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Spectral responses at visible and near-infrared wavelengths of soybean plants to fungicides

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ABSTRACT: *The study evaluated the efficacy and soybean spectral responses to fifteen foliar fungicide mixtures labeled to control Asian soybean rust. Canopy level reflectance was measured using a multispectral camera onboard a multirotor drone before and two hours after each spray. The third application of fungicides improved control of soybean rust and increased yield. Nevertheless, up to three consecutive foliar fungicides applications did not affect the reflectance of soybean plants at visible and infrared wavelengths. Thus, drones can be a viable strategy for data acquisition regardless of the application of the fungicides.*
Key words: *chemical control, remote sensing, digital agriculture, smart farming, RPA.*

Respostas espectrais em comprimentos de onda visível e infravermelho-próximo de plantas de soja a fungicidas

RESUMO: *Esse estudo avaliou a eficácia e as respostas espectrais de plantas de soja a quinze misturas de fungicidas utilizados no controle da ferrugem asiática da soja (FAS). A refletância do nível do dossel foi medida usando uma câmera multiespectral a bordo de um drone multirotor antes e duas horas após cada pulverização. A terceira aplicação de fungicidas melhorou o controle de FAS e aumentou a produtividade. Porém, três aplicações foliares consecutivas de fungicidas não afetaram a refletância de plantas de soja nos comprimentos de onda visível e infravermelho. Assim, drones podem ser uma estratégia viável para aquisição de dados independentemente da aplicação de fungicidas.*
Palavras-chave: *controle químico, sensoriamento remoto, agricultura digital, produção sustentável, VANT.*

Phakopsora pachyrhizi is the causal agent of the Asian soybean rust (ASR), a major disease threat to soybean. It can attack any plant organ across the crop season. Timing applications of fungicides are important to prevent yield losses. And, the use of fungicides of different mechanisms of action can prevent or delay fungal resistance to biocidal chemical compounds (TWIZEYIMANA & HARTMAN, 2017).

Drones hold the potential for disease monitoring and decision-making for the use of fungicides in the correct timing (BAJWA et al., 2017). Because insecticides may affect soybean spectral responses (ALVES et al., 2017), it is necessary to understand the physiological and fungicide residual effects on plants. Therefore, for drones to be useful in disease management, scouting using drones may depend on obtaining aerial images that are not confounded by multiple fungicide applications. This study determined soybean spectral responses and the efficacy of successive applications of foliar fungicides to ASR.

Soybean seeds (cultivar Monsoy 7739) were sown on Dec 20, 2018, over corn straws under a no-tillage system conducted in Rio Verde, state of Goiás, Brazil. Fertilization used 500 kg ha^{-1} of NPK (0-20-20) at planting. Plots consisted of four planting rows spaced by 0.5 m between rows and 6 m in length. Sixty-eight plots were arranged in a randomized complete block design with 17 treatments and four replications per treatment.

The treatments were established by different fungicides applied sequentially in three strategic moments to prevent crop losses from ASR: 1 - plants started flowering at Fev 2, 2019 (R1 growth stage), 2 - beginning of pod formation at Feb 18, 2019 (R3), and 3 - pods were fully developed at Mar 3, 2019 (R4).

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The fungicides used in the study (Table 1) belong to the groups of demethylation inhibitors (tebuconazole, cyproconazole, prothioconazole, and epoxiconazole); quinone oxidase inhibitors (azoxystrobin, trifloxystrobin, picoxystrobin, and pyraclostrobin), and succinate dehydrogenase inhibitors (fluxpyroxade, bixafen, and benzovindiflupir). Plants were treated using a sprayer pressurized with CO_2 calibrated to 150 L ha^{-1}. Two control treatments were established by the absence of fungicide application (T1) and a baseline of plant and disease responses to two fungicide applications (T2), the last was used to determine the benefits of a 3rd fungicide application.

Aerial images were acquired by a multispectral sensor with wavelengths at 450 (blue), 550 (green), 650 (red), and infrared wavelengths at 775 and 825 nm (Sentera Inc., Minneapolis, MN) onboard drone (Inspire 2, DJI Inc., China), before and two hours after the fungicide applications (i.e., R3 and R4 growth stages; Table 1), between 10:00 am to 12:00 pm. Flight altitude was 150 m (3 cm pixel) with 80% frontal and lateral overlaps and less than 20% cloud cover. The images were orthorectified to obtain the arithmetic mean of the pixel values in an area of interest of 1 × 0.5 m from the center of each plot.

A preliminary assessment on fev. 1, 2019, evaluated the potential disease infections before the first application of the fungicides. The severity of ASR was measured at 7, 14, 21, and 28 days after the 3rd application using a diagrammatic scale (GODOY et al., 2006). Phytotoxicity was determined at seven days after applying the fungicides at all three growth stages using a diagrammatic scale (EWRC, 1964). Fungicide control efficiency (ABBOTT, 1925) and the area under the disease progress curve (AUDPC) were calculated using the mean severity of the ASR. The two central rows from each plot (4 m) were harvested individually (Mar 28, 2019). Dry mass was adjusted to grain moisture of 13%; and crop yield was extrapolated (kg ha^{-1}). The ASR severity, plant spectral responses, and yield were analyzed by the F-test. The treatment means were separated by the Tukey test.

In brief, fifteen treatments received three successive fungicides applications within 28 days without any change in the spectral reflectance of soybean in the visible and infrared ranges (Table 2).

Table 1 - Foliar fungicides sequentially applied in different soybean growth stages at recommended doses to control Asian soybean rust in 2019, Rio Verde, GO.

Treat.	----------Soybean Growth Stage----------		
	R1 (flowering)	R3 (beginning of pod)	R4 (fully developed pods)
T1	None	None	None
T2	EFP	AB+DIF	None
T3	EFP	AB+DIF	Picoxystrobin + Ciproconazole 300 mL of product ha^{-1}
T4	EFP	AB+DIF	Mancozebe 1500 g of product ha^{-1}
T5	EFP	AB+DIF	Metominostrobin + Tebuconazole 580 mL of product ha^{-1}
T6	EFP	AB+DIF	Trifloxystrobin + Ciproconazole 200 mL of product ha^{-1}
T7	EFP	AB+DIF	Pyraclostrobin + Fluxapiroxade 350 mL of product ha^{-1}
T8	EFP	AB+DIF	DIF 300 mL prod. ha-¹ + Mancozebe 1500 g of product ha^{-1}
T9	EFP	AB+DIF	Axostrobin + Mancozebe 1500 g of product ha^{-1}
T10	EFP	AB+DIF	Difenoconazole + Ciproconazole 300 mL of product ha^{-1}
T11	EFP	AB+DIF	Mancozebe + Azoxystrobin + Ciproconazole 2000 g product ha^{-1}
T12	EFP	AB+DIF	Picoxystrobin + Tebuconazole + Mancozebe 2250 mL product ha^{-1}
T13	EFP	AB+DIF	Tebuconazole + Chlorotalonil 2500 mL of product ha^{-1}
T14	EFP	AB+DIF	Chlorothalonil 720 100 mL of product ha^{-1}
T15	EFP	AB+DIF	Carbendazim 1500 mL of product ha^{-1}
T16	EFP	AB+DIF	Carbendazim + Tebuconazole 1000 mL of product ha^{-1}
T17	EFP	AB+DIF	Fenpropimorph 300 mL of product ha^{-1} + Copper oxychloride 500 mL of product ha^{-1}

EFP: Epoxiconazole, Fluxapiroxade, and Piraclostrobina at the dose of 0.8 l of product ha^{-1} (Ativum®, BASF Inc.);
AB: Azoxystrobin and Benzovindiflupir at the dose of 0.2 kg of product ha^{-1} (Elatus®, Syngenta Crop Protection Inc.);
DIF: Difenoconazole at the dose of 0.3 l of product ha^{-1} (Score®, Syngenta Crop Protection Inc.).

Table 2 - Soybean reflectance in visible wavelengths and near-infrared at the start of pod formation (R3) and when the pods were fully grown (R4), before and after application of fungicides.

Treat	----------Red----------		--------Green---------		---------Blue--------		---------775 nm---------		-------825 nm-------	
	before	after	before	after	Before	after	before	after	before	after
--Pod formation (R3)---										
T1	91.52	96.67	115.19	117.39	79.79	80.26	94.64	99.43	95.61	97.58
T2	92.25	96.67	115.44	117.29	79.71	80.04	94.40	97.75	95.86	95.7
T3	94.17	97.26	117.54	117.83	81.57	80.34	93.68	95.94	94.65	94.98
T4	94.95	97.8	118.82	118.63	82.07	80.88	91.02	94.69	92.68	93.85
T5	91.43	95.66	114.54	115.98	79.35	78.91	95.24	98.87	95.48	97.97
T6	93.08	97.42	116.07	118.07	80.48	80.79	94.59	98.68	94.96	97.33
T7	94.99	98.31	119.35	119.36	82.66	81.23	94.02	96.23	95.33	95.73
T8	96.32	99.19	120.57	120.02	83.56	81.56	92.01	95.32	94.01	93.76
T9	96.84	100.49	120.93	121.63	83.69	82.95	90.66	96.7	93.55	93.94
T10	93.00	98.14	116.53	119.14	80.53	81.65	95.52	101.64	94.73	99.74
T11	94.52	99.45	118.62	120.93	82.34	82.52	94.67	100.38	94.49	99.36
T12	95.60	99.29	120.24	120.88	83.23	82.51	94.07	96.55	95.60	96.94
T13	96.08	98.83	120.62	120.42	83.54	81.78	92.58	95.61	95.15	93.43
T14	97.25	100.48	121.00	121.82	83.66	83.10	90.07	95.49	93.11	92.09
T15	93.91	98.67	117.55	119.54	81.54	81.70	94.30	98.40	93.37	96.62
T16	94.86	100.08	119.56	121.29	83.03	82.99	92.96	99.07	93.15	97.36
T17	95.94	100.03	119.97	120.87	83.35	82.71	90.27	93.15	91.28	92.61
	F=0.252; P=0.99 CV=34.35%		F=0.28; P=0.99 CV=16.96%		F=0.355; P=0.98 CV=11.60%		F=0.153; P=0.99 CV=43.30%		F=0.51; P=0.93 CV=20.74%	
--Fully grown pods (R4)---										
T1	94.59	105.74	111.31	129.11	75.1	92.99	89.68	99.9	93.19	105.49
T2	94.2	105.24	111.13	128.5	75.19	93.04	90.38	101.55	94.64	107.36
T3	94.10	106.62	111.36	129.77	74.92	94.13	89.08	100.27	93.35	105.95
T4	95.13	108.51	111.95	130.96	75.46	96.5	88.04	97.82	91.67	104.06
T5	93.09	105.6	109.78	128.9	73.84	93.71	90.34	99.77	93.23	106.39
T6	93.53	106.16	110.58	129.21	74.28	93.71	90.97	99.97	94.01	106.42
T7	94.33	105.8	111.29	129.41	74.64	93.72	89.16	99.16	92.61	105.68
T8	95.60	109.21	112.67	132.05	75.59	96.46	87.54	99.05	91.14	105.26
T9	95.79	108.56	112.68	131.94	75.54	96.62	87.53	99.16	90.68	105.46
T10	93.80	105.82	110.61	129.27	74.55	92.92	89.71	100.45	93.41	106.36
T11	94.70	109.83	111.65	132.41	75.08	96.89	89.68	100.48	93.56	106.98
T12	94.84	109.12	111.8	132.06	75.12	96.14	87.78	100.36	91.78	107.06
T13	95.6	108.42	112.78	131.5	75.75	95.89	88.95	100.91	93.11	107.63
T14	96.88	107.73	114.07	131.11	76.85	95.71	87.78	99.92	92.00	106.66
T15	94.9	109.85	111.65	132.69	75.13	97.07	87.19	99.91	91.93	106.12
T16	95.27	107.37	112.27	130.19	75.76	94.51	89.52	99.42	94.19	105.56
T17	96.31	107.6	112.77	131.14	75.95	95.19	86.47	98.44	90.09	104.5
	F=0.43; P=0.96 CV=42.29%		F=0.32; P=0.99 CV=38.34%		F=0.36; P=0.98 CV=60.45%		F=0.19; P=0.99 CV=34.29%		F=0.175; P=0.99 CV=48.15%	

Means differed before and after application. However, means within the same column and vegetation stage did not differ by Tukey test (P > 0.05). Letters from the mean separation test were removed for simplicity.

Thus, foliar fungicides with different mechanisms of action did not affect the subsequent use of remote sensing in the spectral range from 400 to 940 nm (Table 2). There was no significant interaction between fungicides and time (before/after application). There were no symptoms of ASR or injury from other stressors until the end of the study. Fungicides also did not appear to be on plant surfaces.

The ASR severity was low in the first days after the beginning of the evaluations and reached 71% after the 3rd application (Table 3). At 28 days after the 3rd application, there was a difference in the severity of ASR between the treatment that received only two applications (EFP, AB + DIF) and the other treatments that received three fungicide applications. The treatment that only received fungicide in the first two applications (EFP, AB + DIF) did not differ from the other treatments at 7, 14, and 21 days after the 3rd application, and had an average fungicide control efficiency of 69% (Table 3). Ultimately, the 3rd application increased productivity and showed an efficiency of up to 17% more than the treatment received only two applications of fungicide (Table 3). Considering all treatments, the increase in AUDPC significantly reduced yield ($P < 0.01$).

The fungicides used in this study did not appear to have morphophysiological or residual effects on soybean leaves (MAKIO et al., 2007; NANSEN et al., 2010). Similar results were also reported in soybean plants treated with other agrochemicals (ALVES et al., 2017). The increase in reflectance after two hours may be associated with the increase in radiation due to the daily time (MAKIO et al., 2007).

The treatments with tebuconazole (T5 and T13) showed a higher percentage of phytotoxicity (55%) and had similar efficiency in controlling ASR. The treatment picoxystrobin + tebuconazole + mancozebe (T12) increased yield and decreased the disease progress curve (Table 3). Therefore,

Table 3 - Severity of Asian soybean rust, area under the disease progress curve (AUDPC), fungicide control efficiency (FCE), and soybean yield after fungicide application in 2019, Rio Verde, GO.

Treatment	-----------------------------Severity (%)-------------------------				AUDPC	FCE (%)	Yield (kg ha^{-1})
	7 DAA	14 DAA	21 DAA	28 DAA			
T1	2.97 a	31.42 a	53.89 a	71.65 a	868.77 a	-	37.74 e
T2	0.53 b	2.36 b	8.48 b	47.77 b	246.78 b	69%	43.28 de
T3	0.49 b	1.82 b	5.77 b	17.29 cd	116.84 b	86%	50.75 cba
T4	0.55 b	1.47 b	7.54 b	23.01 cd	147.45 b	82%	47.16 bcd
T5	0.55 b	1.76 b	8.00 b	24.03 cd	156.28 b	81%	51.62 abc
T6	0.57 b	1.86 b	7.66 b	18.04 cd	133.78 b	84%	47.77 bcd
T7	0.58 b	3.13 b	8.86 b	28.83 cd	188.89 b	76%	46.52 bcd
T8	0.56 b	2.29 b	7.71 b	20.06 cd	144.19 b	82%	45.62 cd
T9	0.55 b	1.94 b	8.35 b	17.26 cd	136.28 b	83%	48.37 abcd
T10	0.55 b	2.34 b	8.95 b	28.74 cd	183.46 b	76%	48.48 abcd
T11	0.56 b	1.66 b	6.67 b	16.52 cd	120.14 b	85%	52.75 ab
T12	0.52 b	1.66 b	6.81 b	13.09 d	108.75 b	86%	54.23 a
T13	0.51 b	2.87 b	7.35 b	21.95 cd	151.95 b	81%	47.35 bcd
T14	0.49 b	1.84 b	7.13 b	18.84 cd	132.10 b	84%	45.64 cd
T15	0.50 b	2.08 b	8.5 b	32.27 bc	190.53 b	76%	42.40 de
T16	0.52 b	2.98 b	9.94 b	32.29 bc	207.13 b	74%	43.42 de
T17	0.47 b	1.63 b	9.21 b	21.63 cd	154.92 b	82%	45.81 cd
CV (%)	36.15	26.72	38.77	27.31	35.6	-	5.27

Means separated by the same letter in the same column did not differ by Tukey test ($P > 0.05$).

preventive applications of fungicides (i.e., the first two protective applications) were essential to control ASR (TWIZEYIMANA & HARTMAN, 2017). A 3rd application increased disease control for most fungicide treatments. Carbendazim + tebuconazole treatment (T16) and carbendazim (T15) showed the lowest control efficiencies.

Precision agriculture optimized agricultural management practices by considering the distribution of resources according to spatial and temporal variability. Foliar fungicides commonly used to control ASR did not affect the canopy-level reflectance of soybean plants, agreeing with the results from ALVES et al. (2017) that remote sensing can be used for exploring spatial and temporal information regardless of agrochemicals. Planning flight duration and intervals can be especially important because a few hours between images can affect the reflectance of soybean plants regardless of fungicides. The 3rd application of fungicides can be necessary for greater control of ASR and, consequently, greater soybean production.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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