Evaluation of Four Upland Cotton Genotypes for a Rate-Limiting Resistance to Phymatotrichum Root Rot

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ABSTRACT

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Resistance to Phymatotrichum root rot was evaluated in four genotypes of upland cotton reported to possess varying degrees of resistance. Under conditions of controlled soil temperature, inoculum density, inoculum distribution, and host population density, no significant resistance was observed. The four host genotypes had no effect upon the rate of growth of *Phymatotrichum omnivorum* through the soil. Under a regime of increasing soil temperatures, the pathogen grew at a rate best fit by a Gompertz growth model. Wilt symptom progression from plant to plant within rows of the four genotypes did not differ significantly. All symptom progression rates were highly linear. The symptom-expression interval, or the time between fungal contact with the host root system and wilt symptom expression, was highly variable within genotypes but not significantly different between genotypes. The time of initial symptom development did vary among the four genetic stocks and appeared to be influenced by the growth phase and maturity of the hosts. Under the conditions of the investigation, no evidence of host tolerance or rate-limiting resistance was observed.

Phymatotrichum root rot (caused by *Phymatotrichum omnivorum*) causes major economic loss to the cotton producers of Texas, New Mexico, and Arizona (16). Despite the pathogen's restricted edaphic range, 2% of the total cotton yield in Texas has been lost to this disease annually (18). Various management and control schemes for root rot have been attempted with varying degrees of success. Deep plowing of infested fields was an early recommendation that produced some beneficial effects (10,11,17). Soil treatment with salt (9) and deeply placed anhydrous ammonia (13) have had mixed results. Crop rotation schemes have been and continue to be proposed (10,11). To date, the single most effective measure allowing the continued production of cotton in infested areas has been the use of short-season determinate cultivars. The earlier planting dates and shorter production periods of these cultivars allow a proportion of the potential yield of a crop to be set prior to disease onset in most seasons.

Recently, cotton breeding efforts have shifted from escape mechanisms to an emphasis on identification and selection of a true resistance (1–3). Although claims for progress in obtaining resistance have been made (1,2), the potential for resistance breeding has been questioned (9). One view is that significant levels of resistance are unlikely due to the broad nonspecific host range of the pathogen and its generalized mode of attack upon the host plant. A further problem with identifying resistance is that supporting data have come from field evaluations in which physical environment, inoculum density, inoculum distribution, and host population distribution have been highly variable and uncontrolled (7). All the above factors profoundly influence the rate of disease spread—the measure most often used as a criterion for resistance to *P. omnivorum* (1,2). The present investigation was conducted to determine if reported resistance in cotton could be observed under more controlled conditions and to clarify the manner in which potential resistance is manifested.

MATERIALS AND METHODS

The investigation was conducted under greenhouse conditions in soil temperature control tanks at College Station and Temple, TX, in the fall of 1983.

Four cotton genotypes were selected for evaluation of root rot resistance. Three of these genotypes were strains developed in the Multi-Adversity Resistance (MAR) cotton improvement program of the Texas Agricultural Experiment Station and represented superior (CAMAS-2-81), intermediate (CABCS'-1-81), and low (CAHUS-2-81) levels of resistance available in that program. The fourth genotype was a commercial cultivar (Lankart LX571) which had been selected and developed from germ plasm with a long production history in the Blacklands of Texas. The resistance potential of Lankart LX571 was unknown at the time of the test. This cultivar was considered to be a slower developing, later maturing type than the three MAR strains.

Seed from the four genotypes were planted in metal trays containing nonsterile Houston Black clay, a montmorillonitic vertisol. The trays were 27.9 cm deep, 15.2 cm wide, 71.1 cm long, and were filled with soil to a depth of 26 cm. Seeds were hill-planted at a depth of 1.9 cm at intervals of 5 cm. The resulting seedlings were thinned to obtain a uniform row of 12 plants of a single genotype per tray. Soil temperature at the time of planting was 21.1 C. This initial temperature was increased by 2.8-C increments at weekly intervals until a final soil temperature of 29.4 C was attained 4 wk after planting.

At the time of planting, the soil in each tray was infested with 3 g of sclerotia of *Phymatotrichum omnivorum* (Shear) Dug. buried at a depth of 12.7 cm at one end of each tray. The sclerotia were derived from a single isolate of the fungus. Sclerotia were produced in 250-ml flasks containing 1 cm of autoclaved sorghum seed overlying 5 cm of autoclaved Houston Black clay. Plugs taken from a culture of *P. omnivorum* grown on potato-dextrose agar were placed in the flasks. The flasks were sealed and placed in a dark cabinet where they were checked periodically for sclerotia formation. Concurrent with soil infestation, samples of sclerotia were plated on water agar in petri dishes to check viability.

Prior to planting and soil infestation, four 40-cm lengths of clear plastic tubing were placed at 20.3-cm intervals in each tray of the experiment at Temple, TX. The tubes were placed at a slant across the width of the trays and extended the full depth of the trays. The positioning of the tubes corresponded to plant positions 1, 4, 7, and 10 in the planted rows. Sclerotia of *P. omnivorum* were placed in direct contact with the first tube in each tray. Observations of root growth, fungal growth, and root infection were made through the

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tubing by using a borescope as previously described (15).

Measurements and observations included: leaf area increase (square millimeters), time of first fruiting bud initiation (squaring), time of fungal strand appearance at borescope observation portals, time of fungal contact with host roots, and time of wilt symptom development on individual plants. Time intervals for all observations were calculated from the date of seedling emergence. Leaf areas were obtained from measurements made at 3-day intervals on the three tallest plants in a tray. Time of squaring was determined to be the date on which fruiting buds approximately 3 mm long and triangular in shape could be observed. From the above observations, rates of leaf area increase, fungal growth through the soil, and wilt symptom progression in rows were calculated. The time interval between fungal contact with roots and wilt symptom development also was calculated.

The experimental design for the investigation was a randomized complete block consisting of six replicate trays. The design was repeated twice at College Station and once at Temple. Statistical tests included analysis of variance and regression methods.

RESULTS

Fungal growth. The rate of growth by *P. omnivorum* through the soil under the four genetic stocks was not influenced by host genotype, as indicated by the nonsignificant differences in the time of fungal strand appearance at the four borescope tube positions (Table 1). That the time of sclerotial germination was more variable than the subsequent growth rate was indicated by the high coefficient of variability at tube position 1. Combined data from the four genotypes produced a fungal growth curve best fitting the Gompertz model (Fig. 1). As expected, the growth rate of *P. omnivorum* increased as soil temperatures increased.

TABLE 1. Time of *Phymatotrichum omnivorum* strand appearance at borescope observation tubes placed at 20-cm intervals in rows of four cotton genotypes[y]

Genotype	Days until strand appearance at successive observation tubes			
	1	2	3	4
CABCS'-1-81	8.7 a[z]	23.6 a	31.8 a	32.2 a
CAHUS-2-81	6.0 a	28.6 a	36.0 a	31.2 a
CAMAS-1-81	6.5 a	23.2 a	31.0 a	32.0 a
Lankart LX 571	9.2 a	24.3 a	27.3 a	35.0 a
Mean	7.6	24.8	30.9	32.6
C.V.	44.0	19.4	20.3	10.0

[y] Times of strand appearance were calculated from the date of plant emergence.
[z] Days followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Host growth and development. Measurements of the growth and development of the host genotypes indicated Lankart LX571 and CAMAS-1-81 to be slightly slower developing genotypes than CAHUS-2-81 and CABCS'-1-81. The rates of leaf area increase of the four genotypes did not diverge significantly in the College Station test until the last date of measurement; 31 days after emergence. On that date, CABCS'-1-81 had the greatest leaf area (820 cm^2). Lankart LX571 and CAMAS-1-81 had lower leaf areas (750 cm^2 and 728 cm^2, respectively). Significant differences among the genotypes for the date of squaring were obtained in the Temple test and across tests (Table 2). Lankart LX571 and CAMAS-1-81 were the later-squaring genotypes by rank.

Symptom development. Analysis of variance indicated that in the two College Station tests, initial wilt symptom development and disease progression were significantly delayed in comparison to the test at Temple. However, there was no significant genotype-by-test interaction for these parameters. Results for Temple and College Station are reported separately as well as combined in Tables 2 and 3. The differences in disease progression rates in the tests are responsible in part for the lower linear correlations of plant position in rows with time of symptom development obtained across tests (Table 3).

The date of initial wilt symptom development did not differ significantly between genotypes in any test (Table 2). There was a definite trend, however, for initial symptom expression to appear last in the Lankart LX571 cultivar. Across tests, Lankart LX571 was approximately 3 days later than the earliest genetic stock in symptom expression.

Once wilt symptoms began to progress from plant to plant within rows of the four genotypes, no significant differences in rates could be detected. An across-tests analysis of variance of the slopes of symptom progression produced a nonsignificant F value ($F_{3,58}$ =

TABLE 2. The time of squaring and initial wilting induced by *Phymatotrichum omnivorum*, rates of symptom progression in rows, and time intervals for symptom expression in four genotypes of upland cotton in three experiments

Experiment and location	Genotype	Days until squaring[v]	Days until initial symptom[v]	Symptom progression rate[w]	Symptom expression interval[x]
Temple	CAMAS-1-81	30.0 a[y]	32.2 a	1.98 a	20.3 a
	Lankart LX 571	29.7 ab	35.2 a	1.78 a	21.5 a
	CABCS'-1-81	28.9 b	32.5 a	2.13 a	20.3 a
	CAHUS-2-81	27.7 c	34.3 a	2.07 a	19.7 a
	Mean of genotypes	29.1	33.5	2.00	20.5
	C.V.	2.7	12.0	18.6	33.7
College Station I, II[z]	CAMAS-1-81	42.8 a	46.3 a	2.69 a	
	Lankart LX 571	42.1 a	49.2 a	3.26 a	
	CABCS'-1-81	41.9 a	45.2 a	3.28 a	
	CAHUS-2-81	41.7 a	46.5 a	2.40 a	
	Mean of genotypes	42.1	46.7	2.88	
	C.V.	3.2	15.0	30.7	
Across tests	CAMAS-1-81	38.5 a	41.0 a	2.44 a	
	Lankart LX 571	37.9 ab	43.3 a	2.67 a	
	CABCS'-1-81	37.5 bc	40.1 a	2.82 a	
	CAHUS-2-81	37.0 c	41.6 a	2.26 a	
	Mean of genotypes	37.8	41.5	2.54	
	C.V.	3.3	13.4	29.2	

[v] Days until squaring and initial symptom are the number of days after plant emergence.
[w] Slopes of linear regression lines (days per plant).
[x] Time in days from fungal contact with host roots to expression of wilt symptom.
[y] Values within columns for each location followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[z] Means and Duncan's multiple range test were computed from pooled data of two tests.

1.64). The Lankart LX571 cultivar, which developed symptoms later, generally had fewer plants with symptoms on any given date. However, the rate of symptom progression in LX571 was equivalent to that of other genotypes. Symptom progression was linear in both space and time. Plant-to-plant progression sequence was the rule with escape plants being the exception. Rates of symptom progression down rows were found to best fit simple linear models (Table 3, Fig. 2). Significant deviation from a linear model was noted in only one genotype (CABCS'-1-81) in one experiment. On the average, there was a 2-day interval between symptom development on successive plants at Temple and a 2.9-day interval at College Station (Table 2).

The symptom-expression interval, or the time between fungal contact with host root systems and wilt symptom appearance, did not differ significantly among the four genotypes. The across-genotype mean symptom-expression interval was 20.5 days. Although this time interval was highly variable, as indicated by a high coefficient of variance, the range between genotypes spanned only 1.8 days.

DISCUSSION

Given that the growth and dissemination of *P. omnivorum* from primary infection sites is essential to the further development and spread of disease, host factors that limit pathogen growth could greatly retard the spread of root rot. The genotypes observed here displayed no evidence of such a limitation, as no significant differences in the growth rate of *P. omnivorum* in soil under the four genotypes was observed. The possibility exists that *P. omnivorum* could have grown the 71.1-cm row lengths without requiring the intervening plants as sources of nutrition or without interacting with the hosts in some manner. Despite this possibility, the observation that plants died in sequence suggests that a nearby energy source, if not required for fungal growth, was required for successful infection and subsequent symptom expression. The failure to observe differences in rates of symptom progression among the four genotypes suggests that they were equally susceptible hosts. Further, if the time interval required for symptom expression following fungal contact with the host root system is a measure of relative host tolerance, then none of the genetic stocks investigated displayed significant tolerance to the pathogen.

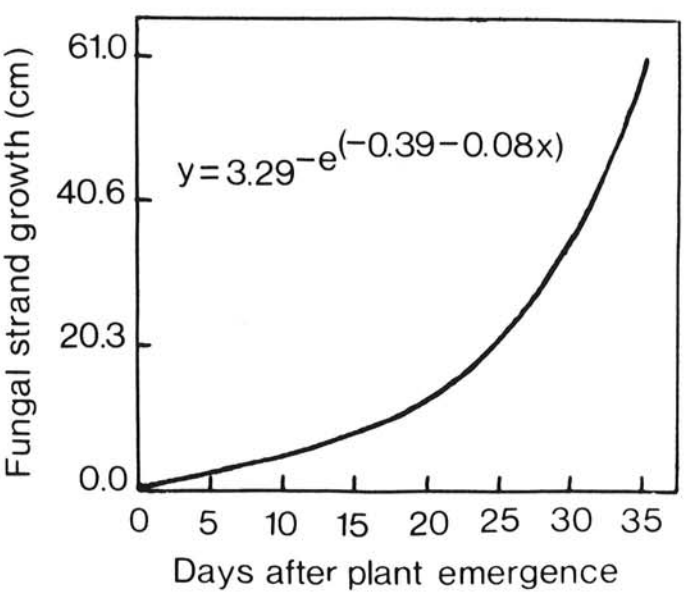

Fig. 1. Growth of *Phymatotrichum omnivorum* in soil under four genotypes of upland cotton. The curve represents growth under controlled conditions. Initial soil temperature was 21.1 C and was increased by 2.8-C increments per week to a final temperature of 29.4 C. Data used in producing the curve best conformed to a Gompertz growth model described by the equation given. Axes have been reversed to conform to convention.

Previous investigations have indicated that the time of initial wilt symptom development of Phymatotrichum root rot is influenced by the host's growth phase and maturity (4–6,12,14). Results from this investigation, though not statistically significant, tend to support the implication of a host-maturity factor in symptom development. Cultivar Lankart LX571, which had one of the slower rates of leaf area increase and later squaring dates, ranked last for the date of initial symptom development.

The failure to observe differences in symptom progression rates, fungal growth rates, or symptom expression intervals within the genotypes of the current investigation requires some reconciliation with the previous reports of resistance of these genotypes. It may be that the levels of resistance observed under field conditions were due to fortuitous combinations of circumstances. There is some

TABLE 3. Tests for significance of relationships between plant position in rows and time of Phymatotrichum root rot symptom development in four cotton genotypes in three experiments

Experiment	Cultivar	Linear *F*	Nonlinear *F*	Linear *r*
Temple	CABCS'-1-81	492.1**[a]	9.35**	0.92
	CAHUS-2-81	232.9**	2.12	0.87
	CAMAS-1-81	275.8**	0.41	0.89
	Lankart LX571	199.7**	1.32	0.93
CS I & II	CABCS'-1-81	280.1**	0.32	0.85
	CAHUS-2-81	234.7**	1.28	0.83
	CAMAS-1-81	108.8**	0.66	0.70
	Lankart LX571	86.4**	0.80	0.71
Across tests	CABCS'-1-81	141.2**	0.59	0.67
	CAHUS-2-81	110.3**	2.19	0.63
	CAMAS-1-81	95.6**	0.65	0.58
	Lankart LX571	61.2**	0.71	0.54

[a] ** F value of regression significant at $P = 0.01$.

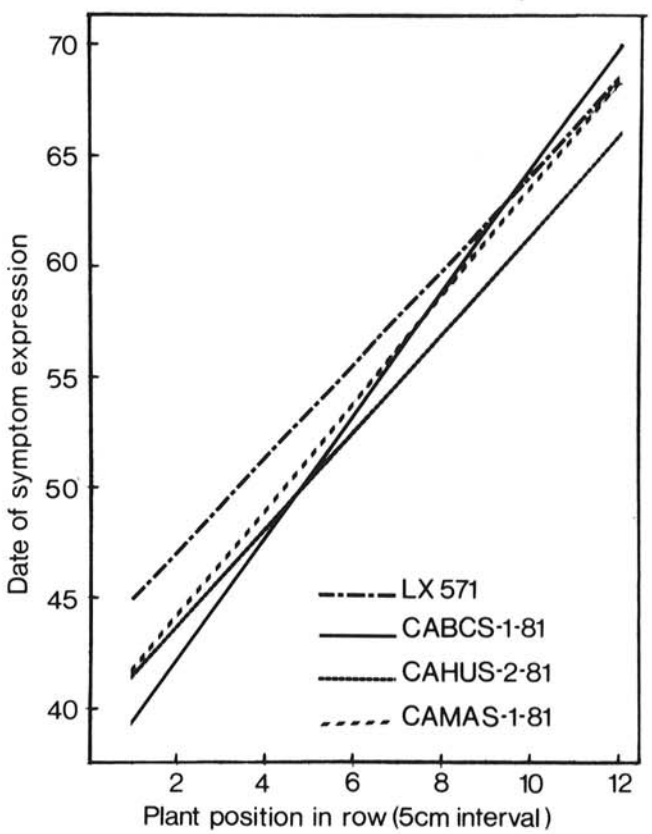

Fig. 2. Predicted linear regression lines for the progression of root rot symptoms in four upland cotton genotypes. The predicted regression lines were produced from pooled data of three experiments.

evidence that past and present field investigations have utilized plot sizes and replication numbers which were not optimal for minimizing variability due to inoculum distribution and density (7). Under the more closely controlled conditions of the present investigation, resistance was not apparent. However, another possibility is that resistance may be due to some factor either not observed or not operating under the conditions of this investigation. Lazo et al (including L. S. Bird) (8) have suggested that genotypes can be bred for ability to establish and support protective microbial populations. In the present investigation, no effort was made to observe, establish, or manipulate any such populations. Plant protection from root rot by beneficial microorganisms remains a viable hypothesis. At present, this possibility needs further examination under controlled conditions.

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