Statistical Procedures for Assessment of Resistance in a Multiple Foliar Disease Complex of Peanut

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ABSTRACT

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Early leaf spot caused by *Cercospora arachidicola*, late leaf spot caused by *Cercosporidium personatum*, and rust caused by *Puccinia arachidis* are the three most important foliar fungal diseases of peanut (*Arachis hypogaea*) worldwide. A 10-parent diallel cross was performed using peanut lines susceptible and resistant to the three major foliar fungi. The objective was to investigate the interrelationship of the multiple disease complex with host genes for resistance. Parents and F_1 hybrid progeny were planted in the field in Thailand in 1985. Leaves of randomly selected plants within plots were evaluated for resistance in the field and in the greenhouse using a detached leaf technique. A correlative structure was observed among traits measured, with no single parameter predominating the disease complex. Late leaf spot lesion number and early leaf spot lesion size were significantly correlated with defoliation in the field. Resistance to rust and late leaf spot was correlated ($r = 0.48$–0.60). Moderate correlation coefficients existed between the two leaf spot diseases. Lesion size and sporulation ratings were moderately correlated for the leaf spots, indicating a genetic and/or physiological relationship within the host involving lesion development for both leaf spots. Principal component analysis and biplots were used to illustrate the correlative nature of parameters of the multiple disease complex and the variation in response among peanut genotypes. Tree diagrams were used to visualize genetic relatedness of resistance among parents and hybrids for the disease complex. Genotypic means, biplots, and tree diagrams aided in relating disease parameters and determining hybrids that reacted similarly to the disease complex. Crosses PI 314817 × ICGS-4 and PI 314817 × (TG 3 × EC 76446[292]) were selected on the basis of parental general combining ability for resistance to all three diseases and biplots for further investigation and selection.

Foliar plant disease epidemics are the product of complex interrelationships among the environment, growth characteristics of both plant and pathogen, and genetics of the host-parasite interaction. Plant breeders may attempt to improve tolerance or resistance of adapted susceptible cultivars via intra- or interspecific crossing. Visual subjective screening for resistance is a fast and effective breeding tool, but it does not enhance understanding of the phenomenon of host resistance. Investigation of individual components of the genetic interrelationship between host and pathogen can help elucidate the mechanisms involved in single or multiple disease epidemics.

Epidemics of each disease may occur singly or in combination and may cause severe leaf necrosis and defoliation. Yield losses may be related primarily to defoliation (4) and are extensive without adequate control. If the role and importance of individual resistance components are known, plant breeders can develop crossing and selection schemes to enhance gains in resistance to the foliar diseases. Numerous studies have been performed on various components of resistance to leaf spot diseases of peanut (*Arachis hypogaea* L.). Field studies (3,8,9,24) and detached leaf procedures (2,3,7,12) have led to an understanding of foliar disease development. Measurement of leaf disease parameters is easiest under controlled environments and is best accomplished by greenhouse inoculation techniques with a detached leaf procedure (15).

Resistance components such as lesion size, spore production, and latent period within peanut leaf spot diseases are moderately to highly correlated (7,12,24). Disease components, as measured with detached leaf procedures, have been generally correlated with disease in the field (7,23). Lesion size, latent period (as measured by incubation time to asexual spore production), degree of sporulation, and percentage of sporulating lesions are often most closely associated with disease ratings in the field. Green and Wynne (8) reported significant correlations between necrotic area, lesion area, and defoliation in the field with corresponding traits measured in the greenhouse for early leaf spot. Relationships among disease parameters of late leaf spot or between leaf spot diseases in the field have not been investigated.

The objectives of this study were to investigate the genetic nature of resistance to the foliar diseases of peanut, determine the relationship between disease parameters measured in the greenhouse and severity of defoliation in the field, investigate the genetic relationship between resistance to the two leaf spot diseases and rust, and identify crosses with resistance to the three diseases.

MATERIALS AND METHODS

Hybrid (F_1) and pure-line parent seed from a 10-parent half-diallel crossing program were planted on 20 August 1985 in single-row plots at Khon Kaen University, Thailand (Table 1). Plants were spaced 20 cm apart within rows and 60 cm between rows. Four of the parents—NC Ac 17090, PI 314817, PI 405132, and (TG 3 × EC 76446[292])—were selected because of their reported resistance to late leaf spot and rust (11).

Plants of the 55 genotypes were sprayed with Azodrin (monocrotophos) and Daconil (chlorothalonil) every week for the first 6 wk after planting to prevent insect damage and infection of leaf spot or rust. Irrigation or fertilization was performed as needed.

At 49 days after planting, the third expanded leaf from lateral stems of nine plants from each of the hybrid and parent genotypes was detached and used in two separate tests in the greenhouse.

A modified detached leaf procedure (15) was used for each of the tests. Four-replicate detached leaves were arranged in a randomized complete block design (RCBD) and inoculated with conidia of *Cercosporidium personatum* (Berk. & Curt.) Deighton

collected from infected leaves in a field used as a leaf spot nursery in Khon Kaen, Thailand. Five other leaves were arranged in a separate RCBD and inoculated with conidia of *Cercospora arachidicola* Hori collected from leaves of a second disease nursery. Conidia (approximately 2×10^4 ml^{-1}) were suspended in water and misted over detached leaves with a hand sprayer. Spray inoculations were performed after leaves had recovered turgor in the moist sand medium (2). Inoculated leaves were allowed to incubate in trays of sand immersed in 2 cm of water. A cloth mesh was suspended approximately 10 cm above incubation trays. Edges of the cloth were submerged in the water reservoir to retain high humidity conducive to leaf spot development. After disease symptoms developed, lesion number, lesion size, latent period (days to 50% of lesions sporulating), and sporulation rating (1 = no spores, 5 = profuse sporulation) were recorded. Leaf area was measured using a Li-Cor leaf area meter (Li-Cor, Ltd., Lincoln, NE) at the end of the test. Percent necrotic area was calculated by multiplying average lesion size with lesion number and dividing by leaf area.

Disease incidence on the hybrids and parents also was recorded in the field. At 50 days, five plants of each genotype were chosen, and the third expanded leaf from the main stem and a lateral stem were tagged with brightly colored yarn. All leaves were free of lesions when they were tagged. Lesions of late leaf spot, early leaf spot, and rust were counted on each leaf at 10 and 15 days after tagging or 18 and 23 days after the last spray. Early and late leaf spot were identified by the brown or black appearance of the lesions and by the predominant sporulation of late leaf spot on the underside of the leaves. Rust consisted of smaller lesions with prominent pustules. A third lesion count, average lesion size, and sporulation rating (1–5) were recorded at 20 days from tagging (28 from last spray) for leaves on the main stem. Defoliation of tagged leaves was monitored daily. Lesion counts at day 15 and day 20 were adjusted to include only new lesions in the 5-day time interval. Disease progress was calculated from the field data by simple linear regression of lesion number on time.

TABLE 1. F_1 hybrid and parent genotypes evaluated for resistance to early leaf spot, late leaf spot, and rust in field and greenhouse at Khon Kaen, Thailand

Cross/parent	Cross/parent
1. Lampang × (TG3 × EC 76446 [292])	24. ICGS-4 × PI 405132
2. NC Ac 17090 × (TG3 × EC 76446 [292])	25. Lampang × ICGS-4
3. Tainan 9 × (TG3 × EC 76446 [292])	26. NC Ac 17090 × ICGS-4
4. Moket × (TG3 × EC 76446 [292])	27. Tainan 9 × ICGS-4
5. NC 2821 × (TG3 × EC 76446 [292])	28. Moket × ICGS-4
6. PI 314817 × (TG3 × EC 76446 [292])	29. NC 2821 × ICGS-4
7. ICGS-4 × (TG3 × EC 76446 [292])	30. PI 314817 × ICGS-4
8. PI 405132 × (TG3 × EC 76446 [292])	31. Lampang × PI 314817
9. ICGSE-5 × (TG3 × EC 76446 [292])	32. NC Ac 17090 × PI 314817
10. Lampang × ICGSE-5	33. Tainan 9 × PI 314817
11. NC Ac 17090 × ICGSE-5	34. Moket × PI 314817
12. Tainan 9 × ICGSE-5	35. NC 2821 × PI 314817
13. Moket × ICGSE-5	36. Lampang × NC 2821
14. NC 2821 × ICGSE-5	37. NC Ac 17090 × NC 2821
15. PI 314817 × ICGSE-5	38. Tainan 9 × NC 2821
16. ICGS-4 × ICGSE-5	39. Moket × NC 2821
17. PI 405132 × ICGSE-5	40. Lampang × Moket
18. Lampang × PI 405132	41. NC Ac 17090 × Moket
19. NC Ac 17090 × PI 405132	42. Tainan 9 × Moket
20. Tainan 9 × PI 405132	43. Lampang × Tainan 9
21. Moket × PI 405132	44. NC Ac 17090 × Tainan 9
22. NC 2821 × PI 405132	45. Lampang × NC Ac 17090
23. PI 314817 × PI 405132	46. Lampang
	47. NC Ac 17090
	48. Tainan 9
	49. Moket
	50. NC 2821
	51. PI 314817
	52. ICGS-4
	53. PI 405132
	54. ICGSE-5
	55. TG3 × EC 76446

Genotypic means were obtained for both field and detached leaf data. Product moment correlations of components were calculated for parameters within tests. The "Stepwise" and "Maximum *R*-square" options of the SAS Institute's Stepwise procedure (20) were performed to determine disease parameters that best explained defoliation of tagged leaves in the field (19). Principal component analysis was performed and represented graphically using a biplot display (6) for parameters of individual diseases and for parameters contributing to multiple field diseases. The vectors (lines) on the biplot correspond with the variables as they are projected onto the plane defined by two principal components. Long vectors indicate variables that are close to the plane being displayed and are well represented. Small angles between vectors indicate high collinearity between variables, and vectors at or near 180° show large negative correlations. Points with numbers represent genotypic means as they are oriented on the plane being presented.

Cluster analysis was performed using euclidean distance between genotypes as defined by the average of the multiple variables. The AVERAGE method was used in the SAS Institute's CLUSTER procedure (20) followed by the TREE procedure which diagrammed the structure of the clustering.

RESULTS

Early leaf spot. Lesion counts at different day intervals and on main and lateral stems in the field were moderately to highly correlated ($r = 0.62$–0.86), whereas the other parameters had low correlation coefficients within field measurements ($r = 0.22$–0.50). Within the detached leaf test, all parameters except lesion number were moderately correlated ($r = 0.53$–0.68). Measurements of lesion size and sporulation were moderately correlated between the field and greenhouse ($r = 0.31$ and $r = 0.39$, respectively). Overall incidence of early leaf spot was low in the field.

Gabriel's (6) biplot helps visualize the principal component analysis. The first three principal components represented 40.1, 21.2, and 10.6% of the variability among disease parameters, respectively. The lines are projections of the variable vectors on the plane defined by two principal components. Latent period, sporulation, and lesion size from the detached leaves (EDLP, EDSP, and EDLS) were the longest vectors, indicating variables that best correspond to the plane of the first two principal components (Fig. 1). This indicated the importance of these components in the overall variability among peanut genotypes for reaction to early leaf spot. Lesion number from detached leaf (EDLN) was not well represented (short vector), but the intermediate lengths of the vectors representing lesion number in the field (ELN10, ELN15, ELN20, ELN10L, and ELN15L) and lesion size in field (ELS) indicated the high correlations among these disease parameters. Vectors EDLS and EDSP showed positive correlation and were negatively correlated with EDLP, as visualized by the vector in the opposite direction. The biplot of the first and third principal components represented only EDLN well and was of minimal value in this case because this parameter often is not considered indicative of resistance (3,7,8,13).

Genotypes represented by point (•) markers were clustered in one general area except for two of the late leaf spot-resistant parents (53 and 55) and crosses 6, 16, 26, 30, and 35 for both biplots (Fig. 1). These hybrids tended to have many small lesions in the field. Entries 51, 32, 31, and 53 had longer latent periods, sporulated less, and had smaller lesions in the detached leaf study, whereas entries 18 and 43 were the reverse (Fig. 1).

There was a general dispersion among genotypes for reaction to early leaf spot in the cluster analysis and tree diagram (Fig. 2). Genotypes connected at short vertical distance from the base had similar measures of disease components. Based on means of lesion size, sporulation, and latent period, genotype 6 was the most resistant in the greenhouse but had high lesion counts in the field. Entries 30 and 35, which are more closely related, had comparatively high lesion counts and small lesions in the field and moderate to low resistance in the greenhouse. PI 314817 and PI 405132 (parents 51 and 53) appeared resistant in the

detached leaf study but less so in the field. The cluster that includes 22, 28, 15, etc. had low field lesion counts and small lesions with long latent periods in the greenhouse.

Late leaf spot. The correlation coefficients for disease parameters of late leaf spot were generally moderate to high. Again, lesion number in the detached leaf experiment (LDLN) generally lacked significant correlation with the field parameters except lesion size (LLS) ($r = 0.43$) and sporulation (LSP) ($r = 0.31$).

Regression coefficients (REG) of lesion counts over days were highly correlated with lesion counts on day 15 (LLN15) and day 20 (LLN20) and were considered to add little additional information to the understanding of the disease. Of parameters measured with detached leaf techniques, latent period was most closely correlated with field variables ($r = -0.27$ to -0.64), although lesion size, sporulation, and necrotic area also were moderately correlated ($r = 0.21$–0.57).

The correlations among late leaf spot disease parameters also were expressed in biplots (Fig. 3). The first three principal components accounted for 52.2, 12.8, and 9.9% of the total variability among disease parameters. Genotypes were not organized in distinct clusters, but resistant parents PI 314817, PI 405132, and (TG 3 × EC 76446[292]) (51, 53, and 55) were outliers in areas of high resistance. Crosses 6 and 30 were closest in spatial distance to these resistant genotypes. Cross 6 is a hybrid between PI 314817 and (TG 3 × EC 76446[292]), whereas 30 is a cross between PI 314817 and ICGS-4. In these cases, the spatial arrangement agreed with genetic relationships between parents and hybrids. Most other crosses corresponded more closely with their susceptible parents.

The small variability of disease reaction between parents 53 and 55 but their spatial distance from all other genotypes could be seen through cluster analysis which used disease parameters measured in both field and greenhouse (Fig. 4). Crosses of interest to the breeder include 6, 23, 29, and 30 because they cluster close to resistant parents and confer similarly high resistance.

Rust. Among genotypes at negative coordinates in the biplots

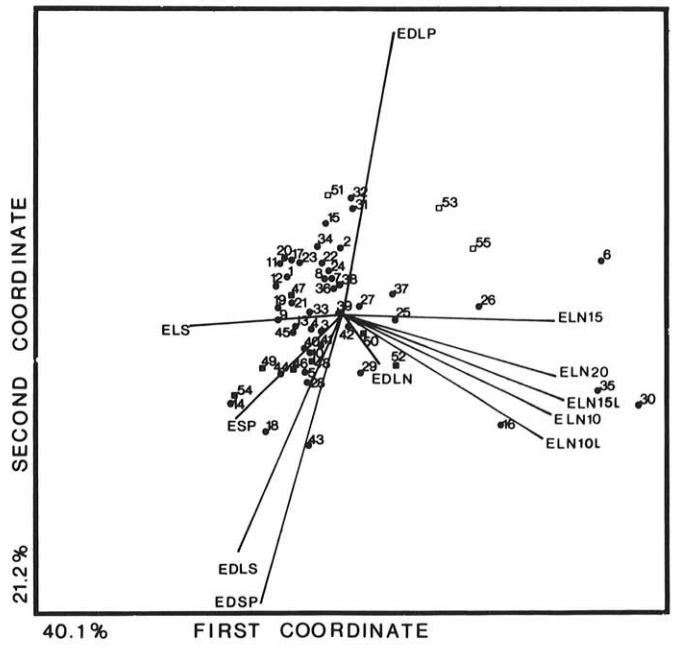

Fig. 1. Biplot display of first and second principal components for early leaf spot disease parameters from the field and greenhouse. Long vectors indicate variables well represented by the plane of the two principal components. Small angles between vectors indicate collinearity, and vectors in the opposite direction are negatively correlated. ● = F_1 hybrids. □ = late leaf spot-resistant parents. ■ = susceptible parents. Numbers = genotypes as in Table 1. ELN10, ELN15, and ELN20 = lesion number on main stem leaves in the field at days 10, 15, and 20. ELN10L and ELN15L = lesion number on lateral stem leaves in the field at days 10 and 15. ELS and ESP = lesion size (mm^2) and sporulation rating (1–5) on main stem leaves in the field at day 20. EDLN, EDLS, EDLP, and EDSP = lesion number, lesion size, latent period (days), and sporulation rating from detached leaves, respectively.

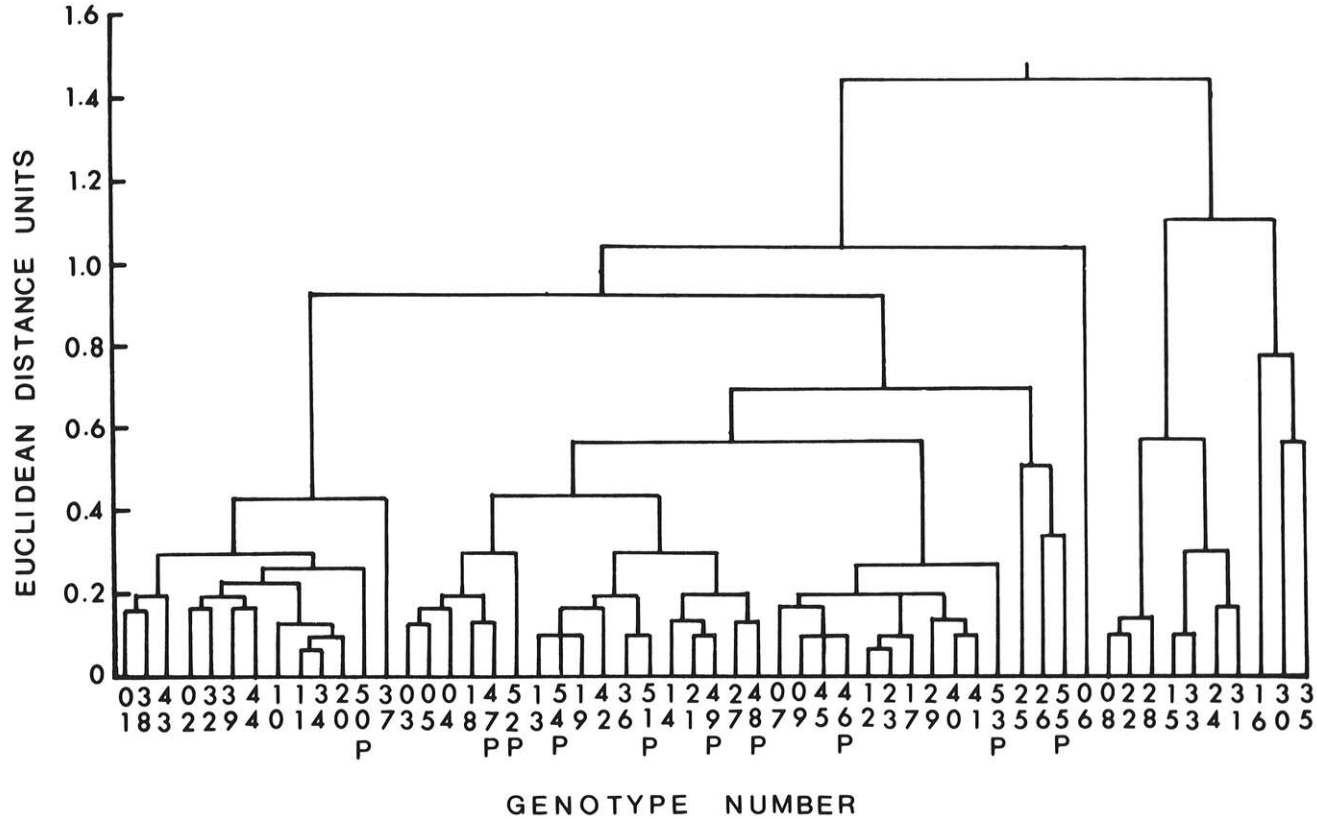

Fig. 2. Tree diagram representing relative euclidean distance among genotypes using early leaf spot disease parameters from field and greenhouse (numbers displayed vertically represent genotypes explained in Table 1). Vertical line height to connecting bar is proportional to distance between genotypes in reaction to disease components. P = parent.

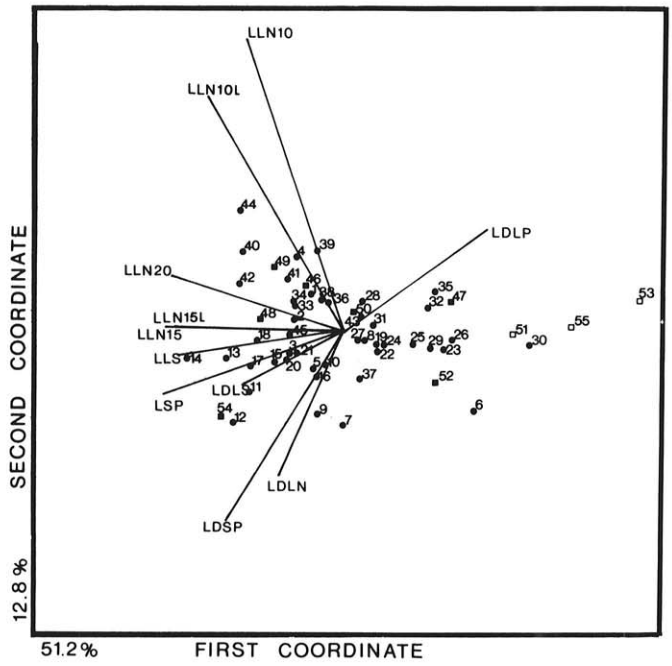

Fig. 3. Biplot display of first and second principal components for late leaf spot parameters from the field and greenhouse. Long vectors indicate variables well represented by the plane of the two principal components. Small angles between vectors indicate collinearity, and vectors in the opposite direction are negatively correlated. ● = F_1 hybrids. □ = late leaf spot-resistant parents. ■ = susceptible parents. Numbers = genotypes as in Table 1. LLN10, LLN15, and LLN20 = lesion number on main stem leaves in the field at days 10, 15, and 20. LLN10L and LLN15L = lesion number on lateral stem leaves in the field at days 10 and 15. LLS and LSP = lesion size (mm) and sporulation rating (1–5) on main stem leaves in the field at day 20. LDLN, LDLS, LDLP, and LDSP = lesion number, lesion size, latent period (days), and sporulation rating from detached leaves, respectively.

were two adapted cultivars of Thailand—Tainan 9 and Moket (parents 48 and 49)—which are highly susceptible to rust (Fig. 5). Lesion counts and regression for resistance to rust were all highly correlated (0.65–0.86). No other disease parameters for rust were recorded in the field, and no detached leaf inoculations were conducted. The regression of lesion number on days did not give additional information and thus was not included in further analysis.

The first principal component accounted for 74.7% of the total variation among genotypes with the second and third accounting for 10.5 and 8.5%, respectively (Fig. 5). Genotypes generally were arranged parallel to the horizontal axis or first principal component. The four rust-resistant lines 47, 51, 53, and 55 and cross 6 were clustered at coordinates of the first principal component that indicate resistance.

Distinct clusters were observed for reactions to rust in the tree diagram (Fig. 6). The rust-resistant parents (47, 51, 53, and 55) were clustered with their progeny 2, 6, 19, 23, and 32. Crosses 30 and 45 which have one resistant parent also were in this group. The distance among genotypes or clusters increases on the vertical axis of the radiogram. The cluster to the left of the resistant genotype cluster thus was spatially closer in reaction than the large cluster to the right. This may help in selection of crosses. For instance, adapted lines 48 and 49 were far removed from the introduced resistant lines; but cross 30 was close to known resistant parents (51, 53, and 55).

Multiple disease complex. Number of rust lesions in the field was moderately correlated with parameters of late leaf spot in the field (Table 2), probably because parents resistant to both diseases were used in the diallel crossing program. However, correlation coefficients between disease parameters of late leaf spot measured in the greenhouse and rust in the field were low.

Significant but low positive correlations occurred between rust and early leaf spot disease parameters measured using the detached leaf technique (Table 2). No significant correlations were observed between early leaf spot and rust in the field. None of the correlations among parameters of the leaf spot diseases and rust indicated

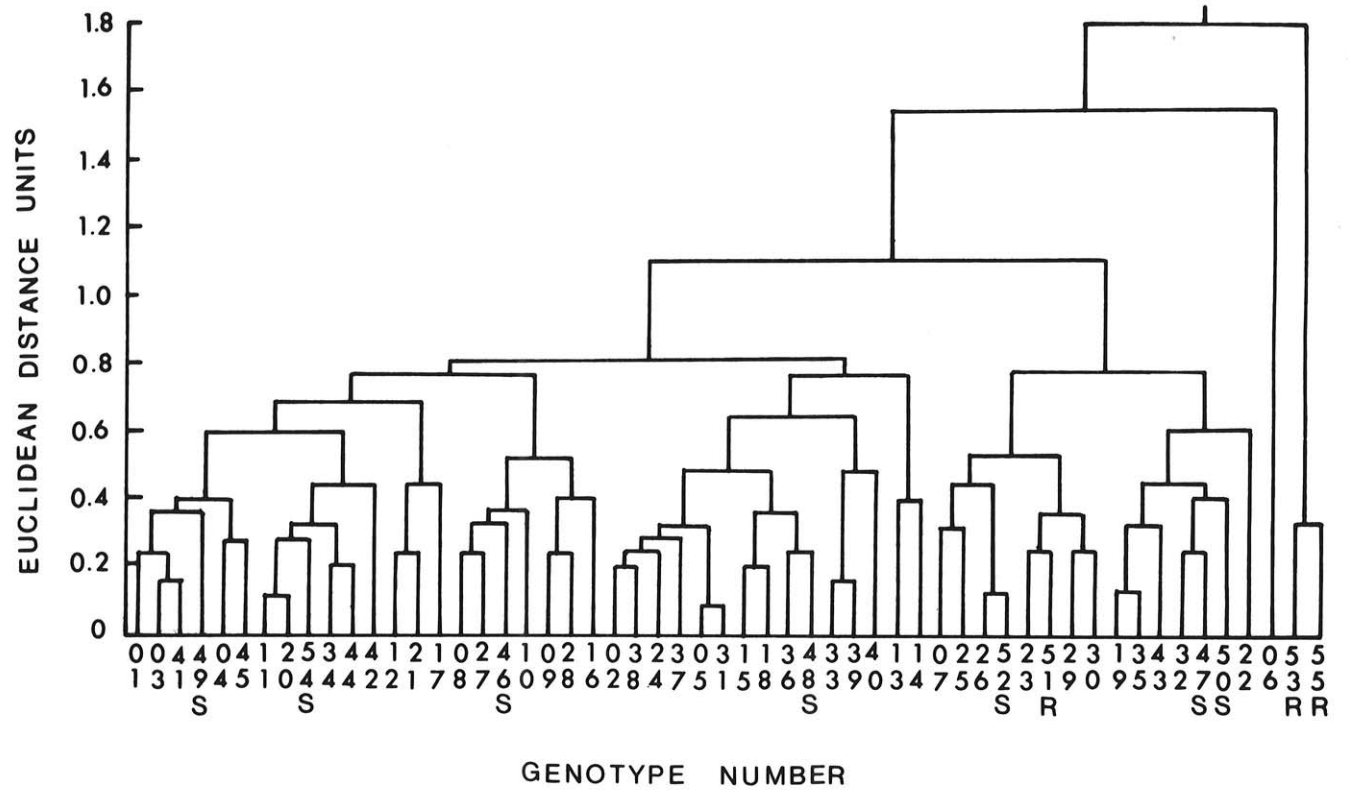

Fig. 4. Tree diagram representing relative euclidean distance among genotypes using late leaf spot disease parameters from field and greenhouse (numbers displayed vertically represent genotypes explained in Table 1). Vertical line height to connecting bar is proportional to distance between genotypes in reaction to disease components. R = resistant parent; S = susceptible parent.

competition for available susceptible plant tissue or unfavorable genetic linkages for resistance.

Lesion counts of early leaf spot were negatively correlated with all parameters of late leaf spot in the field (Table 2), but numbers of lesions of late leaf spot were positively correlated with early leaf spot lesion size and sporulation. Lesion size and sporulation were moderately correlated between diseases in the field and greenhouse.

Parameters of all three diseases were significantly correlated with defoliation in the field. Only early leaf spot lesion number (ELN20) produced coefficients opposite to enhanced defoliation (Table 2).

Stepwise regression on defoliation reduced the full multiple regression model to three variables (Table 3). Backward stepwise gave similar results but retained late leaf spot number, day 20 (LLN20) and early leaf spot lesion number, day 10 (ELN10). This procedure resulted in the following equation:

$$\text{Defoliation in days} = -0.58\ \text{ELS} - 0.21\ \text{ELN10} - 0.12\ \text{LLN10} - 0.05\ \text{LLN15} - 0.014\ \text{LLN20}$$

The first three principal components of the field data accounted for 48.5, 15.9, and 7.4% of the total variation, respectively (Table 4). The fourth component explained only 6.4% of the variability. Disease parameters with the greatest weighting in the first principal component were parameters of late leaf spot and defoliation; early leaf spot lesion number and rust had large weightings for the second principal component.

Resistant lines were well separated from the susceptible parental and F_1 lines in the biplots (Fig. 7). Two crosses, 6 and 30, were grouped close to the resistant parents. These two crosses are potentially useful for further study.

The relatedness of overall reactions can be visualized using tree procedures. The reactions of resistant parents 53 and 55 are well separated from all other genotypes (Fig. 8). Cross 6 is the closest to either 53 or 55 in overall reaction to the three diseases

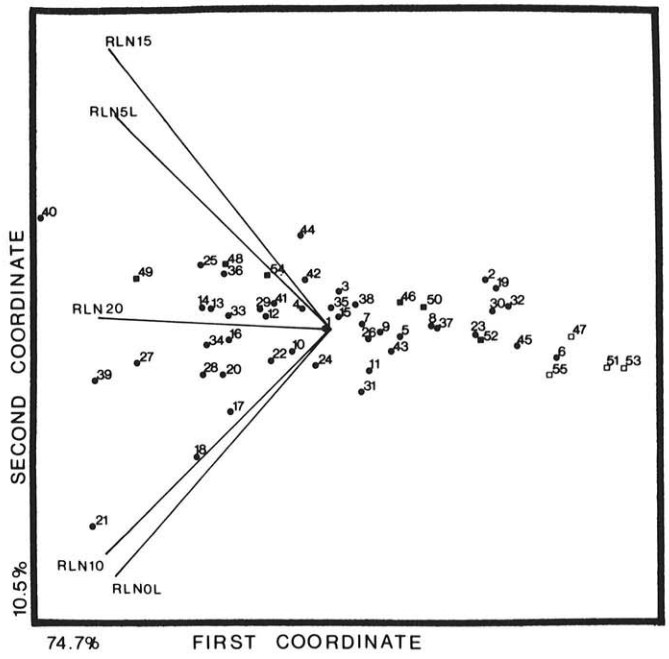

Fig. 5. Biplot display of first and second principal components for rust lesion number. Long vectors indicate variables well represented by the plane of the two principal components. Small angles between vectors indicate collinearity, and vectors in the opposite direction are negatively correlated. ● = F_1 hybrids. □ = late leaf spot-resistant parents. ■ = susceptible parents. Numbers = genotypes as in Table 1. RLN10, RLN15, and RLN20 = lesion number on main stem leaves in the field at days 10, 15, and 20. RLN0L and RLN5L = lesion number on lateral stem leaves at days 10 and 15.

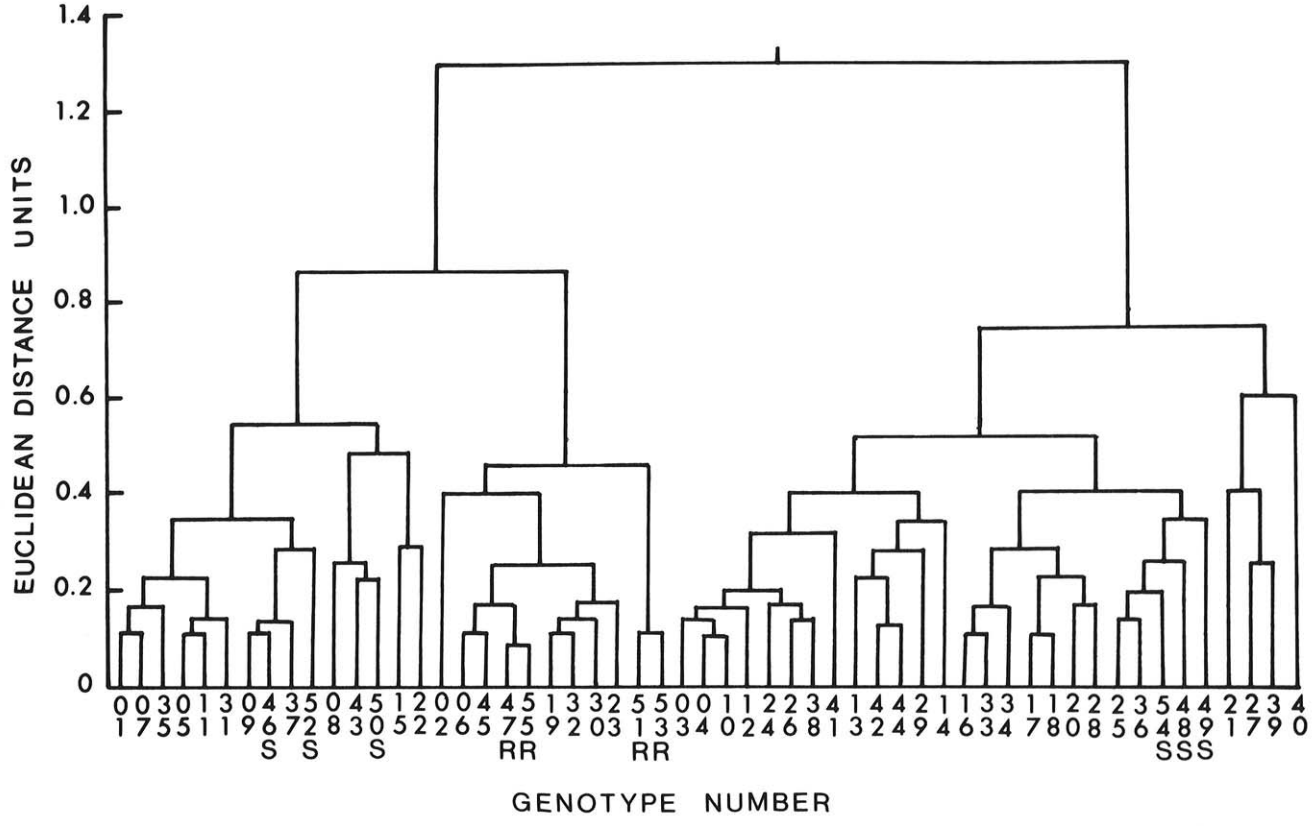

Fig. 6. Tree diagram representing relative euclidean distance among genotypes using rust lesion counts from the field (numbers displayed vertically represent genotypes explained in Table 1). Vertical line height to connecting bar is proportional to distance between genotypes in reaction to disease components. R = resistant parent; S = susceptible parent.

TABLE 2. Product moment correlation coefficients for selected disease parameters of three foliar fungal diseases on peanut in Thailand[a]

	Late leaf spot[b]							
	Field			Detached leaf			Field	
	LLN20	LLS	LSP	LDLS	LDSP	LDLP	RLN20	DEFM
Early leaf spot								
Field								
Lesion number, day 20	−0.48*[c]	−0.47*	−0.53*	−0.35*	0.21	0.32*	−0.10	0.32*
Lesion size	0.49*	0.69*	0.47*	0.39*	0.23*	−0.35*	0.20	−0.50*
Sporulation rating	0.30*	0.31*	0.40*	0.38*	0.24*	−0.38*	0.23	−0.36*
Detached leaf								
Lesion size	0.40*	0.51*	0.49*	0.55*	0.33*	−0.29*	0.28*	−0.42*
Sporulation rating	0.23	0.43*	0.43*	0.27*	0.49*	−0.38*	0.35*	−0.44*
Latent period	−0.18	−0.25	−0.21	−0.08	−0.28	0.12	−0.41*	0.39*
Rust								
Lesion number, day 20	0.60*	0.54*	0.48	0.19	0.27	−0.32*	...	−0.60*
Days to defoliation	−0.74*	−0.67*	−0.66*	−0.35*	−0.38*	0.60*	−0.60*	...

[a] Calculated using genotypic means ($n = 55$).
[b] LLN20 and RLN20 = lesion number at day 20 for late leaf spot and rust, respectively; LLS and LDLS = lesion size (mm^2) in field and detached leaf, respectively; LSP and LDSP = sporulation rating (1–5) in the field and detached leaf, respectively; LDLP = days to 50% lesions sporulating; DEFM = defoliation days to tagged leaf.
[c] * = significant at the 0.05 probability level.

TABLE 3. Stepwise regression results from "maximum R-square" and "stepwise" options[a] of main stem leaf defoliation on 13 disease variables on peanut

Maximum R-square variable[b]				Stepwise variable[b]			
Step	Added (+) or deleted (−)	$C(p)$	R^2	Step	Added (+) or deleted (−)	$C(p)$	R^2
1	+ LLN20	22.9	0.543	1	+ LLN20	22.9	0.543
2	+ LLN15			2	+ LLN15	11.1	0.628
	− LLN20			3	+ LLN10	3.7	0.687
	+ LLN10	5.3	0.664	4	+ ELS	3.3	0.701
3	+ ELS	3.4	0.689	5	− LLN20	3.4	0.689
4	+ LLN20	3.3	0.701		Program terminated		
5	+ ELN10	2.4	0.719				
6	+ ESP						
	− ELS						
	+ LLS	2.1	0.734				
7	+ ELN15	3.3	0.739				
8	+ LSP	4.9	0.741				
9	+ RLN20						
	− LLS						
	+ ELS	6.5	0.743				
10	+ LLS	8.2	0.745				
11	+ RLN10	10.0	0.746				
12	+ ELN20	12.0	0.746				
13	+ RLN15	14.0	0.747				

[a] From: User's Guide: Statistics. Vers. 5th ed. SAS Institute, Inc., Cary, N.C.
[b] All independent variables centered and standardized. ELN10, ELN15, and ELN20 represent early leaf spot lesion number at days 10, 15, and 20, respectively; LLN10, LLN15, and LLN20 represent late leaf spot lesion number at days 10, 15, and 20, respectively; ELS and LLS are lesion size (mm^2) for early and late leaf spot, respectively; ESP and LSP represent sporulation ratings (1–5) for early and late leaf spot, respectively; and RLN10, RLN15, and RLN20 represent rust lesion number at days 10, 15, and 20, respectively.

TABLE 4. Coefficients for the first three principal components of field disease parameters recorded on main stem of 55 peanut genotypes

	Principal components		
Trait	1	2	3
Late leaf spot traits			
Lesion number, day 10	0.22	0.19	0.44
Lesion number, day 15	0.32	0.09	−0.11
Lesion number, day 20	0.32	0.03	0.16
Lesion size	0.32	−0.05	0.12
Sporulation	0.31	−0.08	0.03
Early leaf spot traits			
Lesion number, day 10	−0.19	0.41	0.37
Lesion number, day 15	−0.23	0.41	0.11
Lesion number, day 20	−0.23	0.47	0.09
Lesion size	0.24	−0.21	0.43
Sporulation	0.17	−0.08	−0.06
Rust traits			
Lesion number, day 10	0.25	0.28	−0.47
Lesion number, day 15	0.27	0.33	−0.06
Lesion number, day 20	0.28	0.35	−0.32
Defoliation	−0.32	−0.14	−0.27
Variance as percentage of total	48.45	15.85	7.43
Cumulative variance as percentage of total	48.45	64.30	71.73

assessed in the greenhouse and field. Another cluster includes another resistant parent (51) and a number of its progeny (30, 32, and 23).

DISCUSSION

Host genetics. Nevill (17) concluded that genes governing components of late leaf spot resistance (except defoliation) are recessive. Jogloy (12) found that dominance effects were significant for lesion size and latent period in two crosses from generation means analysis. He reported that lesion number and sporulation had significant dominance effects for one cross but not the other and that additive genetic variance was significant for all parameters in both crosses. Despite these findings, a large portion of the total genetic variance of late leaf spot components is reportedly additive (2,3,12,26). Walls and Wynne (26) concluded that partial resistance expressed by F_1 progenies could not be explained solely by completely recessive genes. They believed that modifier genes were affecting the phenotypic expression of genes at loci controlling resistance.

Using only means and genetic analysis of individual disease parameters may be misleading when they are highly correlated or when variability is low. For this purpose, a better understanding of the importance of the disease parameters and the amount of variability of the total disease among genotypes is necessary. In this study, the multiple disease parameters from leaf spot and rust were used in principal component analysis to help identify the disease parameters that contribute major portions of the genetic variability. The biplots can help depict the relatedness of disease parameters under the two dimensions of principal components that result in the greatest variability and represent the peanut genotypes as they are dispersed within this plain. The comparative strengths and weaknesses of genotypes can be visualized in relation to the most important disease parameters. The total variability among genotypes using all disease parameters can be accomplished through cluster analysis and shown via tree

diagrams. These procedures add additional tools to the analysis of the genetic nature of the host-pathogen interaction.

Biplots (Fig. 3) and the tree diagrams (Fig. 4) of late leaf spot indicated recessive inheritance of resistance in some instances by the extreme separation of resistant parents 53 (PI 405132) and 55 (TG 3 × EC 76446[292]) from all of their progeny. Recessive genes as well as modifier genes may be involved. Parents that show more additive genetic effects such as 51 (PI 314817) and 52 (ICGS-4) may possess more modifier genes or genes for resistance that are not recessive. Progeny of these parents react similarly to late leaf spot (Fig. 4).

Parents 51 and 55 may have similar genes for resistance resulting in a highly resistant hybrid (cross 6) with homozygous recessive gene combinations. Also, the resistance of PI 314817 × ICGS-4 (cross 30) indicated either that both PI 314817 and ICGS-4 possess similar genes for resistance or that there are more additive gene combinations. Cross 8—PI 405132 × (TG 3 × EC 76446[292])—was expected to be more closely associated with its resistant parents (53 and 55). The distance between parents and hybrid cross may indicate that these two homozygous parents possess different sets of genes for resistance. Crosses involving ICGS-4 (parent 52) as a maternal parent (crosses 25–30) are more closely clustered than other crosses to their paternal parent (Figs. 3 and 4). ICGS-4 may in fact possess cytoplasmically inherited genes that confer resistance to late leaf spot.

Nonadditive gene effects have been found to be important in resistance to early leaf spot. Nevill (16) concluded that early leaf spot resistance was recessive. Green and Wynne (8) found that much of the nonadditive gene action was due to epistasis. Conclusions on the genetic nature of early leaf spot host-pathogen reaction in this study are not as clear as with late leaf spot. Clustering within the tree diagram did not correspond with parent-hybrid relationships. Biplots of the first three principal components did show some general clustering of the parents PI 314817 (51) and ICGS-4 (52) with many of their F_1 progeny. The results may be difficult to interpret because of the low amount of the disease in the field in this study.

Bromfield and Bailey (5) concluded that rust resistance is digenic

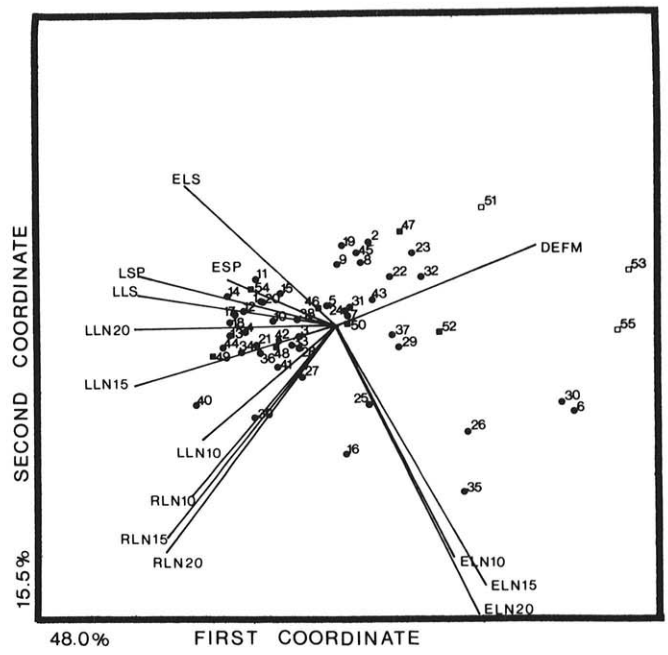

Fig. 7. Biplot display of first and second principal components for multiple disease components recorded in the field. Long vectors indicate variables well represented by the plane of the two principal components. Small angles between vectors indicate collinearity, and vectors in the opposite direction are negatively correlated. ● = F_1 hybrids. □ = late leaf spot-resistant parents. ■ = susceptible parents. Numbers = genotypes as in Table 1. ELN10, ELN15, and ELN20 = lesion number of early leaf spot on main stem leaves at days 10, 15, and 20. LLN10, LLN15, and LLN20 = lesion number of late leaf spot on main stem leaves at days 10, 15, and 20. RLN10, RLN15, and RLN20 = lesion number of rust on main stem leaves at days 10, 15, and 20. ELS and LLS = lesion size (mm^2) of early and late leaf spot. ESP and LSP = sporulation rating (1–3) of early and late leaf spot. DEFM = days to defoliation of main stem leaves.

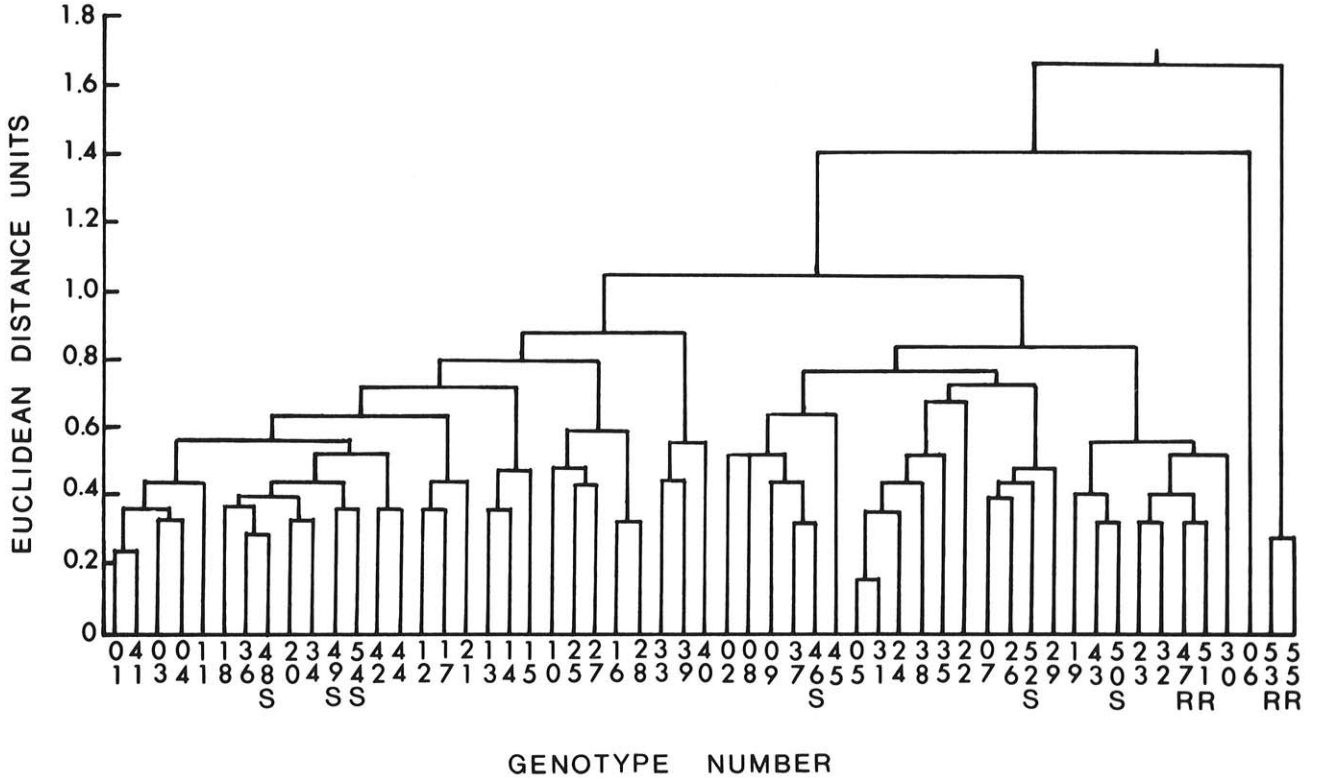

Fig. 8. Tree diagram representing relative euclidean distance among genotypes using multiple (rust, early, and late leaf spot) disease parameters from the field (numbers displayed vertically represent genotypes explained in Table 1). Vertical line height to connecting bar is proportional to distance between genotypes in reaction to disease components. R = resistant parent; S = susceptible parent.

and recessive in nature which has been supported by other studies (17). Nigam et al (18) used the same material in later generations to conclude that more than two genes were involved. Combining ability studies from our diallel cross, biplots, and tree analysis (Fig. 6) indicated that resistance has both additive and nonadditive genetic components for the crosses. Hybrids were generally clustered between parents with some tendency toward the more susceptible parent. From these results and earlier studies (25), it appears that inheritance of resistance to rust may be simpler than either of the leaf spot diseases and that screening for resistance by observation of natural field reaction should be reliable.

Genetic relationship between diseases. Interrelationships of the two leaf spot diseases and rust are not well understood. Peanut rust is not a major disease problem in growing areas of the United States but is prevalent in warm, humid climates of the world and can be the predominant disease or can occur concurrently with either early or late leaf spot (10,22–24).

Temperature and humidity requirements of the two leaf spot diseases are similar (1,21), and competition for infection site(s) may occur (9,14). Comparative resistance, inoculum availability, and microenvironment may all be involved in the predominance of one leaf spot disease over another within a given area, growing season, or specific time period. Anderson et al (2) concluded that inheritance of resistance to early and late leaf spot was independent. Competition for infection sites seemed to occur in this study, but postinfection lesion developments of the two fungi were correlated. Lesion expansion and spore production may be associated with host leaf physiology or linkage of host genes responsible for resistance. Correlations between latent periods of the two diseases were low or nonsignificant (Table 2) and indicated greater genetic dispersion.

Parameters associated with defoliation. Defoliation caused by foliar disease is a major cause of yield losses of peanut (4). The specific disease parameters responsible for defoliation are not clearly understood. If more than one foliar disease is present, it may be difficult to ascertain which most influences premature defoliation.

From correlation results alone, lesion number, lesion size, sporulation of *C. personatum*, and rust lesion number (measured in the field) were most closely associated with defoliation. Conclusions regarding causes of defoliation, however, are not possible due to the high inherent correlation among these parameters. The Stepwise procedures using multiple regression helped determine the disease parameters most responsible for defoliation of leaves in the field. Late leaf spot lesion numbers and early leaf spot lesion size were the most important parameters (Table 3). Most other components of early leaf spot did not contribute to the variability of defoliation. Because of the low lesion count of early leaf spot observed in the field, it was surprising that early leaf spot lesion size significantly contributed to the regression equation. Although rust lesions were at least as numerous as late leaf spot lesions, they had very little effect on days to defoliation. Heavy infestation of rust causes infected leaves to become necrotic and dry, but they remain attached to the plant (23). Therefore, in mixed infection, the damage caused by leaf spot through defoliation would preclude extensive damage to plants by rust. Resistance to rust is required still because yield reduction can be significant from rust infection alone (23).

Identification of resistant crosses. A plant breeder may choose to assess resistance to individual diseases by combining data from multiple diseases and comparing genotypes based on principal components as visualized by Gabriel biplots and cluster analysis through tree diagrams. These techniques tend to reduce multiple parameters into one or few comparative values. These methods provide a unique perspective of the data in lieu of tables of means or combining ability estimates. For early leaf spot (Fig. 1), a breeder may want to choose genotypes that possess prolonged latent periods and reduced sporulation (31, 32, 51, and 53) based on the detached leaf experiments. However, these genotypes are shown not to be similar in total reaction to early leaf spot as the cluster analysis indicates (Fig. 2). A breeder would want to use the variability and not exclude any of the genotypes in subsequent selections within segregating populations for use as parents for resistance.

Within areas of resistance represented in the biplot of late leaf spot disease parameters are the three resistant parents and crosses 6 and 30. A group of genotypes that includes parents 47 and 52 shows some moderate resistance as indicated by the positioning in negative coordinates to lesion number (LLN), sporulation (LSP, LDSP), and lesion size (LLS, LDLS). Cluster analysis (Fig. 4) is consistent with the biplot in this case; genotypes to the right of cross 7 show desirable resistance. Within this group, variability is retained with entries 6, 53, and 55 being outliers. Representatives from within close clusters would be selected for further study.

Biplots and tree diagrams of rust lesion counts (Figs. 5 and 6) easily cluster resistant genotypes (between entries 2 and 53 of Fig. 6). All foliar disease measurements of the field including time to defoliation were included for the biplot of Figure 7.

The first and second principal components represent the majority of the variation among the genotypes (63.5%), thus eliminating most of the extraneous information. All three parents (51, 53, and 55) resistant to late leaf spot and rust diseases lie on the plane showing delayed time to defoliation (DEFM) and opposite to vectors showing high late leaf spot and rust lesion number (LLN10–20, RLN10–20). Selections of desirable genotypes at this point depend on the goals of the breeder. One may choose genotypes 6 and 30 if less defoliation (DEFM), less late leaf spot (LLN, LSP, LLS), and smaller early leaf spot lesion size (ELS) are desired traits. They also have good resistance to rust (RLN) but were less acceptable for early leaf spot lesion number (ELN). The cluster of genotypes between vectors ELS and DEFM may be useful to reduce lesion numbers of rust, late leaf spot, and early leaf spot but have slightly faster defoliation and larger early leaf spot lesion size. The cluster analysis would facilitate further selection once certain key genotypes are identified.

These statistical techniques may be of even greater importance when material from single or multiple populations are assessed via multiple parameters and selections must be made for elite resistant lines or for recurrent selection breeding schemes. Principal component analysis allows the breeder to select based on true sources of variability. The cluster analysis could be adjusted to include only the variability expressed in the first few principal components and simplify the task of selection to an even greater extent.

Early and late leaf spot disease parameters were interdependent, and we could not identify those parameters most responsible for the overall variability among genotypes. The current study supports the notion that a breeder cannot rely on one disease parameter for effective screening. We also could not determine the cause of defoliation in the field, but late leaf spot lesion number and early leaf spot lesion size contributed significantly to the variability of leaf loss. Rust appeared not to contribute to defoliation.

Moderate correlation coefficients were found between parameters measured in the field and greenhouse for resistance to late leaf spot. Latent period was most highly correlated with field disease, but no single parameter should be used in disease resistance assessment. Parameters of rust and leaf spot were significantly correlated; lesion size and sporulation between early and late leaf spot were correlated. A physiological property of the leaf may be involved in general inhibition of sporulation and expansion of lesions.

Nonadditive genetic variability was also evident for early and late leaf spot. Resistance to late leaf spot appeared to be more recessive in nature than resistance to rust. The relative alienation of 53 and 55 from all of their progeny, when crossed with susceptible parents, may indicate the recessive nature of the genes that confer resistance. Hybrids of parent 51 are clustered between parents, indicating a more additive nature to host-disease reactions. The close clustering of susceptible parents ICGS(E)-5 (54) and Moket (49) with their progeny support theories that susceptibility genes are dominant within the host.

Two parents (PI 314817 and ICGS-4) produced progeny resistant to late leaf spot. PI 314817 produced superior progeny

for all disease parameters of all three fungi. Crosses PI 314817 × (TG 3 × EC 76446[292]) (cross 6) and PI 314817 × ICGS-4 (cross 30) gave similar resistance reactions to the three diseases and were clustered closer to resistant parents than most other crosses. Segregating material from these two crosses will be studied further.

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