Techniques

Detection and Measurement of Plant Disease Gradients in Peanut with a Multispectral Radiometer

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ABSTRACT

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Point and line sources of *Cercosporidium personatum* were established in peanut field plot experiments in Plains, GA, in 1986. Disease gradients were quantified by measuring percent leaflet defoliation with respect to distance from the sources and by measuring percent reflectance of 800-nm wavelength radiation off the crop canopy with a hand-held, multispectral radiometer. There was a linear relationship between percent reflectance values and percent defoliation estimates for both point and line source experiments. There was also a linear relationship between percent reflectance values and yield (kg/ha) along the late leaf spot gradient in the line source experiment ($R^2 = 98.2\%$). Disease severity gradients of peanut rust foci were also quantified and compared to percent reflectance values by using linear regression analysis. Percent reflectance values, when used as the independent variable, explained 94.6–96.5% of the variation in peanut rust disease severity along rust gradients. Reflectance of sunlight from peanut canopies at 800 nm provided a rapid and objective measurement of disease intensity and the amount of green leaf area contributing to pod yield. Recording percent reflectance offers a means to quantify the benefits obtained from disease control tactics aimed at protecting and maintaining healthy green leaf area from the effects of plant pathogens.

Additional keywords: remote sensing, spore dispersal.

Disease foci are often observed during the early development of foliar epidemics in field or row crops (14,20). Initial foci (also called point sources) often produce new dispersal units that are deposited throughout the crop, with decreasing numbers as the distance from the source increases. After a period of time when the environment is favorable for infection and colonization, a larger disease focus may be observed. The resulting disease gradient from the center of the point source to the outer edge of the focus can be described by plotting disease intensity (Y)

against distance (X).

Disease intensity and distance measurements are often transformed to obtain a linear relationship. Although several types of transformations have been proposed (14,18), all of them use the slope of the resulting regression line to make comparisons among gradients. Information concerning pathogen spread can be obtained by measuring focal expansion over time and distance. van den Bosch et al (23) described focal expansion in terms of a circular wavelike pattern, and they suggested that disease profiles (i.e., gradients) should be measured as numbers of individuals (sites, leaves, or plants) and not as the percentage of diseased leaf area (severity). However, in some pathosystems, it may be possible to detect and quantify disease gradients in terms of disease severity or incidence of defoliation of plants or plant parts (2,5,10,11,19,25). Others have used spore traps or live plant traps to study pathogen dispersal (4,9,20).

The presence of a disease severity and/or defoliation gradient should also result in a green leaf area (GLA) gradient in which GLA is highest at the edge or "front" of the focus and lowest at or near the center of the focus. Green leaf area gradients have not been studied previously because measurement is very labor intensive and requires destructive sampling. Remote sensing techniques may offer a rapid, objective, and accurate means of estimating GLA without destructive sampling. Ahlrichs and Bauer (1) found that there was a high correlation between leaf area index and percent reflectance measured in the near infrared wavelength range (760–900 nm) in spring wheat canopies. Nutter and Cunfer (17) have shown that there is a strong linear relationship between GLA and percent reflection of sunlight from barley canopies measured in the 800-nm wavelength range using a narrow band, hand-held, multispectral radiometer. Therefore, radiometry may also offer an objective, nondestructive method of detecting and quantifying GLA gradients formed by the expansion of plant disease foci. The purpose of this research was to determine if gradients of late leaf spot (caused by *Cercosporidium personatum* (Berk. & Curt.) Deighton) and peanut rust (caused by *Puccinia arachidis* Speg.) in peanut (*Arachis hypogaea* L.) canopies could be detected and quantified by recording the amount of light reflected from peanut canopies with a multispectral radiometer to indirectly estimate GLA.

MATERIALS AND METHODS

Field experiments. In 1986, three separate field experiments were conducted at the Southwest Branch Experiment Station in Plains, GA, to determine if a hand-held, multispectral radiometer (CROPSCAN, Inc., Fargo, ND) operated at 800 nm could be used to detect and quantify the effects of foliar disease gradients in peanut. The peanut cultivar Florunner, a runner-type peanut grown on more than 90% of the total peanut acreage in Georgia, was used in all three experiments. Florunner is very susceptible to both *C. personatum* and *P. arachidis*.

Late leaf spot point source. A plot 24 rows wide (approximately 97 cm apart) by 96 m long was planted on 22 May in an area not previously planted to peanuts. Rows were oriented along a north-south axis, and the plot was divided into four blocks of equal size (24 rows × 24 m). The year 1986 was extremely dry, and the lack of moisture and initial inoculum prevented late leaf spot epidemics from occurring in this experimental plot until inoculum was introduced and overhead irrigation was applied for extended periods. On 14 July, leaflets containing a total of 50 sporulating lesions were sandwiched between two 20-mesh wire screens (30 cm wide by 20 cm high), and the screens were placed in the center row of each block at midcanopy. Screens were supported upright with 30-cm wooden stakes. To avoid the spread of inoculum by mechanical means, no farm machinery was permitted to enter the fields after this time. Overhead irrigation was applied nightly for the next 3 wk to provide environmental conditions favorable for the establishment of primary and early secondary late leaf spot disease gradients. Disease intensity gradients were measured on 27 August by determining percent defoliation of leaflets on six lateral branch stems systematically selected at 60-cm intervals along the north and south axis of the center row from each point source. Defoliation was defined as the number of missing leaflets divided by total number of nodes × 4, since there is normally one primary leaf with four leaflets at each node.

Just before defoliation measurements, percent reflectance of sunlight in the 800 ± 11.3-nm wavelength range was recorded at 180-cm intervals along the north-south axis of the center row beginning at the north end. Operators walked along the row adjacent to the center row so as not to disturb the center row canopy. The support pole of the radiometer was leaned over the center row and reflectance measurements were taken from a height 1.5 m above the peanut canopy. A bubble spirit level was used to ensure that the support pole of the radiometer was kept at the appropriate angle and height. Reflectance measurements were recorded during a cloud-free period between 1200 and 1400 hours EST. Once reflectance values began to decrease as the point source was approached, reflectance readings were taken every 60 cm. To determine how accurately the radiometer could locate the point source, four different operators recorded percent reflectance in one of the four foci. Reflectance values were later converted to proportions by dividing each reflectance value recorded along the north-south axis by the percent reflectance value recorded for healthy peanut canopies (no defoliation). Reflectance, defoliation, and distance data were fitted to log-log, log-linear, logit-log, and logit-linear models (7,14–16) to determine which gradient model best described defoliation and reflectance gradients as a function of distance from the point source in each of the four blocks.

Late leaf spot line source. A plot 72 rows wide (0.97 cm between rows) and 100 m long was planted on 28 April in an area where peanut was grown the previous year, and, thus, leaf spot inoculum was present. The rows were oriented along an east-west axis, and the plot was divided into two blocks with the first six rows on the north end of the plot acting as a line source of inoculum. One block was sprayed with chlorothalonil (Bravo 500F, 2.5 kg/ha a.i.) beginning 30 days after planting and continuing every 14 days until just before harvest (eight sprays), and the other block was left untreated. Overhead irrigation was applied two to three times per week only to the six line source rows on the north end of the plot from mid-July until digging. This resulted in a line source of lesions of *C. personatum* that was six rows wide and perpendicular to both the sprayed and nonsprayed blocks. Percent reflectance (800 ± 11.3) and percent defoliation were recorded in transects perpendicular to, and every 5 m from, the line source in both sprayed and nonsprayed blocks on 10 September between 1200 and 1300 hours EST. Reflectance was measured before making percent defoliation estimates to avoid disturbing the peanut canopy. Three transects were observed in each block. Peanut pods were dug on 26 September and quadrats 8.3 m long and two rows wide were randomly selected within rows every 5 m away from the line source in both sprayed and nonsprayed blocks to determine if a yield gradient existed in either block.

Peanut rust disease foci. Naturally occurring peanut rust foci, approximately 0.4 m in diameter, were observed in late July in a nonsprayed, dryland Florunner peanut field. Three nonoverlapping foci were chosen for gradient analysis. By 10 September, the three individual rust foci had expanded to more than 3 m in diameter and percent reflectance measurements (800 nm) were made at 0.5, 1.0, 1.5, 2.5, 3.5, 4.5, and 6.0 m from the centers within the row along a north-south axis. Immediately after making percent reflectance readings, five branch stems were sampled from each distance and the percentage of leaf area covered by rust pustules was estimated using the Horsfall-Barrett scale (7). The percentage of severity of leaf area infected was calculated by conversion of the disease ratings using the Elanco conversion tables (Elanco Products Co., Indianapolis, IN). The entire experimental area had not been planted to peanut previously and had not received irrigation; therefore, less than 1% of the leaf area was occupied by late leaf spot lesions. Percent defoliation was also less than 1% at the time rust gradients were quantified.

The relationship between percent reflectance and peanut rust severity for each of the three foci was determined by least squares regression.

RESULTS

Gradients from late leaf spot point sources. A reflectance gradient near the late leaf spot point source was detected by each of four operators (Fig. 1). Each operator located the point source as being 9 m from the location where reflectance measurements were first initiated. The reflectance (GLA) gradients were approximately 5 m in length. Percent reflectance values increased with increasing distance from the point source, while percent leaflet defoliation due to late leaf spot decreased with increasing distance from the point source (Fig. 2A). There was a linear relationship between percent reflectance values and percent defoliation measurements (Fig. 2B) with percent defoliation decreasing 2.8% for each 1% increase in percent reflectance ($R^2 = 95.3\%$, $P \leq 0.001$).

The logit (Y)-linear (X) gradient model (14) best described the relationship between reflectance and distance from the point source and also the relationship between defoliation versus distance. The slopes and intercepts of the four regression equations using the logit reflectance-linear distance model were not significantly different from one another (F-test, $P \leq 0.05$), and these data were pooled to provide the following equation: logit $Y = 0.0243 + 0.0012(X)$, $R^2 = 94.9\%$. The slopes and intercepts of the four regression equations for logit defoliation-linear distance were not significantly different, and these data were also pooled to derive the following equation: logit $Y = -0.2242 - 0.0081(X)$ $R^2 = 96.2\%$.

Gradients from a late leaf spot line source. A defoliation gradient perpendicular to the late leaf spot line source was evident in the nonsprayed block but not in the fungicide-treated block (Fig. 3A). A reflectance (GLA) gradient was detected in the nonsprayed block with values increasing with distance from the line source (Fig. 3B). There was a significant linear relationship between the change in logit Y (proportion of 800 nm of light reflected) and distance from the line source (logit $Y = -0.820 + 0.054(X)$, $R^2 = 86.9\%$, $P \leq 0.001$). A much flatter reflectance gradient was detected in the fungicide-treated block; however, the slope of the regression line relating the change in logit (Y) with the change in distance was not significantly different from zero ($P \leq 0.001$). There was a linear relationship between percent reflectance and percent defoliation measurements (Fig. 3C). On this date, percent defoliation decreased 1.8% for each 1% increase in percent reflectance ($R^2 = 95.9\%$, $P \leq 0.001$). Pod yields sampled from the fungicide-treated block at various distances from the line source did not reveal the presence of a yield gradient; however, a yield gradient was detected in the nonsprayed block (Fig. 4A). Pod yields increased sharply as distance from the line source increased up to 15 m. Beyond 15 m, pod yields gradually increased to a point 35 m from the line source. There was a significant linear relationship ($P \leq 0.001$) between percent reflectance values and pod yield (Fig. 4B). For each 1% increase in percent reflectance, pod yield increased 58.0 kg/ha ($R^2 = 97.6\%$).

Peanut rust disease severity gradients. Peanut rust severity (%) decreased and percent reflectance increased as the distance from the center of rust foci increased (Fig. 5A). There was a significant linear relationship between rust severity and percent reflectance (Fig. 5B, Table 1).

DISCUSSION

Plant disease gradients have traditionally been quantified by measuring disease intensity at specified distances from a source of pathogen propagules (8,15,25). Disease intensity has been assessed as the number of lesions per sampling unit (23), incidence of infected sampling units (18), severity of infection of sampling units (2,10,17), or percent defoliation of sampling units (2). The results of this study show that plant disease gradients can also be detected and quantified by measuring percent reflection of sunlight at 800 nm, which provides an indirect estimate of GLA.

The establishment of point sources of late leaf spot resulted in gradients that could be measured in terms of defoliation gradients and in terms reflectance at 800 nm with a CROPSCAN radiometer. In a related study, Nutter and Cunfer (17) showed that there was a strong relationship between GLA and percent reflectance measured in the 800-nm wavelength band. Waggoner and Berger (24) have suggested that absolute measurements of healthy green leaf area duration may have a better relationship with yield than disease intensity measurements. Hooker (6) showed that losses in corn could be estimated based on the amount of healthy tissue present during the plant reproductive period. In the present study, percent reflectance measurements, which provide an indirect measurement of GLA, explained 98.2% of the variation in yield in the late leaf spot line source experiment. This is the first report showing that defoliation or reflectance (GLA) gradients can also result in yield gradients within peanut canopies.

The GLA gradients obtained from the late leaf spot point and line source studies provide additional insight into the effect of

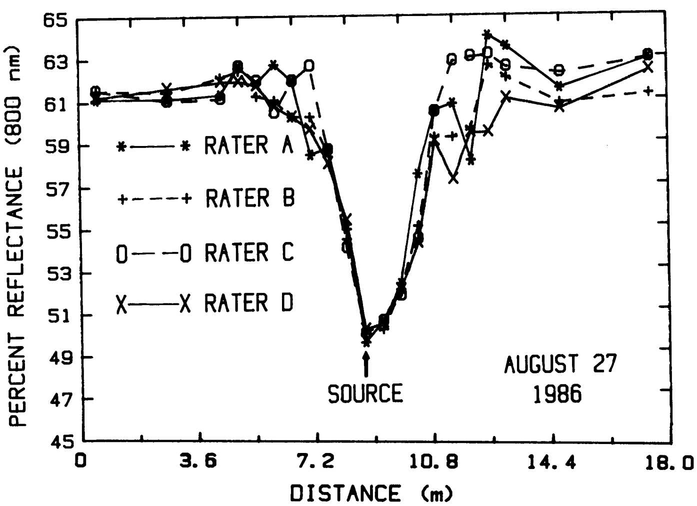

Fig. 1. Percent reflectance (800 nm) values measured by four operators using a CROPSCAN radiometer to determine the location of a *Cercosporidium personatum* point source in one focus.

TABLE 1. Relationship between percent reflectance (800 nm) values and percent peanut rust severity measured from peanut rust disease gradients in Florunner peanut using linear regression analysis

Rust focus number	Intercept[a]	Regression coefficient (slope)[b]	Coefficient of determination (R^2)	F-statistic of overall model	Standard error of the slope estimate
1	103.1	1.78	94.6	<0.0001	0.17
2	104.4	1.81	96.1	<0.0001	0.13
3	102.9	1.80	96.5	<0.0001	0.12

[a]Intercepts were not significantly different as determined by a t-test ($P < 0.05$).

[b]Slopes were not significantly different from each other as determined by t-tests ($P < 0.001$).

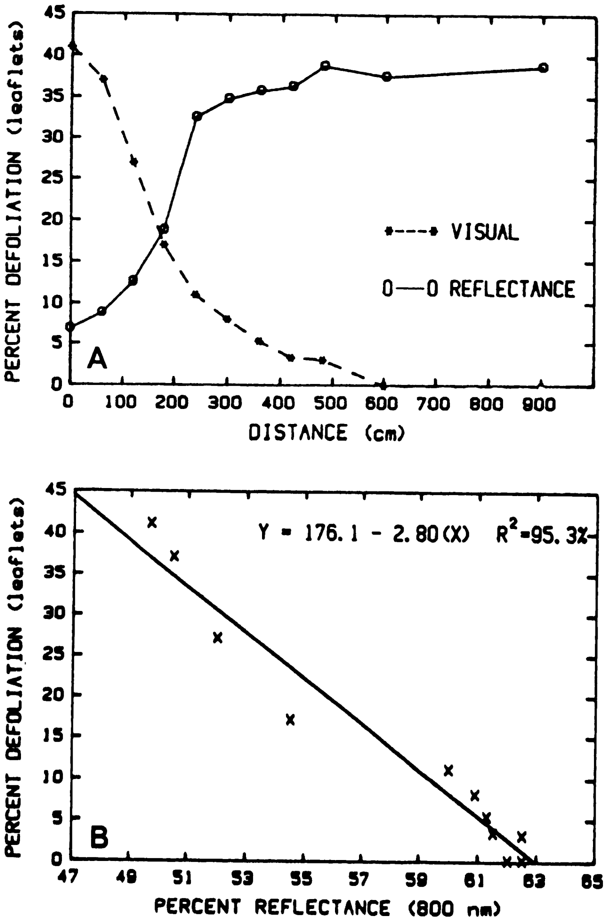

Fig. 2. A, Percent reflectance (800 nm) and percent defoliation (leaflets) measurements as a function of distance from point sources of *Cercosporidium personatum.* Data represent means of four sources; and **B,** linear regression of percent reflectance (800 nm) versus percent defoliation (leaflets) data obtained on 27 August from gradients of *C. personatum* arising from point sources. Data represent means of four sources.

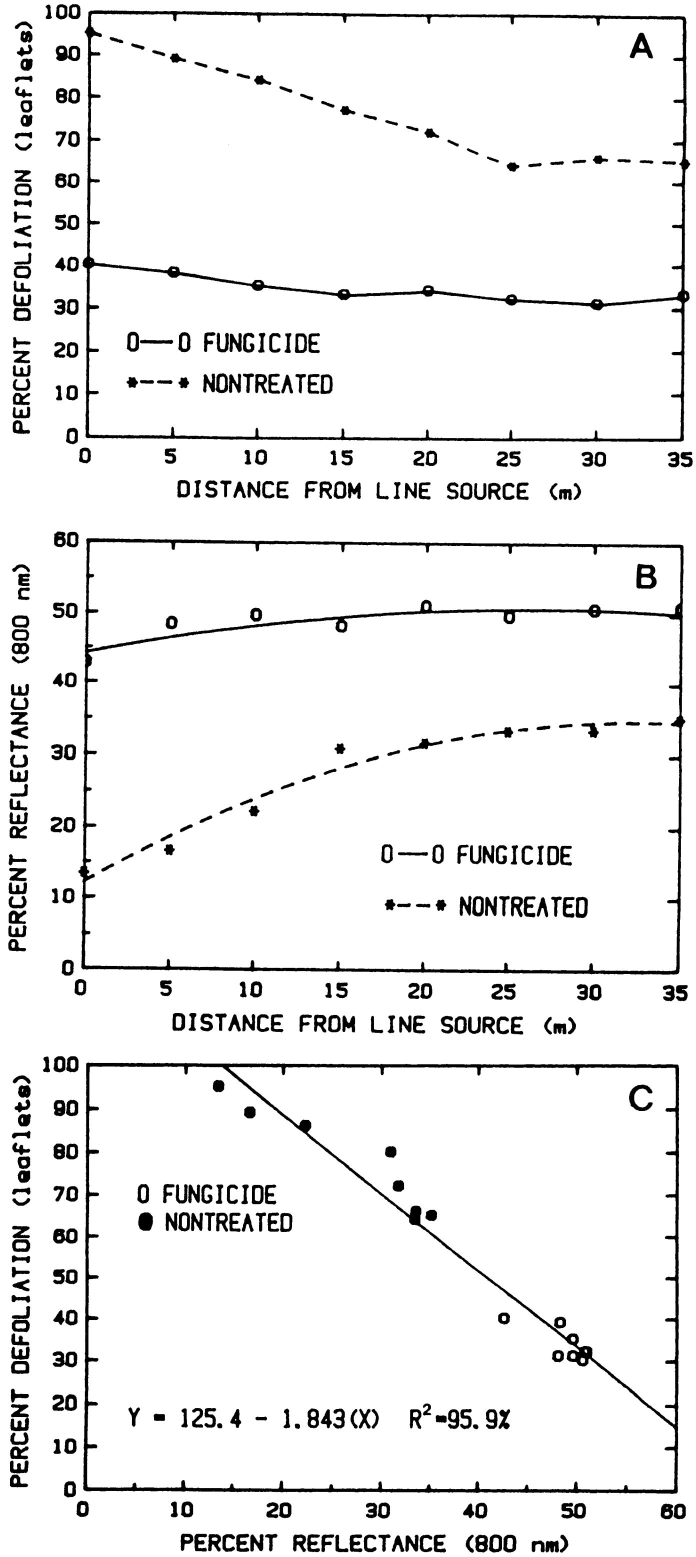

Fig. 3. A, Percent defoliation (leaflets); **B,** percent reflectance (800 nm); and **C,** linear regression of percent reflectance (800 nm) versus percent defoliation (leaflets) data obtained from fungicide-treated and nontreated peanut perpendicular to a line source of *Cercosporidium personatum* spores on September 10.

experimental plot size on the role of secondary inoculum in late leaf spot disease development and yield. GLA and disease gradients were more extensive spatially than the size of plots normally employed for peanut field studies (12,13,21). Based on the lengths of the peanut and line source gradients in this study, interplot interference may pose a hazard in small plots, since there would be a net loss of pathogen propagules from nonsprayed plots and a net gain of propagules in plots in which leaf spot is controlled (3,18).

Whereas late leaf spot infected leaves eventually fall off, peanut leaves infected with peanut rust turn black in color and remain attached to the stem. This difference in symptoms is important because it indicates that reflectance at 800 nm is an estimate of GLA and not merely the presence or absence of plant structures or tissues. This is evidenced by the fact that percent reflectance values increased as either defoliation or peanut rust severity decreased. In fact, percent reflectance values at 800 nm recorded from the centers of peanut rust foci that were 100% necrotic were 2–3% lower than reflectance values from peanut plots with 100% defoliation. This is because plants killed by peanut rust lack chlorophyll pigments and present a better "black body" for absorbing electromagnetic radiation compared with defoliated (bare soil) plots that still reflect some radiation at the 800-nm wavelength.

The establishment of late leaf spot defoliation gradients and the availability of peanut rust disease severity gradients provided a range of disease intensity levels to test the hypothesis that disease intensity gradients can also create GLA gradients. This study shows that the GLA gradients can be detected and quantified by measuring percent reflectance with a hand-held, multispectral radiometer. Gradient models employing transformations to linearize disease intensity (Y) and distance (X) values can also be applied to reflectance and distance measurements. The logit (Y) versus linear (X) model provided the best fit ($R^2 = 94.9\%$) when percent reflectance values were expressed as a proportion of reflectance from healthy plots and used in place of percent

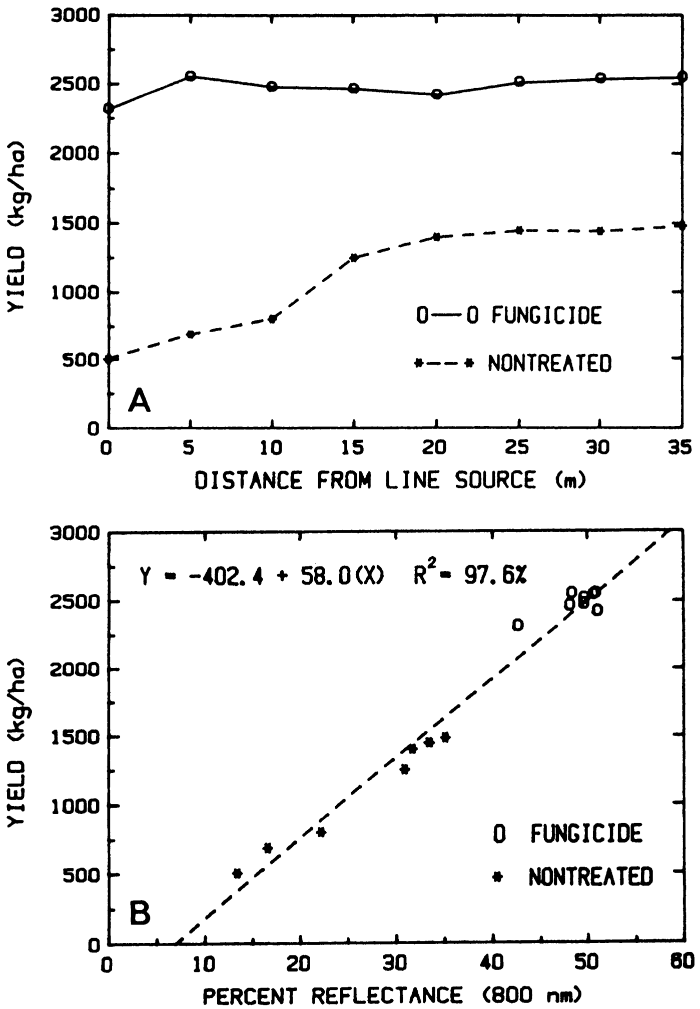

Fig. 4. A, Yield (kg/ha) in fungicide-treated and nonsprayed peanut plots perpendicular to a line source of *Cercosporidium personatum* spores; and **B,** linear regression of yield on percent reflectance (800 nm).

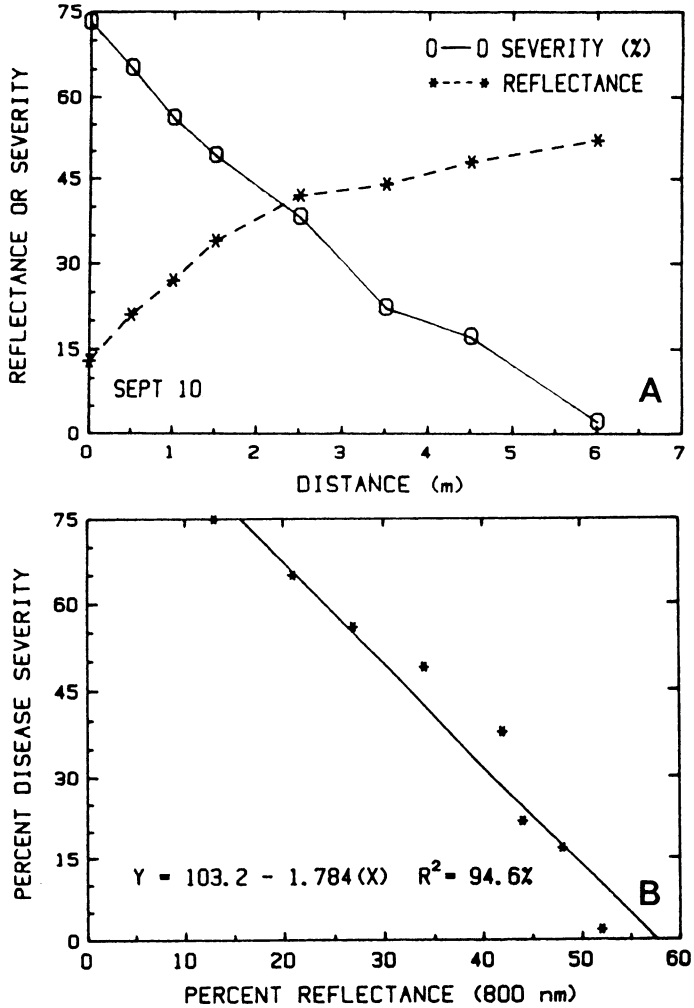

Fig. 5. A, Relationship betwen percent reflectance (800 nm) and percent peanut rust severity measured across a peanut rust disease focus; and **B,** linear regression of percent rust severity on percent reflectance (800 nm).

defoliation (Y) measurements for both the point and line source experiments. The logit (Y) versus linear (X) model was also the best model for describing defoliation (Y) versus distance (X) gradients ($R^2 = 96.0\%$).

Berger and Luke (3) have expressed concern over the possible inadvertent spread of plant pathogens by experimenters entering plots when making assessments. Reflectance measurements can be obtained without assessors disrupting sources of inoculum within the peanut canopy. Measuring gradients within the row of the point source or in transects from the line source resulted in long, narrow plots that provided an efficient means to study gradients. Minogue and Fry (16) have also recommended the use of long, narrow plots as a relatively inexpensive way to study gradients. Thus, radiometric measurements can provide a rapid and objective means of indirectly estimating the effects of disease intensity for the purpose of detecting and measuring foliar disease gradients (or GLA gradients) in peanut.

Regression equations relating percent defoliation (Y) to percent reflectance (X) were different for the point and line source experiments. This was probably due to differences in the absolute amount of GLA resulting from different planting dates, irrigation schedules, and dates of data collection. The line source experiment was planted 24 days earlier, but received less irrigation, and had considerably less vine growth than the point source experiment (average 20 nodes per branch stem vs. 16). Thus, the absolute amount of GLA was higher in the point source experiment. Also, no defoliation had occurred by 27 August at the outer edges of foci in the point source experiment, while percent defoliation was approximately 30% for fungicide-treated peanuts in the line source experiment on 10 September (14 days later). Thus, the higher GLA in the point source experiment required a 2.8% increase in defoliation to obtain a 1% decrease in percent reflectance, while only a 1.8% increase in defoliation was necessary in the line source experiment to obtain the same 1% decline in peanut canopy reflectance. Therefore, to estimate percent defoliation with the radiometer over locations and years, the absolute amount of GLA must also be considered. However, the relative rankings of treatments within an experiment based on reflectance values should remain constant, and, therefore, the acquisition of reflectance data should be useful in evaluating and ranking the efficacy of disease control tactics targeted to protect and maintain GLA such as the use of resistant varieties and experimental fungicides.

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