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The effect of timing of fungicide applications on control of frogeye leaf spot and grain yield of soybeans

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Abstract

Soybean cultivar Samsoy 1, and the breeding lines TGx 849-313D and TGx 996-26E, grown in a field with a heavy epidemic of frogeye leaf spot caused by *Cercospora sojina*, were treated with double foliar applications of the fungicide benomyl. The treatments were made using four application schedules at six different growth stages, starting from V_3 (fully developed leaves, beginning with trifoliate nodes) to R_5 (beginning seed), to determine the effect of the fungicide timing on frogeye leaf spot severity, soybean grain yield and grain quality. Generally, applications at R_1 (beginning bloom) and R_3 (beginning pod) significantly ($P \leq 0.05$) reduced disease severity in the 2 susceptible genotypes,Samsoy 1 and TGx 849-313D. Plot yields of these genotypes were also significantly greater than the untreated controls when the fungicide applications were made at R_1 and R_3. There was no significant difference in disease severity or grain yield, between the untreated control and the different times of application, on the resistant genotype TGx 996-26E. Improved seed germination and lower levels of seed infection by *C. sojina* occurred for all fungicide timings in the susceptible genotypes. The results suggest that fungicide spraying initiated at R_1 and followed up at R_3 is most effective in frogeye leaf spot control and can also result in higher grain yields, than applications made earlier or later in the season. Control of frogeye leaf spot, however, is best achieved by growing resistant cultivars.

Introduction

Frogeye leaf spot caused by *Cercospora sojina*, Hara causes significant yield losses on soybean (*Glycine max* (L.) Merr.) in the humid and subhumid regions of tropical Africa [Dashiell and Akem, 1991; Yorinori, 1987]. The disease first appeared in Nigeria in 1981, in a breeding nursery in Southern Kaduna state [IITA, 1987]. Subsequently, it has caused sporadic, locally severe problems on the soybean varieties grown in the major soybean – producing regions of the country [Dashiell and Akem, 1991; Akem et al., 1992].

Control of frogeye leaf spot has been achieved mainly through the use of resistant cultivars [Akem and Dashiell, 1992; Yorinori, 1987]. However, because of high pathogenic variability of *C. sojina*, many varieties identified to be resistant in Nigeria soon become susceptible to new pathogen races. Presently 12 races of *C. sojina* have been identified in Nigeria [Akem et al., 1993]. Thus effective control of the disease cannot be attained by complete reliance on host plant resistance alone.

Benomyl has proven to be the most effective fungicide for the control of frogeye leaf spot in Nigeria and elsewhere [Dashiell and Akem, 1991; Horn et al., 1978]. Two applications seem to give good control, but the results are often not consistent from location to location and from year to year. Yield response using recommended rates of foliar fungicides on soybeans is highly dependent on the presence of foliar diseases and has ranged from a significant yield increase [Backman et al., 1979; Horn et al., 1979; Ross, 1975] to little increase [Jeffers et al., 1982]. Seed quality response to foliar fungicides has also been variable, although reductions in seed infection by seedborne pathogens have been reported after two applications of benomyl [Ellis et al., 1974]; Jeffers et al., 1982]. One or two applications of benomyl, when needed, would be more economical on a presently low unit-value crop like soybean. In addition, cost of multiple spray applications may not be consistently offset by proportionately higher yields, even though disease control may be improved [Leath and Bowen, 1989]. Thus, there is a need to determine the proper time for fungicide application so as to optimize control and maximize grain yield.

Horn et al. [1979] recommended applications after R_4 (full pod) in some temperate regions to achieve grater control of *C. sojina* and better yields. No information is presently available in Nigeria or tropical Africa on the timing or optimum number of benomyl sprays required to control frogeye leaf spot of soybeans. The purpose of this study was therefore, to (a) determine the effect of applying benomyl at different soybean growth stages on the severity of frogeye leaf spot and (b) determine the yield difference and seed quality associated with fungicide application timing. This information is needed to devise integrated frogeye disease control strategies on soybeans for tropical Africa.

Materials and methods

These investigations wee conducted on the Agricultural Development Project (ADP) research farm at Samaru Kataf near Zonkwa in southern Kaduna state of Nigeria, during the 1991 and 1992 soybean cropping seasons. Choice of this site was influenced by the fact that for 5 years of continuous cultivation, soybean in this area was almost exclusively infected with *C. sojina* at epidemic levels. This judgement was based on yearly disease screening trials conducted in this field for the 5 years of continuous cultivation.

For both years, three soybean genotypes selected on the basis of their agronomic characteristics and host reaction to frogeye leaf spot were used. These were; Samsoy 1, a late-maturing medium height cultivar which is extensively grown in parts of southern Kaduna state and highly susceptible to frogeye leaf spot; TGx 849-313D, a tall medium-maturing genotype, which is susceptible to frogeye leaf spot and TGx 996-26E, a tall early-maturing breeding line with good resistance to frogeye leaf spot.

A split-plot design was used in all experiments in both years, with genotypes as the main plots and fungicide treatments as subplots, in a randomized complete block design with three replications. Seeds were planted at a depth of 3–4 cm in a sandy loam soil at a rate of 30 seeds per meter in six rows, 6 m long with 0.75 m spacing. Seedings were thinned to 20 per meter two weeks after planting.

The following fungicide application schedules were evaluated during the two years of the study: (1) untreated control – no fungicide application; (2) V_3–V_5 [Fehr et al., 1971] = first fungicide application at V_3 (third expanded trifoliate leaf) and second at V_5 (fifth expanded trifoliate leaf); (3) V_5–V_7 = first application at V_5 and second at V_7 (seventh expanded trifoliate leaf); (4) R_1–R_3 = first application at R_1 (flower initiation) and second at R_3 (pod formation); (5) R_3–R_5 = first application at R_3 and second at R_5 (seed formation). Unsprayed guard rows were left between treated rows of each plot. Benomyl, formulated as 50 wp, was applied each time application was needed at 0.50 kg a.i/ha using a knapsack back-sprayer delivering about 200L/ha.

Frogeye leaf spot severity ratings were made at the R_6 (full seed) to R_7 (beginning maturity) growth stages based on the following subjective scale of 0–5; 0, no visible sign of infection; 1.0, one or more spots covering 1–10% of the leaf surface on one or a few plants; 2.0, some spots covering 10–30% of the leaf surface of several plants; 3.0, some spots covering 30–50% of the leaf surface on most or all plants; 4.0, many spots covering 50–80% of leaf surface of all plants; and 5.0, all plants severely infected with loss of 80–100% of the photosynthetic area. Ratings were made to the tenth unit for close approximation of the averages obtained.

The middle four rows of each plot were handharvested at plot maturing in October or November, depending on the genotype, and the grain adjusted to 12% moisture. Yield loss for each genotype and spray schedule was calculated by subtracting the yield of a given genotype nonspray from the yield of that genotype with each spray schedule and represented as a percentage. The percentage seed weight reduction was calculated similarly for each spray schedule using 300-seed weight. The effect of fungicide sprays on seed quality was determined for each genotype by estimating the percentage seed germination. Samples of 100 seeds per treatment, in four replications, were incubated on moist filter paper in petri plates at 27 ± 2 °C and germination counts taken after seven days.

Disease severity, plot yields and seed viability data were subjected to analysis of variance to determine main and split plot effects. Mean separations were performed on disease severity, yield and seed quality with Fisher's least significant difference tests to compare application schedules.

Results

Weather conditions during the cropping seasons of 1991 and 1992 were favorable for the development of *C. sojina*. In 1991, precipitation for July, August and September was 5, 3 and 7% above normal for these months, respectively, and the mean temperature for this time period was warmer than normal, i.e., 1.5 °C above a five year mean.

The overall severity of frogeye leaf spot differed significantly among the soybean genotypes ($P \leq 0.05$) in both years and fungicide treatments (Table 1). Genotype reaction to frogeye leaf spot played a very big role in the interactions as reflected by the large F value for this factor. There was also a significant ($P \leq 0.01$) genotype by fungicide treatment interaction for the two year period. Disease severities were generally higher in 1991 than in 1992, as evidenced by the disease severity values for Samsoy 1 and TGx 996-26E during both years (Table 2). The mean disease severity values were smallest when fungicide was applied in growth stage R1–R3 (Table 2).

Overall, yield of all three genotypes was lower in 1992 than in 1991 (Table 2). In both years the yield increased significantly for the two susceptible genotypes when fungicide was applied at or after growth stage R1 while the yield of the resistant genotype TGx 996-26E was not significantly increased at any growth stage.

Fungicide treatments improved the recorded seed germination rate only for some genotypes and spray schedules in 1992 (Table 3). The seed weight was only significantly increased for Samsoy 1.

Discussion

Benomyl was highly effective in controlling frogeye leaf spot in these experiments. Usually within 7–10 days after treatment, visible differ-

Table 1. Significance of F value from Analysis of Variance for frogeye leaf spot based on disease severity of 3 soybean genotypes and time of fungicide application in 1991 and 1992

Source	d.f	Sum of squares	Mean square	F value
Fungicide Timing (FT)	4	17.3537	4.3384	77.10**
Genotype (Gen)	2	45.1502	22.5751	401.19**
Replication	2	0.1318	0.0659	1.17
Year (Yr)	1	1.1924	1.1924	21.19**
FT X Gen	8	12.3848	1.5481	27.51**
Gen X Yr	2	1.9734	0.9867	17.53**
FT X Gen X Yr	12	1.5080	0.1257	2.23*
Error	55	3.0949	0.0563	
TOTAL	86	82.7892		
CV (%)			13.63	

* Significant at 0.05%
** Significant at 0.01%

Table 2. Effect of timing of double applications of fungicide on severity of frogeye leaf spot and grain yield of soybean genotypes in 1991 and 1992

Fungicide application timing[a]	Samsoy 1		TGx 849-313D		TGx 996-26E	
	DS at R_6–R_7[b]	Yield[c]	DS	Yield	DS	Yield
1991						
V_3–V_5	3.1	1752	2.5	2078	1.2	2296
V_5–V_7	2.7	1884	1.9	2010	1.1	2311
R_1–R_3	1.2	2237	1.1	2327	1.0	2520
R_3–R_5	2.2	2169	1.2	2115	1.0	2479
No treatment	3.9	1504	2.6	1933	1.2	2263
LSD (P = 0.05)	0.6	436	0.3	379	0.1	NS
1992						
V_3–V_5	2.8	1600	1.7	1381	0.3	1449
V_5–V_7	2.7	1362	1.5	1253	0.5	1558
R_1–R_3	1.2	1756	1.0	1774	0.2	1962
R_3–R_5	2.5	1764	1.5	1218	0.5	1839
No treatment	3.6	1212	2.3	1040	0.5	1439
LSD (P=0.05)	0.5	479	0.6	554	NS	NS

[a] Fungicide applied at different growth stages based on Fehr et al., 1971 scale.
[b] Disease severity at R_6–R_7 (full seed – beginning maturity) based on a 0–5 scale, where 0 = no disease and S = severe disease (Dashiell and Akem, 1991).
[c] Yield based on grain weight at 12% moisture.

Table 3. Effect of timing of double applications of fungicide on seed weight and germination of soybean genotypes, 1992

Fungicide application timing[a]	Samsoy 1		TGx 849-313D		TGx 996-26E	
	300 seed (g)	Germination (%)	300 seed (g)	Germination (%)	300 seed (g)	Germination (%)
V_3–V_5	41.7	92.0	40.7	100	46.3	100
V_5–V_7	39.3	86.7	40.3	98.7	47.7	97.3
R_1–R_3	41.9	96.0	40.3	100	46.0	98.0
R_3–R_5	43.7	98.7	39.0	100	46.3	97.3
Control	35.7	96.0	38.0	96.0	45.7	96.0
$LSD_{0.05}$	6.1	3.2	NS	1.6	NS	1.0

[a] Fungicide applied at different growth stages based on Fehr et al., 1971 scale.

ences in lesion color and density could be detected, resulting in lower assessment scores for fungicide – treated plots.

Although disease severity scores were relatively low in nontreated plots by R_3 (podding), significant yield increases ($P \le 0.05$) were detected over the nontreated controls as a result of fungicide application. Yield response to fungicide application was generally greater in 1992 than in 1991, although disease severity values were generally higher in 1991 than in 1992 (Table 2). The lower yield response in 1991 was probably due to the occurrence of a dry spell that was experienced for about two weeks during the grain-filling period

(R_4) in 1992, indicating that low soil moisture became a limiting factor to yield more than or in addition to frogeye leaf spot.

Results of this study indicate that fungicide applications for frogeye leaf spot control on soybeans susceptible to *C. sojina* should be made during the reproductive (R) growth stages of the crop just as infection intensifies. Fungicide applications at R_1 and R_3 provided the greatest disease control on the three genotypes tested, and generally, the highest yield. Early applications attempting to control the disease before the R_1 growth stage under tropical conditions, could result in nonsignificant yield increases. These results agree with previous studies by Horn et al. [1979], on the significance of late-season development of diseases to yield losses in soybean.

In most situations, two fungicide applications appear economical in Nigeria [Dashiell and Akem, 1991]. On an economic basis, the difference in yield when fungicides are first applied at V_5 or at R_1, is significantly larger than when started earlier; (Samsoy 1 = 353 kg/ha and TGx 849-313D = 317 kg/ha in 1991). Late fungicide applications would be beneficial in areas where frogeye leaf spot was the most prevalent disease. In areas where yield losses from early season diseases are expected to be high, fungicide applications would probably be more beneficial if started before flowering. This would be even more appropriate if the same fungicide was effective in controlling both the early and late season disease or diseases.

The significant increase in seed weight in fungicide-treated plots, especially on the very susceptible cultivar Samsoy 1, suggests that reduced pod fill contributes to yield loss in infected plants. This confirm findings of earlier studies [Dashiell and Akem, 1991]. Even though there was some significant effect on seed germination, seed quality was not greatly influenced by fungicide applications. This was confirmed by the low levels of seed infection by *C. sojina* (less than 2%) in each infected control sample when seeds were plated to determine the seedborne incidence of *C. sojina*.

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References

Akem CN and Dashiell KE (1992) Evaluation of soybean cultivars for field resistance to frogeye leaf spot and bacterial pustule in Nigeria. Trop Oil J 1: 3–8

Akem CN, Uwala AC and Dashiell KE (1992) Prevalence of frogeye leaf spot of soybeans in Nigeria. Int'l J of Trop Disease Vol 10: 71–73

Akem CN, Yorinori JT and Dashiell KE (1993) Physiological races of *Cercospora sojina* in soybeans in Nigeria. Trop Oil J 2: 48–56

Backman PA, Rodriguez-Kabana RM, Hammon JM and Thurlow DL (1979) Cultivar, environment and fungicide effects on foliar disease losses in soybeans. Phytopathology 69: 562–564

Dashiell KE and Akem CN (1991) Yield losses in soybeans from frogeye leaf spot caused by *Cercospora sojina*. Crop Prot 10: 465–468

Ellis MA, Ilyas MB, Tenne FD, Sinclair JB and Palm HL (1974) Effect of foliar applications of benomyl on internally seedborne fungi and pod and stem blight in soybeans. Plant Dis Rep 58: 760–763

Fehr WR, Caviness CE, Burmood DT and Pennington JS (1971) Stage of development descriptions for soybeans, *Glycine max* (L) Merrill Crop Sci 11: 929–931

Horn NL, Lee FN and Carver RB (1978) Effects of fungicides and pathogens on yields of soybeans. Plant Dis Rep 59: 724–728

Horn NL, Carver RB, Lee FN and Fort TM (1979) The effect of timing of fungicide applications on the yield of soybeans. Plant Dis Rep 63: 404–406

IITA (1987) Annual Report and Research Highlights 1986. International Institute of Tropical Agriculture, Ibadan, Nigeria, 154 pp

Jeffers DL, Schmitthenner AF and Reichard DL (1982) Seedborne fungi, quality and yield of soybeans treated with benomyl fungicide by various application methods. Agron J 74: 589–593

Leath S and Bowen RL (1989) Effects of powdery mildew, triadimenol seed treatment, and triadimefon foliar sprays on yield of winter wheat in North Carolina. Phytopath 79: 152–155

Ross JP (1975) Effect of overhead irrigation and benomyl sprays on late-season foliar diseases, seed infection and yields of soybeans. Plant Dis Rep 59: 809–813

Yorinori JT (1987) Frogeye leaf spot of soybeans (*Cercospora sojina* Hara). In: World Soybean Production and Utilization Conference IV: pp. 1275–1283. R. Shibbles ed West view Press Inc., Boulder, Colorado, USA