A Comparison of Disease Assessment Methods for Southern Stem Rot of Peanut

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ABSTRACT

Southern stem rot (caused by the soilborne fungus *Sclerotium rolfsii* Sacc.) of peanut (*Arachis hypogaea* L.) traditionally has been assessed based on the percentage of infected 30.5-cm row segments, commonly referred to as disease incidence. Several alternative disease assessment methods were evaluated in four fungicide trials during the growing season (aboveground ratings) and immediately after peanut inversion (belowground ratings). Pearson's correlation coefficients compared disease assessments and yields for all trials. Across all disease assessment methods, belowground assessments at inversion showed a stronger correlation with yield than in-season aboveground assessments. Several of the alternative assessment methods showed a stronger negative correlation with yield than did the traditional disease incidence rating. However, none of the alternative methods were consistently more precise across all assessment dates and trials. There was a significant positive correlation between many of the alternative methods and the traditional disease incidence method. Furthermore, none of the alternative methods was better than the traditional method for detecting differences among fungicide treatments when subjected to ANOVA and subsequent Waller-Duncan mean separation tests (k-ratio = 100). Based on comparisons of the time required to assess disease intensity, the traditional disease assessment method was found to be the most time efficient method of those tested in this study.

Key Words: *Arachis hypogaea*, *Sclerotium rolfsii*.

Southern stem rot, caused by the soilborne fungus *Sclerotium rolfsii* Sacc., is one of the most damaging diseases of peanut (*Arachis hypogaea* L.) in Georgia. *Sclerotium rolfsii* can infect various parts of peanut plants including the crown, pods, pegs, and lateral branches (Aycock, 1996). Consequently, infected plants express a range of symptoms. Such an array of symptoms can make it difficult to consistently assess disease or accurately determine the relationship between disease development and yield. Low correlation between disease assessments and yield can be especially problematic in research plots where different fungicide spray regimes are being evaluated for control of southern stem rot under different environments.

Quantitative assessment of southern stem rot traditionally has been accomplished using a method developed by Rodriguez-Kabana *et al.* (1975). This assessment method involves counting the number of infected 30.5-cm row segments within a plot. The number of infected segments is then divided by the total number of 30.5-cm row segments in the plot and expressed as a percentage of infected segments. This is commonly accomplished using an inverted T-shaped metal rod where the bottom portion is 30.5 cm in length. Disease assessments are based on visible signs and symptoms and may be performed during the growing season (aboveground) or immediately after the plants are inverted exposing belowground signs and symptoms. Although this assessment method is time efficient, correlations between disease assessments and yield are sometimes low, particularly when based on in-season aboveground assessments (Rodriguez-Kabana *et al.*, 1975; Bowen *et al.*, 1992). Numerous factors can contribute to these low correlations, but the fact that mildly infected plants count just as much as a dead plant has led to questions regarding the reliability of this assessment method. It has been suggested that alternative assessment methods based upon disease severity may produce higher correlations with yield than those obtained using the traditional disease incidence method (Bowen *et al.*, 1992; Davis *et al.*, 1996).

This traditional assessment method developed by Rodriguez-Kabana *et al.* (1975) has been commonly referred to and reported in the literature as a disease incidence assessment. Although it may not fit some of the stricter definitions for a disease incidence rating (Campbell and Madden, 1990), it does fit some less stringent definitions (James, 1983). In addition, disease incidence is the standard terminology used in reference to this method in other publications, therefore it will be used in this report.

Alternative assessment methods for southern stem rot in peanut have been employed in the past. Several researchers have measured incidence of southern stem rot based on the percentage of infected plants (Grinstein *et al.*, 1979; Wolf *et al.*, 1997; Marinelli *et al.*, 1998). In a study by Bowen (1998), peanut plants were examined aboveground and belowground throughout the growing season to derive a more accurate estimate of disease incidence. Although these methods produce a more exact measurement of disease incidence than the method developed by Rodriguez-Kabana *et al.* (1975), such assessments can be tedious and require destructive sampling. In a study by Shew *et al.* (1987), methods used to assess disease development in field studies included the number of dead plants per meter of row and the number of dead plants per disease focus. However, the relationship

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between yield and disease intensity was not discussed. Davis *et al.* (1996) used total infected plot length as a measure of aboveground disease, which was used to determine area under disease progress curve (AUDPC) values. However, relationships between yield and disease ratings were somewhat inconsistent in this study. Shokes *et al.* (1996) developed a five-category disease assessment scale for individual plants based on percent of symptomatic tissue. They also calculated AUDPC values; however, relationships between yield and disease intensity were not determined. Therefore, few alternative disease assessment systems have been developed for which the relationship between yield and disease is understood. The objectives of this research were to develop new disease assessment methods for southern stem rot in peanut and to evaluate and compare them based on time efficiency and correlation with yield.

Materials and Methods

Southern stem rot assessment methods were compared in four trials, two in 1999 and two in 2000. The peanut cultivar Georgia Green was planted in all trials (23 seeds/m row) in fields infested with *S. rolfsii* where peanut had been grown previously. Planting dates for the trials in 1999 were 13 May for both trials and 9 May and 10 May for 2000. All plots were sprayed with chlorothalonil (Bravo Ultrex, Syngenta Crop Protection, Greensboro, NC) at 1.3 kg ai/ha on a 2-wk schedule to control foliar diseases. All trials were irrigated as needed and other production practices were conducted according to Univ. of Georgia recommendations. Peanuts were inverted and combine harvested, then dried to 10% moisture prior to weighing and determining yields. Inversion and harvest dates, respectively, for the four trials were as follows: 1999 trial 1, 30 Sept. and 8 Oct.; 1999 trial 2, 30 Sept. and 9 Oct.; 2000 trial 1, 17 Sept. and 27 Sept.; and 2000 trial 2, 11 Sept. and 28 Sept.

Experimental Design. A range of disease levels was produced in these trials by altering the timing of two treatments of azoxystrobin (Abound 2.08F, Syngenta Crop Protection, Greensboro, NC) at 0.34 kg ai/ha. These different timings have produced a wide range of disease levels in previous trials (unpubl. data). Eleven different application timings of azoxystrobin and a nontreated control were included in each experiment for a total of 12 treatments. Initial and second spray dates ranged from 36 to 95 and 61 to 110 d after planting (DAP), respectively. The treatments produced a wide range of disease incidence levels and yields (Table 1), which is desirable to determine how the assessment methods would perform over many different disease levels. Randomized complete block designs were utilized with four or five replications per trial. Individual plots were two rows wide (1.8 m) and 7.6 m long. Plot length was measured after seedling emergence (~25 DAP) and plants outside the 7.6-m length were removed. Disease assessments in this study were made from the crown of the first plant to the last plant within each row (~7.6 m length).

Aboveground Disease Assessments. Aboveground disease incidence and severity were assessed periodically throughout the growing season with variable intervals between assessment dates. Across the four trials, there were a total of 10 disease assessment dates during the growing season. The number of 30.5-cm row segments exhibiting signs or symptoms was counted according to the method developed by Rodriguez-Kabana *et al.* (1975). For each plot, disease incidence (DINC) was expressed as a percentage according to the following formula:

$$\text{DINC} = (\text{no. symptomatic 30.5-cm row segments /no. 30.5-cm row segments in plot})*100 \quad \text{[Eq. 1]}$$

In addition, assessments of disease severity were recorded simultaneously with the assessments for disease incidence. Each infected 30.5-cm row segment was assigned a value of 1 to 4 based on the percentage of symptomatic tissue present (1 = <1-25%, 2 = >25-50%, 3 = >50-75%, and 4 = >75-100%). Individual severity values were summed to derive a total disease severity for each plot (DSEV). Additionally, the mean disease severity (MDSEV) per symptomatic 30.5-cm row segment was determined for each plot using the following formula:

$$\text{MDSEV} = \text{DSEV/no. symptomatic 30.5-cm row segments} \quad \text{[Eq. 2]}$$

Disease intensity (DINT) values were derived using the following formula:

$$\text{DINT} = \text{DINC} * \text{DSEV} \quad \text{[Eq. 3]}$$

Belowground Disease Assessments. Several disease assessment methods were used after peanuts were inverted, prior to harvest. DINC, DSEV, MDSEV, and DINT values were calculated for inverted peanuts as described previously for aboveground assessments. In an alternative assessment method, the number of disease foci per plot were counted (FOCI), and all disease foci were measured and summed over the entire plot, to derive a total infected length (TIL) for each plot. Finally, TIL was divided by FOCI (TIL/FOCI) to derive a mean length of infected row per disease focus (MLPF).

The amount of time needed to conduct the different

Table 1. Incidence of southern stem rot and yield of peanuts in four fungicide trials conducted in Tift County, GA in 1999 and 2000.

		Disease incidence[a]			Yield		
Year	Trial	Min.[b]	Max.	Mean	Min.	Max.	Mean
		% symptomatic -- 30.5-cm segments --			------------kg/ha------------		
1999	1	24	90	62.0	2507	5663	3888
	2	38	90	62.0	2401	5046	3807
2000	1	8	64	24.6	1888	4851	3741
	2	10	72	38.5	3353	5990	4874

[a]Southern stem rot disease incidence based upon percent of infected 30.5-cm row segments after peanut inversion. Disease incidence was defined as ≤ 30.5-cm of plants in a linear row exhibiting signs or symptoms of southern stem rot.

[b]Maximum, minimum, and mean values were determined by examining individual plot values within each individual trial. Each trial consisted of 11 different azoxystrobin (Abound 2.08F at 0.34 kg ai/ha) application timings and a nontreated control replicated four or five times.

disease assessments was evaluated by measuring the time required to rate one replication of the 12 different treatments in each of the four trials using the individual assessment methods. Time required per replication was then converted to mean time required to assess a single plot (7.6 x 1.8 m) according to each individual assessment method.

Calculations of Area Under Disease Progress Curves. For each of the rating methods, both aboveground during the season and belowground after inversion (DINC, DSEV, MDSEV, and DINT), area under disease progress curve (AUDPC) values were calculated using the following formula:

$$AUDPC = \sum_{i=1}^{n} [(X_i + X_{i-1})2](t_i - t_{i-1}) \qquad \text{[Eq. 4]}$$

where n is the number of assessment times, and X_i is the disease intensity at the i^{th} assessment and t_i is the time of the i^{th} assessment (Shaner and Finney, 1977).

Statistical Analysis. Statistical analyses were performed within individual trials due to potential treatment interactions. Data were subjected to analyses of variance using the general linear model procedure and Waller-Duncan mean separation tests (k-ratio = 100) to evaluate differences in fungicide treatments for all rating methods. The number of mean separation groups for each assessment method was compared across the four trials to determine the sensitivity of detecting significant differences. In addition, correlations between yield and disease intensity values generated using the different assessment methods were determined based on Pearson's correlation coefficients (Gomez and Gomez, 1984). For belowground disease ratings, correlations among the different assessment methods were conducted and Pearson's correlation coefficients were determined. Time efficiency values (TEV) for each assessment method were determined using the following formula:

$$TEV = MPCC / \text{time (min)} \qquad \text{[Eq. 5]}$$

where MPCC is the mean Pearson's correlation coefficient, between disease intensity and yield, for each assessment method across the four trials, and time is the time in minutes required to rate a single plot using that particular assessment method.

Results

Aboveground Ratings. Correlations between yield and DINC, DSEV, and DINT were significantly negative ($P \leq 0.05$) for seven of the 10 in-season assessment dates. However, a significant correlation between yield and MDSEV was observed for only five of the 10 assessment dates. Ranges of Pearson's correlation coefficients produced from comparisons involving yield and disease assessment methods over the 10 assessment dates are graphed in Figure 1. More specifically, the DINC method produced Pearson's correlation coefficients ranging from -0.02 to -0.59 (mean = -0.35), DSEV method -0.12 to -0.60 (mean = -0.38), MDSEV method -0.01 to -0.43 (mean = -0.26), and DINT -0.13 to -0.55 (mean = -0.36). However, the DSEV method produced the highest correlation with yield in six of the 10 assessment dates, while the DINC and DINT method each showed the highest correlation in two of the assessment dates.

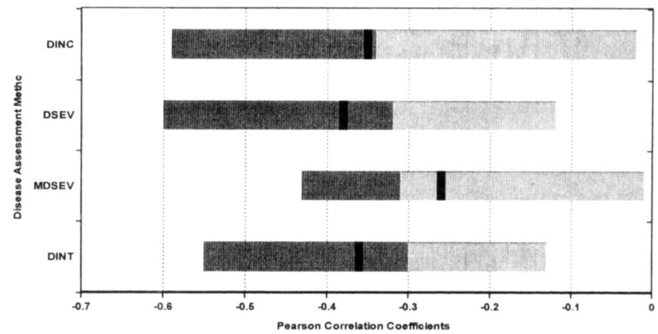

Fig. 1. Ranges of Pearson's correlation coefficients for the relationship between yield and aboveground rating methods for southern stem rot of peanut. Darker gray areas indicate significant correlations ($P \leq 0.05$) and the lighter areas indicate nonsignificant relationships. Black bands indicate the mean of 10 rating dates over four trials. (DINC = disease incidence, DSEV = disease severity, MDSEV = mean disease severity, and DINT = disease intensity).

With regard to the sensitivity of rating methods for differentiating between treatments, differences were found in the numbers of mean separation groups produced per assessment date resulting from analysis of variance and subsequent mean separation tests (data not shown). The DINC method was most sensitive, producing 4.1 separation groups per assessment date followed by DSEV (3.8 groups), DINT (3.4 groups), and MDSEV (2.2 groups).

Belowground Ratings. Correlations between yield and disease assessments were generally stronger for the belowground ratings taken after inversion than for the in-season aboveground ratings. All disease assessment methods except for FOCI showed a significant negative correlation ($P \leq 0.05$) with yield in all four trials. Significant correlations between yield and FOCI were observed in only two of the four trials. Ranges of Pearson correlation coefficients produced from comparisons of yield and belowground disease assessment methods across the four trials are presented in Figure 2. The DINC method produced a range of -0.44 to -0.59 (mean = -0.51), DSEV method -0.50 to -0.63 (mean = -0.56), MDSEV method -0.46 to -0.53 (mean = -0.50), DINT -0.49 to -0.61 (mean = -0.56), FOCI -0.16 to -0.34 (mean = -0.25), TIL -0.41 to -0.61 (mean = -0.55), and MLPF -0.44 to -0.62 (mean = -0.54). The highest correlation coefficients between yield and disease assessment were produced by the DSEV and MLPF methods in two of the four trials.

When correlations between disease rating methods were compared, all rating methods, except for the FOCI method, were significantly correlated ($P \leq 0.01$) to the traditional DINC method for all four trials (Table 2). All rating methods were significantly correlated ($P \leq 0.01$) with each other for all four trials when the FOCI method was not considered. Correlations between DSEV and DINT produced the highest values of Pearson's correlation coefficients (0.98 - 0.99). The FOCI method was not found to be significantly correlated with any of the other disease assessment methods for all four trials.

The average numbers of mean separation groups produced per trial resulting from analysis of variance and

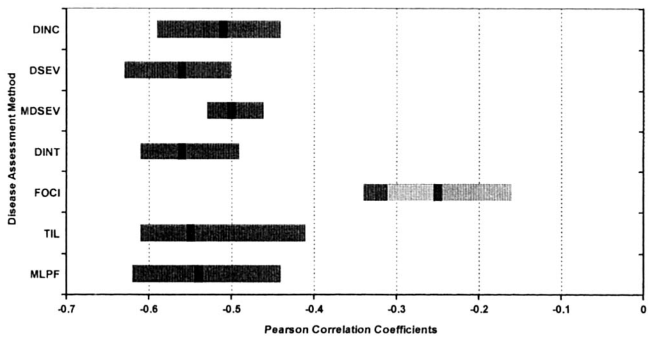

Fig. 2. Ranges of Pearson's correlation coefficients for the relationship between yield and belowground rating methods for southern stem rot of peanut. Darker gray areas indicate significant correlations (P ≤ 0.05) and the lighter areas indicate nonsignificant relationships. Black bands indicate the mean over four trials. (DINC = disease incidence, DSEV = disease severity, MDSEV = mean disease severity, DINT = disease intensity, FOCI = number of disease foci, TIL = total infected length, and MLPF = mean length per disease focus).

subsequent mean separation tests are presented in Figure 3. The DINC, DINT, and TIL methods were most sensitive producing 4.75 separation groups per trial. The DSEV method produced 4.25 separation groups per trial, MLPF 4.0 groups, FOCI 2.5 groups, and the MDSEV method 2.25 groups. The number of mean separation groups per trial for yield was 2.75 groups.

Time Efficiency Values. Time required to rate a single plot for the DINC method was 2.3 min/plot. Comparatively, it required 2.8 min/plot for the DSEV, MDSEV, and DINT methods and 3.0 min/plot for the FOCI, TIL, and MLPF methods. The most time efficient method (lowest negative number) according to this measurement was the DINC method, which produced -0.22 Pearson's correlation coefficient units/minute/plot (Fig. 4). Both the DSEV and DINT methods produced a time efficiency value (TEV) of -0.20. The MDSEV, TIL, and MLPF methods produced a TEV of -0.18 and the FOCI method a TEV of -0.08.

AUDPC Calculations. When AUDPC values were calculated for DINC, DSEV, and DINT across both above- and belowground assessments, there was a significant negative correlation with yield in all four trials for all four methods employed. Ranges of Pearson correlation coefficients produced from comparisons of yield and AUDPC values across the four trials are presented in Figure 5. Values of AUDPC derived from DINC produced a range of correlation coefficients from -0.41 to -0.61 (mean = -0.51). Similarly, AUDPC values derived from DSEV and DINT produced ranges of -0.40 to -0.62

Table 2. Pearson's correlation coefficient and level of significance ranges between seven different disease assessment methods for southern stem rot of peanut from four fungicide trials conducted in Tift County, GA in 1999 and 2000.

Rating method[a]	DINC	DSEV	MDSEV	DINT	TIL	FOCI	MLPF
	Pearson's correlation coefficients range[b] (p-value range)[c]						
DINC	---	0.96 - 0.98 (<0.01)	0.40 - 0.65 (<0.01)	0.94 - 0.98 (<0.01)	0.81 - 0.94 (<0.01)	-0.27 - 0.85 (0.42 - <0.01)	0.64 - 0.85 (<0.01)
DSEV	---	---	0.59 - 0.81 (<0.01)	0.98 - 0.99 (<0.01)	0.83 - 0.94 (<0.01)	-0.33 - 0.78 (0.85 - <0.01)	0.70 - 0.87 (<0.01)
MDSEV	---	---	---	0.52 - 0.74 (<0.01)	0.44 - 0.69 (<0.01)	-0.30 - 0.45 (0.32 - <0.01)	0.43 - 0.70 (<0.01)
DINT	---	---	---	---	0.80 - 0.95 (<0.01)	-0.35 - 0.72 (0.72 - <0.01)	0.68 - 0.89 (<0.01)
TIL	---	---	---	---	---	-0.30 - 0.84 (0.43 - <0.01)	0.69 - 0.90 (<0.01)
FOCI	---	---	---	---	---	---	-0.75 - 0.45 (0.55 - <0.01)
MLPF	---	---	---	---	---	---	---

[a]Rating methods are abbreviated as follows: DINC = disease incidence, DSEV = disease severity, MDSEV = mean disease severity, DINT = disease intensity, TIL = total infected length, FOCI = number of disease foci, and MLPF = mean length per disease focus.

[b]Pearson's correlation coefficients were determined by comparing disease rating values for each rating system within each of the four trials. Presented in the table is the range over these four trials. Each trial consisted of 11 different azoxystrobin (Abound 2.08F at 0.34 kg ai/ha) application timings and a nontreated control and with four to five replications.

[c]One value presented when probabilities were equal for a certain rating method across all trials.

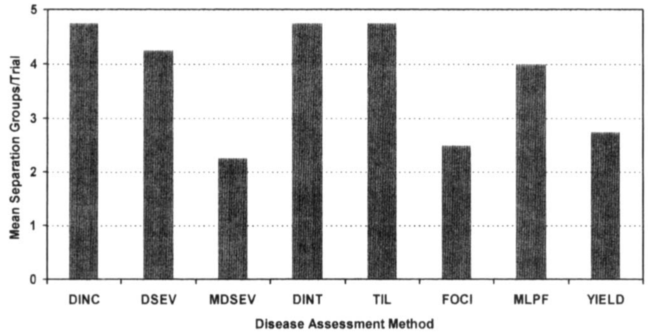

Fig. 3. Number of mean separation groups in four trials according to disease assessment methods conducted on belowground symptoms and signs of southern stem rot in peanut. Analysis of variance and subsequent mean separation tests (Waller-Duncan with a k-ratio = 100) were performed on data for each trial. (DINC = disease incidence, DSEV = disease severity, MDSEV = mean disease severity, DINT = disease intensity, FOCI = number of disease foci, TIL = total infected length, and MLPF = mean length per disease focus).

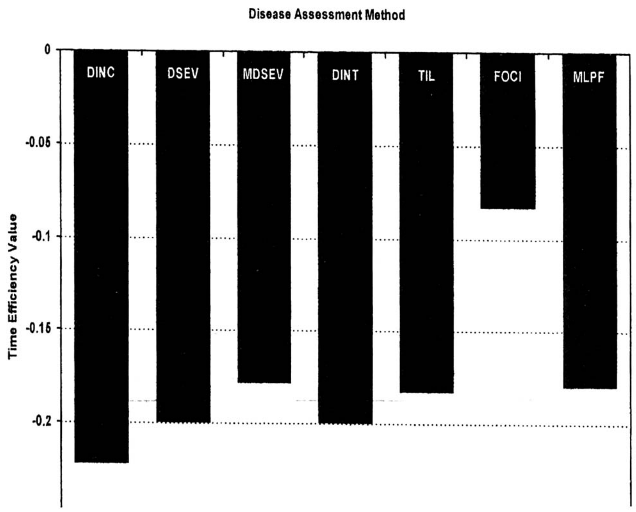

Fig. 4. A comparison of time efficiency values for disease assessment methods for southern stem rot of peanut. Time efficiency values were determined by dividing the mean Pearson's correlation coefficient over four trials (for the relationship between yield and belowground disease assessment) by the amount of time in minutes required to rate a 7.6 x 1.8-m plot. (DINC = disease incidence, DSEV = disease severity, MDSEV = mean disease severity, DINT = disease intensity, FOCI = number of disease foci, TIL = total infected length, and MLPF = mean length per disease focus).

(mean = -0.52) and -0.34 to -0.63 (mean - 0.47), respectively. The highest correlations between yield and AUDPC values derived from disease assessment measurements were produced by the DINT method in two trials and the DINC and DSEV methods in one trial each.

Discussion

Belowground disease assessments recorded after peanut inversion generally were correlated more strongly with yield than the in-season aboveground ratings. This trend was observed regardless of the disease assessment

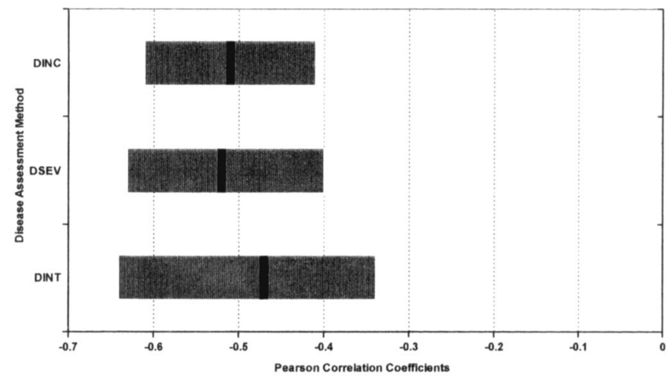

Fig. 5. Pearson's correlation coefficients ranges from the relationship between yield and area under disease progress curve calculations (AUDPC) determined from rating methods for southern stem rot of peanut. Correlations over the entire range for all rating methods were found to be significant ($P \leq 0.05$). Black bands indicate the mean over the four trials. (DINC = disease incidence, DSEV = disease severity, and DINT = disease intensity).

method employed and was noted in all four trials. Based on these studies, the usefulness of in-season, aboveground disease assessments is limited to examining southern stem rot development during the growing season and the effectiveness of fungicide spray regimes on epidemic development. Aboveground disease assessments may be useful for evaluating early generation breeding lines in very small plots that are not conducive to inversion and subsequent harvest. Aboveground disease assessments also serve as an 'insurance rating' in the event that adverse weather conditions or other unforeseen disasters occur jeopardizing the belowground ratings. However, when examining the efficacy of fungicide treatments for season-long disease suppression and its subsequent effect on yield, a belowground disease assessment following peanut inversion is preferable.

All of the disease assessment methods that were designed and tested in this study performed very similarly to the traditional disease incidence method (DINC) based on symptomatic 30.5-cm row segments that was developed by Rodriguez-Kabana *et al.* (1975). One exception to this was the FOCI method in which disease foci were counted without taking their size into consideration. Additionally, correlations between the FOCI method and other disease assessment methods were not significant in many cases. The poor performance of this method emphasizes the need to incorporate disease severity and/or focus length into assessments of southern stem rot in peanut.

When aboveground disease assessments were considered, DSEV was more strongly correlated with yield, but showed a reduced sensitivity for detecting differences between treatments than the traditional DINC assessment. However, differences in disease severity among treatments during the growing season may not relate well to belowground (after inversion) assessments or yield, particularly if there are fungicide applications pending. Since aboveground assessments may not correlate strongly with yield, the use of disease severity scales, such as DSEV, may be desirable to more accurately determine the relationship between yield and aboveground disease assessments. However, alternative aboveground assessment methods varied according to trial and assessment date and were not consistently better than the DINC method. Aboveground ratings are sensitive to environmental conditions that most likely contributed to some of this variation. Aboveground ratings are best taken when conditions favor disease development causing signs and symptoms of southern stem rot to be highly visible. Furthermore, use of alternative aboveground rating systems discussed here requires more time than the traditional method.

Similar trends to those noted in the aboveground assessments were observed in the belowground ratings. Although several of the alternative assessment methods outperformed the DINC method in several trials, none produced consistently higher correlations with yield in all trials. Also, disease assessment values produced by alternative disease rating methods, except for the FOCI method, were strongly correlated with the traditional DINC method. Inconsistent performance by the alternative methods, similarity to the DINC method, and reduced time efficiency make it difficult to recommend these disease assessment methods over the traditional DINC method. Even incorporating aboveground ratings with belowground ratings in the form of AUDPC calculations did not improve correlations between yield and disease assessments.

Correlations between yield and all the disease assessment methods were less than ideal. There are many factors that could have affected yield, including the presence of other diseases or pests, differences in soil texture and fertility, or irrigation variability. The experimental error associated with many peanut tests in Georgia also has increased in recent years due to the impact of spotted wilt, caused by tomato spotted wilt virus. Therefore, the best comparison of disease rating methods is within a given test, and in these comparisons, the traditional DINC rating method appears to offer the best combination of efficiency and accuracy. However, when more accurate disease assessments are warranted, destructive sampling methods, such as those described by Bowen (1998), may be necessary.

The conclusions presented here are based on a wide range of disease intensities and yields. It is worth noting that this range of disease intensity levels was obtained by the differential use of fungicides. Such treatments conceivably could alter the expression of disease symptoms, for example, by controlling aboveground symptoms better than those belowground. Also, all trials reported here were planted with the cultivar Georgia Green and other genotypes may respond differently. These results should be applicable to other fungicide trials, but should be evaluated further before extrapolating to include germplasm evaluations.

In summary, southern stem rot in peanut is a difficult disease to quantitatively assess due to the multitude of symptoms produced by plants infected by *S. rolfsii*. The best correlations with yield resulted from belowground disease assessments taken at peanut inversion prior to harvest. We conclude from this study that the traditional DINC method developed by Rodriguez-Kabana *et al.* (1975) is still the most time efficient method that correlates well with yield for quantitative assessment of southern stem rot of peanut.

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