of sclerotia, but in wet soil M. phaseolina did not survive 12 hours of continuous heat. There was also

evidence that sclerotia of _M. phaseolina_ were also able to tolerate longer total exposure to high temperature, if the exposure was intermittent. This was most apparent at 45 and 40 C where, in dry or wet soil, little or no reduction of sclerotia was observed even after 32 days of two-hour daily heating intervals, but the population of sclerotia steadily declined with the application of continuous heating.

These results also possibly provide a method for predicting the effects of solarization on _P. irregulare_ and _M. phaseolina_. Both fungi were eliminated from wet or dry soil at 55 C or higher in less than 32 days of two-hour daily heating intervals. However, temperatures of 55 C or higher either do not occur or are not consistently achieved during solarization (Katan, 1981). Temperatures of above 45 C for more than two hours daily often occur during solarization and previous studies in Israel and California have reported intervals of more than two hours daily in which temperatures exceeded 50 C (Katan, 1976; Pullman, et al, 1981; Grinstein, 1979). The results in this study indicate that _P. irregulare_ oospores would be completely eliminated from solarized plots within 32 days to the depth where temperatures reach at least 50 C whether the soil is kept moist or not. Even if environmental conditions were not consistent during mid summer solarization, the major portion of _P. irregulare_ would probably be destroyed.

Macrophomina phaseolina, which proved to be the more thermotolerant fungus in this study, would not be as readily affected by solarization as _P. irregulare_. If irrigation either prior to or during solarization was not employed, little reduction of viable sclerotia would occur with maximum daily temperatures under the mulch less than 55 C. Even though high levels of moisture might be maintained, the fungus is not likely to be eliminated under normal solarization conditions and any reduction

that does occur, may not be sufficient to reduce disease (Papavizas, 1975; Young and Alcorn, 1982).

Pathogenicity Studies

Isolates of P. irregulare obtained from diseased tree seedlings were found to be pathogenic. Koch's postulates were fulfilled with one isolate, ESp3, on all individual seedlings of sycamore, scots pine, virginia pine and arborvitae in artificial potting soil. ESp3 was highly virulent; killing all individual seedlings tested. In this study, however, P. irregulare was provided a food base from which to colonize the host plant and few or no antagonistic microorganisms existed in the artificial medium or in pasteurized field soil, so the aggressive nature of the fungus exhibited in the greenhouse may not be representative of the field situation. Future research should include studies designed to determine the pathogenic nature of the fungus in the field on the host species investigated in this study.

Macrophomina phaseolina isolates varied in host range and pathogenicity. Actively growing cultures used to infest artificial potting media were provided with a food base of PDA. Two isolates, not obtained from diseased trees, could not be recovered from any plant in infested media, but three isolates from silver maple and apple were recovered from all tree species. All isolates obtained from diseased tree seedlings proved to be pathogenic to sycamore seedlings in artificial potting media, but varied in their ability to attack other tree species. In artificial potting soil, M. phaseolina isolate SyV2 was the most aggressive and remained pathogenic on sycamore and virginia pine in pasteurized field soil, though low percent recovery of the fungus from trees in field soil suggested that it was less aggressive in

field soil compared to artificial potting soil. However, this may not be the case. Field soil was not infested with actively growing cultures. Thus, conditions may have been less suitable for the fungus to infect the seedlings. Moisture stress, which occurs normally on seedlings at OSDACWFRC, resulted in only a slight increase in the number of either host species colonized. Further study of M. phaseolina at OSDACWFRC is needed to determine the true pathogenic potential of the fungus there.

Distribution

Previous studies of the inoculum pattern of soilborne fungi have revealed clumped arrangements (Smith and Rowe, 1984; Strandberg, 1973; Punja et al, 1985). A study of Pythium aphanidermatum revealed an intensely clumped pattern (Stanghellini et al, 1982) and the pattern of Macrophomina phaseolina was also found to be clumped (Mihail and Alcorn, 1987).

Similarly, using Hill's two-term local quadrat variance method, the patterns of sclerotia of M. phaseolina and propagules P. irregulare in this study were found to be clumped. The patterns of percent soil moisture, mulch coverage, number of pines and reflectance readings were also analyzed using Hill's method. All parameters analyzed were contagious with several parameters revealing sharply defined distributions within the transect.

Regression correlation analysis was quite useful in relating spatial patterns and distributions within this study. In this discussion, the term "pattern" will be used to describe the spatial arrangement across the field or transect studied. The term "distribution" refers only to the variance arrangement across block

sizes analyzed as determined by Hill's method. Variance peaks at specific block sizes indicate the size of clumping that describes the pattern of a parameter (Hill, 1973). Significant correlation of distributions indicate similar clump size and significant positive correlation of patterns indicate that the location of the center of clumps of different parameters were at similar locations within the transect. Significant negative correlation of patterns indicate that the center of a clump of one parameter was close to the spatial center or depression between two clumps of another parameter, in other words, where the population or value of one parameter is at the maximum, the other is near it's minimum.

Some relationships that occurred in this study were expected, but not all. Expected was the strong positive correlation between *Pythium* spp. and percent soil moisture, because *Pythium* spp. generally thrive in moist soil (Lumsden et al, 1976). The distribution and patterns were each significantly positively correlated, indicating a possible cause-effect relationship. The distributions and patterns of *Pythium* spp. and *M. phaseolina* were also strongly positively correlated. The factors that predispose a plant to infection by one pathogen may do likewise for another pathogen. Secondary infection of seedlings could account for this relationship. Another expected relationship was that between the distributions and patterns of percent soil moisture and mulch coverage, both strongly positively correlated. Mulch inhibits the loss of moisture from the soil due to evaporation, maintaining more suitable environmental conditions for growing seedlings.

The distribution of pines was significantly correlated with the distribution of percent soil moisture, but the patterns of these two parameters were significantly negatively correlated. This might be

expected, because where seedlings are most abundant, transpiration and thus uptake of moisture and soil moisture loss would be the greatest.

A very high distribution correlation occurred between mulch coverage and pines, indicating their clump sizes were quite similar and some cause-effect relationship might exist between pines and mulch or between pines, mulch and a third factor. Mulch placed over the beds prior to planting stabilizes the soil and decreases moisture loss, thus creating an environment more suitable for the growth of seedlings, but flooding from heavy rains may displace the mulch or the mulch may not be distributed evenly initially, both result in areas of the bed with reduced mulch coverage. Once the seedlings are established, the soil is more stable and the mulch is less likely to be displaced. A positive pattern correlation between pines and mulch would most likely be expected, since the pines help prevent the displacement of mulch. However in this study, the patterns of pines and mulch coverage were significantly negatively correlated, indicating that where the maximum mulch coverage occurred, the minimum number of pines was found and visa-versa.

Four possible hypothesis might explain the negative pattern correlation that occurred between mulch and pines. First, the pines and mulch have no effect on the distribution of the other and their pattern similarity is purely coincidental. This hypothesis is suspect, however, because the degree of correlation significance is quite high. Secondly, some factor, not assayed in this study has affected the pattern of one of these parameters, resulting in the conditions present at the time of sampling. An environmental component or cultural practice could be involved. Thirdly, the mulch has a negative effect on the germination of seeds and /or the development of seedlings. If any of the above

cases represent the actual condition in the field, it appears that the mulch has no positive effect on the pines and a thorough investigation of the true relationship between this mulch and pines should be undertaken. The fourth possibility is that observations of one parameter taken from the quadrats may have been influenced by the fluxuations of the other parameter, thereby resulting in sampling error. However in this study, this would only be expected to be the case if the density of the pine foliage over some of the quadrats had been great enough to obscure the observation of the mulch. In the transect sampled, even in the quadrats most densely covered by pines, the majority of the mulch or soil surface over the quadrat could easily be viewed.

Sclerotia of M. phaseolina were found adhering to the crown of numerous stunted, chlorotic or dead seedlings at the time of sampling. The sampling procedure for the assay of M. phaseolina involved sampling between rows of seedlings. In this way, the pattern of inoculum resulting from previous crops could be determined and correlated with the size of the current crop. If this fungus was a major factor in the pattern of pines, then a significant distribution correlation and a significant negative pattern correlation would be expected. However, no significant correlation was found between M. phaseolina and pines and it is likely that the fungus was not a factor in the pattern of the pine crop present at the time of sampling. Thus some other factor, possibly environmental or cultural was responsible for the occurrence of M. phaseolina infection on the crop present at sampling. Injury to the established seedlings, which occurs in the field with the pruning process of root undercutting, increases the number of sites on the seedling available for colonization by pathogenic fungi. If performed

at too shallow a depth, undercutting would result in sufficient stress to seedlings to predispose them to attack by M. phaseolina, but may also reduce the root mass sufficiently to result in the premature death of the plant. An investigation should be undertaken to determine if M. phaseolina is the cause of the decline of these seedlings or if the decline of these seedlings due to cultural practices or environmental stress results in an increase in the population of M. phaseolina.

Pythium spp. were isolated from numerous diseased seedlings during this study. Only a slight negative pattern correlation was found between pines and Pythium spp. This indicates that the occurrence of Pythium spp. may have a small effect on the pattern of pines at the time of sampling.

The distribution of 500 nm reflectance correlated most highly with the distribution of soil moisture, mulch and pines, indicating similar clump sizes. The pattern of 500 nm reflectance was significantly negatively correlated with pines. A positive correlation was found between the pattern of 500 nm reflectance and the pattern of percent soil moisture or the pattern of mulch. Reflectance of the 500 nm wavelength of light (blue-green) was probably an indication of mulch that was dark-colored due to absorbed moisture.

The 850 nm reflectance distribution was very highly correlated to the distributions of both Pythium spp. and percent soil moisture, but the pattern of 850 nm wavelength reflectance was significantly negatively correlated with the pattern of Pythium spp. and less so with percent soil moisture. Reflectance of the 850 nm wavelength of light (infrared light) was probably an indication of warm moisture content of the soil on the cool sampling day and would not provide a good method for quick counts of populations of seedlings, at least at the stage of

growth of seedlings found in this study. Reflectance readings of 850 nm will not be useful in the detection of disease of tree populations or population densities at the stage of growth of seedlings found at the time of sampling, but may be useful in seedling beds with more mature densely-foliated seedlings and this should be investigated in future studies.

If performed correctly with efficient methods, the results found in this study are still representative of only one point in time in the particular pine seedling bed studied. A thorough, larger-scale, spatial and temporal study should be conducted to examine the relationships indicated in this study.

Proposed Research

1. Further examination of parsley as a trap crop for the assay of populations of soilborne pathogens in the field should be undertaken.
2. The efficacy of solarization periods longer than four weeks for the control of _S. rolfsii_, _F. oxysporum_ and _P. irregulare_ in seasonal periods when ambient temperature and skycover are not optimal should be determined.
3. Conducting more thorough studies of the efficacy of solarization periods longer than four weeks during seasonal periods when ambient temperature and skycover are optimal for the control of _M. phaseolina_ would be beneficial.
4. A thorough examination of the effects of drip irrigation as opposed to preirrigation in solarization studies for the control of _M. phaseolina_ should be conducted.
5. Studies should be conducted to determine the cause of reduction during solarization in populations of thermophilic antagonists and

saprophytic competitors of phytopathogenic soilborne fungi.

6. A need is indicated for studies of the possible enhancement of thermophilic soilborne phytopathogenic fungi due to the reduction of antagonists and competitors during solarization.

7. More detailed studies of the reduction of weed and nematode populations in Oklahoma using solarization should be performed.

8. A determination should be made of the efficacy of solarization for long-term disease control on crops in which annual solarization is not economically feasible.

9. Studies should be conducted to determine the temporal fluxuations of the percent germination of sclerotia of S. rolfsii over two or more years in fallow fields, in fields with a host crop and in fields with a nonhost crop.

10. The mechanisms involved in the germination of sclerotia of S. rolfsii and development of a consistently reliable procedure for germination of all viable sclerotia of the fungus should be attempted.

11. Investigation of the pathogenic potential of M. phaseolina and P. irregulare on tree seedlings grown at the OSDACWFRC is indicated.

12. A determination should be made of whether the woodchip mulch used at the OSDACWFRC is beneficial or detrimental to the growth of pines seedlings and if found to be detrimental, the cause of the negative effect should be further investigated.

13. A more thorough investigation of the use of radiospectrophotometry for disease detection or population density determinations on pine seedlings at various stages of maturity should be undertaken.

14. A large-scale, spatial and temporal study of nursery tree seedlings during the entire process of growth from planting to harvest should conducted at the OSDACWFRC.

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VITA [2]

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