

## Red leaf blotch (*Dactuliochaeta glycines*) of soybeans (*Glycine max*) and its relationship to yield

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Red leaf blotch of soybeans, caused by *Dactuliochaeta glycines*, was evaluated on soybean plants in field plots located in Zambia. Two experiments were conducted in each of two seasons. Experiment 1 had four cultivars that were either fungicide-sprayed or not sprayed. Disease severity was greatest on leaves at the lowest nodes from early vegetative through the reproductive growth stages. Area under the disease progress curve (AUDPC) values and percentage of nodes defoliated at growth stage R5 were significantly ( $P=0.05$ ) greater in unsprayed plots for all cultivars in both seasons. Yield losses ranged from 8 to 37% while reduced seed size ranged from 21 to 29% for the four cultivars. Number of pods per plant in fungicide-sprayed plots did not differ from those in unsprayed plots. However, the number of seeds per plant and seeds per pod were significantly ( $P=0.05$ ) greater in sprayed than unsprayed plots for some cultivars. In experiment 2, cultivar Tunia was either fungicide-sprayed at different times or not sprayed. The lowest attached leaf had the most variation in the amount of disease while ratings of the most median leaf in the canopy were generally less variable. The AUDPC values calculated from the lowest attached leaf, the mean of all attached leaves, and the median attached leaf differed significantly ( $P=0.05$ ) the number of times plants were sprayed with fungicide. Defoliation and vertical incidence of red leaf blotch from lower to higher nodes were significantly ( $P=0.05$ ) reduced in fungicide-sprayed plots in one season, but not the other. One thousand-seed weight and yield differed significantly ( $P=0.05$ ) with treatment as one application of triphenyltin acetate increased yields by 18% over unsprayed plots in season 1. One thousand-seed weight and yield, regressed on the AUDPC for the median leaf in the canopy, explained 92 and 72% of the variation, respectively.

### INTRODUCTION

Red leaf blotch, caused by *Dactuliochaeta glycines* (Hartman & Sinclair, 1988) of soybeans (*Glycine max*) was first reported in Africa in 1957 (Stewart, 1957). The disease occurs in central and southern Africa where it has been geographically limited to several countries (Hartman *et al.*, 1987). More recently, it was documented in Nigeria (Akem *et al.*, 1992). The source of the primary inoculum is not known; however, the pathogen and disease have been reported on *Neonotonia wightii*, a perennial legume which inhabits grasslands and woodlands of southern Africa (Stewart, 1957; Leakey, 1964). The pathogen is soilborne and produces sclerotia within infected lesions (Hartman *et al.*, 1987). Sclerotia were shown to have the ability to

germinate and form pycnidia or secondary sclerotia (Hartman & Sinclair, 1992).

In experimental trials, losses of up to 37% have been reported in Zambia (Datnoff *et al.*, 1987). Commercial growers in Zambia and Zimbabwe have had to use fungicides to control red leaf blotch because of the unavailability of resistant cultivars. In Zimbabwe, two to three sprays of triphenyltin acetate have been recommended at 14-day intervals for control of red leaf blotch (Muchenje & McClymont, 1990).

Field studies on red leaf blotch including disease development and assessment have been limited. One method of assessment used the lowest attached leaf (Datnoff *et al.*, 1987), while another used the fully expanded leaves from the upper portion of the canopy (Levy *et al.*, 1990). Neither of these assessments accounts for defoliation, which has been reported to be an important component of the disease syndrome

(Javaid & Ashraf, 1978; Hartman *et al.*, 1987). Reliable methods to assess red leaf blotch are critical to evaluate germ plasm adequately, develop meaningful disease progress curves, and further understand the relationship of disease severity parameters to yield components.

The objectives of this study were to rate disease severity on leaves from the lower to upper nodes; measure defoliation; and to quantify the relationship of area under the disease progress curve (AUDPC), defoliation, incidence, and severity based on three types of assessments to 1000-seed weight, number of pods with zero to three seeds, pods per plant, seeds per pod, and yield.

## MATERIALS AND METHODS

### Experiment 1

Four soybean cultivars were planted in a split-plot design in two growing seasons at the Mpongwe Development Project, Copperbelt Province, Zambia. Each experimental plot consisted of four rows, 12 m long, planted on 50-cm centres. There were six and four replicated main plots in the two growing seasons, respectively. Main plots were either sprayed with fentin acetate (Brestan 60 %WP, 0.6 kg a.i./ha) or unsprayed. The fungicide was applied with a Solo mist blower backpack sprayer every 7 to 10 days at V4 through R6 growth stages (Fehr *et al.*, 1971). Cultivars 'Oribi', 'Sable', 'Santa Rosa' and 'Tunia' (all early to medium maturity in Zambia with determinant growth habit) were randomized within each main plot at a planting rate of 40 seeds/m row. Planting dates were 24 and 12 December in 1984 and 1985 (season 1 and 2, respectively). The cropping sequence was soybeans during the rainy season for the previous 5 years and irrigated wheat in the dry season. Disease severity was rated using the Horsfall-Barratt rating system (Horsfall & Barratt, 1945). These values were converted to percentage leaf area diseased using the Elanco Conversion Tables (Elanco Products Co., Indianapolis, IN). Disease severity was assessed at 5- to 14-day intervals from the growth stages V7 to R6. From each plot, five plants were randomly sampled from row 1 or 4 and each leaf on a plant was assessed for disease severity and its node of origin recorded. Defoliation, growth stage, disease occurrence, and total number of leaves and nodes present also were recorded.

Defoliation was converted to a percentage:

$$\left[ \frac{\text{number of nodes without leaves}}{\text{total number of nodes}} \right] \times 100$$
. Spread of the disease from older to younger leaves up the plant or vertical disease progress (VDP) (Pataky & Lim, 1981) was expressed as the percentage of leaves at nodes which had symptoms based on the following formula: 
$$\text{VDP} = \left[ \frac{\text{maximum number of nodes at which leaf symptoms appear}}{\text{maximum number of nodes per plant}} \right] \times 100$$
. The values used for calculating area under the disease progress curve (AUDPC) were the mean severity rating of each leaf on five plants per plot using the formula described by Shaner & Finney (1977).

Number of pods per plant and number of pods with one, two, three, or four seeds were counted on each of five plants per plot at growth stage R6 or R7. Total number of seeds per plant were determined and the percentages of pods with zero, one, two, three and four seeds per pod were calculated.

At maturity, 1 m from each end of the plots was clipped and plants in the two 10-m long centre rows were harvested by hand. Seeds were either dried in an oven for 72 h at 37°C or recorded for percentage seed moisture using a Burrow moisture meter and then weighed. Total yield and 1000-seed weights were adjusted to 13% seed moisture. Seed size was determined by placing 100 seeds in 100 mL of water in a graduated cylinder and recording the amount of water displaced. Percentage yield, and reduction in seed weight and size were calculated within each cultivar by using the formula: 
$$\text{percentage reduction (yield, seed weight, seed size)} = \left[ \frac{\text{value from sprayed plots} - \text{value from unsprayed plots}}{\text{value from sprayed plots}} \right] \times 100$$
.

### Experiment 2

At the Mpongwe Development Project, seeds of cv. Tunia, a commercially grown cultivar in Zambia, were sown in the same size plots as experiment 1 on 23 December 1984 (season 1) and again on 12 December 1985 (season 2).

The experiment consisted of five treatments: (i-iii) sprayed once, twice, and three times; (iv) sprayed weekly (11 and seven times in seasons 1 and 2, respectively) with fentin acetate (Brestan 60% WP) at 0.6 kg a.i./ha using a Solo mist backpack sprayer; and (v) not sprayed. Treatments were arranged in a completely randomized block design with four replications. Plants in plots receiving one application were sprayed at

R5 (Fehr *et al.*, 1971), two applications at growth stages R1 and R5, and three applications at growth stages V7, R1 and R5. Weekly applications began when plants were at growth stage V5–V6 in both experiments.

Disease assessments were estimated visually as described in experiment 1. The mean ratings of the leaf area infected were pooled from each attached leaf on the plant at the time of rating, the mean from the lowest attached leaf, and the mean leaf area infected of the median attached leaf in the canopy. In season 1, assessments were made on 23 February and on 2, 9, 16 and 30 March at growth stages R3, R4, R5, R6 and R7, respectively; in season 2 assessments were made on 2, 10, 20 and 26 February and 5 March at growth stages R2, R3, R4, R5 and R6, respectively. Defoliation and vertical disease progress data were assessed and calculated as previously described.

The number of pods per plant and number of pods with zero, one, two, three or four seeds were counted on each of five plants per plot at growth stage R6 or R7. Total number of seeds per plant was determined and the percentage of pods with zero, one, two or three seeds per pod was calculated.

At maturity, 1 m at the ends of the plots were trimmed and plants in the two 10-m long centre rows were harvested by hand. Seeds were either air- or oven-dried, the latter at 37°C for 72 h. Total yield (kg/ha) and 1000-seed weights (g) were adjusted to 13% moisture based on the moisture recorded with a Burrow moisture meter.

#### Data analysis

Data were analysed by analysis of variance ( $P < 0.05$ ) and treatments were compared by Fisher's least significant differences (FLSD). In experiment 1, which consisted of the four cultivars in fungicide-sprayed and unsprayed plots, VDP of each cultivar was regressed on leaf nodes at various growth stages. Infected leaf area values were transformed by  $\log_{10} + 1$  to stabilize variances. Ordinary-least square regression was used to predict leaf area infected as a function of leaf node position for each treatment and assessment. Regression analysis and predictor variables compared intercepts and slopes of unsprayed plots to other treatments (Freund & Lettell, 1986). Regressions were used to predict 1000-seed weight, seed size, and yield as a function of AUDPC, defoliation, incidence, and leaf area infected taken from the lowest

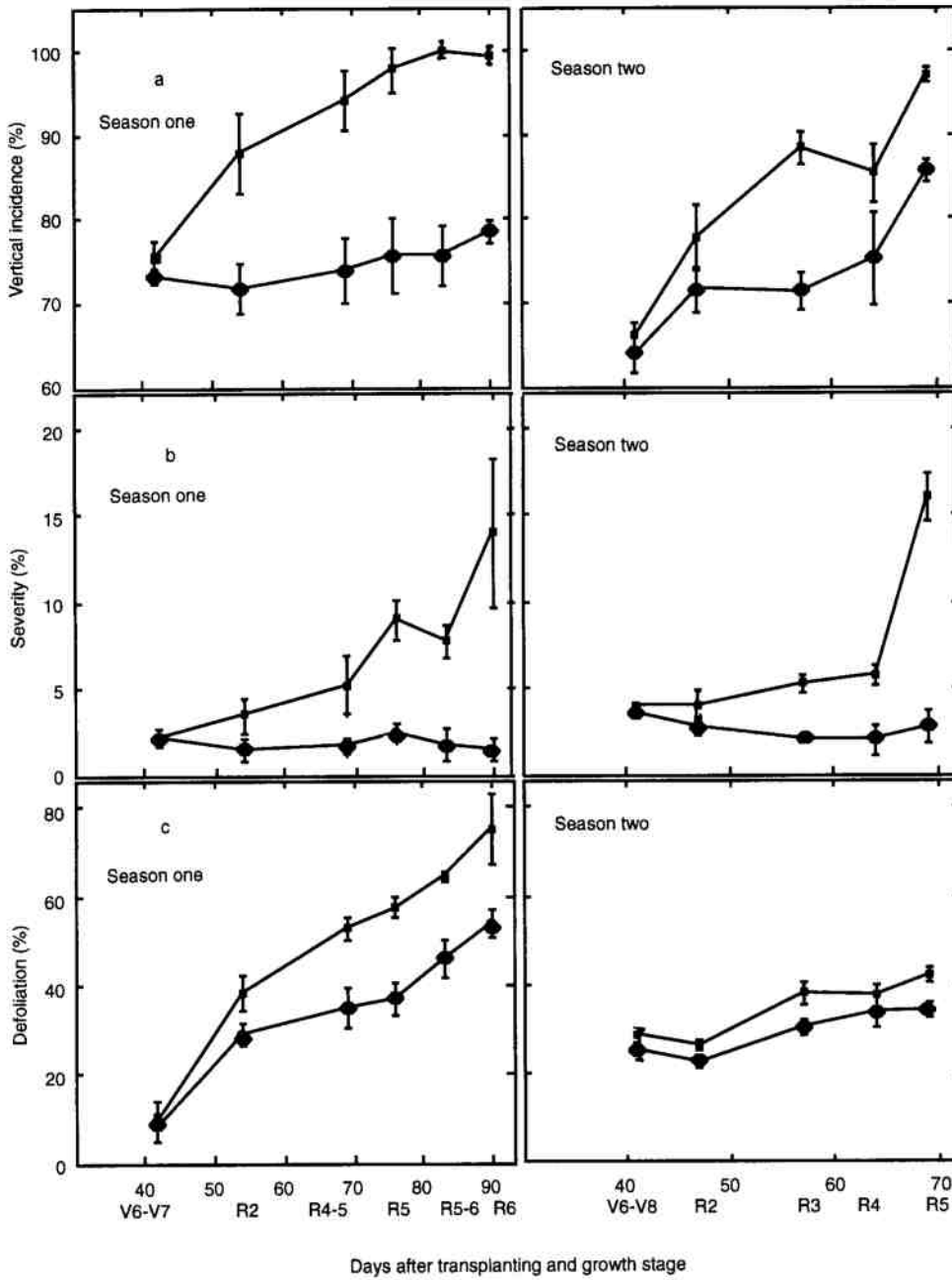
leaf, median leaf, and mean leaf area infected of all leaves at each assessment. *F* statistics were used to compare overall significance ( $P < 0.05$ ) of models and significance of polynomial terms.

## RESULTS

### Experiment 1

Red leaf blotch symptoms appeared first on unifoliolate leaves of all the cultivars. Vertical disease progress of symptoms on leaves approached 100% in each season by growth stage R5 in unsprayed plots while the progress was significantly less in sprayed plots (Fig. 1a). In the first disease rating for both seasons, disease severity was not significantly different in sprayed and unsprayed plots (Fig. 1b). However, by growth stage R2 in season 1 and R3 in season 2, disease severity was significantly greater for plants in unsprayed compared to sprayed plots (Fig. 1b). Disease severity in unsprayed plots was similar for each cultivar in each year with the lower leaves having more disease than upper leaves. Disease severity was greatest on leaves from the lower nodes and linear models best explained the severity of red leaf blotch from lower to upper nodes. Severity values increased rapidly as plants approached full seed development. AUDPC values were significantly higher in unsprayed plots compared to sprayed plots for all cultivars in both seasons (Table 1). Plants in unsprayed plots matured 5 to 7 days before plants in sprayed plots for both seasons. No significant difference occurred in total number of nodes per plant within each cultivar between sprayed and unsprayed plots. Defoliation was significantly greater for plants unsprayed than plants in sprayed plots at most assessments although the differences were greater in season 1 than 2 (Fig. 1c).

Yield, 1000-seed weight, and seed size were significantly lower in unsprayed plots compared to sprayed plots for each cultivar in the first season (Table 2). Yield was reduced in the four cultivars by 34.4 to 37.1% and reduction in 1000-seed weight and seed size was least for Tunia and greatest for Santa Rosa. In the second season, Sable had significantly higher yields and 1000-seed weights in sprayed plots compared with unsprayed plots. No significant difference occurred between sprayed and unsprayed plots for the other cultivars, although sprayed plots always had greater yields.



**Fig. 1** Mean percentage of (a) vertical incidence of red leaf symptoms, (b) disease severity, and (c) defoliated nodes on four soybean cultivars in plots unsprayed (■—■) and sprayed (●—●) with fentin acetate at 0.6 kg a.i./ha in two growing seasons at Mpongwe, Zambia. Vertical bars represent the standard error of the mean.

**Table 1** Comparison of unsprayed and fungicide-sprayed plots for defoliation, disease incidence, severity at R4 growth stage, and area under the disease progress curve (AUDPC) values for four soybean cultivars infected by *Dactuliochaeta glycines*, the causal agent of red leaf blotch, in two growing seasons at Mpongwe, Zambia

Cultivar	Treatment <sup>a</sup>	Defoliation <sup>b</sup> (%)	Incidence <sup>c</sup> (%)	Severity <sup>d</sup> (%)	AUDPC <sup>e</sup>
Season 1					
Oribi	Unsprayed	55.5	98.9	9.5	326.3
	Sprayed	35.8	79.7	3.5	105.8
Sable	Unsprayed	54.8	98.6	10.3	278.1
	Sprayed	33.9	72.6	2.4	96.6
Santa Rosa	Unsprayed	54.3	96.9	6.8	251.2
	Sprayed	41.7	78.8	2.2	65.8
Tunia	Unsprayed	57.0	93.9	7.7	279.5
	Sprayed	36.2	71.1	2.5	95.9
F.L.S.D. <sup>f</sup>		6.5	5.4	2.3	40.8
CV (%) <sup>g</sup>		12.8	5.9	39.1	21.4
Season 2					
Oribi	Unsprayed	43.5	97.4	16.1	163.0
	Sprayed	32.4	86.0	3.1	74.4
Sable	Unsprayed	41.4	97.3	14.2	147.7
	Sprayed	36.4	87.1	3.6	69.0
Santa Rosa	Unsprayed	42.9	95.6	17.5	162.8
	Sprayed	34.4	83.9	1.7	57.8
Tunia	Unsprayed	42.9	95.6	17.5	162.8
	Sprayed	34.7	85.9	1.9	66.8
F.L.S.D.		6.2	9.2	5.0	20.1
CV (%)		12.4	3.2	24.8	14.4

<sup>a</sup> Treatment is based on sprayed plots with 0.6 kg a.i./ha of fentin acetate applied 11 times in six replications in season 1 and seven times with four replications in season 2.

<sup>b</sup> Defoliation based on a percentage of nodes without leaves/total number of nodes.

<sup>c</sup> Incidence is based on a percentage of nodes having leaves with symptoms/maximum number of nodes.

<sup>d</sup> Percentage severity is based on the mean severity of all attached leaves.

<sup>e</sup> Calculated by the formula of Shaner & Finney (1977).

<sup>f</sup> Fisher's least significant difference, ( $P < 0.05$ ).

<sup>g</sup> Coefficient of variation.

The number of pods per plant was not significantly affected by the fungicide treatment regardless of cultivar in either year (Table 3). The number of seeds per pod for all cultivars was significantly greater for sprayed than unsprayed plots in the first, but not the second season. In the first season more seeds per plant were produced in sprayed than unsprayed for Oribi and Santa Rosa. The number of pods without seeds was significantly higher in unsprayed plots than in sprayed plots for all cultivars except Sable in the first season; in the second season, Oribi and Sable had significantly more pods without seeds in unsprayed than sprayed plots. The number of pods with one seed was significantly higher only for Sable in the first season, while Tunia had significantly more pods with two seeds in sprayed

plots compared to unsprayed plots in the second season. Pods with three seeds were significantly higher only for Sable in sprayed versus unsprayed plots in the first season. Few pods had four seeds in the first season and none were recorded in the second season.

## Experiment 2

Initial (33 and 23 plants per m row) and final stand counts (29 and 21 plants per m row) in seasons 1 and 2, respectively, did not differ significantly between treatments. The days to maturity differed as plants in unsprayed plots matured 4 to 10 days earlier than plants in fungicide-sprayed plots.

In season 1, the leaf area infected from plants

**Table 2** Comparison of unsprayed and fungicide-sprayed plots for yield, 1000-seed weight and seed size and their respective losses for four soybean cultivars infected by *Dactuliochaeta glycines*, the causal agent of red leaf blotch, in two growing seasons at Mpongwe, Zambia

Cultivar	Treatment <sup>a</sup>	Yield		1000-seed weight		Seed size <sup>c</sup>	
		Actual (kg/ha)	Loss <sup>b</sup> (%)	Actual (g)	Loss (%)	Actual (ml)	Loss (%)
Season 1							
Orbi	Unsprayed	1307	37.1	135.7	29.7	36.5	25.2
	Sprayed	2061		193.9		48.8	
Sable	Unsprayed	1340	34.9	151.4	29.9	39.7	23.2
	Sprayed	2080		216.4		51.8	
Santa Rosa	Unsprayed	1229	34.2	124.9	31.7	32.8	28.7
	Sprayed	1933		183.5		46.0	
Tunia	Unsprayed	1548	34.4	163.5	25.7	41.7	21.3
	Sprayed	2356		220.4		53.0	
F.L.S.D. <sup>d</sup>		306	NS <sup>f</sup>	11.8	6.0	1.6	2.6
CV (%) <sup>e</sup>		12		6.0	16.7	3.3	8.6
Season 2							
Orbi	Unsprayed	1060	10.6	154.0	-2.8	#	-
	Sprayed	1193		150.8		=	
Sable	Unsprayed	963	25.9	155.3	11.0	=	-
	Sprayed	1323		175.3		=	
Santa Rosa	Unsprayed	1070	7.7	135.5	-4.6	=	-
	Sprayed	1160		129.5		=	
Tunia	Unsprayed	945	11.6	165.5	5.7	=	-
	Sprayed	1078		176.5		=	
F.L.S.D.		183	NS	18.3	NS		
CV (%)		12		7.7			

<sup>a</sup> Treatment is based on sprayed plots with 0.6 kg a.i./ha of fentin acetate applied 11 times in six replications in season 1 and seven times with four replications in season 2.

<sup>b</sup> Losses based on comparisons within replications to sprayed plots.

<sup>c</sup> Measurement of seed size is the displacement of 100 seeds in 100 mL of water and is expressed in mL.

<sup>d</sup> Fisher's least significant difference. ( $P < 0.05$ ).

<sup>e</sup> Coefficient of variation.

<sup>f</sup> Not significant.

<sup>#</sup> Data not recorded.

in unsprayed plots increased over time, but in season 2 the leaf area infected varied over time with the lowest rating occurring at 60 days after transplanting (Fig. 2a and b). In both experiments, the lowest attached leaf had the most variation in the amount of disease along with the greatest severity. At each assessment time, the leaf area infected significantly decreased from the lower to the upper leaves in the canopy for each treatment. For example, at growth stage R5 for both experiments, plants that were fungicide-sprayed had more disease on leaves lower in the canopy than on leaves that were located in the upper canopy (Fig. 3a and b).

The AUDPC values calculated from the

lowest, median, and mean leaf differed significantly in plants from fungicide-sprayed compared to unsprayed plots in season 1, with the lowest values from plots sprayed the maximum number of times (Table 4). The AUDPC values for the median and the lowest leaf, and the mean AUDPC in plots sprayed once were significantly less than those from unsprayed plots. In season 2, the highest AUDPC value occurred in unsprayed plots, but did not differ significantly from plots sprayed seven times, except for values from the lowest leaf.

Defoliation was significantly greater after growth stage R3 from plants in unsprayed plots compared to plants in plots sprayed three or 11

