EVALUATION OF TOLCLOFOS-METHYL FOR CONTROL OF POD ROT OF PEANUT CAUSED BY *RHIZOCTONIA SOLANI* IN OKLAHOMA

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PREFACE

Evaluation of fungicides for control of peanut pod rot incited by Rhizoctonia solani in Oklahoma was investigated in this study. Sensitivities to tolclofos-methyl and pentachloronitrobenzene of naturally occurring isolates of R. solani and Rhizoctonia-like fungi were assessed in a defined medium containing the fungicides. Microplot and greenhouse studies were employed to evaluate the efficacy of tolclofos-methyl in reducing populations of R. solani, reducing peanut pod rot and in increasing yield.

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LITERATURE REVIEW

Characteristics of *Rhizoctonia solani*

Rhizoctonia solani Kuhn is a ubiquitous soilborne plant pathogenic fungus (Baker, 1970). Isolates are capable of attacking a broad range of host plants including horticultural crops, field crops, ornamentals and turf grass. *Rhizoctonia solani* causes seed decay, damping-off, stem cankers, root rots, fruit decay and foliage diseases.

Major morphological characteristics of *R. solani* include rapidly growing hyphae of large diameter (about 5 microns) increasing in dark (brown) pigmentation with age, branching at nearly right angles near the distal septum of cells, formation of a septum at each hyphal branch, and more than two nuclei per hyphal cell. Some isolates produce sclerotia and/or monilioid cells. *Rhizoctonia solani* produces the basidiomycetous teliomorphic state *Thanatephorous cucumeris* (Frank) Donk (Talbot, 1970). *Rhizoctonia solani* does not develop conidia, clamp connections or rhizomorphs.

Rhizoctonia solani is made up of a collection of non-interbreeding populations recognized and identified through the anastomosis group (AG) concept (Anderson, 1982). The basis of the AG concept is that hyphal fusion occurs only

between isolates from the same AG. Presently, there are seven AG's: AG 1, AG 2, AG 3, AG 4, AG 5, AG 6, and AG B1 (Kuninaga and Yokosawa, 1980). Isolates in AG 4 can infect a broad range of plant hosts especially legumes, such as peanut.

Genetically, AG 4 isolates arising from single basidiospores and cultured in the laboratory, are homokaryons, whereas field isolates are heterokaryons. Isolates belonging to different AG's are not able to anastomose with one another and do not form heterokaryons (Anderson, 1982). Following anastomosis of heterokaryotic field isolates, new isolates that are morphologically and pathogenically different from their parents can arise (Bolkan and Butler, 1974). The process of heterokaryosis is essential for pathogenicity and virulence of the AG 4 isolates. An avirulent isolate, after heterokaryon formation, may carry genes for virulence (Anderson, 1982). In addition, heterokaryosis is a mechanism for the formation of R. solani isolates with increased tolerance to pentachloronitrobenzene (PCNB) (Meyer and Parmeter, 1968).

Ecology and Epidemiology

Rhizoctonia solani can survive in soil by parasitism of a host, saprophytic colonization of organic matter in soil, and/or formation of persistant structures (Garrett, 1970; Papavizas, 1970). Hyphae are generally considered to be resistant to lysis imposed by soil microorganisms, and

capable of high saprophytic ability and active growth from a food source through the soil (Papavizas, 1970). In the absence of colonizable hosts or organic debris, the fungus maintains itself in soil by the formation of sclerotia. Isolates of R. solani differ in their abilities to use the survival mechanisms available to them. For instance, cultivated fields in several states, including Florida, Texas, New Mexico and Nebraska, contained a number of distinct R. solani isolates with various saprophytic and pathogenic potentials on several crops, including bean, radish, beet, wheat, and soybean (Papavizas and Davey, 1962).

Soil temperature plays an important role in the parasitism of R. solani in the soil, affecting the growth of the organism, it's ability to survive and it's potential for infection. Benson and Baker (1974a) reported an optimum temperature of 30 C at -0.27 bars (approx. field capacity) for growth of R. solani. They considered growth of the organism to be a measure of its inoculum potential and found survival of the fungus was optimal at 15 C in dry soil. Papavizas et.al. (1975) reported there was a significant (P=.05) positive correlation between soil temperature and inoculum density, and that 5-15 C was the most conducive to survival of two isolates of R. solani. Low winter temperatures were partly responsible for reduced activity of the fungus. Martin and Lucas (1984) reported the optimum temperature for radial growth of R. solani and Rhizoctonia-like

fungi on potato dextrose agar was 28 C. All of these isolates were from diseased turfgrass.

High soil moisture promoted a pod rot of peanut in Israel; however, if the topsoil was allowed to dry completely, disease was retarded, but not prevented (Frank, 1974). Papavizas (1970) has discussed the influence of soil moisture on the growth of R. solani in soil. He reported that the saprophytic capability of R. solani increases at soil moisture contents of 20-60% of the moisture holding capacity. At saturation, however, growth of R. solani was reduced due to reduced soil aeration and an increase in carbon dioxide concentration. In addition, high soil moisture contents may increase bacterial populations which cause lysis of R. solani mycelium. At 30% saturation there was no bacterial lysis of the hyphae; however, bacterial lysis increased with increasing soil moisture. In Oklahoma, temperature extremes during the growing season and the low moisture holding capacity of many of the soils may affect the disease potential of R. solani.

Nutrients for energy and precursors for the synthesis of new structural materials are essential for growth and host infection by fungi. General nutrient sources are available to the fungus: endogenous nutrients within the the fungal propagule, plant tissue including plant seed and root exudates, and organic debris in the soil or organic matter in solution (Garrett, 1970). Weinhold et al. (1969) found that without the proper nutrients available, growth and

infection by R. solani were decreased. They found a direct relationship between the amount of available carbon or nitrogen and virulence, and that the amount of nutrients required for infection was greater than for vegetative growth. Kamal and Weinhold (1967) reported a decrease in virulence after a short period in the soil, and related this decrease to the loss of nutrients necessary to support the metabolic activity of the fungus.

The application of soil fungicides may affect nutrient availability and disease. Farley and Lockwood (1969), found that PCNB reduced nutrient competition in soil by inhibiting PCNB-sensitive fungi and actinomycetes, which selectively favored the increase of fungi, tolerant to PCNB. Littlefield (1967) performed tracer studies using ^{32}P and suggested a relationship between the ability of the thallus of R. solani to translocate nutrients and susceptibility to fungicides. The ability of fungicides to inhibit or kill the fungus depended on how the toxicant was moved through the organism and where it was stored.

Inoculum densities of R. solani in natural field soils are generally much lower than for several other soilborne phytopathogenic fungi. For instance, Weinhold (1977) sampled 60 cotton and potato fields in California and found 77% contained less than 2 propagules (p)/100g soil with a range of 0 - 15 p/100g soil. A similar range was found for 33 peanut soils sampled in Texas and New Mexico (Woodward and Jones, 1983). The inoculum density of R. solani needed

to cause disease depends on several factors, including the susceptibility of the host, the virulence of the isolate, and soil environmental factors regulating the host-pathogen interaction. Nonetheless, inoculum densities required for 50% or greater disease incidence (or severity) are generally 1-10 p/100g soil. For instance, Benson and Baker (1974b) showed that 0.76 p/g soil caused a 50% damping-off of radish seedlings. Emergence of cotton seedlings was decreased 50% in soil at 5 p/100g soil (Weinhold, 1977) and 10 p/100g soil completely kill 'Tamnut 74' peanut seeds (Woodward and Jones, 1983). Garcia and Mitchell (1975) found that 10 sclerotia/g autoclaved soil caused a small, but significant (P=.05) increase in pod rot of 'Florunner' peanut beyond those grown in noninfested soil.

Several methods used to artificially infest soil with R. solani are discussed in some detail by Papavizas and Lewis (1986). In regard to use of artificially-infested soil, Benson and Baker (1974b) found that after inoculating natural soil with cultures grown on cornmeal sand or chopped potato soil at three different inoculum densities (37, 85, and 127 p/100g soil) of R. solani there was a brief increase followed by a rapid and drastic decline (up to 90%) in the number of detectable propagules. Thereafter, throughout the experiment, inoculum densities (ID) remained low, but were proportional to the original infestation density. They concluded that the initial ID did not affect the rate at which propagules of R. solani became inactive in the soil.

Recently, Papavizas and Lewis (1986) reviewed several methods for isolating R. solani, including the beet seed colonization method (Davey and Papavizas, 1962), paper disk method (Herr, 1975), debris particle method (Davey and Papavizas, 1962), wet sieving (Weinhold, 1977) and the multiple pellet soil sampler (MPSS) (Henis, et al., 1978). They reported the MPSS method depends on the assumption that the fungal propagules are randomly distributed in the soil. As R. solani may not be randomly distributed in natural soil, Papavizas and Lewis question the use of the MPSS method for natural soils. However, random distribution may be assumed for artificially-infested soil. Use of the MPSS in naturally-infested soils is justified if care is taken in sampling to obviate errors in pathogen clumping (Dr. Ralph Baker, personal communication).

Pod Rot And Other Diseases Of Peanut Caused By Rhizoctonia solani

Rhizoctonia solani (AG 4) can attack all parts of the peanut plant and at all stages of development (Porter, et al., 1982). Diseases include pre- and post-emergence damping-off; root and stem rots; foliage, limb and peg blights; and pod rot.

Pods may be attacked by R. solani during any stage of their development. Often the tips of pegs are infected first and infection spreads into the developing pod. When pods are dug, this weakened junction breaks, leaving pods in

the soil. Symptoms on mature pods include brown to black, sunken lesions which often result in a complete blackening of the hull, accompanied by various stages of hull and kernel decay (Garren, 1970; Moore and Wills, 1974).

Although pod rot can be caused by individual fungi such as R. solani, the disease is most often considered to have a complex etiology. Frequently found with R. solani in peanut soils are other phytopathogenic fungi such as Pythium myriotylum (Garren, 1970; Frank, 1972) and Fusarium solani (Frank, 1972). In addition to fungi, plant-parasitic nematodes (Garcia and Mitchell, 1975b) also have been implicated in enhancing pod rot disease severity. Among the abiotic factors, soil fertility, particularily the role of calcium nutrition in the disease, has received study (Csinos and Gaines, 1986).

Control Measures For Peanut Pod Rot

Presently, genetic resistance in peanut cultivars to pod rot caused by R. solani is limited. Some work has been done in Texas (Godoy et al., 1984) in which the cultivars Toalson and Tx AG-3 were shown to have less Rhizoctonia pod rot than Florunner. Although the cultivars Spanco and Okrun are considered to be less susceptible to pod rot (Dr. Ron Sholar, personal communication) in Oklahoma, the cultivars were not tested specifically against R. solani.

Several fungicides such as benomyl, captan, carboxin, and pentachloronitrobenzene (PCNB) are available with

activity against R. solani. However, for decades, PCNB has been the primary chemical used as a soil fungicide to control diseases caused by R. solani in several crops.

Studies have been conducted to determine how PCNB controls R. solani in soil. Ko and Oda (1972) found that PCNB does not kill R. solani, but is a strong growth suppressor (fungistat). At inoculum densities of 5% R. solani, damping-off of radish seedlings was 100%; however, with the addition of PCNB (100 ug/g) damping off was reduced to 4% but the R. solani populations remained the same. Bristow et al. (1973) found that PCNB accumulated in plant tissue, and control of damping-off of bean seedlings was directly related to the concentration of PCNB accumulated. They reported that increasing the amount of organic matter reduces the amount of PCNB that accumulates in the plant tissue, thus decreasing protection.

Pentachloronitrobenzene has been used for the control of southern blight, seedling diseases, and pod rot of peanut in Oklahoma for several decades. Yet these diseases still pose serious economic problems to many Oklahoma peanut growers. The extensive use of PCNB coupled with no significant decreases in these diseases suggested increased fungal resistance to PCNB. In a preliminary report, some isolates of R. solani taken from Oklahoma peanut fields were shown to have EC_{50} values of 375 - 1000 ppm PCNB (75% ai WP) in amended media. These PCNB tolerant isolates were pathogenic to Florunner and Spanco cultivars, both grown extensively in

Oklahoma (Filonow, 1984).

Recently, a combination of metalaxyl (effective against Pythium spp.) and PCNB called, Ridomil-PC, was labeled for use in Oklahoma for pod rot control of peanut. Although Ridomil-PC was effective in reducing Pythium spp. populations in treated soils, R. solani populations increased in some treated soils (Filonow et al., 1987).

Tolclofos-methyl (O, O-dimethyl-O (2, 6-dichloro-4-methylphenyl)-phosphothioate; trade name: Rizolex) is an organothiophosphate with curative and slightly systematic action and a reported high degree of toxicity (0.01 ug a.i./ml agar) towards R. solani (Sumitomo Chemical Co. Technical Report). Van Bruggen and Arneson (1984) found that tolclofos-methyl decreased hyphal growth of 9 isolates from New York State on amended media. Successive transfers of some fungal colonies resulted in adaptive growth at concentrations up to 400 ug tolclofos-methyl/ml agar which did not alter the pathogenicity of the isolates.

Csinos (1985) compared PCNB to tolclofos-methyl on two R. solani isolates and reported that tolclofos-methyl reduced peanut limb rot damage at lower concentrations (8.4 kg /ha) than PCNB (11.2 kg/ha).

The reported high activity of tolclofos-methyl against R. solani plus the research of others indicated that this fungicide may have potential for control of peanut pod rot caused by R. solani. Due to the frequent presence of this fungus in Oklahoma peanut fields, coupled with occasional

severe yield losses from pod rot, evaluation of tolclofos-methyl for control of pod rot under Oklahoma growing conditions was considered worthwhile.

This thesis is presented in an alternative format which deviates from the traditional thesis outlined in the Thesis Writing Manual provided by the Graduate College of Oklahoma State University. The thesis is composed of four chapters; a literature review and two manuscripts written according to the format style established by the American Phytopathological Society for manuscripts submitted to the professional journals of PHYTOPATHOLOGY or PLANT DISEASE. The fourth chapter is an appendix which includes data not given in the manuscripts.

Chapter II, entitled "Sensitivity of Rhizoctonia solani isolates and Rhizoctonia-like fungi from Oklahoma Peanut Fields to Tolclofos-methyl and Pentachloronitrobenzene" is a report of the sensitivities of 17 R. solani isolates and 3 Rhizoctonia-like isolates from diseased peanut pods from Oklahoma peanut fields, to tolclofos-methyl or pentachloronitrobenzene (PCNB) in amended media. Chapter III entitled "Evaluation of Tolclofos-methyl for control of peanut pod rot caused by Rhizoctonia solani in Oklahoma" describes a study involving two field experiments and several greenhouse experiments to evaluate the effectiveness of tolclofos-methyl in reducing soil populations of R. solani, reducing pod rot severity and increasing yields. These manuscripts will be submitted to PLANT DISEASE.

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CHAPTER II

Sensitivity of *Rhizoctonia solani* Isolates and Rhizoctonia-like Fungi From Oklahoma Peanut Fields to Tolclofos-methyl and Pentachloronitrobenzene

ABSTRACT

Radial hyphal growth of 17 *Rhizoctonia solani* AG4 isolates and 3 Rhizoctonia like fungi (RLF) from peanut fields in Oklahoma was measured on a defined medium containing 20.0 g/L glucose and 2.0 g/L asparagine amended with tolclofos-methyl (50 WP) or pentachloronitrobenzene (PCNB, 75 WP). In some experiments, asparagine was omitted from the medium. Fungicides were added to the media to give 0.01, 0.1, 0.5, 1.0 and 10.0 ug a.i./ml agar. Growth was measured after 72 h at 25 C and percent inhibition of radial growth calculated based on a comparison to growth on fungicide-free media. Fifteen of the 17 *R. solani* isolates and all RLF were considered sensitive to tolclofos-methyl, showing 66-100% inhibition at 1.0 ug/ml. Two *R. solani* isolates were relatively insensitive, showing 56-79% growth inhibition at 10.0 ug/ml. One of these isolates had an EC_{50} =9.00 ug/ml. PCNB was not as inhibitory as tolclofos-methyl. No fungal isolate was 100% inhibited at 10.0 ug PCNB/ml, and some isolates were considered resistant to PCNB (EC_{50} =>5000 ug/ml). There appeared to be no clearcut evidence for the

presence in isolates of fungicide cross-resistance. Growth enhancement beyond that on fungicide-free media was observed for several isolates on media amended with concentrations of 0.01-0.5 ug/ml of either fungicide. Omission of asparagine from the growth medium generally increased the inhibitory effect of either fungicide.

INTRODUCTION

Pod rot of peanut (*Arachis hypogaea* L.) can be a serious disease in many of the peanut producing areas of the world. Peanut pods develop below the ground and there are no above ground symptoms, therefore, the disease often remains undetected until the peanut is harvested. Peanut pod rot is thought to be a complex disease caused by several soilborne fungi including species of *Pythium*, *Fusarium*, *Sclerotinia*, and *Rhizoctonia solani*, anastomosis group 4. Nematodes also may be involved in some fields. In addition to causing pod rot of peanut, *R. solani* can cause a damping off, root and stem rot, and a blight of foliage, pegs and limbs.

Pentachloronitrobenzene (PCNB) has been one of the main fungicides for control of diseases of peanut caused by *Sclerotium rolfsii* and *R. solani*; however, it has not always been effective. For instance, Csinos et al. (1984) reported that PCNB did not significantly reduce pod rot of Florunner and Early Bunch peanut in several field trials in Georgia. Field observations from Oklahoma (Filonow, unpublished) have shown that PCNB does not effectively control peanut pod rot. This may be due in part to the development of resistance to PCNB by isolates of *R. solani*. There are numerous reports of *R. solani* acquiring resistance to organic fungicides such

as PCNB, (Shatla and Sinclair, 1963; Meyer and Parmeter, 1968; Katara and Grover, 1974), captan, dichlone, maneb, and thiram (Elsiad and Sinclair, 1964; Abdalla, 1975) and to some systemic fungicides such as oxycarboxin and carboxin (Grover and Chopra, 1970). For example, Kataria and Grover (1974), through successive transfers, increased resistance of R. solani to PCNB at 24 times the lethal dose (ED_{50} of 1.5uM).

Tolclofos-methyl (O, O-dimethyl-O (2, 6-dichloro-4-methylphenyl)-phosphorothioate) (Rizolex) developed by Sumitomo Chemical Co., Ltd., Japan, is an organothiophosphate fungicide with curative and slightly systemic action and a reported high degree of toxicity (EC_{50} of 0.01 ug/ml agar) towards R. solani (Sumitomo Chemical Technical Information). Csinos (1985), reported that tolclofos-methyl (TME) reduced limb rot and increased peanut yields in field trials at 3/4 the recommended rate of PCNB. Radial hyphal growth in water agar containing TME was inhibited at 0.1 ug/ml, whereas PCNB inhibited growth at 1.0 ug/ml. Van Bruggen and Arneson (1984) reported that R. solani isolates were adapted to increasing concentrations of TME up to 400 ug/ml.

The molecular structures of TME and PCNB have in common a chlorinated benzene ring, and cross-resistance by some fungi to several substituted aromatic hydrocarbons is common (Georgopolus and Zaracovitis, 1967). Thus, the possibility exists that the effectiveness of TME for field use may have been diminished where long-term use of PCNB had been made.

Therefore, before large scale field testing of TME in Oklahoma, it appeared useful to assess R. solani isolates from several peanut production areas in the state for their sensitivity to TME. Moreover, as binucleate, Rhizoctonia-like fungi (RLF) often are isolated from peanut pods and soils in Oklahoma (Filonow, unpublished observation), the sensitivity of RLF to TME was also of interest.

Reduced sensitivity of a fungus to a fungicide is often seen in nutrient-rich media (Georgopolus and Zaracovitis, 1967). Wicks and Volle (1978) showed that three Botrytis cinerea isolates grew and sporulated on PDA amended with chlorothalonil, but not on water agar. Therefore, the nutrient status of the growth medium may have an important effect on the growth of a fungus under fungicide stress. Weinhold et al. (1969) had previously shown that the presence of glucose and/or asparagine in a defined medium influenced the growth rate and the virulence of R. solani mycelium. Thus, I sought to assess the effect of these nutrients, in a preliminary way, on the sensitivity of R. solani and RLF to TME and PCNB.

The objectives of this research were (1) to assess in defined medium the growth sensitivities of R. solani isolates and RLF from Oklahoma peanut fields to TME and PCNB and (2) to determine the effect of nutrients on these sensitivities. A peliminary report on this research has been published (Inskeep and Filonow, 1986).

MATERIALS AND METHODS

Peanut pods showing typical symptoms of pod rot were obtained from growers fields in the major peanut producing counties in Oklahoma. Pieces of peanut hulls were surface disinfested for 45 sec in 10% household bleach, rinsed in sterile water, blotted on sterile paper towels and incubated on potato dextrose agar (PDA) at 24-26 C for 2-3 days. Hyphal tips of actively growing mycelium whose morphology resembled *R. solani* were transferred to PDA. Most of these isolates were identified as *R. solani* according to the following characteristics: rapidly growing, large diameter hyphae branching at nearly right angles near the distal septum of cells, formation of a septum at each hyphal branch, multi-nucleate cells, brown pigmentation of hyphae, presence of monilioid cells and production of sclerotia. Some isolates were not *R. solani* as their hyphae were of smaller diameter, had a yellow pigmentation, and binucleate cells. These isolates did not produce sclerotia. They were classified as Rhizoctonia-like fungi (RLF). The *R. solani* isolates were tested for anastomosing group by pairing isolates with known tester isolates on PDA. Pure cultures of these fungi were stored on PDA pieces in sterile water and in sterile soil.

Rhizoctonia solani (AG 4) isolates were obtained from

the following counties in Oklahoma: Bryan Co. -- isolates Rs 13, Rs 42, Rs 43 and Rs 50; Caddo Co. -- isolates Rs 12, Rs 4, Rs 14, Rs 44, Rs 45, Rs 20, and Rs 7; Hughes Co. -- isolates Rs 46, Rs 47, Rs 30, and Rs 48; Okfuskee Co. -- Rs 49; and Payne Co. -- Rs 25. Isolates RLF 19 and RLF 11 were obtained from Caddo Co., whereas, RLF 32 was obtained from Hughes Co.

The R. solani isolates and RLF were maintained on PDA. Three days prior to an experiment, a 7 mm diameter plug from the margins of a PDA culture was transferred to a mineral salts medium (Weinhold et al., 1969) containing 20 g agar/L (Sigma Chemical Co., St. Louis). After 3 days, 7 mm diameter plugs from the margins of this culture were transferred to the center of 9 cm diameter Petri dishes containing the mineral salts agar plus 20.0 g/L glucose and 2.0 g/L asparagine (hereafter referred to as Weinhold's glucose asparagine medium or WGA medium). In other experiments, the asparagine was omitted from the medium (hereafter referred to as Weinhold's glucose medium or WG medium). Omission of asparagine was done to assess the affect of the carbon to nitrogen ratio and/or availability of asparagine on hyphal growth and fungicide efficacy. Appropriate amounts of TME (50% WP) or PCNB (75% WP) were dissolved in 95% ethanol, and 10 ml aliquots were added to WG or WGA media to achieve concentrations of 0.01, 0.1, 0.5, 1.0 and 10.0 ug a.i./ml of agar for each fungicide. The fungicides were added to the media (55 C) and the amended

media were thoroughly mixed prior to dispensing 15-20 ml into the plastic Petri dishes. Media containing 10 ml of 95% ethanol per liter without fungicides served as the controls. Three dishes for each fungicide concentration and the control, at both nutrient levels (glucose alone or glucose plus asparagine), were used for each isolate. Radial growth of a colony was measured after 72 h incubation at 25 C in the dark. Growth was circular and uniform in most cases, and when it was irregular, growth was measured at the widest point. Percent inhibition of growth was calculated for each isolate as compared to a control.

All experiments were repeated at least once. Results reported here are the means of all replicates for each treatment, combined from all experiments. Treatments were compared by analysis of variance and least significant difference (P=.05) determined. The effective concentration to give 50% inhibition of radial growth (EC_{50}) was determined using computer-generated log-probit plots of fungicide concentration and percent growth inhibition for each isolate:fungicide:nutrient level. Only positive inhibition values were used in generating log-probit plots.

RESULTS

Fungal isolates varied in their radial growth after 72 h on WGA and WG containing no fungicide (Table 1). The slowest growing isolate was Rs 20 (15.9 mm) and the fastest growing were RLF 11, RLF 32 and Rs 45 with radial growth of 39.5 mm each. All of the RLF isolates and 41% of the R. solani isolates grew less on WG than on WGA. There were significant (P=0.0001) 1st order interactions between the isolates and nutrient level, isolate and fungicide concentration, nutrient level and fungicide concentration, and significant (P=0.0001) 2nd order interactions between isolate, nutrient level and fungicide concentration indicated by analysis of variance procedures.

The majority of the isolates did not show a progressively linear decrease in hyphal growth with increasing concentrations of TME or PCNB when grown on WGA (Table 2). Surprisingly, several R. solani isolates showed increased (negative percent inhibition) hyphal growth, some of which was substantial, on TME amended media. Moreover, some of these isolates showed increased hyphal growth on PCNB amended media as well. For instance, in WGA amended with 0.01 ug/ml of either fungicide, isolates Rs 13, 20, 46, and 44 were stimulated to grow 32.7-50.9% greater by the TME or 35.0-68.1% greater by the PCNB than their respective

controls. Some of the isolates, e.g. Rs 44, had enhanced growth on WGA plus 0.01 or 0.5 ug TME/ml agar, but not at 0.1 ug/ml. Also, enhanced growth of isolates Rs 4, 12, 7, 13, 44, and 48 was observed at 0.01-0.5 ug PCNB/ml WGA. Growth enhancement at these fungicide concentrations were greatest when asparagine was in the medium (Table 2) than when asparagine was omitted (Table 3). Also, growth enhancement was observed for RLF on the medium without asparagine (Table 3) but not on the WGA medium (Table 2).

In addition to growth enhancement, several isolates exhibited polymodal growth responses to increasing fungicide concentrations in WGA. Isolate Rs 14, for example, showed 11.1%, 8.4%, 53.0%, 6.9%, and 55.8% inhibition at 0.01, 0.1, 0.5, 1.0, 10.0 ug TME/ml WGA, respectively. Similar examples of this effect were observed with PCNB. On the other hand, lack of asparagine in the growth medium appeared to obviate the effect, in that growth inhibition for most isolates increased as fungicide concentration increased (Table 3).

All of the isolates were able to grow to a varying extent, on WGA plus TME at 0.5 ug/ml (Table 2). In fact, 35% of all isolates showed enhanced growth at this concentration. However, a doubling of the TME concentration effectively reduced the growth of most of these isolates. Ninety five percent of all isolates had EC_{50} values of 0.05-0.93 ug/ml WGA. Eight of the 20 isolates (40%) were able to grow to some extent at 10.0 ug TME/ml WGA. Isolates Rs 14

and Rs 50 were the least affected by TME of all the isolates, showing 55.8% and 78.5% growth inhibition, respectively, at 10.0 ug TME/ml agar. Isolate Rs 14 had an EC_{50} of 9.00 ug/ml WGA (Table 4). When asparagine was not in the medium, growth of 90% of the isolates at 10.0 ug TME/ml was completely inhibited. Omission of asparagine generally increased EC_{50} values of the isolates, although some EC_{50} values were decreased (Table 4).

Pentachloronitrobenzene did not completely inhibit growth of any isolate at 10.0 ug/ml. The range of inhibition for all isolates at 10.0 ug PCNB/ml WGA (Table 3) was 3.0-84.4%. In a preliminary test, 6 fungal isolates were inhibited 25-81% on WGA containing 100 ug/ml. Forty five percent of all isolates had EC_{50} values of $\geq$50 ug PCNB/ml (Table 4). Generally, hyphal growth inhibition was more pronounced at this concentration in the medium without asparagine (Table 3). A notable exception to this was Rs 25 which was less inhibited (28.5%) at 10.0 ug PCNB/ml of WG than in the WGA medium (50.7% inhibition). Several of the *R. solani* isolates showed increased EC_{50} values of > 5000 ug/ml WG (Table 4)

It was of interest to see if TME was fungitoxic or fungistatic to hyphal growth. The same plugs of fungi which showed no growth on WGA plus TME at 0.5-10.0 ug/ml agar were transferred to WGA medium without TME. All isolates grew (5-40 mm) in 72 h, suggesting that TME is a fungistatic compound. A similar test was done for isolates transferred

from PCNB at 10.0 ug /ml agar. All of these isolates also grew in 72 h, confirming that PCNB also is a fungistatic compound.

At the beginning of these studies, isolates Rs 14 and 50 could be maintained on Weinhold's agar. During the study growth of these isolates on the medium without added nutrients progressively decreased with repeated transfers. Ultimately, they did not survive on this medium. Attempts to maintain these fungi on PDA also were futile, as were attempts to recover the isolates from sterile water or sterile soil cultures by transfer to PDA or to a selective medium specific for Rhizoctonia spp. (Ko and Hora, 1971). Thus, data for these isolates were not taken for their growth on TME or PCNB amended medium without asparagine.

DISCUSSION

Rhizoctonia solani (AG 4) isolates evaluated in this study came from several different fields in the major peanut growing counties of Oklahoma. Of the 17 isolates evaluated in this study, 15 were considered to be sensitive to TME as judged by their >50% inhibition of hyphal growth at 1.0ug TME/ml agar. Csinos (1985) in Georgia, also reported that field isolates of *R. solani* were very sensitive (>50% inhibition at 0.1 ug/ml) to TME, although only two isolates were tested. Two *R. solani* isolates from Oklahoma were considered to be relatively insensitive to TME, as they showed little inhibition at 1.0 ug/ml and were only 56-79% inhibited at 10.0 ug/ml. This suggests the potential for the development of resistance to TME in Oklahoma peanut fields; however, as reported by Van Bruggen and Arneson (1984) some isolates of *R. solani* which were adapted to 400 ug TME/ml PDA had slower growth rates in nonameded PDA, although their pathogenicity to bean plants was not reduced compared to fungicide-sensitive isolates. Thus, the ecological fitness of *R. solani* isolates resistant to TME should be considered in evaluating the potential of widespread resistance to this fungicide. Nothing, however, is known about the ecological fitness of *R. solani* isolates used in this study, although all isolates were pathogenic to

peanut seedlings (Filonow, unpublished observation). Until more is known about their ability to survive, these resistant isolates do not appear at the present to pose a threat to use of TME in Oklahoma peanut fields. However, monitoring fields for TME resistance in R. solani would be advised if TME were labeled for use in Oklahoma.

The R. solani isolates tested in this study were about 10-5000 times more sensitive to TME than to PCNB, depending on the growth medium used in the assay. Eight of 17 isolates had EC_{50} values $\geq$ 50 ug PCNB/ml WGA. An EC_{50} of > 50 ug/ml was used by Martin et al. (1984) in reporting isolates of R. solani and RLF which were resistant to PCNB. It appears that PCNB-resistant isolates of R. solani are present in Oklahoma peanut fields, but their contribution to plant disease has not been assessed.

The RLF as a group were not considered more sensitive to TME or PCNB than the R. solani isolates, although only 3 RLF were tested. This may be fortunate. Although little is known of their ecology and importance in peanut soils, they may play a role in restricting colonization of peanut pods by fungal pathogens, as suggested by Garcia and Mitchell (1975) for avirulent isolates of R. solani. Natural field resistance to fungicides with activity against R. solani would then be a favorable trait for RLF used as biocontrol agents or in understanding the ecology of RLF in soil.

In this study, TME was shown to be a fungistat in that agar plugs of R. solani that were 100% inhibited at various

concentrations, grew out when placed on fungicide-free medium. Pentachloronitrobenzene was also found to be fungistatic as previously reported (Elsiad and Sinclair, 1964; Ko and Oda, 1972). Sclerotial production by the isolates under fungicide stress was not studied; however, Csinos (1985) reported that $\geq$ 0.1 ug TME/ml PDA suppressed sclerotia formation by 40% or more.

There appeared to be no clearcut relationship between the sensitivities of *R. solani* isolates and RLF to TME and PCNB, suggesting that cross-resistance between these fungicides has not developed in Oklahoma peanut fields. More study may be needed, however. The data given in this thesis may be useful for the future evaluation of fungicide cross-resistance by monitoring field isolates.

There was great variability among the individual fungal isolates tested as to their sensitivity to TME which was evident at 0.01-0.5 ug/ml. For example, growth of several isolates was enhanced by TME beyond that shown on fungicide free medium, whereas other isolates were not. This growth enhancement by low concentrations of fungicide was not an artifact of method or isolate origin, in that several of the isolates from diverse geographical areas showed this effect. In fact, there did not appear to be any relationship between the origin of any isolate and its sensitivity to the fungicides. Such variability would be expected in *R. solani* as a result of heterokaryosis (Meyer and Parmeter, 1968) which has been implicated in altered chemical tolerance by *R.*

solani. The polymodal response of several isolates to the fungicides was similar to that first reported by Dimond et al. (1941) for thiram. This response may be due to alterations in cell membrane permeability, storage of non-dissociated fungicide or detoxification by the fungus (Horsfall, 1956).

Omission of asparagine from the growth medium in which these studies were conducted, greatly influenced the responses of the fungal isolates to both fungicides. Generally, a lack of asparagine made the isolates more sensitive to the fungicides, although there were a few individual isolates that did not respond in this manner. One implication of this finding relates to the exclusive use of nutrient rich media such as PDA in fungicide bioassays without the use of a nutrient-limited medium. Nutrient rich media may encourage optimal growth which may overcome fungitoxic effects seen on nutrient-limited media (Georgopolus and Zaracovitis, 1967; Wicks and Volle, 1978). Another implication relates to the effect deletion of asparagine had on the growth enhancement effect. Without asparagine the effect was markedly reduced, suggesting that nitrogen may be important in the expression of the enhancement effect. Possibly the use of nitrogen containing fertilizers may influence the fungicide sensitivitiy of certain R. solani isolates in agricultural soils.

Table 1. Mean radial growth of *Rhizoctonia solani* isolates and Rhizoctonia-like fungi (RLF) on a defined growth medium with and without asparagine

Isolate	Mean Radial Growth, mm[z]	
	Asparagine[y]	No asparagine
RLF 11	39.5	34.5
RLF 32	39.5	34.4
Rs 45	39.5	30.9
Rs 30	38.1	27.4
Rs 49	37.6	34.4
Rs 48	35.1	32.2
Rs 42	32.7	29.1
RLF 19	32.1	30.8
Rs 43	27.4	22.8
Rs 47	27.1	30.5
Rs 4	26.9	30.7
Rs 25	26.3	22.8
Rs 7	25.0	26.8
Rs 12	23.5	24.8
Rs 13	22.7	28.1
Rs 44	22.4	24.7
Rs 14	21.7	ND[x]
Rs 46	20.5	25.9
Rs 50	18.6	ND
Rs 20	15.9	26.1
LSD (P=0.05)	5.5	10.6

[z]Values are the means of 6 replications.
[y]Asparagine (2g/L) was added to Weinhold's agar containing 20g/L glucose.
[x]ND=not determined. Isolates died before experiments were conducted.

Table 2. Inhibition of radial growth of Rhizoctonia solani isolates and Rhizoctonia-like fungi (RLF) on a defined growth medium with asparagine and amended with tolclofos-methyl (TME) or PCNB

	Percent inhibition at each concentration[z]									
	TME, ug/ml[y]					PCNB, ug/ml				
Isolate	0.01	0.1	0.5	1.0	10.0	0.01	0.1	0.5	1.0	10.0
Rs 47	-4.8	35.7	94.7	96.1	100.0	3.7	2.3	58.9	36.0	72.6
Rs 43	-3.0	20.0	71.1	96.2	99.6	8.5	13.9	41.9	38.0	56.9
Rs 49	-4.2	15.9	83.6	80.2	90.9	3.8	6.9	53.8	63.0	70.1
Rs 4	2.2	21.2	-37.4	94.6	100.0	-12.3	-9.5	-54.2	17.5	49.8
Rs 12	6.3	32.8	-3.9	97.6	98.9	4.5	6.7	-20.1	39.0	58.7
Rs 7	-14.8	22.4	-34.3	98.0	99.0	-17.4	-8.6	-52.5	29.2	41.9
Rs 13	-32.7	30.7	-17.0	98.8	100.0	-35.0	-26.4	-49.6	21.7	41.8
Rs 20	-50.9	1.1	-85.5	98.2	100.0	-68.1	-46.1	-92.8	24.3	61.0
Rs 46	-37.4	14.5	-49.0	98.7	100.0	-43.9	-29.4	69.0	31.4	46.2
Rs 44	-35.0	29.1	-27.4	100.0	100.0	-38.9	-33.9	-64.0	21.2	46.0
Rs 25	5.9	36.1	24.6	89.4	97.8	6.6	5.9	0.3	16.7	50.7
Rs 42	17.9	43.1	28.1	95.1	98.8	6.8	10.2	4.4	25.8	76.8
Rs 45	7.6	28.9	14.1	100.0	100.0	0.0	0.0	0.0	9.5	75.9
Rs 48	10.1	52.3	24.1	100.0	100.0	4.3	-1.4	-0.9	26.8	84.4
Rs 30	18.2	26.4	16.3	90.6	100.0	10.3	8.7	2.7	19.8	75.8
Rs 14	11.1	8.4	53.0	6.9	55.8	1.5	8.2	38.6	12.3	3.0
Rs 50	14.1	-20.3	-14.8	-79.8	78.5	-4.7	-16.7	2.9	33.3	25.4
RLF 19	11.4	30.4	9.5	100.0	100.0	3.7	4.3	3.4	15.5	63.5
RLF 11	10.7	18.1	9.8	85.8	100.0	5.6	8.6	11.4	16.5	74.8
RLF 32	26.3	24.1	11.9	66.4	100.0	13.3	13.0	0.0	9.2	60.0
LSD (P=0.05)				13.2	11.6				14.0	14.8

[z]Radial growth was measured after 72 h at 25 C and percent inhibition of growth calculated on a comparison to growth on a fungicide-free Weinhold's agar containing 20g/L glucose and 2g/L asparagine. Values are the means of six replications.

[y]TME or PCNB were added as 50 WP or 75 WP, respectively.

Table 3. Inhibition of radial growth of Rhizoctonia solani isolates and Rhizoctonia-like fungi (RLF) on a defined growth medium without asparagine and amended with toclofos-methyl (TME) or PCNB

	Percent inhibition at each concentration [z]									
	TME, ug/ml [y]					PCNB ug/ml				
Isolate	0.01	0.1	0.5	1.0	10.0	0.01	0.1	0.5	1.0	10.0
Rs 47	17.1	65.0	98.9	100.0	100.0	12.7	8.1	23.9	39.4	80.7
Rs 43	-8.7	49.9	77.8	100.0	100.0	-0.9	-3.0	0.6	10.0	50.4
Rs 49	0.0	62.1	78.7	79.9	89.4	5.4	8.7	18.4	57.9	77.6
Rs 4	7.2	36.0	89.3	97.8	100.0	-7.7	-4.4	-2.4	6.4	62.9
Rs 12	7.7	35.7	49.3	98.1	100.0	-6.2	-1.4	7.5	13.9	58.5
Rs 7	11.8	42.0	65.2	96.1	100.0	-2.3	0.2	24.9	33.5	61.3
Rs 13	18.8	44.3	66.4	94.8	100.0	3.0	7.2	57.2	36.9	50.8
Rs 20	5.6	46.0	94.1	100.0	100.0	-2.2	5.4	39.9	50.3	74.4
Rs 46	-0.7	46.0	92.8	100.0	100.0	7.9	5.0	32.4	39.6	63.4
Rs 44	6.8	49.9	94.8	100.0	100.0	-9.1	-5.2	33.5	35.8	65.8
Rs 25	-2.7	20.8	72.4	89.7	94.5	0.8	2.5	12.3	40.7	28.5
Rs 42	18.2	75.9	90.2	100.0	100.0	22.9	24.0	52.2	59.0	83.3
Rs 45	-5.9	80.9	96.6	98.4	100.0	2.9	-0.3	11.4	57.5	59.8
Rs 48	6.4	76.1	100.0	100.0	100.0	8.7	14.1	12.0	23.3	81.9
Rs 30	4.0	24.6	11.0	81.9	100.0	2.9	-0.9	-0.2	-0.6	63.9
Rs 14	ND [x]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Rs 50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
RLF 19	-3.7	73.5	100.0	100.0	100.0	5.5	4.7	5.9	35.7	69.2
RLF 11	-0.4	4.9	4.1	89.6	100.0	0.6	-2.1	-4.2	1.4	67.7
RLF 32	-3.6	7.8	45.5	68.2	100.0	-2.7	-7.1	5.2	6.1	75.0
LSD (P=0.05)				NS						16.8

[z] Radial growth was measured after 72 h at 25 C and percent inhibition of growth calculated on a comparison to growth on fungicide-free Weinhold's agar containing 20g/L glucose but no asparagine. Values are means of six replications.

[y] TME or PCNB were added as 50 WP or 75 WP, respectively, to the medium.

[x] ND=not determined. Isolates died before experiments were conducted.

Table 4. EC50 values of isolates of *Rhizoctonia solani* and Rhizoctonia-like fungi (RLF) on a defined growth medium with and without asparagine and amended with tolclofos-methyl (TME) or PCNB

	EC50, ug/ml agar[z]			
	Asparagine[y]		No asparagine	
Isolate	TME	PCNB	TME	PCNB
Rs 14	9.00	95.00	ND[x]	ND
Rs 50	0.93	>100.00	ND	ND
Rs 43	0.74	8.30	ND	ND
Rs 25	0.73	98.00	0.001	>5000.00
Rs 49	0.70	9.80	0.089	0.07
Rs 4	0.60	9.60	0.099	9.60
Rs 30	0.50	30.00	0.780	8.50
Rs 45	0.40	8.80	0.005	7.00
Rs 7	0.38	>100.00	0.099	0.88
Rs 42	0.20	9.40	0.056	0.82
Rs 47	0.20	5.00	0.099	9.65
Rs 13	0.18	>100.00	0.096	>5000.00
Rs 48	0.10	3.00	0.097	8.90
Rs 12	0.10	50.00	0.099	>5000.00
Rs 46	0.10	87.00	ND	>5000.00
Rs 20	0.09	6.00	0.096	>5000.00
Rs 44	0.05	50.00	0.093	630.00
RLF 32	0.74	86.00	0.900	8.50
RLF 11	0.72	9.50	0.975	56.00
RLF 19	0.10	22.00	0.380	7.00

[z] Concentration (ug a.i. fungicide per ml of medium) required for 50% inhibition of radial growth after 72 h at 25 C as determined by probit analysis.
[y] Weinhold's agar with 20g/L glucose and containing 2g/L asparagine or no asparagine.
[x] ND=not determined. Isolates died before experiments were conducted

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CHAPTER III

Evaluation of Tolclofos-methyl For Control of Peanut Pod Rot Caused by Rhizoctoni solani in Oklahoma

ABSTRACT

Field microplots planted to peanut cv. Florunner and Spanco were artificially infested 3-4 weeks after planting at inoculum densities (ID) of 12.0-1627.5 propagules (p)/ 100g soil in 1985 and 211.2-1353.5 p/100g soil in 1986 with Rhizoctonia solani AG4. Tolclofos-methyl (50 WP) was applied at 11.2 kg a.i./ha at flowering. Rhizoctonia solani soil populations were monitored on a monthly basis. One week following placement in pegging zones, the highest ID's of R. solani had decreased by >90%. Thereafter, ID's in plots fluctuated from 0 to 84.8 p/100g soil in 1985 and 0-157.5 p/100g soil in 1986. At harvest, each pod was rated for disease severity and yields were determined. Tolclofos-methyl effectively reduced R. solani soil populations by harvest; however, it did not significantly (P=0.05) reduce pod rot or significantly (P=0.05) increase yields. In the greenhouse, steam pastuerized soil was infested with R. solani at 12.0-388.5 p/100g soil and planted to Florunner and Spanco. Tolclofos-methyl (50 WP) or pentachloronitro-benzene (PCNB, 75 WP) were applied at 5.6 kg /ha as a

preplant and at-flowering as a soil drench. Inoculum densities of treated soil were reduced by harvest, but PCNB was significantly (P=Ø.Ø5) more effective in reducing pod rot and increasing yield than tolclofos-methyl.

INTRODUCTION

Rhizoctonia solani Kuhn (Anastomosis Group 4) is a common soilborne fungus which causes many severe plant diseases including damping-off, root rot, and peg and limb rot of peanut (*Arachis hypogaea* L.). It is also involved in the disease complex which causes pod rot of peanut. In Oklahoma, *R. solani* was isolated from soil and rotted pods in 35% and 29% of 42 and 48 samples in 1984, and 1985, respectively (Filonow, unpublished). For several decades, control of plant diseases caused by *R. solani* on several crops has been achieved mainly through the application of pentachloronitrobenzene (PCNB). On peanut, PCNB is labelled for use against *Sclerotium rolfsii* with a maximum allowable rate of 11.2 kg a.i./ha. Control with PCNB is often erratic. Field observations (Csinos, et al. 1984; Filonow, unpublished) have also shown a lack of pod rot control with PCNB. Moreover, *R. solani* isolates from cotton (Elsiad and Sinclair, 1963) and peanut (Filonow, 1984) have been shown to have resistance to PCNB. Thus, there is a need for a soil fungicide which is effective in reducing pod rot caused by *R. solani*. Recently, tolclofos-methyl (trade name: Rizolex) was introduced into the USA for experimental evaluation for control of *R. solani*. Tolclofos-methyl is an organothiophosphate with curative and slightly systemic

action and a reputed high degree of toxicity (ED50=0.01 ug a.i./ml agar) towards R. solani (Sumitomo Chemical Co. technical report; Ohtsuki and Fujinami, 1982). There are not many published reports of tolclofos-methyl use on peanut. Csinos et al (1984) showed that tolclofos-methyl did not reduce pod rot on Florunner and Early Bunch peanuts in field tests, but did reduce peanut limb rot damage (Csinos, 1985).

The chemical structure of tolclofos-methyl is similar to PCNB and considering the long-term use of PCNB in Oklahoma peanut fields, the possibility of cross resistance in R. solani isolates to tolclofos-methyl exists. Van Bruggen and Arneson (1984) showed that R. solani isolates from bean and beet roots could be adapted to high (400ppm) concentrations of tolclofos-methyl in agar. Csinos, (1985), on the other hand, showed that tolclofos-methyl at 0.1 and 1.0 ug/ml agar reduced hyphal growth of R. solani isolates from Georgia more than PCNB.

A principle of plant pathology states that the effectiveness of a fungicide increases when there is a decrease of the inoculum potential (Kenaga, 1974). Taken another way, this means that fungicides work best at lower inoculum densities of the pathogen, and it implies that fungicides should be evaluated against increasing inoculum densities of a pathogen to get an adequate appraisal of the fungicide's effectiveness.

The objectives of this study were to (1) evaluate the

efficacy of tolclofos-methyl in decreasing inoculum densities of *R. solani* in soil and (2) to compare the efficacy of tolclofos-methyl to PCNB in reducing pod rot.

MATERIALS & METHODS

Microplots Experiments

Microplots were 2.4 x 2.4 x 0.3 m wooden boxes of sandy loam soil located on the Plant Pathology Farm, Stillwater, OK. Soils were rototilled and fumigated with 0.68 kg Bromogas® (methylbromide) per microplot, under a plastic tarp for three days. The tarps were removed and the soil was allowed to aerate prior to planting. Each microplot was planted with Florunner and Spanco peanut, with two 0.9 m rows per cultivar and 9 plants per row. Plants were sown as seedlings to ensure a full stand of mature plants. Peanut seeds (*Arachis hypogaea* L.), were surface disinfested in 10% household bleach for 2 minutes, rinsed 3 times in sterile water, and germinated on sterile, moistened paper towel for 48 hours.

A randomized complete block design was used in the experiments in a 2 x 2 x 4 factorial arrangement of treatments with four replications per treatment. Treatments consisted of four inoculum densities of *R. solani* infested soil, two cultivars and fungicide treated and nontreated plants. In 1985, inoculum densities were 12.0 propagules (p)/100g soil, 122.6 p/100g soil and 1627.5 p/100g soil. In 1986, the inoculum levels were 211.2 p/100g soil, 627.3

p/100g soil and 1354.95 p/100 g soil. Non-inoculated plots served as controls. The soil in the pegging zone of the plants was removed, leaving a trench about 15cm wide by 10cm deep in 1985, and about 31cm wide and 10cm deep in 1986, alongside each row. The trenches were filled with steam pasteurized soil infested with R. solani at the inoculum densities given above. In 1985, approximately 2.3 kg of infested soil was applied in each trench at flowering, whereas, in 1986, 4.5 kg of each infested soil was applied. Inoculum was prepared using coarse sand mixed with ground corn meal (CMS) (95% sand + 5% cornmeal; water 20% v/w). This mix was autoclaved in 22.9 x 33.0 x 5.1 m baking pans and/or 1 liter Erlenmeyer wide mouth flasks for 1 hour on 3 consecutive days. The mix was inoculated with mycelial mats of four isolates (7, 9, 13, and 20) of R. solani grown for 2 weeks at 25 C in 50 ml of potato dextrose broth in 250 ml Erlenmeyer flasks. The CMS cultures were inoculated 2-3 months prior to use. The CMS inoculum was added to steam-pasteurized soil and mixed in a cement mixer. The ID of infested soil was then determined (Henis et al., 1978), and appropriate dilutions of this mixture made to achieve the required inoculum density. The final inoculum density in each dilution was determined.

Tolclofos-methyl (50 WP) was applied about two weeks after infestation, on 18 July 1985, and 24 July 1986, at a rate of 11.2 kg a.i./ha as a spray over the plants. Plots were irrigated to move the fungicide into the soil.

Nontreated rows served as controls.

Soil samples from the pegging zones of plants were taken prior to planting, after soil infestation, after fungicide application, and at harvest. Soil samples were analyzed to determine R. solani populations using a multiple pellet soil sampler (MPSS) technique (Henis et al., 1978). Ten plates of a modified selective medium for R. solani (Ko and Hora, 1971) were used for each sample to determine inoculum density. The R. solani selective media was modified by using 1.0 ml/L Ridomil (2 EC) instead of 0.18 g/L Dexon. Plates were incubated at 25 C and examined after 48 h for R. solani colonies. Rhizoctonia solani was positively identified with a light microscope at 100x.

Peanut cultivars were harvested at maturity. Spanco was harvested 25 October 1985 and 2 October 1986. Florunner was harvested 4 November 1985, and 5 October 1986. After harvesting, peanuts were hand washed and rated for disease severity. Each pod from every treatment was visually rated using a scale from 1 to 5 where 1=no visible lesions, 2=1-25%, 3=26-50%, 4=51-75% and 5=>75% lesions. Pods falling into disease classes 3-5 were totaled, divided by the total number of pods and multiplied by 100 to give percent pod rot. To correlate percent recovery of R. solani to pod rot, after rating, a subsample of 10 diseased pods from each replication was used for isolation of R. solani. Twenty five to thirty pieces about 3 mm square from these pods were surface disinfected in 10% household bleach for 1 minute,

rinsed 3 times in sterile water and plated on potato dextrose agar (PDA). Pods were oven dried at 50 C and weighed for calculation of total yield. Yields were determined by extrapolating the row size to an actual peanut field. Final inoculum densities of R. solani in soil at harvest were determined.

Greenhouse Experiments

Steam-pasteurized sandy loam soil was infested with CMS inoculum of R. solani isolate 7 as discussed above. Preliminary greenhouse studies showed that 750-1500 p/100g soil completely inhibit peanut seedling emergence; therefore, inoculum densities of 12.0, 57.0, and 388.5 p/100g soil were used. Non-infested soil served as the control. Soils were placed in 18 cm diameter x 30 cm plastic pots, arranged in a completely randomized block design with a 2 x 3 x 4 factorial arrangement of treatments, with 5 replications. The experiment was duplicated and run concomittantly. Split applications of tolclofos-methyl (50 WP) and PCNB (75 WP) at 5.6 kg/ha were made, as a soil drench, at preplant and pegging. Plants receiving no fungicide were the controls. Surface disinfested seedlings of 'Florunner' and 'Spanco' were planted at a depth of about 5 cm, with one cultivar per pot. Pots were watered every 2-3 days as necessary, and were fertilized with Hoaglands Solution supplemented with (7 g/L) ammonium nitrate every 10-12 days.

Cultivars were harvested ca. 160 days after planting.

Plants were washed, pegs counted, and the pods and roots were separated. Root fresh weights were taken and each pod was individually rated for disease severity based on the scale of 1-5, as described previously. The frequency of R. solani colonization of pods was determined by plating 5 pieces from diseased peanut pods, one piece from one pod, for each plant, surface disinfested (as above), on PDA at 25 C for 48 h. Yields were determined based on the oven dried (50 C) weight of the pods. The inoculum densities of soils in pots were determined after each fungicide application and at harvest, using the MPSS method.

All data were analyzed by analysis of variance, least significant difference, and nonparametrically using the Kruskall Wallis test. Critical values to compare rank means between disease classes were calculated as described by Dunn (1964) using an experimentwise error of 15%. Spearman's rank correlation for each pair of criteria was computed for each treatment (Daniel, 1978). Nonparametric data is reported in the Appendix.

RESULTS

Microplot Experiments

In 1985, R. solani was detected in fumigated and non-infested soils at all sampling dates (Table 1). Soils planted to Florunner or Spanco peanut had 3.75-84.75 p/ 100 g soil or 3.75-25.5 p/100g, respectively. However, in 1986 (Table 2), initial assays of noninfested soils showed no detectable R. solani, although later in the growing season R. solani was detected in the control soils. In addition, inoculum densities in noninfested soils planted to either cultivar for both years, were greater at harvest than at the initial sampling (July).

One week following initial inoculation of plants with infested soils, the inoculum densities of these soils markedly declined. This effect was noticed in both experiments (Table 1 and 2) and was most pronounced for the highest inoculum density. For instance, in 1985, less than 10% of the original inoculum density (1627.5 p/100g soil) was recovered one week after peanut rows were inoculated with the soil in the pegging zone.

There appeared to be no consistent pattern of R. solani recovery from infested soils during the season in either experiment. In some cases, R. solani inoculum densities

decreased as the season progressed, and in other cases the densities increased. There was no apparent relationship between the original infestation densities in 1985 and 1986 and the inoculum densities of nontreated soils planted to either cultivar at harvest. When the means for all treatments were compared, there was no significant (P=Ø.Ø5) correlation between the original infestation densities and harvest inoculum densities in 1985 (Table 3), but there was a significant correlation (P=Ø.Ø5-Ø.1Ø) in 1986 (Table 4).

At harvest, nontreated soils planted to Florunner peanut generally had greater inoculum densities of R. solani than soils planted to Spanco (Table 1 and 2), although these differences were not significant (P=Ø.Ø5). This was also true when means of all cultivar treatments were compared. For instance, at harvest in 1985, the mean inoculum density of R. solani in nontreated soil planted to Florunner was 32.7 p/1ØØg soil compared to 14.85 p/1ØØg in soil planted to the Spanco cultivar. In 1986, nontreated soil with Florunner peanut had 65.85 p/1ØØg soil, whereas soil with Spanco peanut had 37.Ø5 p/1ØØg soil.

Generally, tolclofos-methyl applications decreased the inoculum densities in treated soils compared to nontreated soils (Table 1 and 2), although only a few of these reductions were significant (P=Ø.Ø5). In a few cases, inoculum densities in treated soils were significantly (P=.Ø5) increased, such as in the 1986 harvest sample of soil at the 1627.5 p/1ØØg soil infestation level and planted to Florunner

peanut (44.25 p/100g for the control compared to 135.45 p/100g for treated soil). Tolclofos-methyl also reduced the inoculum densities of R. solani in the treated control (noninfested) soils, although there was an exception to this in the July, 1985 sample. The effectiveness of tolclofos-methyl in reducing inoculum densities of R. solani was also observed when the mean of all treated plots for both cultivars was compared to the mean from all nontreated plots (Fig. 1). There appeared to be no consistent, significant (P=0.05) interactive effect of tolclofos-methyl and cultivar on inoculum density in treated plots.

Generally, pod rot severity was not significantly (P=0.05) decreased by the use of tolclofos-methyl except in 1985 with Florunner at the 1627.5 p/100g soil infestation density (Table 5) and in 1986 except for Florunner at the 627.0 p/100g soil infestation density (Table 6). In fact, the percent pod rot was often increased by the application of tolclofos-methyl. This lack of disease control appeared not to be related to inoculum density, as no significant (P=0.05) disease control was observed at the lowest inoculum densities in soils, although there was a reduction in pod rot of Spanco peanut at 211.5 p/100g soil in 1986. In both experiments, pod rot was significantly (P=0.05) greater on the Florunner cultivar grown in treated soil than on the Spanco cultivar. This was also true for these cultivars grown in nontreated soil. For instance, in 1985, the mean amount of pod rot for Florunner in tolclofos-methyl treated

plots was 19.6% compared to 6.0% for Spanco and in 1986, mean pod rot was 34.2% for Florunner and 18.9% for Spanco. Pod rot showed no relationship to the original infestation densities (Table 3 and 4); however, it was significantly (P=0.01-0.05) correlated to the inoculum density at harvest in 1986, (Table 4), but not in 1985 (Table 3).

There appeared to be no consistent, positive relationship between the colonization of pods, as indicated by the frequency of R. solani recovered on PDA, and pod rot severity. Of interest was the significant (P=0.01-0.05) negative correlation between R. solani recovery from pods and pod rot in 1985 (Table 3). Although there was a significant (P=0.01-0.05) correlation between the original infestation density of R. solani in soil with recovery of the fungus from pods in 1986 (Table 4), a significant correlation was not seen in 1985.

Several other fungi and bacteria were isolated from pods at harvest. Species of Fusarium, Trichoderma, Penicillium, Rhizopus, and Aspergillus were frequently isolated, although the frequency of their isolation was not determined. In addition, Sclerotinia minor was also noted on many of the pods from treated and nontreated plots. Rhizoctonia-like fungi were also occasionally isolated.

Yields were not significantly (P=0.05) increased by use of tolclofos-methyl. In fact, yields from some tolclofos-methyl treated plots were significantly depressed beyond the nontreated plots. The mean yield from tolclofos-methyl

treated soils planted to Florunner (2339 kg/ha) was not significantly (P=Ø.Ø5) greater than that of Spanco (2195 kg/ha) in 1985, nor in 1986 (1267 kg/ha for Florunner and 1187 kg/ha for Spanco). Yield showed a significant negative correlation with pod rot in 1985 (Table 3, P=Ø.Ø1-Ø.Ø5), and in 1986 (Table 4, P=<Ø.Ø1).

Results indicate that root weights were higher for Spanco, although not significantly, in both 1985, and 1986. However, in 1985, there was a significant (P=.Ø5) increase in root weight for the treated Spanco plants at 123.Ø p/1ØØg soil. In 1986, there were no significant differences between the root weights of the treatments.

Greenhouse Experiments

Inoculum densities of R. solani quickly declined after initial artificial infestation in all infested soils planted to either cultivar. At harvest, most pots contained soil with little or no detectable R. solani (Table 7 and 8). In only one of the noninfested controls, was R. solani found (11.1 p/1ØØg soil). In general, inoculum densities of soils treated with tolclofos-methyl or PCNB were reduced at harvest, and there was no apparent difference between tolclofos-methyl and PCNB in this respect, as generally they reduced the soil inoculum densities to zero in both experiments. There appeared to be no consistent relationship between the original infestation density of soil and the recovery of R. solani from pods at harvest. Recovery of

R. solani was generally greatest from pods taken from soils containing 388.5 p/100g or 57.0 p/100g soil but there was no recovery from pods from soil at 12.0 p/100g soil. Recovery of R. solani from pods from soils treated with tolclofos-methyl or PCNB was significantly (P=0.05) reduced, although the effectiveness of PCNB in this regard was greatest in treated soils planted to the Spanco cultivar. For instance, in soils at 388.5 p/100g soil, planted to Florunner peanut and treated with PCNB, R. solani recovery from pods was reduced from 10% in the control to 4% with PCNB. In soils with similar inoculum densities and planted to Spanco, recovery was reduced from 20% (Table 7) and 16% (Table 8) to 0%.

Pod rot was found on cultivars planted in noninfested soil (Table 9); however, the severity (6.7%) in the control pots was significantly (P=0.05) less than on peanut in infested soil (14.1-17.2%). There was no apparent relationship between the original infestation density and pod rot severity at harvest.

Pod rot severity was significantly (P=0.05) increased by tolclofos-methyl but was reduced by PCNB, although not significantly (Table 10 and 11). For instance, in one experiment (Table 10) the mean pod rot severity for all nontreated pots was 11.0% as compared to 21.8% for tolclofos-methyl treated ones and 7.9% for PCNB treated pots. Experiment 2 followed a similar trend (Table 11). Spanco peanut were slightly more diseased than Florunner in

experiment 2, but Florunner had more disease in experiment 1; however, the differences were not significant (P=Ø.Ø5).

Pod yields were significantly (P=Ø.Ø5) increased by PCNB in experiment 1 as compared to the nontreated control; however, tolclofos-methyl did not increase yields significantly (Table 1Ø and 11).

Root weights were significantly (P=Ø.5) greater for Spanco in both experiments (Table 1Ø and 11). PCNB significantly increased root weight in experiment 1 (9.8 g) as compared to the check (8.5 g), but did not increase root weight significantly more than tolclofos-methyl (9.Ø g). In experiment 2, there were no significant differences between root weights for any of the treatments.

DISCUSSION

One week after placement of artificially infested soil in the pegging zones of plants in the microplots, the highest inoculum densities of R. solani abruptly and drastically decreased by > 90%. This occurred in 1985, and 1986. A similar decrease in inoculum occured in the greenhouse experiments. These reductions in the soil populations may be due in part to the high temperatures in Oklahoma, and the need to water heavily for pod set, which adversely affect the survival of the fungus. Benson and Baker (1974) reported a similar severe reduction with R. solani in artificially infested soil and concluded that moderate soil temperatures (30C) and dry soils favored establishment of the fungus. They also found that the initial inoculum level had no effect on how quickly R. solani became inactive in the soil.

Another explanation for this drastic population reduction may be that microfloral antagonists to R. solani in the bulk of microplot soil moved into the artificially infested soil reducing R. solani populations. In addition, artificially grown inoculum of R. solani may be ecologically less fit than natural field isolates capable of more frequent anastomosis encounters with a R. solani (AG) population of diverse genetic nature. The reduction may also be

blamed on the fact that the infestation inoculum most likely contained sclerotia and hyphal fragments. Upon contacting natural soil these hyphal fragments died off quickly. However, the generally recognized resistant nature of R. solani hyphae to lysis (Papavizas, 1970) argues against this. Finally, in the microplots, artificially infested soil may have become diluted by the adjacent noninfested soil. This explanation, however, cannot account for the abrupt decline in inoculum densities of greenhouse soils.

I found that, although R. solani populations were reduced, the inoculum densities present were sufficient to cause severe pod rot disease. The inoculum densities reported in my work are representative of those found in peanut soils (Woodward and Jones, 1983) and other agricultural soils (Weinhold, 1977) and most likely typify the carrying capacity (Chuang and Ko, 1981; Zadoks and Schein, 1979) of agricultural soil for R. solani (AG 4).

Tolclofos-methyl was effective in reducing populations of R. solani by harvest; however, it was not effective in reducing pod rot disease or in increasing yield. This lack of disease control may be due to other fungi, such as Rhizopus, Pythium sp., Penicillium, Trichoderma, Fusarium sp., Sclerotinia sp., and Aspergillus, which were detected in soil samples and/or isolated from pods. They may have recolonized the microplots through irrigation water, inadvertent contamination from sampling, and/or contaminated seed. Some of these organisms have been implicated in

peanut pod rot and are reportedly (Sumitomo Technical Report) not sensitive to tolclofos-methyl, e.g. Pythium spp. and Fusarium spp. Although the overall R. solani populations were decreased by tolclofos-methyl, the amount of inoculum that was present was enough to cause significant disease (Garcia and Mitchell, 1975, Woodward and Jones, 1983). There was a difference between the varieties in terms of their overall capacity to maintain populations of R. solani around the plant and on the pods. Soil around the Spanco cv. supported higher populations of R. solani, although pod rot was lower for this variety. Florunner peanut had higher yields, but the amount of pod rot was also higher. Results from the greenhouse experiments indicated PCNB was more effective than tolclofos-methyl in reducing pod rot and increasing yield. The inability to recover R. solani from soil or pods at harvest made it difficult to assess the effect of tolclofos-methyl on inoculum density in greenhouse experiments.

Although tolclofos-methyl was not effective in reducing pod rot or in increasing yield in these experiments, it was effective in reducing soil populations of R. solani. Its effectiveness in reducing hyphal growth of R. solani isolates from Oklahoma peanut fields (Chapter II) suggests that it may be useful in managing R. solani populations in peanut soils. Further research should focus on the timing of tolclofos-methyl delivery, split applications, and the use of different formulations.

Table 1. Populations of Rhizoctonia solani in microplots planted to Florunner and Spanco peanut before and after tolclofos-methyl treatment in 1985

Cultivar	p/100g[z]	Treatment[y]	R. solani, p/100g soil[x] July	August	Sept.	Oct.
Florunner	0	-	37.95	3.75	6.75	84.75
		+	1.17	1.95	0.00	25.50
	12.0	-	2.25	7.50	7.20	82.95
		+	7.50	12.75	0.00	2.25
	123.0	-	4.20	5.70	9.45	27.45
		+	10.20	5.70	1.50	8.70
	1627.5	-	60.00	7.95	3.00	31.20
		+	11.25	2.70	0.00	0.00
Spanco	0	-	12.75	3.75	3.75	25.50
		+	40.50	5.25	1.20	6.75
	12.0	-	6.75	16.20	6.75	25.50
		+	2.25	5.25	2.25	3.00
	123.0	-	12.45	6.45	6.75	6.75
		+	9.45	3.45	0.00	15.45
	1627.5	-	58.50	15.00	6.75	37.95
		+	39.45	5.70	0.00	0.00
LSD (P=0.05)			32.25	10.95	6.30	31.95

[z]Inoculum densities in soil used in infestation of microplots.
[y]Nontreated denoted by - and + = 11.2 kg a.i./ha tolclofos-methyl applied at flowering.
[x]R. solani inoculum densities in soil samples from pegging zones. The July sample was one week after infestation of microplots and the October sampling was done at harvest. Values are means of four replications.

Table 2. Populations of Rhizoctonia solani in microplots planted to Florunner and Spanco peanut before and after tolclofos-methyl treatment in 1986

Cultivar	p/100g[z]	Treatment[y]	R. solani, p/100g soil[x] July	August	Sept.	Oct.
Florunner	0	-	0.00	0.00	45.00	76.50
		+	0.00	0.00	17.70	44.25
	211.5	-	15.75	93.75	63.75	76.50
		+	31.50	40.50	67.20	42.75
	619.5	-	19.50	96.75	73.50	70.95
		+	10.50	97.20	74.70	36.45
	1354.5	-	5.70	64.95	57.45	44.25
		+	11.70	70.50	66.45	135.45
Spanco	0	-	0.00	4.50	37.95	20.70
		+	0.00	0.00	16.50	4.20
	211.5	-	1.50	48.75	60.00	66.45
		+	12.45	93.00	115.50	18.75
	619.5	-	46.95	101.25	75.75	67.95
		+	37.95	63.00	17.25	32.70
	1354.5	-	9.95	150.75	81.45	35.70
		+	29.70	30.45	26.70	44.70
LSD (P=0.05)			20.85	70.35	62.10	46.95

[z]Inoculum densities in soil used in infestation of microplots.
[y]Nontreated denoted by - and + = 11.2 kg a.i./ha tolclofos-methyl applied at flowering.
[x]R. solani inoculum densities in soil samples from pegging zones. The July sample was one week after infestation of microplots and the October sampling was done at harvest. Values are means of four replications.

Table 3. Correlation coefficients among pod rot, yield, initial infestation inoculum, Rhizoctonia solani recovery from peanut hulls and inoculum density for all microplots at harvest in 1985

	Podrot	Yield	Infestation[z] Inoculum	Inoculum[y] Density	Percent[x] Recovery
Podrot	1.00	...	...	...	...
Yield	-0.35**	1.00	...	...	...
Infestation Inoculum	0.07	0.07	1.00	...	...
Inoculum Density	0.16	0.17	-0.13	1.00	...
Percent Recovery	-0.25*	0.00	-0.18	0.18	1.00

[z]Inoculum densities in soil used in infestation of microplots.
[y]R. solani inoculum densities in soil samples from pegging zones of plants at harvest.
[x]The frequency of recovery of R. solani from hull pieces plated on PDA, expressed as a percent of total pieces plated.
* significance at P=.01-.05.
** significance at P<.01.

Table 4. Correlation coefficients among pod rot, yield, initial infestation inoculum, Rhizoctonia solani recovery from peanut hulls and inoculum density for all microplots at harvest in 1986

	Podrot	Yield	Infestation[z] Inoculum	Inoculum[y] Density	Percent[x] Recovery
Podrot	1.00	...	...	...	...
Yield	-0.36***	1.00	...	...	...
Infestation Inoculum	0.17	-0.27**	1.00	...	...
Inoculum Density	0.31**	0.07	0.21*	1.00	...
Percent Recovery	0.13	0.08	0.32**	0.14	1.00

[z]Inoculum densities in soil used in infestation of microplots.
[y]R. solani inoculum densities in soil samples from pegging zones of plants at harvest.
[x]The frequency of recovery of R. solani from hull pieces plated on PDA, expressed as a percent of total pieces plated.
* significance at P=.05-.10.
** significance at P=.01-.05.
*** significance at P<.01.

Table 5. Effect of tolclofos-methyl on Florunner and Spanco peanut grown in soil infested with increasing inoculum densities of Rhizoctonia solani in microplots at harvest in 1985

Cultivar	p/100g[z]	Treatment[y]	Podrot %	Yield kg/ha	Root Wt[x] g	Percent[w] Recovery
Florunner	0	-	17.75	2982	93	15.0
		+	18.31	2131	91	4.0
	12.0	-	19.73	2109	74	19.0
		+	21.22	2461	84	9.0
	123.0	-	19.73	2195	64	7.0
		+	13.65	2365	77	7.0
	1627.5	-	18.24	2648	89	4.0
		+	28.54	1824	79	6.0
Spanco	0	-	4.95	2106	429	2.0
		+	7.81	2243	484	8.0
	12.0	-	5.38	2103	416	17.0
		+	3.82	2107	409	16.0
	123.0	-	5.54	1979	378	18.0
		+	10.44	2118	495	6.0
	1627.5	-	1.54	2644	498	12.0
		+	8.34	2261	502	1.0
LSD (P=0.05)			7.35	590	75	15.2

[z]Inoculum densities in soil used in infestation of microplots.
[y]Nontreated denoted by - and + = 11.2 kg a.i./ha tolclofos-methyl
[x]Fresh weight of roots.
[w]The frequency of R. solani recovery from hull pieces on PDA, expressed as a percent of total pieces plated.

Table 6. Effect of tolclofos-methyl on Florunner and Spanco peanut grown in soil infested with increasing inoculum densities of Rhizoctonia solani in microplots at harvest in 1986

Cultivar	p/100g[z]	Treatment[y]	Podrot %	Yield kg/ha	Root Wt[x] g	Percent[w] Recovery
Florunner	0	-	34.27	1496	98	20.5
		+	27.59	1455	93	5.5
	211.5	-	25.69	1751	109	13.5
		+	35.92	1086	97	9.8
	619.5	-	30.58	1378	104	22.3
		+	45.56	1091	81	25.8
	1354.5	-	34.25	930	111	18.3
		+	39.73	946	103	19.5
Spanco	0	-	14.55	1453	122	24.8
		+	15.47	1041	120	2.8
	211.5	-	23.53	1218	115	16.3
		+	12.53	1127	129	22.3
	619.5	-	21.99	1335	121	25.3
		+	19.57	1099	117	12.5
	1354.5	-	20.02	1023	119	35.8
		+	23.66	1228	128	29.5
LSD			11.86	463	30	16.6

[z]Inoculum densities in soil used in infestation of microplots.
[y]Nontreated denoted by - and + = 11.2 kg a.i./ha tolclofos-methyl.
[x]Fresh weight of roots.
[w]The frequency of R. solani recovery from hull pieces on PDA, expressed as a percent of total pieces plated.

Table 7. Effect of tolclofos-methyl (TME) and PCNB on the populations of Rhizoctonia solani in soil planted to Florunner and Spanco peanut in the greenhouse at harvest:Experiment 1

Cultivar	p/100g[Z]	Treatment[Y]	Inoculum[X] Density	Percent[W] Recovery
Florunner	0	Nontrt	11.10	8.0
		TME	0.00	0.0
		PCNB	0.00	0.0
	12.0	Nontrt	0.00	0.0
		TME	0.00	0.0
		PCNB	0.00	0.0
	57.0	Nontrt	0.00	8.0
		TME	0.00	0.0
		PCNB	0.00	4.0
	388.5	Nontrt	5.10	10.0
		TMR	0.00	0.0
		PCNB	0.00	4.0
Spanco	0	Nontrt	0.00	0.0
		TME	0.00	0.0
		PCNB	0.00	0.0
	12.0	Nontrt	0.00	0.0
		TME	0.00	0.0
		PCNB	0.00	0.0
	57.0	Nontrt	0.00	0.0
		TME	0.00	0.0
		PCNB	0.00	0.0
	388.5	Nontrt	55.80	20.0
		TME	0.00	0.0
		PCNB	12.90	0.0

[Z]Inoculum density in soil used in infestation of microplots.
[Y]Nontrt = Nontreated. Fungicides applied at pre-plant and at flowering at 5.6 kg a.i./ha per application as soil drenches.
[X]Inoculum density (p/100g soil) at harvest.
[W]The frequency of R. solani recovery from hull pieces on PDA, expressed as a percent of total pieces plated.

Table 8. Effect of tolclofos-methyl (TME) and PCNB on the populations of Rhizoctonia solani in soil planted to Florunner and Spanco peanut in the greenhouse at harvest:Experiment 2

Cultivar	p/100g[Z]	Treatment[Y]	Inoculum[X] Density	Percent[W] Recovery
Florunner	0	Check	0.00	0.0
		TME	11.70	0.0
		PCNB	0.00	0.0
	12.0	Check	0.00	0.0
		TME	0.00	0.0
		PCNB	0.00	0.0
	57.0	Check	6.60	10.0
		TME	0.00	0.0
		PCNB	0.00	0.0
	388.5	Check	6.90	0.0
		TME	0.00	0.0
		PCNB	0.00	0.0
Spanco	0	Check	0.00	17.0
		TME	0.00	0.0
		PCNB	0.00	0.0
	12.0	Check	0.00	0.0
		TME	0.00	0.0
		PCNB	0.00	0.0
	57.0	Check	11.40	8.0
		TME	0.00	0.0
		PCNB	0.00	0.0
	388.5	Check	11.40	16.0
		TME	0.00	0.0
		PCNB	12.90	0.0

[Z]Inoculum density in soil used in infestation of microplots.
[Y]Nontrt = Nontreated. Fungicides applied at pre-plant and at flowering at 5.6 kg a.i./ha per application as soil drenches.
[X]Inoculum density (p/100g soil) at harvest.
[W]The frequency of R. solani recovery from hull pieces on PDA, expressed as a percent of total pieces plated.

Table 9. Effect of inoculum density on pod rot and yield in the greenhouse

p/100g soil[z]	Yield[y] g	Pod rot[x] %
0	7.2	6.7
12.0	7.0	17.2
57.0	6.8	14.1
388.5	4.0	15.8
LSD (P=0.05)	0.7	7.7

[z]Inoculum densities of soils in pots.
[y]Values are means of all plants for both experiments grown in soil at the stated inoculum density. Pod weights were determined after 48-72 h at 60 C.
[x]Values are means of pod rot ratings for all plants from both experiments grown in soil at the stated inoculum density.

Table 10. Effect of tolclofos-methyl (TME) on pod rot, yield and root weight: greenhouse experiment 1

Treatment[z]	Pod rot[y] %	Yield[x] g	Root Weight[w] g
TME	21.8	5.9	9.0
PCNB	7.9	6.8	9.8
Nontreated	11.0	5.9	8.5
LSD (P=.05)	10.3	0.6	8.5
Spanco	12.4	6.3	10.9
Florunner	14.7	6.1	7.3
LSD (P=.05)	9.2	0.6	3.0

[z]TME and PCNB were applied at pre-plant and at flowering at 5.6 kg a.i./ha per application.
[y]Values are means of pod rot ratings for all plants grown in pots receiving the stated treatments.
[x]Values are means of all plants grown in pots receiving the stated treatments.
[w]Values are means of all plants grown in pots receiving the stated treatments.

Table 11. Effect of tolclofos-methyl (TME) on pod rot, yield and root weight: greenhouse experiment 2

Treatment[z]	Pod rot[y] %	Yield[x] g	Root Weight[w] g
TME	23.1	6.1	10.9
PCNB	6.9	6.5	11.6
Nontreated	10.0	6.5	10.8
LSD (P=.05)	9.3	0.6	1.7
Spanco	16.6	6.1	12.9
Florunner	10.1	6.6	9.4
LSD (P=.05)	9.2	1.3	2.7

[z]TME and PCNB were applied at pre-plant and at flowering at 5.6 kg a.i./ha per application.
[y]Values are means of pod rot ratings for all plants grown in pots receiving the stated treatments.
[x]Values are means of all plants grown in pots receiving the stated treatments.
[w]Values are means of all plants grown in pots receiving the stated treatments.

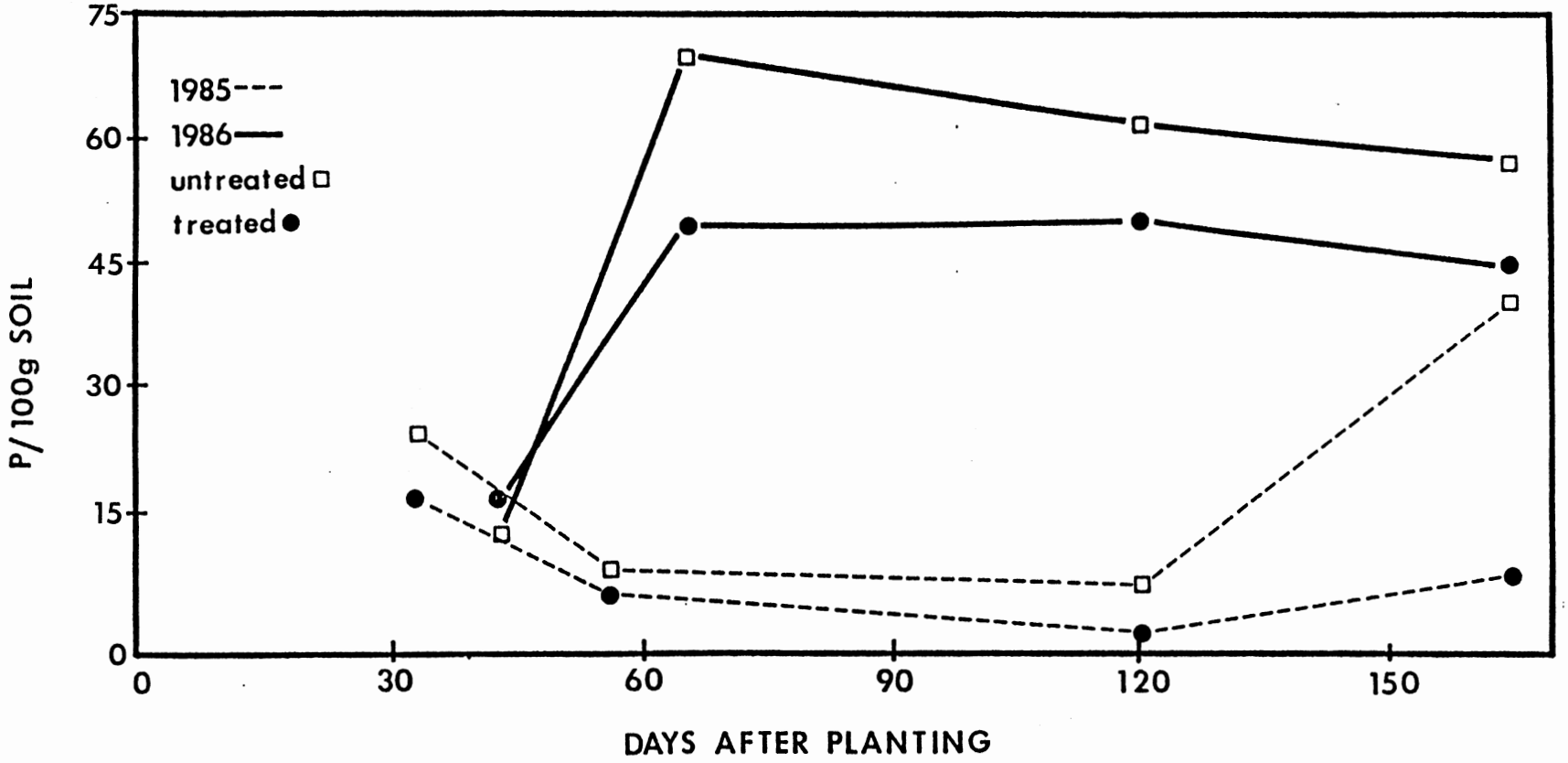

Fig. 1. Effect of tolclofos-methyl (TME) on inoculum densities of Rhizoctonia solani in soil from the pegging zones of all untreated or TME-treated peanut cultivars in the microplots during 1985 and 1986. In 1985 and 1986, TME (11.2 kg/ha) was applied 60 days after planting.

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CHAPTER IV

APPENDIX

A. Nonparametric analysis of disease severity classes for pod rot of peanut from microplots.

Tolclofos-methyl was evaluated for control of pod rot of peanut caused by R. solani in Oklahoma. Field experiments were conducted in both 1985, and 1986 (both described in Chapter III). For both seasons, peanut pods were examined and placed into a disease class (described in Chapter III) based on percent of the pod covered with lesions. The percentage of pods that fell into each of the five disease classes were ranked using nonparametric analysis. The purpose of using nonparametric analysis was to compare distributions of pods in each class or as each disease class as a category rather than a parameter. Rank means for the five disease classes for both field seasons are given in Table 1 and 2.

In some cases, there was a significant difference in the ranks for an individual disease class between the treated and the check. For instance, in 1985, Florunner at 1627.5 p/100g soil infestation inoculum density, rank means for the treated plots were significantly lower for disease class 2, but were significantly higher for disease class 3

and 5. Also, Spanco had significantly higher rank means for the treated plots from disease class 3, 4, and 5 at the 1627.5 p/100g soil inoculum density. In 1986, Florunner had significantly lower rank means for disease classes 2 and 3 and significantly higher rank means for disease class 5 at the 1354.5 p/100g soil infestation inoculum density. However, there were no significant differences between rank means for the treated and nontreated plots for Spanco at 1354.5 p/100g soil.

In 1985, Spanco had significantly higher rank means for the treated plots in disease class 4, at every inoculum density except the check, and in disease class 5, at every inoculum density; however, this was not true for 1986.

Tolclofos-methyl was effective in significantly reducing soil populations of R. solani; however, it did not significantly reduce pod rot. Control of pod rot of peanut caused by R. solani through the use of soil fungicides was inconsistant. Further studies are needed to address the behavior of this fungus when it encounters a fungicide in the soil.

Table 1. Nonparametric analysis of disease severity classes for pods from the 1985 microplot experiments

Cultivar	p/100g[z]	Treatment[y]	Percent in disease classes[x]				
			1	2	3	4	5
Florunner	0	-	20.0	15.0	12.0	17.0	20.0
		+	16.0	21.0	24.0*	19.0	16.0
	12.0	-	18.0	17.0	18.0	15.0	19.0
		+	18.0	19.0	18.0	21.0	17.0
	123.0	-	17.0	18.0	17.0	16.0	21.0
		+	19.0	18.0	19.0	20.0	15.0
	1627.5	-	19.0	22.0	13.0	15.0	13.0
		+	17.0	14.0*	23.0*	21.0	23.0*
Spanco	0	-	22.0	14.0	14.0	17.0	11.0
		+	14.0*	22.0*	22.0*	19.0	25.0*
	12.0	-	16.0	19.0	18.0	14.0	14.0
		+	20.0	17.0	18.0	22.0*	22.0
	123.0	-	16.0	23.0	16.0	15.0	14.0
		+	20.0	13.0*	20.0	21.0*	22.0*
	1627.5	-	19.0	17.0	12.0	11.0	13.0
		+	17.0	19.0	24.0*	25.0*	23.0*

[z]Inoculum densities of R. solani in soil used in infestation of microplots.
[y]Nontreated soil denoted by - and + = 11.2 kg a.i./ha tolclofos-methyl applied at flowering.
[x]Percent of pods in disease classes 1-5, where 1=no disease and 5=>75% discoloration of pod surface. * indicates that value is significantly (P=0.05) different from the value of no treatment. Critical value = 7.6.

Table 2. Nonparametric analysis of disease severity classes for pods from the 1986 microplot experiments

Cultivar	p/100g[z]	Treatment[y]	Percent in disease classes[x] 1	2	3	4	5
Florunner	0	-	14.0*	18.0	24.0	20.0	17.0
		+	22.0	18.0	12.0*	16.0	19.0
	211.5	-	20.0	25.0	13.0*	14.0	17.0
		+	16.0	11.0*	23.0	22.0*	19.0
	619.5	-	20.0	18.0	12.0*	17.0	16.0
		+	16.0	18.0	24.0	19.0	20.0
	1354.5	-	20.0	21.0	22.0	20.0	15.0
		+	16.0	15.0*	14.0*	16.0	21.0*
Spanco	0	-	18.0	17.0	16.0	19.0	18.0
		+	18.0	19.0	20.0	17.0	18.0
	211.5	-	15.0	15.0	22.0	21.0	19.0
		+	21.0	21.0	14.0*	15.0	17.0
	619.5	-	18.0	17.0	20.0	15.0	20.0
		+	18.0	19.0	16.0	21.0	16.0
	1354.5	-	18.0	20.0	18.0	15.0	19.0
		+	18.0	16.0	18.0	21.0	17.0

[z] Inoculum densities of *R. solani* in soil used in infestation of microplots.
[y] Nontreated soil denoted by - and + = 11.2 kg a.i./ha tolclofos-methyl applied at flowering.
[x] Percent of pods in disease classes 1-5, where 1=no disease and 5=>75% discoloration of pod surface. * indicates that value is significantly (P=0.05) different from the value of no treatment. Critical value = 7.6.

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Master of Science

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