Effect of Crop Sequence, Previous Peanut Blackhull Severity, and Time of Sampling on Soil Populations of Thielaviopsis basicola

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New Mexico State University Agricultural Experiment Station Journal Article 664.

The author thanks Stephen Kendall and Hassalletta French for technical assistance; John Booth and Donald Kindsey for professional advice; and Melchor Ortiz for help in statistics.

Research supported in part by Grant 12-14-100-9944(34), U.S. Department of Agriculture, Agricultural Research Service.

Accepted for publication 3 May 1978.

ABSTRACT

HSI, D. C. H. 1978. Effect of crop sequence, previous peanut blackhull severity, and time of sampling on soil populations of Thielaviopsis basicola. Phytopathology 68: 1442-1445.

The selective medium of Tsao and Bricker (19) was used to determine soil populations of *T. basicola*. No significant differences in the mean populations were found between or within fields that had been cropped in four different sequences. However, blackhull severity was higher in fields under peanut monoculture than in fields under a rotation of peanuts and sorghum. Blackhull severity was lower in the middle portion of each field than at either end. The mean populations of *T. basicola* were significantly greater in fields where blackhull had been severe, than in those with low disease incidence. Populations of *T. basicola* during June and August were significantly lower than those determined in December or March.

Thielaviopsis basicola (Berk. & Br.) Ferraris [recently named *Chalara elegans* (12)] causes fruit discoloration (blackhull) on Valencia peanuts (*Arachis hypogaea* L. subsp. *fastigiata* Waldron var. *fastigiata*) (2, 3, 6, 8, 11). In New Mexico, severity of peanut blackhull was enhanced by low seasonal and soil temperatures, high late-season rainfall, excessive irrigation or heavy rain immediately following irrigation during the late growing season, fine-textured soil with poor drainage, and crop sequences of peanuts following peanuts or cotton (*Gossypium hirsutum* L.) (4, 5, 7). Acidified soil (12), a crop sequence of peanuts following grain sorghum (*Sorghum vulgare* Pers.) (5, 6), and high air and soil temperatures (7) suppressed the disease.

The principal soil groups in the peanut-growing area in New Mexico include Amarillo, Springer, Arvana, Portales, and Arch (15). Their textures range from coarse (sandy loam) to medium fine (loam). The soil pH values vary from 7.6 to 8.4 and average 8.0. Most of the soils are rather calcareous. A pH of 8.0 and low temperatures (15 to 16 C) were found to be optimum for the growth of several New Mexico strains of *T. basicola* (10). These conditions, however, do not favor peanut plant growth (14).

This paper reports the effects of crop sequence, previous blackhull severity, location in the field, and time of sampling on soil populations of *T. basicola*.

MATERIALS AND METHODS

Sixteen peanut fields with four different crop sequences were selected. Within each crop sequence, two fields were chosen for histories of high blackhull severity and two for low disease incidence. The fields were used as replicates for statistical analysis of the data. The fields were near Portales, the principal peanut-producing area in New Mexico, and all were furrow-irrigated. Peanuts were usually planted the latter part of May and the common New Mexico Valencia cultivar was used in all fields.

Soil samples (about 900 g each) were collected at three locations (upper end, mid-portion, and lower end of the irrigation flow pattern) in each field. The samples were taken at depths of 0-10 cm. At each location, 12 small samples were taken at 10- to 15-m intervals across the field to obtain a representative sample. The small samples were thoroughly mixed to form a composite sample and air dried prior to sieving and assaying. Soil samples were taken from plant beds during early June and late August 1972, and from plowed fields in December 1972 and March 1973.

Soils were assayed by the dilution plate technique (18). One random 10-g subsample was removed from each composite sample and placed in a sterile 250-ml Erlenmeyer flask. Five ml of sterile distilled water was added to each flask and the wet soil samples were left overnight in covered flasks (D. L. Lindsey, *unpublished*). After 24 hr, 95 ml of sterile distilled water were added to each pre-wetted soil sample. The first dilution of 10^{-1} (w/v) was mixed for 30 min on a wrist-action shaker. Further dilutions to 10^{-2} and 10^{-3} were made from the continuously agitated soil suspensions. A 1-ml portion of each dilution was pipetted to each 9-cm-diameter petri plate before 15 ml of cooled molten agar medium (43 to 45 C) were added. Assay plates were counted and examined microscopically on the 5th day of incubation through the 10th day or longer. A total of 30 petri plates were so treated. Counts from the 30 plates were averaged and this

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average constituted a single observation.

The medium used was a modified RB-M2 medium with the following composition: 10.0 g glucose, 0.5 g peptone, 0.5 g yeast extract, 0.5 g KH_2PO_4, 0.5 g $K_2HPO_4 \cdot 7H_2O$, 18.0 g agar, 0.05 g rose bengal, 0.03 g nystatin, 0.6 g pentachloronitrobenzene (PCNB), 0.03 g streptomycin,and one liter of sterile distilled water. In this study PCNB was used at 0.6 g instead of 0.5 g as in the original modified RB-M2 medium (19). This modified RB-M2 agar was suggested by Tsao and Bricker (19); it combines desirable features of Gilpatrick's RB-M2 (17) and Papavizas' VDYD-PCNB (13).

Least significant differences at $P = 0.05$ were calculated for any two mean values under consideration. Analysis of variance for factors affecting soil populations of *T. basicola* was calculated for the entire experiment.

RESULTS

Populations of *T. basicola* in natural field soils were determined by dilutions of 10^{-2} and 10^{-3}. Results of the two dilutions showed similar trends and agreed closely. For brevity, only results of the 10^{-3} dilution are presented in this paper. Individual assays ranged from 0 to 5,000 propagules/g soil. Only field averages are given in Table 1. Mean populations of *T. basicola* ranged from 66 to 759 propagules/g soil. Fields having high previous disease severity nearly always yielded higher populations of *T. basicola* than those having low previous disease severity. Soil populations of *T. basicola* did not differ significantly between either the two high-disease-incidence fields or the two low-disease-incidence fields within the same crop sequence. For that reason, the data from the two high-disease-incidence fields of the same crop sequence were combined as were those from the two low-disease-incidence fields (Table 2).

The analysis of variance for factors affecting soil population of *T. basicola* is shown in Table 3. Populations of *T. basicola* differed significantly between prior disease incidence (P) or among times of sampling (T) but not among three different locations (L) in the fields. Pathogen populations from fields having different crop sequences (C) differed at $P = 0.09$, but did not at $P = 0.05$. First-order interactions that showed significantly different populations of *T. basicola* were C × T, P × T, and L × T, all of which involved time of sampling. The second-order interaction that showed significantly different pathogen populations was C × L × T.

Mean populations of *T. basicola* from fields with different crop sequence histories did not differ significantly at $P = 0.05$ (Table 2). There also were no significant differences in populations of *T. basicola* between soil samples taken from three different locations in the field. Samples taken during June and August had significantly fewer propagules than samples taken during December and March. August sampling sometimes yielded higher populations than the June sampling. There were little or no differences in population counts between the December and March samplings. Previous history of blackhull severity in the field definitely affected populations of *T. basicola* in the field soils; fields with a record of blackhull severity yielded significantly higher pathogen populations than those with low disease incidence. With only one exception, such significant differences held true for soil samples either from different locations in the fields or from fields following different crop sequences. Taking all factors (including sampling time) into consideration, soil samples from high-disease-incidence fields yielded an average of 410 propagules/g soil whereas those from low-disease-incidence fields only 208 propagules/g soil (Table 2). Mean difference between high and low disease incidences exceeds the level needed for significance at $P = 0.01$.

TABLE 1. Soil population of *Thielaviopsis basicola* as affected by previous blackhull severity, crop sequence, and time of sampling, Portales, New Mexico, 1972 and 1973

Field	1970 or 1971 blackhull[a] incidence (%)	Crop sequence			Propagules/g soil: Time of sampling				
		1970	1971	1972	6/72	8/72	12/72	3/73	Avg.
A	25	P[b]	S	S	245[c]	533	478	589	461
B	31	P	S	S	200	356	511	578	411
C	8	P	S	S	111	311	245	256	231
D	8	P	S	S	66	333	200	222	205
E	36	P	S	P	233	311	478	589	403
F	27	P	S	P	345	322	759	589	504
G	5	P	S	P	89	155	367	311	231
H	6	P	S	P	67	133	333	278	203
I	30		P	S	200	467	378	511	389
J	33		P	S	389	389	456	456	423
K	11		P	S	111	278	222	233	211
L	14		P	S	133	189	156	234	178
M	38		P	P	200	191	578	444	353
N	37		P	P	222	215	400	489	332
O	14		P	P	89	200	278	278	211
P	8		P	P	100	55	322	278	189

[a]Percent of the peanut pods that showed more than 25% black discoloration.
[b]Abbreviations for crops: P = peanuts and S = sorghum.
[c]Each figure is the average of three locations (upper end, mid-field, lower end relative to irrigation flow) in the field.

DISCUSSION

Results from this study indicate that nonhost plants (eg, sorghum) had little influence on populations of *T. basicola* in field soils. Previous observations (5) indicated that peanuts following grain sorghum had considerably less blackhull than peanuts following peanuts or cotton. The differences in disease severity between crop sequences were apparently not due to the differences in pathogen populations. Bateman (1) previously found nonhost plants (wheat and corn) did not influence populations of *T. basicola*. He observed only a small increase in pathogen numbers in nonrhizosphere soil planted with host plants (tobacco and bean), and this occurred only after disease was well advanced. In the present study, it is possible that sorghum roots could have loosened soil texture, thus raising soil temperatures to the detriment of *T. basicola*. Furthermore, sorghum rhizosphere soils could have higher numbers of microorganisms or compounds inhibitory to growth of *T. basicola* than the peanut or cotton rhizosphere soils. Such differences in the microflora between crop sequences would not be detected with selective media favoring *T. basicola*.

Pod samples taken from upper or lower ends of the irrigated fields frequently had more blackhull than those taken from the mid-field. These differences in disease severity between locations could not be explained by the differences in populations of *T. basicola* because all three locations in this study showed essentially the same number of propagules/g soil (Table 2). The differences in disease severity between locations were probably due to differences in soil temperatures. Flooding and consequently lower soil temperatures frequently occurred at the lower and upper ends of the fields that received furrow irrigation. Low soil temperatures favor the growth and development of *T. basicola*.

Soil samples taken during the growing season had lower populations of *T. basicola* than those taken during winter fallow periods. Conditions in June and August could have stimulated the germination of chlamydospores in the decaying endocarpic tissues of the nonharvested fruits and subjected the mycelia and endoconidia to lysis as a result of microbial activities. In the cold months (December and March in the High Plains of New Mexico) *T. basicola* exists and survives in soil mainly as chlamydospores as in California (19), and these spores become the origin of nearly all colonies developing from natural soils in the selective agar medium. Freshly produced mycelia and endoconidia and some

TABLE 2. Summary of populations of *Thielaviopsis basicola* in soil sampled at four different times at three locations in fields having high or low incidence of peanut blackhull and following different crop sequences at Portales, New Mexico, in 1972 and 1973

Crop sequence			Location in the field	Propagules per gram of soil								
				Avg. for soil sampling time					Average for disease incidence			Avg. for crop
1970	1971	1972		6/72	8/72	12/72	3/73	LSD[a] ($P = 0.05$)	High	Low	LSD ($P = 0.05$)	sequence
P[b]	S	S	U.E.[c]	133	384	350	392	93	396	233	143	
			M.	150	383	375	409	147	438	221	161	
			L.E.	183	383	350	434	172	475	200	242	
			Av.	156	383	359	411	243	437	218	172	328
P	S	P	U.E.	200	284	403	483	117	435	250	155	
			M.	108	200	592	425	157	446	217	205	
			L.E.	242	208	458	417	89	479	183	154	
			Av.	183	231	484	442	124	454	217	161	336
	P	S	U.E.	175	300	333	400	124	413	192	152	
			M.	250	292	267	342	NS[d]	396	179	112	
			L.E.	200	400	309	333	76	408	213	164	
			Av.	208	331	303	359	78	406	195	101	301
	P	P	U.E.	217	185	433	375	128	405	200	124	
			M.	67	92	392	434	164	313	179	NS	
			L.E.	175	220	359	308	84	310	221	77	
			Av.	153	165	394	372	126	343	200	89	272
Average for locations in the field			U.E.	181	288	380	413	97	412	219	40	
			M.	144	242	407	403	173	398	199	70	
			L.E.	200	303	369	373	113	418	204	149	
LSD ($P = 0.05$)				NS	NS	NS	NS		NS	NS		NS
Average for sampling time				175	278	385	396	74				
Average for disease incidence									410	208	65	

[a]LSD ($P = 0.05$) = least significant difference at $P = 0.05$.
[b]Abbreviations for crops: P = peanuts and S = sorghum.
[c]Abbreviations for location in the field: U.E. = upper end, M. = mid-field, and L.E. = lower end (relative to irrigation flow).
[d]The abbreivation NS = not significant (for the differences between means).

TABLE 3. Analysis of variance for factors affecting populations of *Thielaviopsis basicola* in New Mexico field soils

Source of variation	DF	Mean squares	F-value	PR > F
Crop sequence (C)	3	40,103.56	3.10	0.0892
Prior incidence level (P)	1	1,960,814.63	151.58	0.0001
C × P	3	20,300.42	1.57	0.2709
Fields/C × P (Error a)	8	12,935.63		
Locations (L)	2	4,975.40	0.74	0.4912
L × C	6	4,399.10	0.66	0.6846
L × P	2	1,842.15	0.28	0.7629
L × C × P	6	12,602.04	1.88	0.1460
(L × F) / (C × P) (Error b)	16	6,692.85		
Time (T)	3	516,425.71	44.09	0.0001
T × C	9	56,640.90	4.84	0.0009
T × P	3	46,073.74	3.93	0.0205
T × C × P	9	3,727.85	0.32	0.9609
(T × F) / (C × P) (Error c)	24	11,711.81		
T × L	6	12,413.31	2.62	0.0282
T × L × C	18	12,585.86	2.65	0.0037
T × L × P	6	7,065.22	1.49	0.2020
T × L × C × P	18	5,243.51	1.13	0.3762
(T × L × F) / (C × P) (Error d)	48	4,743.82		
Total	191			

transformed endoconidia "secondary chlamydospores" (16) can develop into colonies in soil dilution plates (14). In this study, nonviable mycelia and endoconidia probably outnumbered the viable ones and thus populations of *T. basicola* during the growing season were lower than those during the winter fallow periods.

Previous incidence or severity of blackhull affected the populations of *T. basicola*. Fields with high blackhull severity conceivably left more infected pod tissues and propagules than those with less disease. It is also possible that differences in incidence of blackhull are due to undefined field conditions, favorable or unfavorable, for growth and development of the pathogen.

It is conceivable that even the best selective media can only offer an estimate of the real populations of *T. basicola* from limited soil samples and that a wider range of populations could be detected with some other media such as those reported recently by Maduewesi et al. (9). However, the relationships among the several variables considered in this study would likely remain unchanged because of the size and scope of this experiment, adequately replicated in plates, location, sampling times, and number of sampled fields.

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