A Field Evaluation of Eight Fungicides in the Control of Soybean Rust in Zimbabwe during 2002-03.

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SUMMARY

The "Australasian-type" of soybean rust, *Phakopsora pachyrhizi,* has been known to devastate soybeans since the beginning of last century, however, until fairly recently it has been restricted to South East Asia, India and Australia. In the late 1990s, it entered Africa and thereafter moved down the continent to severely reduce commercial production, until good fungicidal control limited yield loss. In 2001, *P. pachyrhizi* was discovered in South America where it has since caused acute yield loss. This presence alerted the United States Department of Agriculture to develop a coordinated national action plan to counter soybean rust prior to its arrival in the United States. Under this plan, fungicide studies are to be done in countries where soybean rust is prevalent by international collaborators, so that suitable fungicides may be registered for control of the pathogen once it arrives.

This report details an experiment involving an evaluation of eight fungicides, either sprayed two or three times on a soybean cv. planted at three sites in Zimbabwe during the 2002-03 summer. This season was unfortunately characterized by below-normal rainfall. Rust symptoms were only appeared at two sites on unsprayed plants after early pod-fill (a late infection), and on plants sprayed twice with mancozeb, propiconazole, BAS500, BAS516 and chlorothalonil at the Gwebi site. The disease did not cause net yield loss at these sites, but did cause significant kernel mass reductions. Spraying was shown to effectively improve the kernel mass, with BAS500, BAS516, tetraconazole, and chlorothalonil, the most consistently effective. Despite these results, no firm conclusion can be drawn about the relative fungicide efficacies - the late arrival of *P. pachyrhizi*, the low resulting rust severity and the lack of correlation between the yield components negating a determination. The experiment needs to be repeated under higher disease pressure in wetter conditions, as previously experienced at these sites.

INTRODUCTION

Phakopsora pachyrhizi Sydow. & Sydow , the cause of the "Australasian-type" of soybean rust, has been known to devastate small-scale soybean production in South East Asia, India and Australia since just after the turn of last century. In 1994, it was found on soybeans and several legumes growing in Hawaii. With an ability to infect many natural legumes, to acutely disrupt chlorophyll biosynthesis and defoliate plants, and thereby, severely reduce yields, it poses a major threat to any soybean production. It was first confirmed on soybeans in central Africa in 1997, and thereafter rapidly moved southwards to large-scale commercial plantings in Southern Africa, where it initially devastated many fields. Subsequently, extensive and timely fungicide control has limited its impact.

Soybean rust was reported for the first time in the Western Hemisphere from eastern Paraguay in March 2001 (Morel, pers.comm.), and shortly thereafter, in Paraná State, Brazil (Yorinori, pers.comm.). Severe, localized yield losses were recorded in both countries upon harvest. The presence of *P. pachyrhizi* in South America caused the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA, APHIS) to formulate a national strategic action plan in advance of the arrival of *P. pachyrhizi* to continental United States. Soybean scientists meeting at the USDA, APHIS headquarters in July 2002 believed that the entry of this pathogen was imminent and that it posed a significant threat to the soybean industry.

P. pachyrhizi can be effectively controlled by timely chemical applications. Temporary registration of azoxystrobin (Quadris® 2.08SC; Syngenta) was granted under the action plan, so that United States soybean farmers could control this disease in the short term. Further data of product efficacy, however, was required to obtain full registration of this and other fungicides. This report details a field experiment whereby eight chemicals were evaluated in the 2002-03 summer at three sites in Zimbabwe, Africa, as part of an international collaboration.

MATERIALS AND METHODS

i) Soybean Rust Severity Assessment

The method established by the International Working Group on Soybean Rust was followed to assess soybean rust infection and severity (Shanmugasundaram, 1977). The rating was modified from the three-digit to a two-digit notation, hence

First digit - denotes the leaf position of the plant infected (vertical displacement)

- 1: bottom third of leaves measured from ground level
- 2: middle third of leaves measured from ground level
- 3: upper third of leaves measured from ground level

Second digit - denotes the density of rust lesions on the examined leaves (severity)

1: no lesions
2: light lesion density
3: medium lesion density
4: heavy lesion density

ii) Field Experimentation

The efficacies of eight chemicals in the control of *P. pachyrhizi* were investigated at the Rattray Arnold Research Station (RARS), Enterprise; Gwebi Variety Testing Center (Gwebi VTC); and the Banket Research Station (Banket RS), Banket, Zimbabwe during the 2002/03 summer. The clay soil was ploughed, rolled and kongskilde-harrowed at RARS to provide an even tilth, whereas the loam soil was ploughed and/or disced at the other two sites. Prior to planting, compounded fertilizer at 30 kg NO_3^{2-}, 90 kg $P_2O_5^-$ and 40 kg K_2O^- ha^{-1} (500 kg '6-18-15' ha^{-1}) (RARS); at 15 kg NO_3^{2-}, 54 kg $P_2O_5^-$ and 30 kg K_2O^- ha^{-1} (300 kg '5-18-10' ha^{-1}) (Gwebi VTC); and at 18 kg NO_3^{2-}, 45 kg $P_2O_5^-$ and 36 kg K_2O^- ha^{-1} (300 kg '6-15-12' ha^{-1}) (Banket RS) was incorporated as per the requirements of soil analysis.

Seeds of the soybean cv., 'Soprano' (indeterminate growth habit) were mixed with a slurry of a commercialized *Bradyrhizobium japonicum* isolate ('Grasslands 1491'), sown into furrows at approx. 50-mm intervals, and then lightly covered with soil on November 25, 2002 (Gwebi VTC), December 11, 2002 (Banket RS) and December 12, 2002 (RARS). All plots consisted of four rows, each 6.0 m long (RARS and Gwebi VTC), or 5.5 m (Banket RS), and 0.9 m apart, and were laid out in a randomized complete blocks design, replicated four times.

The following chemicals were tested:

(i) 50 g flusilazol + 100 g carbendazim ha^{-1} (400 m*l*/ha Punch® Xtra, DuPont de Nemours);
(ii) 1.6 kg mancozeb ha^{-1} (2.0 kg Dithane® M-45 ha^{-1} ; Rohm & Haas);
(iii) 189 g propiconazole ha^{-1} (756 m*l* Tilt® 250EC ha^{-1}; Syngenta);
(iv) 157.5 g azoxystrobin ha^{-1} (630 m*l* Quadris® 2.08SC ha^{-1}; Syngenta);
(v) 168 g BAS 500 ha^{-1} (600 m*l* Headline® EC ha^{-1}; BASF);
(vi) 168 g BAS 516 UD F ha^{-1} (442 g of unnamed 38% WG formulation ha^{-1}; BASF);
(vii) 100 g tetraconazole ha^{-1} (800 m*l* Eminent® 12SL ha^{-1}; Sipcam Agro);
(viii) 1.44 kg chlorothalonil ha^{-1} (2.0 *l* Echo® 720 ha^{-1}; Sipcam Agro), and

compared with plants within unsprayed plots, which provided controls.

Each fungicide was mixed separately with 400 *l* ha^{-1} water and sprayed onto selected plots at either 50 and 70 days after planting (dap) (2 sprays), or at 50, 70 and 90 dap (3 sprays) using a hand-operated Jacto® PJ16 knapsack sprayer fitted with a 3-bar pressure regulator and a Lurmark® F110/1.6/3 flood-jet attachment.

Plants in became naturally-infected with *P. pachyrhizi* during early March 2003. The vertical distribution of the disease, the severity and the physiological age of the plants (Richie, Hanway and Thompson, 1985) were assessed as above at 4-day intervals from 90 to 110 dap.

After physiological maturity, plants within a 5.0 m x 0.9 m area in each plot were hand-harvested, bagged in hessian sacks, sun-dried and threshed. The moisture contents of the harvested seed were measured separately using a Burrows Digital Moisture Computer 700. The yields of the harvested kernels (yield; YLD) and 100-seed samples (100-seed mass; HSM) from each plot were then weighed and the resulting values adjusted to reflect a 11% seed moisture content.

iii) Statistical Analysis

The areas under the disease progress curves (AUDPCs) of the vertical distribution and severity for each treatment were calculated. Analyses of variance for a randomized complete blocks design (Little and Hills, 1978) were then applied to the various disease assessments, the AUDPCs, and the YLDs and HSMs recorded. Significant differences within the variances of the treatments were determined by pertinent F-tests, and the L.S.D.s between the means calculated. Correlations between the YLDs, HSMs and the AUDPCs were then established to determine the relative disease impact.

RESULTS

The summer season in Zimbabwe was characterized by poor rainfall during November and December 2002, erratic showers fell during January and early February 2003, and then more 'normal' amounts were received up to April 2003. Generally, meteorological stations recorded seasonal amounts 30 – 50% below the expected long-term (> 100

year) means. Little rain fell at the Banket RS site throughout the season. Sprinkler irrigation supplemented the plant water requirements at the RARS and Gwebi VTC sites, but with no irrigation capacity at Banket RS, the plants suffered moisture stress throughout their growth, they became stunted, and the yields harvested were well below normally expected in this area.

Rust was first recorded on the lower trifoliate leaves of the unsprayed plants at RARS and Gwebi VTC at 106 dap, when the plants were in their R6 growth stage. Infection had moved up to the middle canopy leaves by 110 dap, on which only a mild severity developed. No rust symptoms were noticed on sprayed plants at RARS, but a low severity did develop in the lower canopy leaves sprayed with mancozeb and BAS516 at Gwebi VTC. Consequently, the AUDPCs for the vertical displacement (Table 1) and rust severity (Table 2) of unsprayed plants were significantly greater ($\boldsymbol{P} \leq 0.001$) than those of sprayed plants.

Similar results were obtained at these sites in respect to harvested grain. Spraying did not significantly improve the YLD at either site (Figs 1 and 2), - and the harvest could not be correlated with the HSMs, or the disease severities recorded (Appendices 1 and 2). However, spraying significantly increased the HSM ($\boldsymbol{P} \leq 0.001$), but an additional spraying did not generally improve the kernel mass (Figs 4 and 5). At RARS, BAS500, BAS516, tetraconazole, chlorothalonil and azoxystobin (three sprays) produced the heaviest kernels (Fig. 4), as did, flusilazol + carbendazim, BAS500, BAS516, tetraconazole, chlorothalonil, azoxystrobin (two times) and mancozeb (three times) at Gwebi VTC (Fig. 5). Propiconazole only marginally improved the HSM above that produced by the unsprayed plants. The HSMs were negatively correlated with disease severities recorded (Appendices 3 and 4), which indicated a very close cause-and-effect relationship between these factors.

No rust infection was noticed on plants up to the R7 growth stage (110 dap) at the Banket RS site and no benefit in YLD or HSM resulted from spraying, or with any specific fungicide (Figs 3 and 6).

DISCUSSION

The 2002-3 summer in Zimbabwe was characterized by a below-normal rainfall, with an erratic distribution. Soybean rust was not a constraint to commercial production, and early, widespread spraying further reduced the potential spore load in the atmosphere. At two of sites used, symptoms were recorded only on unsprayed plants during pod-fill, and the infection progressed only to the middle canopy leaves by normal leaf-drop. In wetter weather, initial rust symptoms would have been expected during early pod development, and to have developed rapidly to affect the upper canopy leaves. In this experiment, spraying generally prevented symptom development in the lower leaves, but due to the low severity, net yield remained unaffected, and thus, no broad conclusions could be drawn about fungicide efficacy, or the relative benefits of the specific chemicals applied.

Kernel mass was reduced with disease progress - showing that even under these conditions, *P. pachyrhizi* had a substantial effect on the metabolism of plants, late in their development. However, the very low correlation between net yield and kernel mass indicated that sub-optimal plant growth had occurred at all three sites during the season. Thus, the reader is cautioned from concluding that the increases in kernel mass, which followed spraying with certain chemicals, were indicative of effective disease control.

This experiment needs to be repeated under conditions of higher rust severity, in wetter seasons. The Banket site lacked irrigation capacity, and thus, if repeated, another site would be sought where supplementary irrigation could be applied.

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Table 1. The vertical displacement of soybean rust between 90 and 110 days after planting (Shanmugasundaram, 1977; expressed as the area under the disease progress curve (AUDPC)) on soybean cv., 'Soprano', either sprayed two or three times with one of eight fungicides, or left unsprayed, at the Rattray Arnold Research Station, Enterprise and the Gwebi Variety Testing Center, Mount Hampden, Zimbabwe during the 2002-03 season.

Treatment	Site	Rattray Arnold Research Station			Gwebi Variety Testing Center		
	No. of sprays	Two	Three	Mean	2 sprays	3 sprays	Mean
unsprayed		14.0	12.0	13.0	10.5	12.5	11.5
flusilazol + carbendazim		6.0	6.0	6.0	6.0	6.0	6.0
mancozeb		6.0	6.0	6.0	11.5	6.0	8.8
propiconazole		6.0	6.0	6.0	7.5	6.0	6.8
azoxystrobin		6.0	6.0	6.0	6.0	6.0	6.0
BAS 500		6.0	6.0	6.0	7.5	6.0	6.8
BAS 516		6.0	6.0	6.0	11.0	6.0	8.5
tetraconazole		6.0	6.0	6.0	6.0	6.0	6.0
chlorothalonil		6.0	6.0	6.0	8.0	6.0	7.0
Mean		6.9	6.7	6.8	8.2	6.7	7.5
S.E. $_{(treatment)}$ (±)			0.27			1.00	
L.S.D. (1%)			1.0			3.8	
C.V. (%)			8.0			26.8	

Table 2. The severity of soybean rust between 90 and 110 days after planting (Shanmugasundaram, 1977; expressed as the area under the disease progress curve (AUDPC)) on soybean cv., 'Soprano', either sprayed two or three times with one of eight fungicides, or left unsprayed, at the Rattray Arnold Research Station, Enterprise and the Gwebi Variety Testing Center, Mount Hampden, Zimbabwe during the 2002-03 season.

Treatment	Site	Rattray Arnold Research Station			Gwebi Variety Testing Center		
	No. of sprays	Two	Three	Mean	2 sprays	3 sprays	Mean
unsprayed		12.0	10.0	11.0	10.5	12.5	11.5
flusilazol + carbendazim		6.0	6.0	6.0	6.0	6.0	6.0
mancozeb		6.0	6.0	6.0	10.5	6.0	6.0
propiconazole		6.0	6.0	6.0	7.5	6.0	8.2
azoxystrobin		6.0	6.0	6.0	6.0	6.0	6.0
BAS 500		6.0	6.0	6.0	7.5	6.0	6.8
BAS 516		6.0	6.0	6.0	10.5	6.0	8.2
tetraconazole		6.0	6.0	6.0	6.0	6.0	6.0
chlorothalonil		6.0	6.0	6.0	7.5	6.0	6.7
Mean		6.7	6.4	6.6	8.0	6.7	7.4
S.E. $_{(treatment)}$ (±)			0.27			0.89	
L.S.D. (1%)			1.0			3.4	
C.V. (%)			8.3			24.1	

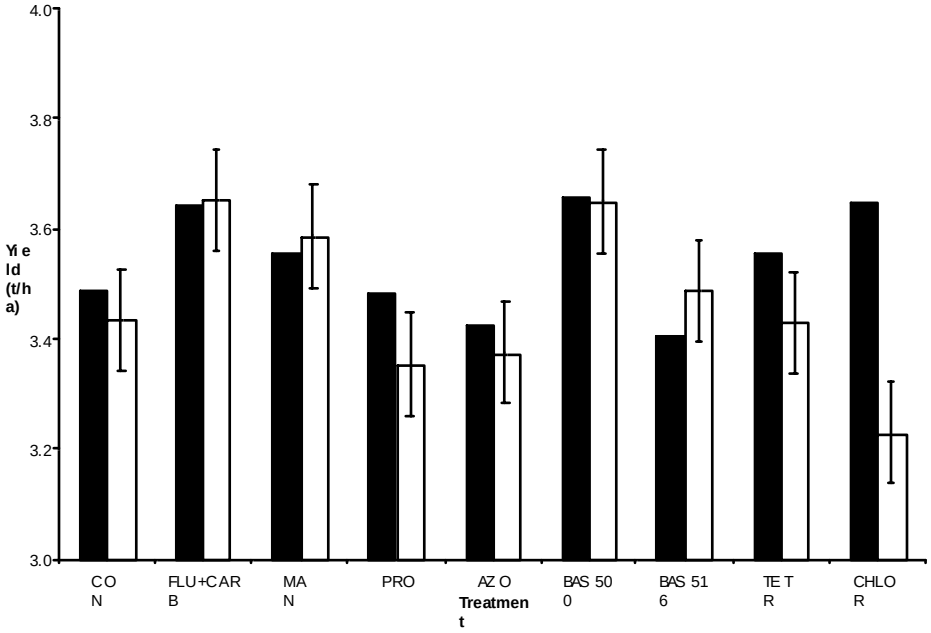

Fig. 1 Yield (t ha^{-1} at 11% moisture content) of soybean cv., 'Soprano' either sprayed two (black) or three times (white) with one of eight chemicals, or left unsprayed at the Rattray Arnold Research Station, Enterprise, Zimbabwe in the 2002-03 season (I = S.E. $_{(treatment)}$; CON = unsprayed; FLU+CARB = flusilazol + carbendazim; MAN = mancozeb; PRO = propiconazole; AZO = azoxystobulin; TETR = tetraconazole; CHLOR = chlorothalonil)

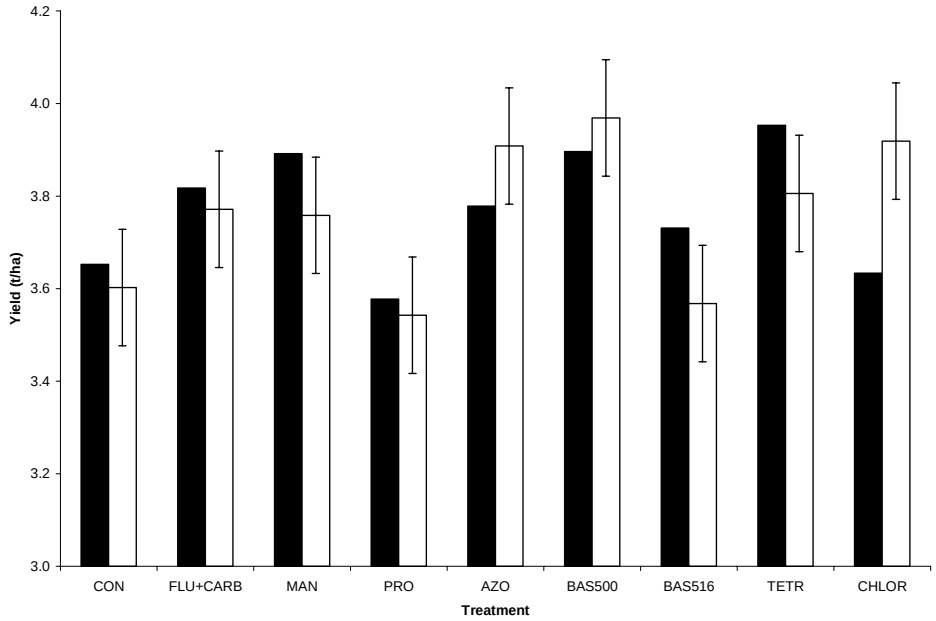

Fig. 2 Yield (t ha^{-1} at 11% moisture content) of soybean cv., 'Soprano' either sprayed two (black) or three times (white) with one of eight chemicals, or left unsprayed at the Gwebi Variety Testing Center, Mount Hampden, Zimbabwe in the 2002-03 season (I = S.E. $_{(treatment)}$; CON = unsprayed; FLU+CARB = flusilazol + carbendazim; MAN = mancozeb; PRO = propiconazole; AZO = azoxystobulin; TETR = tetraconazole; CHLOR = chlorothalonil)

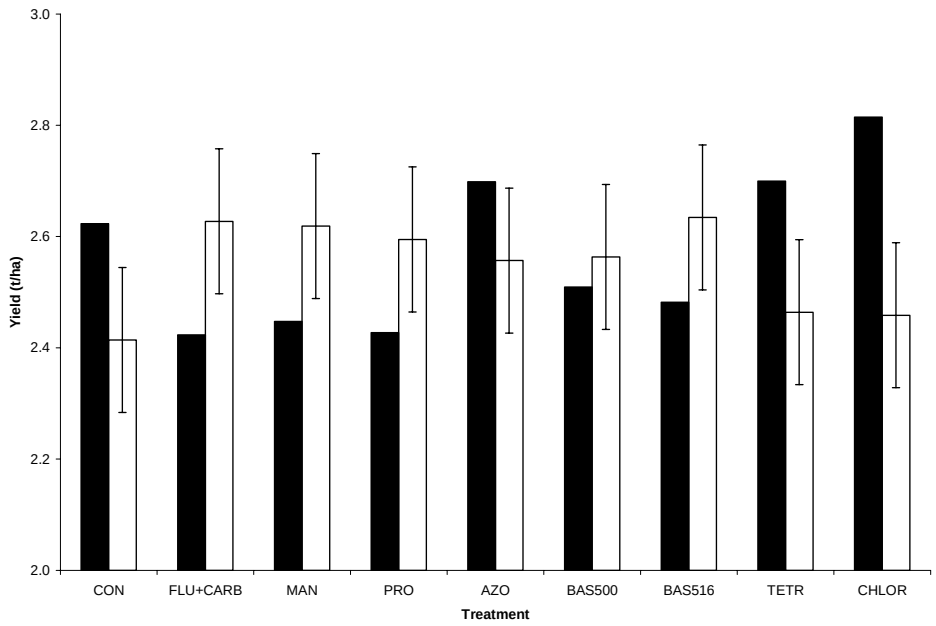

Fig. 3 Yield (t ha^{-1} at 11% moisture content) of soybean cv., 'Soprano' either sprayed two (black) or three times (white) with one of eight chemicals, or left unsprayed at the Banket Research Station, Banket, Zimbabwe in the 2002-03 season (I = S.E. $_{(treatment)}$; CON = unsprayed; FLU+CARB = flusilazol + carbendazim; MAN = mancozeb; PRO = propiconazole; AZO = azoxystobulin; TETR = tetraconazole; CHLOR = chlorothalonil)

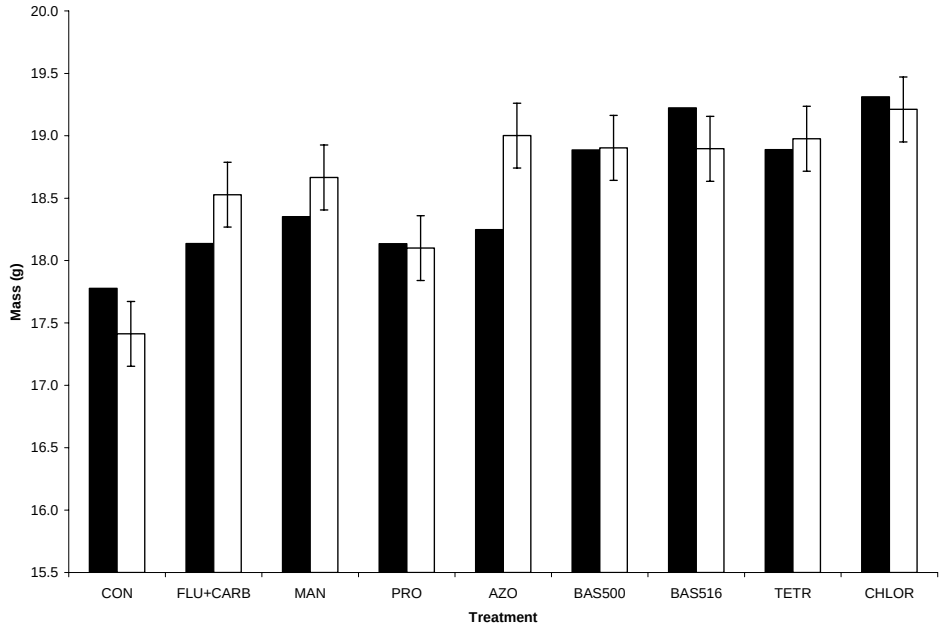

Fig. 4 Kernel mass (g, at 11% moisture content) of soybean cv., 'Soprano' either sprayed two (black) or three times (white) with one of eight chemicals, or left unsprayed at the Rattray Arnold Research Station, Enterprise, Zimbabwe in the 2002-03 season (I = S.E. $_{(treatment)}$; CON = unsprayed; FLU+CARB = flusilazol + carbendazim; MAN = mancozeb; PRO = propiconazole; AZO = azoxystobulin; TETR = tetraconazole; CHLOR = chlorothalonil)

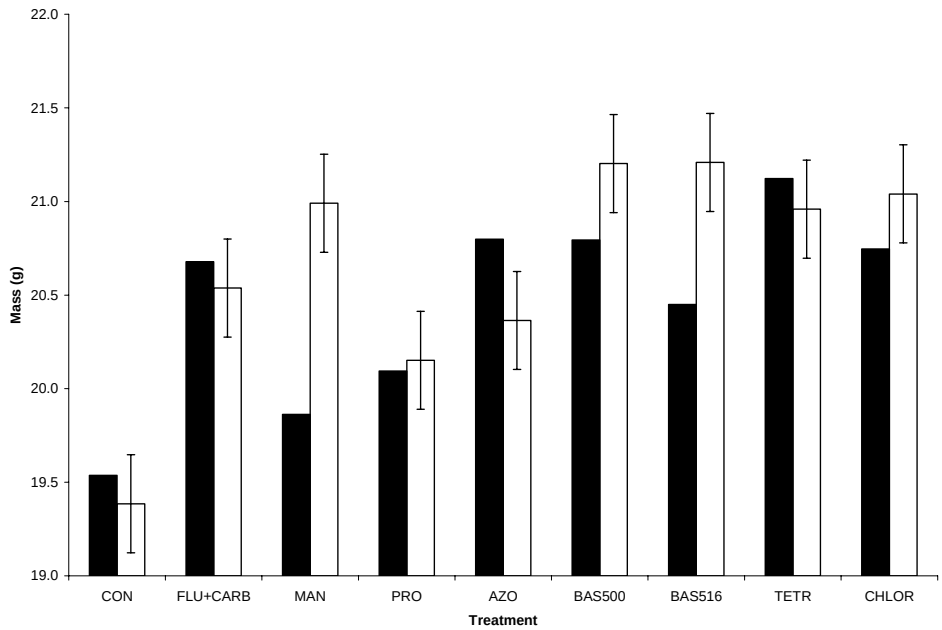

Fig. 5 Kernel mass (g, at 11% moisture content) of soybean cv., 'Soprano' either sprayed two (black) or three times (white) with one of eight chemicals, or left unsprayed at the Gwebi Variety Testing Center, Mount Hampden, Zimbabwe in the 2002-03 season (I = S.E. $_{(treatment)}$; CON = unsprayed; FLU+CARB = flusilazol + carbendazim; MAN = mancozeb; PRO = propiconazole; AZO = azoxystobulin; TETR = tetraconazole; CHLOR = chlorothalonil)

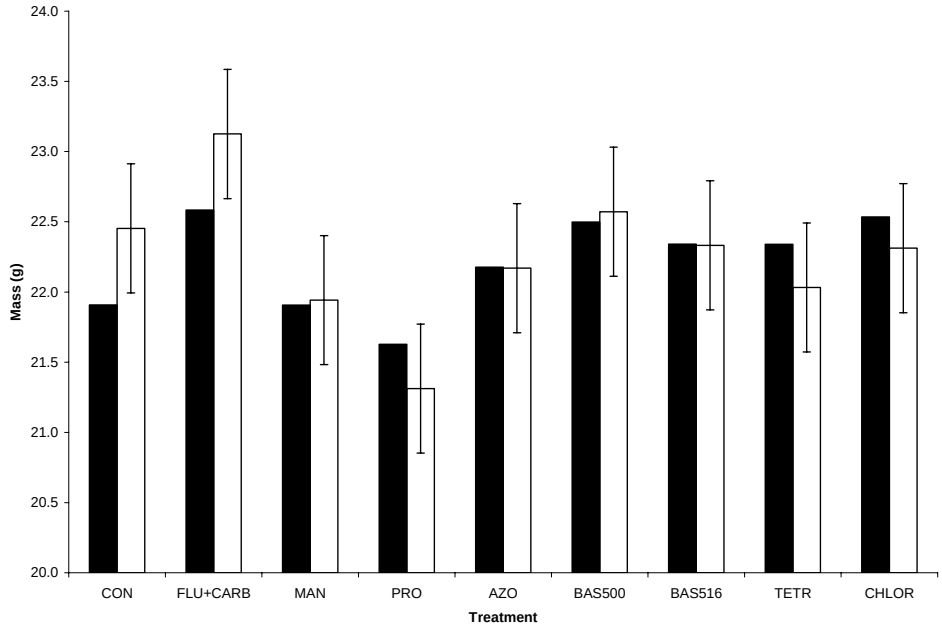

Fig. 6 Kernel mass (g, at 11% moisture content) of soybean cv., 'Soprano' either sprayed two (black) or three times (white) with one of eight chemicals, or left unsprayed at the Banket Research Station, Banket, Zimbabwe in the 2002-03 season (I = S.E. $_{(treatment)}$;CON = unsprayed; FLU+CARB = flusilazol + carbendazim; MAN = mancozeb; PRO = propiconazole; AZO = azoxystobulin; TETR = tetraconazole; CHLOR = chlorothalonil)

Appendix 1. Coefficients of correlation (r) between kernel yield (Yield), the 100-seed mass (HSM), the severity of soybean rust at 106 and 110 days after planting (dap), and the area under the disease progress curve (AUDPC), for soybean cv. 'Soprano', either sprayed two or three times with one of eight fungicides or left unsprayed at the Rattray Arnold Research Station, Enterprise, Zimbabwe in 2002-03 (d.f.=34).

Number of sprays		Yield	100-seed mass
2 times	HSM	$0.2061^{NS(1)}$	
	106 dap	-0.0877^{NS}	$-0.4043^{*(2)}$
	110 dap	-0.0877^{NS}	-0.4043^{*}
	AUDPC	-0.0877^{NS}	-0.4043^{*}
3 times	HSM	0.0115^{NS}	
	106 dap	-0.0888^{NS}	-0.4258^{**}
	110 dap	-0.0530^{NS}	-0.6227^{***}
	AUDPC	-0.0806^{NS}	-0.5493^{***}

(1): ^{NS} = correlation not significantly greater than zero.
(2): *, **, *** = correlation significantly greater than zero at $\boldsymbol{P}\leq 0.05$, $\boldsymbol{P}\leq 0.01$ and $\boldsymbol{P}\leq 0.001$, respectively.

Appendix 2. Coefficients of correlation (r) between kernel yield (Yield), the 100-seed mass (HSM), the severity of soybean rust at 106 and 110 days after planting (dap), and the area under the disease progress curve (AUDPC), for soybean cv. 'Soprano', either sprayed two or three times with one of eight fungicides or left unsprayed at the Gwebi Variety Testing Center, Mount Hampden, Zimbabwe in 2002-03 (d.f.=34).

Number of sprays		Yield	100-seed mass
2 times	HSM	$0.0428^{NS(1)}$	
	106 dap	-0.0737^{NS}	$-0.5595^{***(2)}$
	110 dap	-0.0737^{NS}	-0.5595^{***}
	AUDPC	-0.0737^{NS}	-0.5595^{***}
3 times	HSM	0.2604^{NS}	
	106 dap	-0.2290^{NS}	-0.5960^{***}
	110 dap	-0.1516^{NS}	-0.6074^{***}
	AUDPC	-0.2010^{NS}	-0.6096^{***}

(1): ^{NS} = correlation not significantly greater than zero.
(2): *** = correlation significantly greater than zero at $\boldsymbol{P}\leq 0.001$.

Appendix 3. Coefficients of correlation (r) between kernel yield (Yield) and the 100-seed mass (HSM) for soybean cv. 'Soprano', either sprayed two or three times with one of eight fungicides or left unsprayed at the Banket Research Station, Banket, Zimbabwe in 2002-03 (d.f.=34).

	Yield	
	2 sprays	3 sprays
HSM	$0.0438^{NS(1)}$	0.1403^{NS}

(1): ^{NS} = correlation not significantly greater than zero.