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Study of rhizosphere microflora in relation to varietal resistance or susceptibility of tomato to Fusarium oxysporum (Schlecht) f. sp. lycopersici (Saccardo) Snyder and Hansen

Donny D. McFall

To the Graduate Council:

I am submitting herewith a thesis written by Donny D. McFall entitled "Study of rhizosphere microflora in relation to varietal resistance or susceptibility of tomato to Fusarium oxysporum (Schlecht) f. sp. lycopersici (Saccardo) Snyder and Hansen." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.

Leander F. Johnson, Major Professor

We have read this thesis and recommend its acceptance:

James W. Hilty, Howard E. Reed

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

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and recommend its acceptance:

Howard E Reed

James W. Kelly

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

STUDY OF RHIZOSPHERE MICROFLORA IN RELATION TO VARIETAL RESISTANCE OR SUSCEPTIBILITY OF TOMATO TO *FUSARIUM OXYSPORUM* (SCHLECHT) F. SP. *LYCOPERSICI* (SACCARDO) SNYDER AND HANSEN

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee

Donny D. McFall

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ABSTRACT

The relationship of soil microflora (fungi, actinomycetes, and bacteria) isolated from the rhizosphere of four different tomato varieties on the resistance and susceptibility of these varieties to Fusarium oxysporum lycopersici was studied under laboratory conditions. Four tomato varieties chosen for their resistant and susceptible qualities to Fusarium oxysporum lycopersici were used: Better Boy, Manapal, Bonny Best, and Ponderosa.

The density of fungi, actinomycetes and bacteria in the rhizospheres and their antagonistic effect on F. oxysporum f. lycopersici were determined. The numbers of actinomycetes and bacteria were higher in the rhizospheres of the resistant varieties, Better Boy and Manapal, than in the susceptible ones, Bonny Best and Ponderosa. The numbers of fungi did not differ appreciably among varieties. The quantity of antagonism of actinomycetes and bacteria was significantly greater ($P < .05$) in the rhizospheres of the resistant varieties, Better Boy and Manapal than in the susceptible ones, Bonny Best and Ponderosa.

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CHAPTER I

INTRODUCTION

Since the early 1900's, soil microbiologists have been aware that the soil immediately adjacent to the root cortex of higher plants is a zone of intense microbial proliferation. In this zone, called the rhizosphere, early researchers discovered a marked increase in the numbers of fungi, bacteria, actinomycetes, protozoa, and nematodes. Later it was found that certain microorganisms are preferentially stimulated in the rhizosphere. Among the factors involved in influencing the composition of microorganisms in the rhizosphere are age and kind of plant, soil type, soil moisture and temperature, mineral fertilization, organic soil amendments, and foliar application of fertilizer and pesticides (11, 12).

During the past decade, using sophisticated methods of chromotographic and spectophometric analysis, soil chemists have shown that higher plants' roots exude various substances such as amino acids, sugars, vitamins, organic acids, nucleotides, flavones, enzymes, glycosides, auxins, and saponins. This exudate represents a localized increase in substrates that can be utilized in metabolic pathways of certain native microflora (12, 13).

Root exudates affect growth, physiology and parasitism of organisms in the rhizosphere. They stimulate germination of certain fungal spores and induce hatching of nematode eggs. Some of these responses have been shown to be specific, in that the organisms respond to the exudate of only one species (13, 14).

Recently, in the study of root diseases, emphasis has been placed on the differential effect of root exudates of resistant and susceptible host varieties on the activity of pathogens in the rhizosphere (5, 10, 16). There is evidence from these studies that varieties resistant to soil-borne fungal pathogens either directly exert less 'rhizosphere effect' on the pathogen or indirectly inhibit the microbe by promoting the activity of nonpathogens which are antagonistic to the pathogenic species (5, 10).

Studies by Lochhead et al. in 1940 demonstrated the difference in rhizosphere effect exerted by varieties of flax resistant and susceptible to wilt caused by Fusarium oxysporum f. lini (Bolley) Snyder and Hansen (9). They noted not only an increase in microorganisms in the rhizosphere of the susceptible varieties but also a qualitative difference in the flora composition. Timonin (18) suggested that the difference was due to the exudation of greater amounts of HCN in the rhizospheres of resistant flax. Timonin (17) noted a similar quantitative and qualitative rhizosphere effect in later studies of tobacco varieties resistant and susceptible to black root rot caused by Thielaviopsis basicola. Confirmation of the finding that susceptible varieties support larger numbers of microorganisms in the rhizosphere than corresponding resistant varieties has subsequently been reported with other species of crop plants (5, 10).

In an investigation performed by Subba-Rao and Bailey (15) five varieties of tomato, two susceptible and three resistant to wilt caused by Verticillium albo-atrum (Reinke and Berth), were studied

with respect to quantitative changes in fungal flora of their rhizoplane, the qualitative nature of their root exudates, and the possible interaction of all of these factors with the pathogen. More fungi occurred in the rhizospheres of the two susceptible varieties than in the rhizospheres of the resistant varieties. A third resistant variety had similar numbers of fungi as the susceptible varieties.

The objective of this study was to determine (1) the total populations of actinomycetes, fungi, and bacteria in the rhizospheres of four tomato varieties, (2) the populations of actinomycetes, fungi, and bacteria antagonistic to Fusarium oxysporum lycopersici in the rhizospheres of these varieties, and (3) if differences in total or antagonistic populations related to resistance or susceptibility of these varieties to Fusarium wilt disease.

CHAPTER II

MATERIALS AND METHODS

The tomato varieties used in this investigation were Better Boy, Manapal, Bonny Best, and Ponderosa. The first two are resistant to Fusarium wilt, the latter two are susceptible.

Fusarium oxysporum (Schlecht) f. sp. lycopersici (Saccardo) Snyder and Hansen Race I cultures were obtained from Dr. John Paul Jones, University of Florida, Bradenton, Florida. Immediately upon arrival, they were transferred to 10-ml potato dextrose agar slants.

Four plants per variety were grown in the greenhouse in four-inch pots filled with unsterilized clay silt-loam field soil. The soil was watered with tap water every 24 hours; approximately the same quantity was added to each pot. Samples of rhizosphere soil were obtained as follows: Four plants per variety were carefully removed from the pots and shaken individually to remove clumps of soil adhering to the root system. Three grams of rhizosphere soil were carefully removed from each plant, mixed thoroughly, and transferred individually to aliquots of 297-ml distilled water in 500-ml Erlenmyer flasks fitted with rubber stoppers. The flasks containing the rhizosphere soil-water solution were then shaken on a Burrell Shaker at a constant speed for 30 minutes. The flasks were removed and serial dilutions were made. Manual agitation during this process of serial dilution insured adequate distribution of the soil particles. One-ml aliquots of the 1:1,000 dilution of each sample were

pipetted into 20 sterile petri plates per variety and approximately 20-ml soil extract agar plus yeast extract medium (6) was added. Counts of fungi were taken on the sixth day after plating from 20 replicate plates per variety. Counts of actinomycetes and bacteria were made on the eighth day after plating from 20 replicate plates per variety. Afterwards, 25 colonies of fungi from each replicate were selected at random and transferred into test tubes containing slanted potato dextrose agar media (6). This procedure was repeated with actinomycetes and bacteria with the following exceptions: actinomycete colonies were transferred into test tubes containing oatmeal agar; bacteria were transferred into slants containing Czapek's mineral agar plus one gram of dextrose and one gram yeast extract per liter of solution (6).

Tests for antagonism of 400 isolates each of bacteria and actinomycetes were made against a pathogenic isolate of Fusarium oxysporum lycopersici by the following procedure: An isolate of a single bacterium or actinomycete was streaked on four sides near the edge of a 10-cm petri dish containing Czapek's dextrose yeast extract agar medium and incubated at 28°C. for 48 hours (6). An agar disk approximately 5mm in diameter from an actively growing culture of Fusarium oxysporum lycopersici was placed in the center of the petri dish. This procedure allowed approximately 25mm between each isolate and the Fusarium disk. After incubation for eight days at 28°C., the distance from the foremost edge of the Fusarium culture to the center of each actinomycete culture was determined and recorded in

millimeters. Nonantibiotic actinomycetes and bacteria overgrown by the Fusarium were discarded without further testing (6).

The fungi were tested by streaking the fungal isolates about the edge of a 10-cm petri dish containing Czapek's dextrose yeast extract agar medium (6). An agar disk approximately 5mm in diameter from an actively growing culture of Fusarium oxysporum lycopersici was placed at the opposite outer edge of the same petri dish. This left approximately 70mm between each fungus culture and the Fusarium disk. After seven days of incubation at 28°C., inhibition zones were measured and recorded in millimeters (6). Nonantibiotic fungi overgrown by the Fusarium were discarded without further testing.

The procedure described above was repeated for three separate plantings of tomatoes: May 1974, September 1974, and February 1975.

The data collected under laboratory conditions were taken and coded on IBM cards. Analysis of variance was conducted on each study to determine the significance of date, variety, replication, and interaction of variety with Fusarium oxysporum lycopersici. In the above analysis where variety was found to be a significant source of variation in the study, Duncan's new Multiple Range Test was used to evaluate which means were significantly different.

CHAPTER III

RESULTS

Means of population densities and antagonistic effects of microorganisms isolated from the four varieties of tomatoes are listed in Tables I, II, and III. Data collected under laboratory conditions were taken and coded on IBM cards with the format listed in Table IV. Analyses of these data are outlined in Tables V-XXII.

I. NUMBERS OF ORGANISMS PER GRAM OF RHIZOSPHERE SOIL

Means of numbers of fungi per variety are listed in Table I. Analysis of these data is presented in Table V. There was no significant difference at the five percent level of probability in numbers per gram among the varieties. There was a significant difference ($P < .001$) among the three planting dates.

Means of numbers of actinomycetes are listed in Table II. Analysis of these data is presented in Table XI. There were significantly more actinomycetes at the five percent level of probability in the rhizospheres of the resistant varieties (Better Boy and Manapal) than in the susceptible ones (Bonny Best and Ponderosa). Date of planting was a significant ($P < .001$) source of variation. There was a significant interaction between varieties and dates of planting ($P < .001$).

Means of numbers of bacteria per variety are listed in Table III. Analysis of these data is presented in Table XVII. There were

TABLE I

POPULATION DENSITIES AND ANTAGONISM TO *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* OF FUNGI ISOLATED FROM RHIZOSPHERES OF FOUR VARIETIES OF TOMATOES

	Means* of Three Planting Dates					
Variety	Number Per Gram of Soil	Number of Antagonistic Propagules/ Gram of Soil	Percent of Total That Were Antagonistic	Average Inhibition Zone (mm)/ Antagonist	Antibiotic Index**	Antibiotic Potential***
Better Boy	110,050[a]	4,625[a]	18.5[a]	19.5[a]	3.58[a]	395,314[a]
Manapal	114,450[a]	5,500[a]	22.0[a]	18.3[a]	4.60[a]	530,376[a]
Bonny Best	106,657[a]	3,375[a]	13.5[a]	21.0[a]	2.99[a]	277,660[a]
Ponderosa	101,100[a]	4,375[a]	17.5[a]	21.3[a]	3.72[a]	325,238[a]

*Means followed by the same letter are not significantly different at the 5 percent level of probability.

**Total zone of inhibition divided by number tested.

***Antibiotic index multiplied by number per gram soil.

TABLE II

POPULATION DENSITIES AND ANTAGONISM TO FUSARIUM OXYSPORUM F. SP. LYCOPERSICI OF ACTINOMYCETES ISOLATED FROM THE RHIZOSPHERE OF FOUR VARIETIES OF TOMATOES

	Means* of Three Planting Dates					
Variety	Number Per Gram of Soil	Number of Antagonistic Propagules/ Gram of Soil	Percent of Total That Were Antagonistic	Average Inhibition Zone (mm)/ Antagonist	Antibiotic Index**	Antibiotic Potential***
Better Boy	11,633,333[a]	2,083,333[a]	8.33[a]	12.40[a]	1.50[a]	12,673,000[b]
Manapal	10,266,667[b]	4,083,333[a]	16.33[a]	12.37[a]	2.78[a]	24,575,333[a]
Bonny Best	6,466,667[c]	2,666,667[a]	10.67[a]	9.10[ab]	1.70[a]	9,353,333[c]
Ponderosa	6,266,667[d]	1,250,000[a]	5.00[a]	4.79[b]	0.67[a]	4,107,667[d]

*Means followed by the same letter are not significantly different at the 5 percent level of probability to Duncan's Multiple Range Test.

**Total zone of inhibition divided by number tested.

***Antibiotic index multiplied by number per gram soil.

TABLE III

POPULATION DENSITIES AND ANTAGONISM TO FUSARIUM OXYSPORUM F. SP. LYCOPERSICI OF BACTERIA ISOLATED FROM THE RHIZOSPHERE OF FOUR VARIETIES OF TOMATOES

	Means* of Three Planting Dates					
Variety	Number Per Gram of Soil	Number of Antagonistic Propagules/ Gram of Soil	Percent of Total That Were Antagonistic	Average Inhibition Zone (mm)/ Antagonist	Antibiotic Index**	Antibiotic Potential***
Better Boy	24,100,000[a]	1,333,333[a]	5.33[a]	9.20[a]	1.07[a]	25,180,167[a]
Manapal	19,100,000[b]	1,583,333[a]	6.33[a]	8.21[a]	1.12[a]	16,394,333[b]
Bonny Best	13,750,000[c]	750,000[a]	3.33[a]	5.98[ab]	0.48[ab]	3,591,333[c]
Ponderosa	14,733,333[d]	250,000[a]	1.00[a]	2.29[b]	0.15[b]	1,593,333[d]

*Means followed by the same letter are not significantly different at the 5 percent level of probability.

**Total zone of inhibition divided by number tested.

***Antibiotic index multiplied by number per gram soil.

TABLE IV

FORMAT USED FOR IBM CARDS

Data	Code	IBM Cards Column Numbers
Date		1
May 1974	1	
September 1974	2	
February 1975	3	
Variety		2
Better Boy	1	
Manapal	2	
Bonny Best	3	
Ponderosa	4	
Replication		3
1	1	
2	2	
3	3	
4	4	
Response	Actual Number	4-20

TABLE V

ANALYSIS OF VARIANCE OF THE NUMBER OF FUNGI PER GRAM OF SOIL

Source	Degrees of Freedom	Sum of Squares ($\times 10^6$)	Mean Squares ($\times 10^6$)	F Value
Date	1	27,730.13	27,730.13	304.029***
Variety	3	761.78	253.93	2.784
Replication	3	744.58	248.19	2.721
Interaction (Date X Variety)	3	692.34	230.77	2.530
Residual	21	1,915.39	91.21	
Total	31	31,844.20		

***$P < .001$.

TABLE VI

ANALYSIS OF VARIANCE OF THE NUMBER OF ANTAGONISTIC FUNGI PER GRAM OF SOIL

Source	Degrees of Freedom	Sum of Squares (X 10^6)	Mean Squares (X 10^6)	F Value
Date	1	11.28	11.28	2.052
Variety	3	18.34	6.11	1.112
Replication	3	7.84	2.61	0.475
Interaction (Date X Variety)	3	35.09	11.70	2.128
Residual	21	115.41	5.50	
Total	31	187.96		

TABLE VII

ANALYSIS OF VARIANCE OF THE PERCENT ANTAGONISM OF FUNGI

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Date	1	180.50	180.50	2.052
Variety	3	293.50	97.83	1.112
Replication	3	125.50	41.83	0.475
Interaction (Date X Variety)	3	561.50	187.17	2.128
Residual	21	1,846.50	87.93	
Total	31	3,007.50		

TABLE VII

ANALYSIS OF VARIANCE OF THE ANTIBIOTIC INDEX FOR FUNGI

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Date	1	19.28	19.28	4.892*
Variety	3	10.60	3.53	0.896
Replication	3	4.45	1.48	0.376
Interaction (Date X Variety)	3	26.30	8.77	2.224
Residual	21	82.76	3.94	
Total	31	143.39		

*$P < .05$.

TABLE IX

ANALYSIS OF VARIANCE OF THE AVERAGE ZONE (MM) PER ANTAGONISTIC FUNGUS

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Date	1	134.28	134.28	7.926**
Variety	3	47.52	15.84	0.934
Replication	3	42.44	14.15	0.834
Interaction (Date X Variety)	3	14.96	4.99	0.294
Residual	21	355.76	16.94	
Total	31	594.94		

**$P < .01$.

TABLE X

ANALYSIS OF VARIANCE OF THE ANTIBIOTIC POTENTIAL OF FUNGI

Source	Degrees of Freedom	Sum of Squares ($X 10^6$)	Mean Squares ($X 10^6$)	F Value
Date	1	18,390.55	18,390.55	0.297
Variety	3	290,410.99	96,803.66	1,565
Replication	3	40,383.83	13,461.28	0.217
Interaction (Date X Variety)	3	350,496.93	116,832.31	1.889
Residual	21	1,298,298.11	61,823.72	
Total	31	1,997,980.40		

TABLE XI

ANALYSIS OF VARIANCE OF THE NUMBER OF ACTINOMYCETES PER GRAM OF SOIL

Source	Degrees of Freedom	Sum of Squares (X 10^8)	Mean Squares (X 10^8)	F Value
Date	2	2,396,466.67	1,198,233.30	12.704***
Variety	3	2,635,300.00	878,433.33	9.314***
Replication	3	512,100.00	170,700.00	1.809
Interaction (Date X Variety)	6	3,345,400.00	557,566.67	5.911***
Residual	33	3,112,300.00	94,312.12	
Total	47	12,001,566.70		

***$P < .001$.

TABLE XII

ANALYSIS OF VARIANCE OF THE NUMBER OF ANTAGONISTIC ACTINOMYCETES PER GRAM OF SOIL

Source	Degrees of Freedom	Sum of Squares ($X 10^8$)	Mean Squares ($X 10^8$)	F Value
Date	2	697,916.67	348,958.33	3.607*
Variety	3	512,291.67	170,763.89	1.765
Replication	3	155,625.00	51,875.00	0.536
Interaction (Date X Variety)	6	642,083.33	107,013.89	1.106
Residual	33	3,191,875.00	96,723.48	
Total	47	5,199,791.67		

*$P < .05$.

TABLE XIII

ANALYSIS OF VARIANCE OF THE PERCENT ANTAGONISM OF ACTINOMYCETES

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Date	2	1,116.67	558.33	3.607*
Variety	3	819.67	273.22	1.765
Replication	3	249.00	83.00	0.536
Interaction (Date X Variety)	6	1,027.33	171.22	1.106
Residual	33	5,107.00	154.76	
Total	47	8,319.67		

*$P < .05$.

TABLE XIV

ANALYSIS OF VARIANCE OF THE ANTIBIOTIC INDEX FOR ACTINOMYCETES

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Date	2	25.87	12.93	3.074*
Variety	3	27.25	9.08	2.159
Replication	3	5.51	1.84	0.436
Interaction (Date X Variety)	6	21.36	3.56	0.846
Residual	33	138.80	4.21	
Total	47	218.79		

*$P < .05$.

TABLE XV

ANALYSIS OF VARIANCE OF THE AVERAGE ZONE (MM) PER ANTAGONISTIC ACTINOMYCETE

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Date	2	268.94	134.47	2.627
Variety	3	466.22	155.41	3.036*
Replication	3	57.94	19.31	0.377
Interaction (Date X Variety)	6	702.31	117.05	2.287*
Residual	33	1,688.81	51.18	
Total	47	3,184.22		

*$P < .05$.

TABLE XVI

ANALYSIS OF VARIANCE OF THE ANTIBIOTIC POTENTIAL OF ACTINOMYCETES

Source	Degrees of Freedom	Sum of Squares ($X 10^8$)	Mean Squares ($X 10^8$)	F Value
Date	2	1,386,125.23	693,062.61	0.297
Variety	3	27,126,070.30	9,042,023.40	3.884*
Replication	3	5,863,285.95	1,954,428.60	0.839
Interaction (Date X Variety)	6	11,969,253.80	1,994,875.60	0.857
Residual	33	76,806,370.10	2,327,465.80	
Total	47	123,151,105.00		

*$P < .05$.

TABLE XVII

ANALYSIS OF VARIANCE OF THE NUMBER OF BACTERIA PER GRAM OF SOIL

Source	Degrees of Freedom	Sum of Squares ($X 10^8$)	Mean Squares ($X 10^8$)	F Value
Date	2	8,531,516.67	4,265,758.30	7.747**
Variety	3	8,055,425.00	2,685,141.70	4.876**
Replication	3	1,041,025.00	347,008.33	0.630
Interaction (Date X Variety)	6	8,354,550.00	1,392,425.00	2.528*
Residual	33	18,170,075.00	550,608.33	
Total	47	44,152,591.70		

*$P < .05$.

**$P < .01$.

TABLE XVIII

ANALYSIS OF VARIANCE OF THE NUMBER OF ANTAGONISTIC BACTERIA PER GRAM OF SOIL

Source	Degrees of Freedom	Sum of Squares ($X 10^8$)	Mean Squares ($X 10^8$)	F Value
Date	2	255,416.68	127,708.33	7.825**
Variety	3	128,958.33	42,986.11	2.634
Replication	3	78,958.33	26,319.44	1.612
Interaction (Date X Variety)	6	87,916.67	14,652.78	0.897
Residual	33	538,541.67	16,319.44	
Total	47	1,089,791.67		

**$P < .01$.

TABLE XIX

ANALYSIS OF VARIANCE OF THE PERCENT ANTAGONISM OF BACTERIA

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Date	2	434.00	217.00	8.690**
Variety	3	200.00	66.67	2.669
Replication	3	120.00	40.00	1.601
Interaction (Date X Variety)	6	150.00	25.00	1.001
Residual	33	824.00	24.97	
Total	47	1,728.00		

**$P < .01$.

TABLE XX

ANALYSIS OF VARIANCE OF THE ANTIBIOTIC INDEX FOR BACTERIA

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Date	2	12.94	6.47	7.228**
Variety	3	7.83	2.66	2.973*
Replication	3	4.84	1.61	1.803
Interaction (Date X Variety)	6	5.24	0.87	0.976
Residual	33	29.54	0.90	
Total	47	60.55		

*P < .05.

**P < .01.

TABLE XXI

ANALYSIS OF VARIANCE OF THE AVERAGE ZONE (MM) PER ANTAGONISTIC BACTERIUM

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Date	2	994.23	497.12	13.010***
Variety	3	338.70	112.90	2.954*
Replication	3	177.66	59.22	1.549
Interaction (Date X Variety)	6	303.56	50.59	1.324
Residual	33	1,260.84	38.21	
Total	47	3,075.00		

*P < .05.

***P < .001.

TABLE XXII

ANALYSIS OF VARIANCE OF THE ANTIBIOTIC POTENTIAL OF BACTERIA

Source	Degrees of Freedom	Sum of Squares ($X 10^8$)	Mean Squares ($X 10^8$)	F Value
Date	2	38,075,432.70	19,037,716.00	4.247*
Variety	3	44,597,571.40	14,865,857.00	3.136*
Replication	3	7,651,770.77	2,550,590.30	0.569
Interaction (Date X Variety)	6	33,263,811.70	5,543,968.60	1.236
Residual	33	147,921,187.00	4,482,460.20	
Total	47	271,509,774.00		

*$P < .05$.

significantly more organisms at the 5 percent level of probability in the rhizospheres of the resistant varieties (Better Boy and Manapal) than in the rhizospheres of the susceptible ones (Bonny Best and Ponderosa). There was a significant difference ($P < .01$) among dates of planting. Interaction was significant ($P < .05$) between varieties and dates.

II. NUMBERS OF ANTAGONISTIC PROPAGULES PER GRAM OF SOIL

Means of numbers of antagonistic fungi per variety are listed in Table I, page 8. Analysis of these data is presented in Table VI, page 13. There was no significant difference in numbers per gram of soil among the varieties.

Means of numbers of antagonistic actinomycetes per variety are listed in Table II, page 9. Analysis of these data is presented in Table XII, page 19. There was no significant difference at the 5 percent level of probability in numbers among varieties.

Means of numbers of antagonistic bacteria per variety are listed in Table III, page 10. Analysis of these data is presented in Table XVIII, page 25. There was no significant difference in numbers of antagonistic organisms among the varieties.

III. PERCENT OF TOTAL ORGANISMS THAT WERE ANTAGONISTIC

Means of percent total antagonistic fungi per variety are listed in Table I, page 8. Analysis of these data is presented in Table VII, page 14. There was no significant difference at the 5 percent

level of probability in the total percent antagonism among the varieties.

Means of the percent total antagonistic actinomycetes per variety are listed in Table II, page 9. Analysis of these data is presented in Table XIII, page 20. There was no significant difference at the 5 percent level of probability in total percent antagonism among varieties. There was a significant difference among dates of planting ($P < .05$).

Means of the percent total antagonistic bacteria per variety are listed in Table III, page 10. Analysis of these data is presented in Table XIX, page 26. There was no significant difference at the 5 percent level of probability in total percent antagonism among varieties. There was a significant difference among dates of planting ($P < .01$).

IV. AVERAGE INHIBITION ZONE (MM) PER ANTAGONIST

Means of the average inhibition zone (mm) for fungi per variety are listed in Table I, page 8. Analysis of these data is presented in Table IX, page 16. There was no significant difference at the 5 percent level of probability in the average zone per microorganism among varieties. There was a significant difference among dates of planting ($P < 01$).

Means of the average inhibition zone (mm) for actinomycetes are listed in Table II, page 9. Analysis of these data is presented in Table XV, page 22. The average inhibition zone per microorganism

was greater at the 5 percent level of probability in the resistant varieties than in the susceptible ones. Interaction was significant ($P < .05$) between varieties and dates.

Means of the average inhibition zone (mm) for bacteria are listed in Table III, page 10. Analysis of these data is presented in Table XXI, page 28. There were significantly larger inhibition zones per antagonist in the rhizospheres of Better Boy, Manapal, and Bonny Best than in Ponderosa. There was a significant difference among dates of planting ($P < .001$).

V. ANTIBIOTIC INDEX

The antibiotic index is defined as the average zone of inhibition (mm) per organism tested. Means of the antibiotic indices of fungi are listed in Table I, page 8. Analysis of these data is presented in Table VIII, page 15. There was no significant difference at the 5 percent level of probability in the antibiotic indices among the varieties. Date of planting was found to be a significant ($P < .05$) source of variation.

Means of the antibiotic indices of actinomycetes are listed in Table II, page 9. Analysis of these data is presented in Table XIV, page 21. There was no significant difference in the antibiotic indices among the varieties. There was a significant difference among dates of planting ($P < .05$).

Means of the antibiotic indices of bacteria are listed in Table III, page 10. Analysis of these data is presented in Table XX,

page 27. The antibiotic indices were significantly higher at the 5 percent level of probability in the rhizospheres of the resistant varieties than in the susceptible ones.

VI. ANTIBIOTIC POTENTIAL

The antibiotic potential is defined as the antibiotic index multiplied by the number of organisms per gram of soil. Means of the antibiotic potentials of fungi are listed in Table I, page 8. Analysis of these data is presented in Table X, page 17. There was no significant difference in the antibiotic potentials among the varieties.

Means of the antibiotic potentials of actinomycetes are listed in Table II, page 9. Analysis of these data is presented in Table XVI, page 23. There were significantly more pronounced antibiotic potentials at the 5 percent level of probability in the rhizospheres of the resistant tomato varieties (Better Boy and Manapal) than in the susceptible ones (Bonny Best and Ponderosa).

Means of the antibiotic potentials of bacteria are listed in Table III, page 10. Analysis of these data is presented in Table XXII, page 29. There were significantly larger antibiotic potentials at the 5 percent level of probability in the rhizospheres of the resistant varieties (Better Boy and Manapal) than in the susceptible ones (Bonny Best and Ponderosa). There was a significant difference among dates of planting ($P < .05$).

CHAPTER IV

DISCUSSION

Analysis of the numbers of fungi, actinomycetes, and bacteria isolated from the rhizosphere of two resistant and two susceptible tomato varieties refute earlier studies on other species of crop plants.

Data obtained from this study revealed no significant variations in the number of fungi among varieties. However, the numbers of actinomycetes and bacteria were found to be higher in the rhizosphere of the two resistant varieties, Better Boy and Manapal.

Timonin (17) observed that tobacco varieties susceptible to black root rot caused by Thielaviopsis basicola supported higher numbers of soil microorganisms than resistant ones. Confirmation of the finding that susceptible varieties support larger numbers of microorganisms in the rhizosphere than corresponding resistant varieties has been subsequently reported with other species of crop plants. This was observed by Harper (5) and Rombouts (10) in studies of Panama disease caused by Fusarium oxysporum f. cubense, Snyder and Hansen, by Lochhead (8) with flax varieties susceptible and resistant, respectively, to wilt caused by Fusarium oxysporum f. lini, by Buxton (2,3) with peas varying in resistance to strains of Fusarium oxysporum f. pisi, Snyder and Hansen, by Agnihothrudu (1) in a comparison of pigeon pea strains susceptible and resistant,

respectively, to wilt caused by Fusuarium udum and Subba-Rao with tomato varieties susceptible and resistant, respectively, to wilt caused by Verticillium.

These data reveal that both the susceptible and resistant tomato varieties responded indifferently in relation to the quantity of antagonistic fungi found in the rhizosphere. However, in a similar study involving soil microflora of potatoes, a higher percent of antagonistic fungi was found in the susceptible plant (16).

Timonin (17, 18) in a study of resistance of the flax variety Bison to Fusarium wilt showed that hydrocyanic acid was secreted in appreciable quantities by roots of resistant varieties, but only in traces by susceptible varieties. Trichoderma viride, a fungus antagonistic to Fusarium, was shown to be actually stimulated by the presence of hydrocyanic acid. Subba-Rao and Bailey (15) found an association between the high incidence of Trichoderma viride to Verticillium wilt in two resistant varieties of tomatoes, but not in a third, from the rhizosphere of which Trichoderma was absent.

The average inhibition zone between the test organism Fusarium oxysporum f. lycopersici and antagonistic actinomycetes tested was greater in the resistant varieties (Better Boy and Manapal). Agnihothrudu (1) observed that species of antagonistic streptomyces isolated from the rhizospheres of pigeon pea varieties resistant to Fusarium udum were highly inhibitory to Fusarium udum. In the rhizosphere of the potato variety "Up To Date" which is resistant to black scurf, Sudha (16) found a higher percentage of antagonistic

actinomycetes. It has been shown by work done with tobacco plants that varieties resistant to Fusarium wilt support a higher population of antagonistic actinomycetes in the rhizosphere than do varieties susceptible to the disease (17).

Bacteria isolated from rhizospheres of resistant varieties and tested in vitro against the pathogen exhibited a more pronounced quantitative antibiotic effect than ones obtained from the susceptible plants. The average zone per bacterium tested and the average zone per antagonistic bacterium were found to be higher within resistant varieties. The results stated above were in agreement with those of preceding authors. Harper (5) and Rombout (10) recorded bacteria that were highly antagonistic to Fusarium oxysporum cubense isolated from rhizosphere of the banana plant resistant to Panama disease.

CHAPTER V

SUMMARY AND CONCLUSION

This work indicates that both the density and antibiotic potentials of rhizosphere microflora were affected both quantitatively and qualitatively by tomato variety. Evidence pointing to this fact includes: (1) the numbers of actinomycetes and bacteria were higher in the rhizospheres of the resistant varieties, Better Boy and Manapal, than in the susceptible ones, Bonny Best and Ponderosa; (2) the amount and degree of antagonism of actinomycetes and bacteria were shown to be significantly higher ($P < .05$) in the rhizospheres of the resistant varieties, Better Boy and Manapal, than in the susceptible ones, Bonny Best and Ponderosa; and (3) the results obtained revealed that no significant differences in numbers of fungi occurred between the resistant and susceptible tomato varieties tested.

Results of this study suggest that an interaction among variety, number, and antagonistic rhizosphere microflora and the Fusarium wilt pathogen, Fusarium oxysporum f. lycopersici, does exist and that further research involving these interactions is desirable. In depth rhizosphere studies of other tomato varieties are necessary to determine how the rhizosphere microflora and pathogen are associated.

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