INTERACTION OF <u>VERTICILLIUM</u> <u>DAHLIAE</u> KLEB. WITH

TRIFLURALIN OR VERNOLATE IN SPANISH

PEANUTS (<u>ARACHIS</u> <u>HYPOGAEA</u> L.)

By

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CHAPTER I

INTRODUCTION

Due to the high cost of labor and fuel, farmers are treating more acres with herbicides to control weeds this decade than previously. With the development and use of herbicides in peanuts the possibility exists that there could be an interaction between herbicides, plants, and plant pathogens. It is well known that trifluralin can injure peanuts under cool wet conditions (41) (42). Vernolate can also injure seedlings under certain conditions (87). Because of this there is the possibility that these herbicides could change the plant metabolism in some way making the peanut plant either more or less susceptible to Verticillium dahliae Kleb. Several researchers have indicated herbicides are interacting with V. dahliae in several other crops (13) (18) (38) (69) (83) (86).

Many agricultural chemicals used for weed control find their way into the soil environment. A knowledge of the effect of these materials on soil microorganisms is obviously desirable so that harmful effects to the soil environment can be minimized and beneficial effects can be utilized.

The objectives of this study were (a) to determine if there is an interaction between trifluralin or vernolate and V. dahliae in peanuts (Arachis hypogaea L. cv. 'Pronto') when peanuts are inoculated

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with _V. dahliae_ by a stem injection technique, (b) to determine

if there is an interaction between trifluralin or vernolate and

V. dahliae in peanuts cv. 'Pronto' when peanuts are inoculated

with _V. dahliae_ by a soil infestation technique, (c) to determine

if trifluralin or vernolate has a direct effect on mycelia growth,

and (d) to determine if trifluralin or vernolate has an effect

on the inoculum density of _V. dahliae_ in soil.

CHAPTER II

LITERATURE REVIEW

Trifluralin

Trifluralin is the common name for a,a,a-trifluro-2, 6-dinitro-N,N-dipropyl-p-toluidine. The trade names for trifluralin are: "Treflan, Trefanocide, and Elancolon". Trifluralin is used in peanuts to control most annual grasses and many small seeded broadleaf weeds, as they germinate. At recommended application rates, season-long weed control is usually obtained (1) with the degradation of trifluralin being nearly complete after 48 weeks (36) (60) (73).

Trifluralin at 0.6 to 1.1 kg/ha is the recommended rate for annual grass and broadleaf weed control. For best results trifluralin is incorporated into the top 5 to 8 cm within 24 hours after application (1). Some of the common implements used to incorporate trifluralin are: PTO-driven rotary cultivator, tandem disc, bed conditioner, rolling cultivator, and ground driven rotary hoe (22) (40).

Trifluralin does not directly inhibit seed germination. Its mode of action is to inhibit root growth and the most obvious symptom is inhibition of lateral or secondary root formation. Shoot growth can also be inhibited but this is probably a secondary effect caused by root growth being limited (2) (4). In most species, trifluralin is translocated only slightly from roots to other plant parts (73).

Trifluralin has been shown to inhibit the growth of peanut roots under certain environmental conditions. Hawxby et al. (41) observed that peanut (Starr) seedlings were injured by trifluralin under cool field conditions. In the laboratory, accumulation of trifluralin was greatest at 21 C which suggests that trifluralin injury due to low temperatures may result from increased uptake of trifluralin. A greenhouse-grown peanut seedling study showed that peanut roots were injured the most by trifluralin at 21 and 38 C. The least injury occurred at 32 C (41) (42). This correlates well with what was observed in the field. The time of peanut planting in Oklahoma is often during a cool wet period, indicating that at this time of the year trifluralin could be phytotoxic to peanuts.

Trifluralin can also cause peanut injury when the cation exchange capacity and organic matter levels are low. Cargill (17) found that when the percent organic matter was 0.0, trifluralin at 0.56 kg/ha injured the roots and stunted peanut plants. As the organic matter level was increased, the phytotoxicity of trifluralin to peanut plants was reduced. As the CEC increased, the amount of root injury from trifluralin at 0.56 kg/ha also decreased.

Vernolate

Vernolate is the common name for s-propyl dipropylthiocarbamate. The trade name for vernolate is "Vernam". Vernolate is used to control annual grasses, many broadleaf weeds as well as yellow (Cyperus esculentus L.) and purple (Cyperus rotundus L.) nutsedge in peanuts.

The recommended application rate for vernolate is 2.2 to 4.5 kg/ha. At recommended application rates, vernolate does not give season-long weed control (12). The half life of vernolate in soil at 21 to 27 C is approximately 10 days.

Vernolate needs to be incorporated into the top 5 to 8 cm of soil immediately after application. If vernolate is applied to dry soil, incorporation may be done by overhead irrigation (1).

Knowledge on the mode of action of thiocarbamate herbicides is rather limited. The mode of action of vernolate is not known. It is known, however, that vernolate inhibits growth in the meristematic region of grass leaves (1). Two major phytotoxic symptoms produced by thiocarbamates in broadleaf weeds are: (a) growth is generally inhibited and (b) leaves may be cupped with necrotic tissue around the edges (2). Bourke and Fang (11) found that vernolate is readily translocated from roots to upper foliage in peanuts. The application of vernolate at recommended rates (2.5 to 3.6 kg/ha) has been shown not to have an adverse effect on the yield of peanuts (19) (37); however, in the early stages of growth, the highest rate did slightly stunt peanut plants. This agrees well with the findings of Smith (88), who reported that vernolate was safe on peanuts when applied at 2.24 kg/ha, but was toxic when applied at 3.36 kg/ha.

Verticillium dahliae

Verticillium dahliae Kleb. is a soil borne pathogen which causes Verticillium wilt of peanuts. Verticillium dahliae has a wide range of hosts (87). The occurrence of V. dahliae has been reported in North,

Central and South America, Australia, Africa, Europe, Syria, USSR and
Iran (74) (81) (89).

Verticillium dahliae has the following morphological
characteristics (87):

> "Vegetative hyphae septate, hyaline, thin-walled, 2-4u
> diameter. Conidiophores verticillately branched septate.
> Seldom with secondary branches, hyaline, usually shorter
> (80-160u long), tapering from 3-5u at base to 2.0-2.5u at base
> of terminal whorl, bearing one-three whorls 30-40u apart,
> often with only one or two whorls on each conidiophore.
> Phialides frequently borne directly from long horizontal
> hyphae at irregular intervals. Phialides straight to slightly
> curved with septum at base, 14-26u long tapering from 2.5u at
> base to lu at apex, terminal phialides 30-44u long.
> Conidia continuous (rarely one septate), hyaline
> elliptical, borne singly at the apices of the phialides and
> often aggregated in heads, first-formed conidia mainly 3-5.5u
> x 1.5-2u. Resting mycelium septate, becoming dark brown, then
> budding to form abundant black, thick-walled microsclerotia
> about 30-60u diameter." (pg. 460)

Yield reduction in peanuts caused by Verticillium wilt has not been
well documented; however, in Australia, Verticillium wilt reduced the
yield of individual plants by 14 to 60% (74). In Oklahoma, yield
reductions of 63% have been reported in severely infested, irrigated
fields (91).

Tripp (90) gives the following description of the disease symptoms
caused by V. dahliae in peanuts:

> "Wilt symptoms are usually seen about flowering time as a
> dull green or chlorotic discoloration of portions of lower
> leaflets. As the disease progresses, many leaflets over the
> entire plant become withered and brown and fall from the
> plant. Infected plants may progressively lose foliage until
> they die, but unless unusually dry weather prevails, infected
> plants do not die rapidly or exhibit severe overall wilting.
> If adequate moisture is present, infected plants remain alive
> but are stunted with sparse foliage and are relatively
> unproductive. Brown to black vascular discoloration can be
> found in the root, stem and petioles in advanced stages of the
> disease." (pg. 455)

There are several means by which V. dahliae can survive adverse environmental conditions in soil. In early studies using V. albo-atrum Reinke and Berth, Schnathorst (75) (76) (77) suggested that there were several types of mycelia and hyaline microsclerotia capable of surviving in dried laboratory cultures. Conidia were found non viable after being air dried for 20 minutes. Therefore, conidia have been ruled out as being a factor in the survivial of V. dahliae under adverse conditions. Schnathorst and Fogle (80) later found that the viable dormant propagules of V. dahliae capable of surviving in soil were: hyaline hyphal fragments, hyaline torulose hyphae, hyaline globular cell masses or hyaline microsclerotia, or dormant cells in a hyaline matrix within or outside the host tissue.

Several researchers have reported that V. dahliae is capable of surviving in soil for at least 4 years (50) (74). Wilhelm (93) found that cultures of V. albo-atrum (a closely related species) 13 years old were still capable of growth. A soil that was known to be infested with V. albo-atrum was planted to grains and pasture for 14 years. After 14 years, a few viable propagules were found. This strongly suggests that V. dahliae is capable of long term survival in soil without the presence of susceptible hosts. Krikun (54) reported that V. dahliae was capable of infecting wheat, (triticum aestirum L.) but no symptoms occurred. It is well documented that many weed hosts propagate V. dahliae (30) (50).

Germination of conidia and microsclerotia of V. albo-atrum is greatly reduced in the presence of soil. Schreiber (84) found the average germination in the presence of soil for conidia and

microsclerotia was 12.2 and 11.5%, respectively. Germination in the absence of soil was 92.4 and 91.7%, respectively. Soil fungistasis was overcome when microsclerotia were in contact with a nonhost wheat and a host plant tomato (Lycopersicon esculentum Mill.). The increase in germination was an average of 29.7% for wheat and 83.3% for tomato. These findings indicate that a nonhost will stimulate germination of microsclerotia in soil but not to the degree a host plant will. Similar results to Schreiber's study have been reported for V. dahliae (8) (9) (62).

Sewell (82) reported that propagules of Verticillium need to be in close proximity to roots in order for infection to take place. Hyphae were not found more than 2 mm away from germinating propagules in soil. Garber (34) reported that Verticillium could penetrate virtually all of the underground plant parts of cotton (Gossypium hirsutum L.). If the root density doubled at a given inoculum density, Huisman and Ashworth (46) suggested that the probability of a root coming in contact with an infectious propagule would double.

In studying disease development, it is important to have an understanding of inoculum density. Using the Anderson air sampler technique, Schnathorst (80) found that 200 to 4000 V. dahliae propagules per gram of soil caused severe disease symptoms in cotton. It was suggested that the amount of variation found was due to the differences in the virulence of cultures found in naturally infested soils. Ashworth et al. (3) reported that by using the wet sieve technique 3.5 or more microsclerotia/gram were needed to obtain 100% incidence of Verticillium wilt in cotton fields. A disease incidence of 20 to 50% occurred in soils containing 0.3 to 1 microsclerotia/gram of soil.

There is a considerable difference in inoculum densities found between the Anderson air sampler and the wet sieve technique. Comparative studies have shown that the Anderson air sampler gives results 3 to 10 times higher than the wet sieve technique (15) (80).

Evans and McKeen (31) reported that 6 to 12 microsclerotia per gram of soil of V. dahliae caused severe wilt of eggplants (Solanum melongena L.) in two studies. The method used to estimate the number of microsclerotia was root bioassay, described by Evans et al. (32). Butterfield and Devay (14) reported that 10 propagules per gram of soil of V. dahliae would cause 100% incidence of disease in cotton.

The optimum temperature for growth of V. dahliae is between 22.5 and 25.0 C (79). Verticillium dahliae grows moderately well at 30 C, a little at 32 C, and none at 35 C (47). Ludbrook (60) reported that the maximum soil temperature at which V. dahliae caused disease symptoms in cotton was 30 C as compared to 28 C for V. albo-atrum.

Control of Verticillium wilt should be aimed at the initial inoculum (25). Verticillium wilt is a single cycle vascular wilt disease which seldom produces new inoculum that is effective during the same growing season. Epidemics build up because of increases in primary inoculum over several years. Any control measure which reduces the primary inoculum will have an effect on the rate of disease development (61).

Some of the various methods that can be used to reduce losses caused by Verticillium wilt in the field are: flooding, resistant cultivars, clean fallow, rotation, chemical control, irrigation timings, and proper amounts of major and minor elements (7) (15) (33) (43) (49) (52) (53) (55) (56) (57) (58) (65) (66) (71) (74) (78) (79) (67).

Interactions

In recent years many herbicides have been found to interact with plant pathogens (53). Both decreases and increases in plant resistance have been reported with the application of trifluralin depending on the host plant and pathogens. Jacobsen and Hopen (48) reported that dinoseb and trifluralin increased resistance of peas (Pisum arvense) to Aphanomyces root rot. Duncan and Paxton (20) reported that trifluralin increased rot of soybeans (Glycine max (L.) Merr.) caused by Phytophthora megasperma Drechs. by as much as 80%. Grinstein et al. (38) reported that trifluralin reduced the incidence of Rhizoctonia solani Kuehn. in eggplant, tomato, and pepper (Capsicum annuum L.) but had no effect on beans (Phaseolus vulgaris L.). El-Khadern et al. (23) reported that trifluralin and dinitramine (n4,n4-diethyl-a,a,a-trifluro-3,-5-dinitrotoluene-2, 4-diamine) increased preemergence and postemergence damping off caused by Rhizoctonia solani in cotton seedlings. Few references were found that dealt with vernolate and plant pathogens. However, vernolate was found to have no effect on the growth of Sclerotinia and the Rhizobium inoculant used in peanuts (70) (92).

Nilsson (69) reported that when trifluralin, alachlor [2-chloro-2, 6-diethyl-N-(methoxymethyl) acetanilide], and nitrofen (2,4-dichlorophenyl p-nitrophenyl ether) were applied to rape (Brassica napus L.), there was an increase in wilt disease caused by V. dahliae. The rape plant roots were inoculated by spraying 10 ml of a spore suspension containing 100,000 spores/ml when the third leaf began to develop. This was done 5 days before the herbicides were applied to the

the rape. In this experiment, the effect of the herbicides on entry
of V. dahliae into the plant was bypassed.

Hubbeling and Chaudhury (44) reported that a spore suspension of a
virulent isolate of V. dahliae survived for 1 week in a 5% solution of
chloramben (3-amino-2,5-dichlorobenzoic acid). All of the single spore
cultures taken from this treated suspension showed a decrease in
pathogenicity when compared to the original isolate. These findings
indicate that amiben has a mutagenic effect on V. dahliae.

Grinstein et al. (38) found that trifluralin and nitralin
[4-(methylsulfony)-2,6-dinitro-N,N-dipropylaniline] increased plant
resistance to Verticillium wilt. They planted eggplants and tomatoes
in soil treated with trifluralin and nitralin. After emergence,
seedlings were removed from this soil and inoculated with conidia
from V. dahliae. Plants were then planted in herbicide free soil.
Reduction in percentage of diseased seedlings from the control was
55 and 57% for nitralin and trifluralin, respectively.

Grinstein et al. (38) suggested several possibilities for
increased resistance of eggplants and tomatoes to Verticillium wilt. In
vitro, V. dahliae was not inhibited at herbicide concentrations found
in plant tissue and used in soil. However, the degradation products in
the plant tissue might be fungitoxic to V. dahliae. The second
possibility is that the virulence of the pathogen may be altered by the
herbicides. Another possibility is that trifluralin and nitralin alters
plant defense mechanisms in some way making them more resistant to
Verticillium wilt. If this is true then the mechanism may be
nonspecific because several herbicides have increased resistance of
eggplants and tomatoes to several diseases.

Sewell and Wilson(83) reported that the incidence of Verticillium wilt of hop (Humulus lupulus L.) was reduced when weed control was accomplished with paraquat (1,1-dimethyl-4,4-bipyridinium ion) at 9.88 lb ai/ha sprayed as multiple applications between July and February with a March application of simazine [2-chloro-4, 6-bis(ethylamino)-s-triazine] at 2.25 kg ai/ha. The control was normal tillage which stopped after June and allowed a tall dense weed growth to develop between the rows until winter plowing. The study was initiated in 1963 and ended in 1969. The reduction in disease severity was not noticed every year, only in years when the incidence of disease was high which was the first 4 years.

Sewell and Wilson (83) suggested three possible reasons for the reduction in the incidence of Verticillium wilt of hop: (a) the eradication of alternative weed hosts by the herbicide treatments reduced the build up of inoculum through the year (35) (39) (94); (b) cultivation severs infected roots which may aid in the build up and spread of the disease (82); or (c) the application of simazine was suggested to inhibit root growth which would reduce the amount of inoculum the roots came in contact with (46).

Several workers have reported that trifluralin, alachlor, prometryn [2,4-bis(isopropylamino)-6-(methylthio)-s-triazine], fluometuron [1,1-dimethyl-3-(a,a,a-trifluoro-m-tolyl) urea], diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], dipropetryn [2-(ethylthio)-4,6-bis(isopropylamino)-s-triazine], MSMA (monsodium methanearsonate), and DSMA (disodium methanearsonate) do not have an effect on the severity of Verticillium wilt in cotton grown in the field unless stands are reduced early in the growing season. Treatments that

had high plant populations had less severe disease symptoms than treatments with low plant populations (18) (86). This is in agreement with Blank et al. (10) who reported that higher cotton populations reduced the incidence of Verticillium wilt in cotton.

Erwin (13) reported that the growth retardants, N,N-dimethylpyrrolidinium iodide (DPII) and N,N-dimethylopyrrolinium iodide (DPYI), reduced the severity of Verticillium wilt and also reduced the number of propagules of V. albo-atrum in cotton plants. These chemicals were found not to be fungitoxic to V. albo-atrum in vitro. DPYI and DPII stimulated the production of hemigossypol in cotton plants. Hemigossypol is a phytoalexic in cotton (5) (6). This chemical may play a part in increased resistance of cotton to Verticillium wilt (95).

In 1974, Erwin (28) reported that CHE 8728 (tributyl[(5-chloro-2-thienyl)methyl] phosphonium chloride), a non-fungitoxic growth retardant used in cotton, increased cotton seed and lint yield by 13 to 20% over the control (24). The area in which these experiments were carried out usually approached 100% incidence of Verticillium wilt. The number of propagules/g of cotton petiole was determined at three times during the growing season. On all dates, CHE 8728 decreased the number of propagules when compared to the control. On the average, there was 16,633 propagules/g for the control and 1,767 propagules/g for the CHE 8728 treatments. Foliar symptoms were reduced also. Erwin and co-workers reported similar results in other studies (27), (29).

Increased resistance of cotton to Verticillium wilt was attributed to a change in the metabolism of the cotton (29). The application of CHE 8728 might produce phytoalexins like hemigossypol which may play a part in increasing resistance of cotton to Verticillium wilt (27).

Cycocel or chlorocholinechloride (CCC) a nonfungitoxic (27) growth regulator, reduced the number of Verticillium propagules in two varieties of cotton. The SJ-2 variety had an average of 25,000 propagules/g of tissue for the control and 2,200 propagules/g in plants treated with CCC. The SJ-3 variety had 19,000 propagules/g for the control and 2,800 propagules for the CCC treatment. An application rate of 10 g/ha CCC increased cotton seed and lint yields on Verticillium infested fields by 16.8 and 10.7% for the SJ-2 and SJ-3 varieties, respectively (26). The rate of CCC was an important factor in increasing yield (27). At 10 g/ha and 24.7 g/ha, there was an increase in yield, at 74 g/ha, there was a decrease in yield. Again, increased resistance was attributed to a change in the metabolism of the plant (27). Sinha and Wood (85) reported that CCC stimulated tylose formation in vessels. The formation of tyloses may slow the spread of V. dahliae within the plant.

Verticillium wilt symptoms have also been suppressed by the use of these non-fungitoxic growth regulators: 2-napthyloxy acetic acid, gibberellic acid, and 1,1-dimethyl-pyperidinium chloride (BAS 083) (21) (27).

CHAPTER III

METHODS AND MATERIALS

One growth chamber experiment and seven greenhouse
experiments were conducted to evaluate the interaction of two
herbicides (trifluralin and vernolate) with V. dahliae in Spanish
peanuts cv. 'Pronto'. Untreated peanut seeds were germinated in
flats of masonry sand. Seedlings were then transferred to 1 l pots
(one plant per pot) containing 1.5 kg of a herbicide treated soil
mixture. The soil media for all experiments was 1:1 w/w mixture of
masonry sand and sandy loam soil. The pH of the soil mix was 6.7.
The soil mix in peanut experiment I was pasturized with a portable
Soil King pasturizer[1] by heating the soil until it reached 83 C.
Pasturized soil was not used in any other experiments. The soil
mixture was treated with either trifluralin at 0, 0.375, 0.5, 0.75
and 1.0 ug/g or vernolate at 1.0, 1.5, 2.0, 3.0 and 4.0 ug/g of
soil and thoroughly mixed into 6 kg of mix. This was then divided
equally into 4 pots. The methods used for inoculating peanut
plants with V. dahliae will be discussed later. Peanut plants
were fertilized with Miracle Gro by applying 1 level tablespoon of
Miracle Gro to 3785 ml of water and then applying 40 ml of this
solution to each plant every 2 weeks. The guaranteed analysis for
Miracle Gro is as follows: N=18%, P205=18%, K20=21%, Mg=.5%,

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[1]Made by Soil King Products Co., Model 400 Deluxe.

Cu=.05%, Fe=.1%, Mn=.05%, and Zn=.05%. The light intensity for the growth chamber was 300 microeinsteins. Peanut experiments II through VIII were conducted in a greenhouse with a temperature range of 21 to 32 C. The light intensity for the greenhouse on a clear day was 1300 microeinsteins. When the experiments were terminated, the following plant measurements were taken: percent moisture of total above ground herbage, percent moisture of leaflets, relative water content of leaflets, and total fresh weight of above ground herbage.

Percent moisture of leaflets was obtained by sampling eight randomly selected leaflets from each plant. Relative water content was obtained from the same eight randomly selected leaflets by the following formula:(68)

$$\frac{\text{Fresh Wt. of Leaflets - Dry Wt. of Leaflets}}{\text{Saturated Wt. of Leaflets - Dry Wt. of Leaflets}} \times 100$$

Leaflets were placed in petri plates half filled with water for 2 days and then weighed to obtain the saturated weight of leaflets.

Unless otherwise mentioned, the experiments were conducted as a randomized complete block design with a 2x2x3 factorial arrangement of treatments. To determine the effect of V. dahliae on peanuts at each herbicide rate, an orthogonal comparison was made by subtracting the measured treatment effects with pathogen from the measured treatment effects without pathogen for the

control, and then comparing this value to the difference between the measured treament effects with pathogen and the measured treatment effects without pathogen for the herbicide rates. This was done to determine if any of the herbicide rates caused V. dahliae to develop wilt symptoms differently than the control. All data were analyzed with analysis of variance. Treatment affects were compared using LSD's at the 0.05 level.

V. dahliae Stem Injection Experiments

Peanut Experiment I.

A complete treatment list is shown in Table I. Peanut experiment I was conducted with four replications. Potted cultures were grown in a growth chamber with 12 hour days and a temperature range of 21 to 28 C. Forty days after planting, a 0.5 ml aliquot of a conidial suspension containing 500,000 conidia/ml of V. dahliae was injected with a hypodermic needle into the peanut plants 1 cm above ground level (64). Treatments not receiving injection were similarily treated but with sterile distilled water. Seventy days after inoculation, the experiment was terminated and plant measurements taken.

Peanut Experiment II.

Treatments were the same as in peanut experiment I except that it was conducted with eight replications. Peanuts were injected with the pathogen 28 days after planting and the experiment terminated 56 days after injection.

TABLE I

TREATMENT LIST FOR PEANUT EXPERIMENTS I THROUGH IV

	Treaments		
Herbicide	Rate		Pathogen
	Study 1 & II	Study III & IV	
	(ug/g)		
Trifluralin	0	0	No Pathogen
" "	0	0	With Pathogen
" "	0.5	0.375	No Pathogen
" "	0.5	0.375	With Pathogen
" "	1.0	0.75	No Pathogen
" "	1.0	0.75	With Pathogen
Vernolate	0	0	No Pathogen
" "	0	0	With Pathogen
" "	2.0	1.5	No Pathogen
" "	2.0	1.5	With Pathogen
" "	4.0	3.0	No Pathogen
" "	4.0	3.0	With Pathogen

Peanut Experiments III and IV.

A complete treatment list for both experiments III and IV is shown in Table I. Both experiments were conducted with eight replications. Peanuts were injected with the pathogen 28 days after planting and the experiments terminated 56 days after injection. Analysis of variance tables were pooled to determine if the experiments could be combined. There was a significant run by treatment interaction therefore, the experiments were not combined but, analyzed as two separate experiments.

Verticillium dahliae Infested Soil Experiments

Soil used in these experiments was artificially infested with V. dahliae. Verticillium dahliae was grown on potato dextrose agar plates for 28 days. The contents of twenty plates were placed into a blender with 1 l of sterile distilled water and blended for five minutes. This suspension was thoroughly mixed into 9.07 kg of soil. The soil was then allowed to air dry at 22 C for 10 days. Soil not receiving the pathogen was similarly treated but with sterile potato dextrose agar.

The number of propagules/g of soil was determined by using a soil dilution plating technique on ethanol water agar. The final inoculum density used in the experiments was obtained by diluting the infested soil with masonry sand and uninfested soil to obtain the proper inoculum density. Inoculum densities of 300 and 600 propagules/g of soil were used. Soil not receiving

the pathogen was similarly mixed with masonry sand and soil. Herbicide treatments were then applied to the infested soil and non-infested soil in the same manner as it was in experiments I through IV.

Peanut Experiment V.

Treatments were the same as in experiments III and IV except that the inoculum density used was 0 and 300 propagules/g of soil. Peanut experiment V was conducted with eight replications. Plant measurements were taken 103 days after planting.

Peanut Experiment VI.

Herbicide rates used were the same as in peanut experiment V. However, the inoculum density used was 0 and 600 propagules/g of soil. This experiment was conducted with eight replications. Plant measurements were taken 90 days after planting.

Peanut Experiment VII.

A complete treatment list for experiment VII is shown in Table II. Peanut experiment VII was conducted as a randomized complete block design with eight replications. The inoculum density used was 0 and 300 propagules/g of soil. Plant measurements were taken 95 days after planting.

TABLE II

TREATMENT LIST FOR PEANUT EXPERIMENTS VII AND VIII

Herbicide	Rate (ug/g)	Pathogen
1. Control	-	No Pathogen
2. Control	-	With Pathogen
3. Trifluralin	1	No Pathogen
4. Trifluralin	1	With Pathogen
5. Vernolate	4	No Pathogen
6. Vernolate	4	With Pathogen

Peanut Experiment VIII.

The design and herbicide rates used were the same as in experiment VII. The inoculum density used was 0 and 600 propagules/g of soil. Plant measurements were taken 90 days after planting.

Mean Diameter Growth of V. dahliae Experiments IX and X

Czapek dox agar was autoclaved and allowed to cool to 45 C in a water bath. Herbicides were applied immediately before pouring of the petri plates. This was done so that the herbicides would not be broken down by high temperatures. The petri plates were 100 by 15 mm style dishes. One day after plates were poured, 3 mm diameter plugs of V. dahliae were placed in the middle of plate. Colony diameter was measured at 1, 2, and 3 weeks of growth. Mean diameter of each culture was determined by measuring 3 randomly selected diameters, with a vernier caliper. The 3 diameters were then averaged to give the mean diameter growth for each colony.

Herbicide rates used were trifluralin at 0.5, 1.0, 10.0 and 100.0 ug/g and vernolate at 2, 4, 10, and 100 ug/g. A non-herbicide treated check was also included. Two experiments were conducted as a randomized complete block design. Experiment IX was conducted with eight replications and experiment X with four replications.

All data were analyzed with analysis of variance. Analysis of variance tables were pooled to determine if the experiments could be combined. There was a significant run by treatment interaction

therefore, the experiments were not combined but analyzed as two separate experiments. Treatment effects were compared using LSD's at the 0.01 level.

Survival of <u>V</u>. <u>dahliae</u> in Herbicide Treated Soil

Two greenhouse experiments were conducted to determine if the number of viable microsclerotia in soil was affected by the application of either trifluralin or vernolate. Soil infestation and herbicide applications were done in the same manner as was previously discussed. Experiments were conducted as randomized complete block design with four replications. Treatment effects were compared using LSD's at the 0.05 level of significance. The temperature range in the greenhouse was 21 to 32 C.

A modified Ashworth-Huisman (45) technique developed by Martin et al. (63) was used to determine the number of microsclerotia per 5 g of soil. Soil samples were taken and allowed to air dry for at least a week. Five g of air dried soil was washed through nested screens with 125 and 37 um openings. The residue that was retained on the 37 um screen was surface sterilized for 10 seconds in a 0.5% solution of NaOCL. The residue was then washed into a beaker where residue and water totaled 15 to 20 ml. Residue and water was plated onto 10 agar plates. These plates were prepared as follows: 2 g of agar was dissolved in 250 ml of water and autoclaved. After cooling to 48 C, 2.5 ml of the following solution was added: .133 g streptomycin sulfate (77%) and 0.05 g tetracycline (99%) was dissolved in 5 ml distilled water and 6 ml 95% ethanol. Plates were incubated as 21 C for 2 weeks at which time soil residue was

washed from the agar surface. Colonies of <u>V. dahliae</u> were counted
and each colony was assumed to have originated from 1
microsclerotia. The total number of colonies on the 10 agar plates
equals the number of microscleotia per 5 g of soil.

Experiment XI.

Soil was artificially infested with <u>V. dahliae</u> at 300
propagules/g of soil. A complete list of all treatments is shown
in Table III. Treatments that were designated to have peanuts were
planted with three peanuts per pot. This was done to determine if
root exudates have an effect on the growth of <u>V. dahliae</u> in herbicide
treated soil. Treatments were assayed for number of microsclerotia/
5 g of soil on the day of initiation and 1 week after initiation of
the experiment.

Experiment XII.

Soil was artificially infested with <u>V. dahliae</u> at 600
propagules/g of soil. Treatments were trifluralin at 1 ug/g,
vernolate at 4 ug/g and a control. Peanuts were grown in all pots.
After a 90 day incubation period soil samples were taken and
assayed for number of microsclerotia/5g of soil.

TABLE III

TREATMENT LIST FOR INOCULUM DENSITY EXPERIMENT XI

	Rate (ug/g)	Peanuts Present	
		Yes	No
Soil Sampled First Day			
Dry Soil	-		X
H20	-		X
Trifluralin	1		X
Vernolate	4		X
Soil Sampled After One Week			
H20	-	X	X
Trifluralin	1	X	X
Vernolate	4	X	X

CHAPTER IV

RESULTS AND DISCUSSION

Peanut Experiment I

Significant interactions between herbicides, rates, and pathogen were not observed for relative water content and percent moisture of leaflets (Table IV). A highly significant pathogen by herbicide rate interaction was observed for percent moisture of above ground herbage. Trifluralin at 1.0 ug/g significantly increased disease severity. Trifluralin at 0.5 ug/g and vernolate did not have an affect on the disease severity.

A pathogen by herbicide rate interaction was not observed for fresh weight of above ground herbage (Table IV). This was probably due to the variability in the weight of individual plants. However, there was a highly significant herbicide by rate interaction observed (Table IV). Trifluralin at 0.5 and 1.0 ug/g significantly reduced fresh weight. Vernolate did not affect fresh weight of peanut plants.

Peanut Experiment II

The results of peanut experiment II were similar to the results of peanut experiment I (Table V). Again there was not a pathogen by herbicide rate interaction observed for percent moisture

TABLE IV

EFFECT OF TWO HERBICIDES ON SPANISH PEANUTS
STEM INJECTED WITH V. DAHLIAE
(PEANUT EXPERIMENT I)

Herbicide	Rate (ug/g)	Inoculated With Pathogen 1/	Moisture of Above Ground Herbage	Change	Moisture of Leaflets	Change	Relative water Content of Leaflets	Change	Total Fresh Weight of Above Ground Herbage (grams)
						%			
Untreated	0	No	77.8		76.9		84.7		26.4
				4.6		13.7		39.3	
Untreated	0	Yes	73.2		63.2		45.4		19.8
Trifluralin	0.5	No	73.8		75.2		85.7		8.9
				3.8		11.4		38.0	
Trifluralin	0.5	Yes	70.0		63.8		47.7		8.6
Trifluralin	1.0	No	70.9		74.1		80.1		4.0
				18.0		21.7		48.0	
Trifluralin	1.0	Yes	52.9		52.4		32.1		1.8
Vernolate	2.0	No	77.6		78.5		92.1		26.9
				8.5		15.1		41.2	
Vernolate	2.0	Yes	69.1		63.4		50.9		18.9
Vernolate	4.0	No	76.2		76.4		87.4		23.0
				10.6		22.6		52.6	
Vernolate	4.0	Yes	65.6		53.8		34.8		14.0
LSD (0.05)			7.0 2/	9.9	8.3 2/	11.8 3/	12.4 2/	17.6 3/	6.6 2/

F Ratios

Source of Variability:

Herbicide	4.30*	0.20	0.03	43.02*
Pathogen	26.47*	71.71*	223.10*	13.83*
Rate	10.88*	4.21*	4.52*	22.67*
Herbicide x Pathogen	0.25	0.10	0.06	2.97
Herbicide x Rate	3.11	0.17	2.33	9.90*
Pathogen x Rate	3.41*	2.25	1.57	0.37
Herbicide x Rate x Pathogen	1.17	0.13	0.22	0.39

1 = Peanuts were inoculated with V. dahliae by a stem injection method with a 0.5 ml suspension containing 500,000 conidia/ml.

2 = Herbicide free checks were the result of 8 replications while all other treatments were the result of 4 replications. the corresponding LSD is for comparison of the herbicide free checks to any herbicide treatment.

3 = This LSD is for comparison of the herbicide free check to any herbicide treatment.

* F ratio is significant at the 0.05 level.

TABLE V

EFFECT OF TWO HERBICIDES ON SPANISH PEANUTS
STEM INJECTED WITH <u>V. DAHLIAE</u>
(PEANUT EXPERIMENT II)

Herbicide	Rate (ug/g)	Inoculated With Pathogen 1/	Moisture of Above Ground Herbage	Change	Moisture of Leaflets	Change	Relative water Content of Leaflets	Change	Total Fresh Weight of Above Ground Herbage (grams)
						%			
Untreated	0	No	72.9		73.6		86.2		31.5
				3.3		12.5		40.7	
Untreated	0	Yes	69.6		62.1		45.5		25.8
Trifluralin	0.5	No	71.2		72.2		81.0		8.9
				9.6		9.6		36.8	
Trifluralin	0.5	Yes	61.6		62.6		44.2		4.6
Trifluralin	1.0	No	69.9		70.5		71.3		3.4
				20.1		12.5		42.8	
Trifluralin	1.0	Yes	49.8		58.0		28.5		2.2
Vernolate	2.0	No	74.1		73.6		82.1		26.4
				5.8		14.0		45.1	
Vernolate	2.0	Yes	68.3		59.6		37.0		16.7
Vernolate	4.0	No	73.8		72.4		82.6		13.8
				7.3		9.2		33.1	
Vernolate	4.0	Yes	66.5		63.2		49.5		8.8
LSD (0.05)			4.4 2/	6.2 3/	4.4 2/	6.3 3/	8.1 2/	17.6 3/	3.0 2/

F Ratios

Source of Variability:

Herbicide			21.51*		1.13		8.56	113.97*
Pathogen			63.19*		125.34*		430.58*	55.36*
Rate			12.21*		0.58		5.69*	325.06*
Herbicide x Pathogen			6.98*		0.07		0.417	4.11*
Herbicide x Rate			9.39*		1.51		8.57	40.52*
Pathogen x Rate			8.43*		0.23		0.25	2.58
Herbicide x Rate x Pathogen			3.39		1,365		2.05	1.74

1/ Peanuts were inoculated with <u>V. dahliae</u> by a stem injection method with a 0.5 ml. suspension containing 500,000 conidia/ml.

2/ Herbicide free checks were the result of 16 replications while all other treatments were the result of 8 replications. The corresponding LSD is for comparison of the herbicide free checks to any herbicide treatment.

3/ This LSD is for comparison of the herbicide free check to any herbicide treatment.

* F ratio is significant at the 0.05 level.

of leaflets and relative water content of leaflets. There was a highly significant pathogen by herbicide rate interaction observed for percent moisture of above ground herbage. In this experiment, trifluralin at 0.5 and 1.0 ug/g significantly increased disease severity. As the rate of trifluralin increased disease severity increased. Vernolate did not significantly increase the severity of Verticillium wilt.

Again there was not a pathogen by herbicide rate interaction observed for fresh weight of above ground herbage (Table V). There was a herbicide by rate interaction observed. With an increase in trifluralin or vernolate, fresh weight was reduced.

Peanut Experiment III

A highly significant pathogen by herbicide rate interaction occurred for all three moisture measurements (Table VI). All 3 moisture measurements showed that trifluralin at 0.75 ug/g significantly increased disease severity. Vernolate at 1.5 ug/g significantly increased disease severity as measured by percent moisture of leaflets but did not for any other measurements. Vernolate at 3 ug/g did not affect disease severity.

As in peanut experiment I and II there was a herbicide by rate interaction but not a pathogen by herbicide rate interaction for fresh weight of above ground herbage (Table VI). A very significant reduction in fresh weight was obseved for trifluralin at 0.375 and 0.75 ug/g. Vernolate at 1.5 and 3 ug/g resulted in only a slight reduction in fresh weight.

TABLE VI

EFFECT OF TWO HERBICIDES ON SPANISH PEANUTS STEM INJECTED WITH V. DAHLIAE (PEANUT EXPERIMENT III)

Herbicide	Rate (ug/g)	Inoculated With Pathogen 1/	Moisture of Above Ground Herbage	Change	Moisture of Leaflets	Change	Relative water Content of Leaflets	Change	Total Fresh Weight of Above Ground Herbage (grams)
						%			
Untreated	0	No	76.4		77.1		94.1		30.8
				3.0		4.6		27.3	
Untreated	0	Yes	73.4		72.5		66.8		23.5
Trifluralin	0.375	No	75.4		74.9		94.1		14.2
				2.8		3.9		20.7	
Trifluralin	0.375	Yes	72.8		71.0		73.4		10.4
Trifluralin	0.75	No	74.8		75.1		95.1		12.7
				13.2		15.0		51.4	
Trifluralin	0.75	Yes	61.6		60.1		43.7		5.5
Vernolate	1.5	No	76.2		77.3		97.3		25.1
				3.1		9.1		32.6	
Vernolate	1.5	Yes	73.1		68.2		64.7		20.8
Vernolate	3.0	No	76.2		75.4		94.6		24.6
				3.6		8.4		37.8	
Vernolate	3.0	Yes	72.6		67.0		56.8		18.7
LSD (0.05)			3.0 2/	4.2 3/	2.8 2/	4.0 3/	8.4 2/	11.8 3/	4.0 2/

F Ratios

Source of Variability:

Herbicide			10.14*		4.50*		0.18		84.45*
Pathogen			45.57*		130.32*		275.77*		39.36*
Rate			9.95*		23.09*		9.18*		57.84*
Herbicide x Pathogen			5.47*		0.55		0.08		0.34*
Herbicide x Rate			7.66*		2.84		1.99		9.41*
Pathogen x Rate			6.29*		10.07*		8.75*		1.04
Herbicide x Rate x Pathogen			4.96*		6.63*		3.46*		0.20

1/ Peanuts were inoculated with V. dahliae by a stem injection method with a 0.5 ml. suspension containing 500,000 conidia/ml.

2/ Herbicide free checks were the result of 16 replications while all other treatments were the result of 8 replications. The corresponding LSD is for comparison of the herbicide free checks to any herbicide treatment.

3/ This LSD is for comparison of the herbicide free check to any herbicide treatment.

* F ratio is significant at the 0.05 level.

Peanut Experiment IV

Peanut experiment IV did not show a pathogen by herbicide rate interaction for percent moisture of leaflets and relative water content of leaflets (Table VII). However, a highly significant herbicide by rate by pathogen interaction occurred for percent moisture of above ground herbage. Trifluralin at 0.75 ug/g significantly increased disease severity. When the pathogen was present at this rate plants were severely wilted.

Again there was not a pathogen by herbicide rate interaction observed for fresh weight of above ground herbage (Table VII). As in the previous studies, a herbicide by rate interaction did occur for peanut experiment IV. Trifluralin at 0.375 and 0.75 ug/g and vernolate at 3 ug/g significantly reduced fresh weight of above ground herbage.

Peanut Experiment V

Peanut experiment V did not show a significant pathogen by herbicide rate interaction for any of the moisture measurements (Table VIII). However, when an unprotected LSD was used, there was a significant reduction in disease severity for trifluralin at 0.75 ug/g for all three moisture measurements. The reason the protected LSD was not significant was probably due to the variability with in the experiment. Vernolate did not significantly affect the severity of Verticillim wilt.

A significant pathogen by herbicide rate interaction was observed for the fresh weight of above ground herbage (Table VII).

TABLE VII

EFFECT OF TWO HERBICIDES ON SPANISH PEANUTS
STEM INJECTED WITH V. DAHLIAE
(PEANUT EXPERIMENT IV)

Herbicide	Rate (ug/g)	Inoculated With Pathogen 1/	Moisture of Above Ground Herbage	Change	Moisture of Leaflets	Change	Relative water Content of Leaflets	Change	Total Fresh Weight of Above Ground Herbage (grams)
Untreated	0	No	72.8		73.5		87.1		28.6
Untreated	0	Yes	66.0	6.8	62.2	11.3	49.4	37.9	18.2
Trifluralin	0.375	No	72.1		73.5		85.2		12.5
Trifluralin	0.375	Yes	61.6	10.1	61.6	11.9	45.8	39.4	7.6
Trifluralin	0.75	No	71.2		72.5		82.5		8.1
Trifluralin	0.75	Yes	49.3	21.9	56.7	15.8	27.9	54.6	3.6
Vernolate	1.5	No	73.1		72.2		86.1		28.1
Vernolate	1.5	Yes	66.5	6.6	62.8	9.4	54.4	31.7	20.4
Vernolate	3.0	No	72.5		72.6		84.9		22.9
Vernolate	3.0	Yes	68.3	4.2	61.7	10.9	49.1	35.8	19.7
LSD (0.05)			4.6 2/	6.5 3/	4.9 2/	6.9 3/	11.3 2/	16.0 3/	5.0 2/

F Ratios

Source of Variability:

Herbicide			17.76*		0.53*		4.42		70.41*
Pathogen			75.28*		103.64*		219.27*		33.23*
Rate			4.96*		1.16*		3.08		23.08*
Herbicide x Pathogen			10.09*		0.66		1.16		0.02
Herbicide x Rate			6.81*		0.56		1.59		16.55*
Pathogen x Rate			2.84*		0.45		1.19		2.59
Herbicide x Rate x Pathogen			6.38*		0.70		2.36		0.27

1/ Peanuts were inoculated with V. dahliae by a stem injection method with a 0.5 ml. suspension containing 500,000 conidia/ml.

2/ Herbicide free checks were the result of 16 replications while all other treatments were the result of 8 replications. The corresponding LSD is for comparison of the herbicide free checks to any herbicide treatment.

3/ This LSD is for comparison of the herbicide free check to any herbicide treatment.

* F ratio is significant at the 0.05 level.

TABLE VIII

EFFECT OF TWO HERBICIDES ON SPANISH PEANUTS INFECTED WITH <u>V. DAHLIAE</u> BY ARTIFICIALLY INFESTING THE SOIL (PEANUT EXPERIMENT V)

Herbicide	Rate (ug/g)	With Pathogen 1/	Above Ground Herbage	Change	Moisture of Leaflets	Change	Relative water Content of Leaflets	Change	Total Fresh Weight of Above Ground Herbage (grams)
						%			
Untreated	0	No	72.6		74.7		86.6		29.2
				9.0		12.9		41.2	
Untreated	0	Yes	63.6		61.8		45.4		20.2
Trifluralin	0.375	No	71.3		71.8		84.2		11.1
				5.0		4.3		16.3	
Trifluralin	0.375	Yes	66.3		67.5		67.9		13.0
Trifluralin	0.75	No	69.5		71.8		83.9		5.8
				1.5		1.0		14.1	
Trifluralin	0.75	Yes	68.0		70.8		69.8		6.4
Vernolate	1.5	No	72.8		72.9		89.4		28.0
				9.1		17.5		54.3	
Vernolate	1.5	Yes	63.7		55.4		35.1		20.5
Vernolate	3.0	No	73.0		72.2		88.4		21.6
				6.8		9.2		35.3	
Vernolate	3.0	Yes	66.2		63.0		53.1		16.9
LSD (0.05)			4.5 2/	6.3 3/	6.4 2/	9.1 3/	16.1 2/	22.7 3/	5.2 2/

F Ratios

Source of Variability:

Herbicide			0.00		5.46*		3.61		59.14*
Pathogen			40.48*		40.34*		78.74*		14.23*
Rate			0.33		0.96		1.41		32.34*
Herbicide x Pathogen			3.02		7.83*		8.82*		4.22*
Herbicide x Rate			0.18		0.61		0.85		7.16*
Pathogen x Rate			1.76		2.41		1.60		3.16*
Herbicide x Rate x Pathogen			0.25		0.74		1.25		1.11

1/ Peanuts were inoculated with <u>V. dahliae</u> by infesting the soil to an inocuulum density of 300 propagules/g.

2/ Herbicide free checks were the result of 16 replications while all other treatments were the result of 8 replications. The corresponding LSD is for comparison of the herbicide free checks to any herbicide treatment.

3/ This LSD is for comparison of the herbicide free check to any herbicide treatment.

* F ratio is significant at the 0.05 level.

There was not a significant difference between no pathogen and with pathogen for trifluralin at 0.375 and 0.75 ug/g and vernolate at 3 ug/g. However, there was a very significant decrease in fresh weight between no pathogen and with pathogen for the control and vernolate at 1.5 ug/g. As in peanut experiments II through IV, trifluralin and vernolate at 3 ug/g significantly reduced fresh weight of peanut plants.

Peanut Experiment VI

A highly significant pathogen by herbicide rate interaction occurred with trifluralin at 0.375 ug/g and vernolate a 3 ug/q for all three moisture measurements (Table IX). Both herbicides reduced disease severity. This interaction was also observed with trifluralin at 0.75 ug/g for percent moisture of leaflets and relative water content of leaflets but not for percent moisture of above ground herbage. The results of the fresh weight of above ground herbage was similar to peanut experiment V (Table VIII).

Peanut Experiment VII

Trifluralin at 1 ug/g significantly reduced disease severity as measured by all three moisture measurements (Table X). Vernolate also significantly reduced disease severity as measured by percent moisture of above ground herbage and percent moisture of leaflets but not for relative water content of leaflets. However, for this measurement vernolate showed a tendency to reduce disease severity.

As in previous experiments trifluralin significantly reduced

TABLE IX

EFFECT OF TWO HERBICIDES ON SPANISH PEANUTS INFECTED WITH V. DAHLIAE BY ARTIFICIALLY INFESTING THE SOIL (PEANUT EXPERIMENT VI)

Herbicide	Rate (ug/g)	Inoculated With Pathogen 1/	Moisture of Above Ground Herbage	Change	Moisture of Leaflets	Change	Relative water Content of Leaflets	Change	Total Fresh Weight of Above Ground Herbage (grams)
Untreated	0	No	72.9	5.0	72.2	10.5	92.5	41.3	32.1
Untreated	0	Yes	67.9		61.7		51.2		23.6
Trifluralin	0.375	No	73.7	0.9	73.5	3.2	88.7	10.4	13.8
Trifluralin	0.375	Yes	72.8		70.3		80.6		15.1
Trifluralin	0.75	No	71.2	2.0	71.2	2.0	88.7	15.4	7.5
Trifluralin	0.75	Yes	69.2		69.8		73.3		7.4
Vernolate	1.5	No	73.2	2.7	72.9	9.5	91.5	30.0	30.5
Vernolate	1.5	Yes	70.5		63.9		61.1		23.5
Vernolate	3.0	No	71.4	-0.1	71.4	0.8	91.54	8.8	23.0
Vernolate	3.0	Yes	73.2		70.6		82.7		25.9
LSD (0.05)			2.3 2/	3.3 3/	4.0 2/	5.6 3/	12.2 2/	17.2 3/	4.8 2/

F Ratios

Source of Variability:

	Moisture Herbage	Moisture Leaflets	Relative water	Total Fresh Weight
Herbicide	1.68	0.90	0.26	82.42*
Pathogen	22.27*	41.13*	72.41*	8.52*
Rate	5.06	5.86	6.51	37.13*
Herbicide x Pathogen	0.46	0.04	0.55	0.29
Herbicide x Rate	4.97*	1.99	2.51	16.94*
Pathogen x Rate	5.03*	7.75*	9.10	6.38*
Herbicide x Rate x Pathogen	1.39*	2.14	1.87	2.39

1/ Peanuts were inoculated with V. dahliae by infesting the soil to an inoculum density of 300 propagules/g.

2/ Herbicide free checks were the result of 16 replications while all other treatments were the result of 8 replications. The corresponding LSD is for comparison of the herbicide free checks to any herbicide treatment.

3/ This LSD is for comparison of the herbicide free check to any herbicide treatment

* F ratio is significant at the 0.05 level.

TABLE X

EFFECT OF TWO HERBICIDES ON SPANISH PEANUTS INFECTED WITH V. DAHLIAE BY ARTIFICIALLY INFESTING THE SOIL (PEANUT EXPERIMENT VII)

Herbicide	Treatments Rate (ug/g)	Infested With Pathogen 1/	Moisture of Above Ground Herbage	Change	Moisture of Leaflets	Change	Relative water Content of Leaflets	Change	Total Fresh Weight of Above Ground Herbage (grams)
Untreated	0	No	76.1		78.1		91.4		26.8
				14.0		22.9		49.7	
Untreated	0	Yes	62.1		55.2		41.7		15.4
Trifluralin	1.0	No	69.5		72.7		79.5		4.4
				0.6		3.1		7.4	
Trifluralin	1.0	Yes	68.9		69.6		72.1		6.5
Vernolate	4.0	No	75.6		76.4		92.3		29.0
				4.8		6.6		26.6	
Vernolate	4.05	Yes	70.8		69.8		65.7		19.0
LSD (0.05)			5.1	7.2	7.9	11.1	20.6	29.1	5.8

1/ Peanuts were infected with V. dahliae by infesting the soil to an inoculum density of 300 propagules/g.

fresh weight of above ground herbage. There was not a significant difference in fresh weight due to the pathogen for trifluralin at 1 ug/g. However, the control and vernolate at 4 ug/g showed a significant reduction in fresh weight when the pathogen was present.

Peanut Experiment VIII

As in peanut experiment VII, all 3 moisture measurements showed that trifluralin at 1 ug/g significantly reduced disease severity (Table XI). Vernolate also showed a significant reduction in disease severity but not to the degree that trifluralin did.

The results of total fresh weight of above ground herbage was the same as in the peanut experiment VII (Table X). Again, trifluralin at 1 ug/g significantly reduced the effects of V. dahliae.

Mean Diameter Growth of V. dahliae

Experiment IX.

Mean diameter growth of V. dahliae was measured at weekly intervals for three weeks. Trifluralin at 10 and 100 ug/g significiantly reduced growth of V. dahliae for the entire length of the experiment (Table XII), thus, indicating that at these high rates, trifluralin does have a fungitoxic effect on V. dahliae. Vernolate at 100 ug/g produced a significant reduction in growth one week after initiation of the experiment but by the end of the experiment there was not a significant difference from the control.

TABLE XI

EFFECT OF TWO HERBICIDES ON SPANISH PEANUTS INFECTED WITH V. DAHLIAE BY ARTIFICIALLY INFESTING THE SOIL (PEANUT EXPERIMENT VIII)

Herbicide	Treatments Rate (ug/g)	Infested With Pathogen 1/	Moisture of Above Ground Herbage	Change	Moisture of Leaflets	Change	Relative water Content of Leaflets	Change	Total Fresh Weight of Above Ground Herbage (grams)
Untreated	0	No	73.2		72.9		86.4		35.2
Untreated	0	Yes	61.6	11.6	51.4	21.5	31.7	54.7	17.0
Trifluralin	1.0	No	69.5		71.6		77.5		8.0
Trifluralin	1.0	Yes	69.9	-0.4	70.7	0.9	78.1	-0.6	10.4
Vernolate	4.0	No	73.5		72.2		85.6		35.4
Vernolate	4.05	Yes	68.6	4.9	65.0	7.2	54.8	30.8	21.7
LSD (0.05)			3.7	5.2	5.2	7.4	12.9	18.2	5.5

1/ Peanuts were infected with V. dahliae by infesting the soil to an inoculum density of 600 propagules/q.

TABLE XII

MEAN DIAMETER GROWTH OF V. DAHLIAE ON
HERBICIDE TREATED CZAPEK DOX AGAR
(EXPERIMENT IX)

| Treatments | | Weeks After Transfers | | |
Herbicide	Rate (ug/g)	One Week	Two Weeks	Three Weeks
		--------------------(cm)------------------		
Control	-	2.023	4.399	6.180
Trifluralin	0.5	2.010	4.384	6.129
Trifluralin	1.0	1.981	4.464	6.194
Trifluralin	10.0	1.616	3.961	5.771
Trifluralin	100.0	0.944	2.484	3.711
Vernolate	2.0	2.033	4.490	6.323
Vernolate	4.0	2.011	4.430	6.294
Vernolate	10.0	2.034	4.538	6.260
Vernolate	100.0	1.731	4.263	6.124
LSD (0.01)		0.066	0.065	0.091
CV %		2.72	1.17	1.16

At the end of the three week incubation period, there was a significant increase in growth with vernolate at 2 and 10 ug/g.

However, the greatest increase over the control was only 0.143 cm which is not of practical significance. Trifluralin at 0.5 and 1.0 ug/g did not significantly effect growth of V. dahliae.

Experiment X.

Trifluralin at 10 and 100 ug/g significantly reduced growth of V. dahliae when measured at one and three weeks after initiation of the experiment (Table XIII). Two weeks after initiation of the experiment, trifluralin at 10 ug/g did not significantly reduce growth. However, at 100 ug/g, growth was significantly reduced. As in experiment IX, vernolate at 100 ug/g produced a significant reduction in growth one week after initiation of the study but by the end of the study there was not a significant difference from the control. None of the herbicide treatments significantly increased growth of V. dahliae.

Inoculum Density

Experiment XI.

The soil mix, before any herbicide or moisture treatments were applied, had an inoculum density of 9 microsclerotia per 5 g of soil. The application of H2O, trifluralin at 1 ug/g or vernolate at 4 ug/g had no significant effect on the number of microsclerotia when sampled immediately after application (Table XIV). Soil samples taken one week after initiation of the experiment showed no significant effect on numbers of microsclerotia for any of the treatments when

TABLE XIII

MEAN DIAMETER GROWTH OF V. DAHLIAE ON HERBICIDE TREATED CZAPEK DOX AGAR (EXPERIMENT X)

Treatments		Weeks After Transfers		
Herbicide	Rate (ug/g)	One Week	Two Weeks	Three Weeks
		----------------cm----------------------		
Control	-	2.270	4.375	6.660
Trifluralin	0.5	2.260	4.278	6.518
Trifluralin	1.0	2.200	4.335	6.598
Trifluralin	10.0	2.075	4.290	6.415
Trifluralin	100.0	1.453	3.210	4.990
Vernolate	2.0	2.238	4.285	6.515
Vernolate	4.0	2.293	4.310	6.570
Vernolate	10.0	2.253	4.010	6.570
Vernolate	100.0	2.113	4.518	6.648
LSD (0.01)		0.103	0.472	0.172
CV %		2.45	5.71	1.36

TABLE XIV

EFFECT OF TWO HERBICIDES ON THE INOCULUM DENSITY
OF <u>V. DAHLIAE</u> IN SOIL WITH A BEGINNING
INOCULUM DENSITY OF 300 PROPAGULES/G
OF SOIL (EXPERIMENT XI)

	Rate (ug/g)	Peanuts Present Yes	No
Soil Sampled First Day			
Dry Soil	x	x	9
H20	x	x	15
Treflan	1	x	15
Vernam	4	x	14
Soil Sampled After One Week			
H20	x	18	24
Treflan	1	19	9
Vernam	4	17	25

LSD 0.05 = 12

Note: Results are given as number of microsclerotia/5g of soil.

peanut plants were present. Trifluralin at 1 ug/g significantly reduced the number of microsclerotia when peanuts were not present. Vernolate at 4 ug/g had no effect on the number of microsclerotia.

Experiment XII.

The soil mix at the beginning of this experiment had an inoculum density of approximately 20 microsclerotia per 5 g of soil. After 3 months, the inoculum density of the control doubled (Table XV). At this time, trifluralin at 1 ug/g and vernolate at 4 ug/g had significantly fewer microsclerotia than the control. Two possible explanations for this are: (1) Trifluralin is hindering the development of microsclerotia in the soil; and (2) because of reduced root mass, root exudates are not produced in as great a quantity as that of a non-herbicide treated plant. For this reason, microsclerotia in the soil may not be stimulated to germinate and increase the inoculum density of the soil.

TABLE XV

EFFECT OF TWO HERBICIDES ON THE INOCULUM DENSITY OF V. DAHLIAE IN SOIL WITH A BEGINNING INOCULUM DENSITY OF 600 PROPAGULES/G OF SOIL (EXPERIMENT XII)

Treatments		
Herbicide	Rate	Microsclerotia/5 g of soi
	(ug/g)	
Control	-	44.0
Trifluralin	1	21.5
Vernolate	4	8.0
LSD (0.05)		20.2

CHAPTER V

SUMMARY

Growth chamber and greenhouse experiments were·conducted to
investigate the interaction between two herbicides and V. dahliae
in 'Pronto' peanuts. The herbicides used were trifluralin and
vernolate.

In general, growth chamber and greenhouse experiments showed
that disease severity caused by V. dahliae was increased when
peanut plants were grown in soil treated with trifluralin at either
0.75 or 1.0 ug/g and subsequently stem injected with the pathogen. This
was true in all experiments where treatment effects were assessed by
measuring percent moisture of above ground herbage. Percent moisture
of leaflets and relative water content measurements were not as
sensitive to this interaction as percent moisture of above ground
herbage. Only in peanut experiment III did the high rate of
trifluralin significantly increase disease severity when treatment
effects were assesed by measuring percent moisture of leaflets and
relative water content of leaflets. Also, total top growth was not
a good indicator of the pathogen by herbicide rate interaction.

When soil was artificially infested with V. dahliae the
results were just the opposite of the experiments in which peanuts
were stem injected with V. dahliae. In general when trifluralin
was applied at 0.75 and 1.0 ug/g, disease severity was

significantly reduced. In peanut experiment VI, trifluralin at
0.375 ug/g even reduced disease severity. In general vernolate also
reduced disease severity when applied at 3 and 4 ug/g. The two
inoculum densities used showed no apparent difference in the results.
In these studies the results of percent moisture of leaflets and
relative water content of leaflets correlated very well with percent
moisture of above ground herbage. Also, total growth correlated
well with the moisture measurements for trifluralin but now always
for vernolate.

Laboratory experiments were conducted to determine if trifluralin
or vernolate reduces growth of V. dahliae. It was found that only
extremely high rates of trifluralin and vernolate significantly
reduced growth of V. dahliae. These rates being trifluralin at
10 and 100 ug/g and vernolate at 100 ug/g. From this information,
it is highly unlikely that either herbicide siginificantly re-
duces growth of V. dahliae in plant tissue or in the soil.

Two laboratory experiments were also conducted to evaluate the
effect of trifluralin and vernolate on the number of viable
microsclerotia per 5 g of soil. It was found that when trifluralin
at 1 ug/g and vernolate at 4 ug/g are applied to V. dahliae infested
soil and incubated for 3 months, the number of viable microsclerotia
are significantly reduced. Since peanuts were grown in these
cultures, it is possible that, because of reduced root mass, root
exudates are not produced in as great a quantity as that of a non-
herbicide treated plant. For this reason, microsclerotia in the
soil may not be stimulated to germinate and increase the inoculum
density of the soil. Another possibility is that trifluralin is
hindering the development of microsclerotia in the soil.

When cultures were incubated for one week, trifluralin at 1
ug/g significantly reduced the number of microsclerotia when peanut
plants were not present. However, when peanuts were present there
was no significant difference.

The results of the artificially infested soil experiments agree
with what Sewell and Wilson (83) reported. They suggested that when
root growth is inhibited, the amount of inoculum the roots come in
contact with is reduced. Huisman and Ashworth (46) suggested that
if the root density doubled at a given inoculum density the
probability of a root coming in contact with an infectious
propagule would double. Since trifluralin and vernolate reduces
root growth, it is possible that the roots are not in contact
with a sufficient number of propagules to incite disease symptoms.

Although increased resistance was obtained with trifluralin at
high rates it would not be practical to use these high rates
because of the significant reduction in top growth. However, it is
possible that under field conditions lower rates may obtain the same
results. Because of the lower number of microsclerotia found in
herbicide treated soil, long term use may reduce inoculum levels.
More research into this area is necessary to determine if this is
possible. Vernolate did not reduce top growth in some studies
and only slightly in other studies but still the benefits are
questionable. Again field research is necessary to determine if
trifluralin or vernolate could reduce Verticillium wilt severity
under field conditions.

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VITA 2

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