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Spray volume deposits and fungicide efficacy on soybean rust (*Phakopsora pachyrhizi*)

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Abstract

Soybean rust (SBR), caused by *Phakopsora pachyrhizi*, is one of the most destructive fungal diseases affecting soybean yields in many countries. Fungicide application methods that provide better SBR control efficacy may reduce soybean losses due to this disease. We investigated the effects of spray volumes applying the fungicide pyraclostrobin plus epoxiconazol at 133 + 50 g a.i. ha^{-1} by a conventional sprayer (CS) and an air-assisted sprayer (AAS). Field experiments were conducted comparing the effects of spray volumes of 110, 160, and 210 L ha^{-1} and two application techniques (CS and AAS) on spray deposits and SBR control. Fungicide efficacies were measured by disease severity, thousand seed weight, and yield. Correlations between disease severity and yield were also assessed. All treatments were applied with an Advance 2000 AM18 sprayer. In general, SBR disease and yield did not differ significantly when fungicide applications were applied with AAS compared to CS. Increasing the spray volume from 110 to 210 L ha^{-1} did not increase spray deposit coverage on soybean leaves. Low disease severity was obtained by fungicide applications using a spray volume of 210 L ha^{-1}. Safe recommendations of ground spray volumes for SBR control should be between 160 and 210 L ha^{-1}, using hydraulic nozzles.

Keywords: *Glycine max,* pesticide application technology, spray deposits, spray rate.
Abbreviations: SBR_Soybean rust; CS_conventional sprayer; AAS_air assisted sprayer; RH_relative humidity; ºC_degree Celsius; DLA_disease leaf area; AUDPC_area under the disease progress curve; TSW_thousand seed weight; ha_hectare; kg_kilogram; LSD_least significant difference; L:_liter; Rs_response ratio.

Introduction

Soybean [*Glycine max* (L.) Merrill] is an economically important crop cultivated by producers all over the world (Murithi et al., 2016). Soybean rust (SBR), caused by the fungus *Phakopsora pachyrhizi* Syd & P. Syd., is the most challenging disease of soybean in Brazil (Godoy et al., 2016). This is not only because the disease spreads rapidly, but also because it can cause significant yield reductions where management practices are poor (Prado et al., 2015).

Despite many studies on the development of soybean cultivars resistant to *P. pachyrhizi* (Langenbach et al., 2016; Vuong et al., 2016; Childs et al. 2018), control has been difficult due to the variability and dynamic plasticity of the rust population, varying in virulence and genetic composition (Murithi et al., 2016). One of the most effective management strategies to suppress SBR severity and to reduce yield loss is the application of sequential fungicides (Scherm et al., 2009; Costa et al., 2015; Prado et al., 2015). The correct choice of fungicides, as well as many other parameters, is essential for satisfactory SBR control (Mueller et al., 2009). Optimal control of SBR is achieved with fungicides belonging to the triazole and quinone outside inhibitor (QoI) fungicide groups, particularly the combination triazole-QoI (Scherm et al., 2009; Mueller et al., 2009).

Fungicides are typically diluted in water and distributed over crops in the form of liquid spray atomized by hydraulic nozzles. The purpose of pesticide applications is to reach the target and to obtain coverage, resulting in the optimal efficacy of the applied product (Jensen and Olesen, 2014), while at the same time avoiding pesticide losses by drift.

Symptoms associated with SBR are initially observed in the lower soybean canopy and then move upwards as the disease progresses (Zhu et al., 2008). To provide fungicide coverage on the leaves in the lower canopy is not an easy task, especially in dense canopies. Air-assisted spraying (AAS) is a technique that may improve droplet penetration and increase the amount of coverage of the lower canopy compared to conventional spraying (CS) (Zhu et al., 2008). However, there are few reports that prove fungicide application efficacy using ASS against SBR. For example, Christovam et al. (2010) did not observe significant differences in SBR disease severity and yield between fungicides applied with AAS and CS. Perhaps the greater amount of fungicide deposited in the lower canopy by ASS is not sufficient to improve SBR control efficacy compared with CS application.

The correct pesticide application volume is important for providing adequate spray coverage and uniform deposits on the target surface. Over the last several years, ground application spray volume reduction has been routinely practiced by Brazilian farmers to increase the operational sprayer capacity, consequently reducing production costs (Bayer et al., 2011). The reductions in application cost may be more economical in large agricultural areas, such as for soybeans. For example, a sprayer calibrated to deliver a greater spray volume can only cover a limited area per tank load (Fergunson et al., 2016); the frequency of refilling a sprayer will therefore increase, and more time will be spent performing the application.

Despite the benefits of applying fungicide with a low spray volume, as mentioned previously, it is necessary to know how far the spray volumes can be reduced without affecting the efficacy of the pesticide applications (Garcera et al., 2011). In previous studies, the range of spray volumes from 60 to 187 L ha^{-1} did not impact the amount of deposits throughout the soybean canopy (Derksen et al., 2008; Prado et al., 2015); however, a reduction of the spray volume appears to not work well when fungicides are applied to control SBR. According to Prado et al. (2015) and Costa et al. (2015), control of SBR is reduced and yield losses increase as the spray volume decreases. Excessive reductions in spray volume should be carefully considered to avoid inadequate control of SBR and increased yield losses.

Although the importance of adequate fungicide coverage of the soybean canopy to minimize damage caused by *P. pachyrhizi* is well-recognized, the literature contains few studies detailing the most adequate spray volumes, and there is controversy surrounding the efficiency of AAS. In this context, the need to clarify the efficiency of AAS and to determine the most suitable spray volume to apply fungicides for SBR management is the main purpose of this study. Thus, the main objectives of this research were to assess the efficiency of different application spray volumes applied by CS and AAS on spray deposits through the soybean canopy and on the control of SBR disease.

Results

Quantification of spray deposit

Figure 1 presents the average spray deposits among the top, middle, and bottom canopy sections in 2009/10 and 2010/11. Spray deposit differences were observed in two crop seasons from different canopy sections of the soybean plant (F = 224.8; $P < 0.0001$ - 2009/10 and F = 53.7; $P < 0.0001$ - 2010/11).

Significant differences between spray deposits were observed in 2009/10. Greater spray deposits (69%) were detected on leaves from the top canopy, followed by the middle (22%) and bottom canopy (9%). In 2010/11, the spray deposits from the top canopy were significantly different from those on the middle and bottom canopies. No significant difference was detected on leaves from the middle and bottom canopies. As in 2009/10, more spray deposits were detected from the top canopy (70%), followed by the middle (19%) and the bottom canopy (11%) (Fig. 1).

The increase in spray volume from 110 to 210 L ha^{-1} did not result in significant differences in spray deposits, regardless of the crop season (F = 2.6; $P = 0.0849$ - 2009/10 and F = 0.6; $P = 0.5299$ - 2010/11). Differences in application techniques were not identified (F = 2.6; $P = 0.1156$ - 2009/10 and F = 0.03; $P = 0.8637$ - 2010/11). Regardless of the crop season, the spray deposits in the treatments using AAS did not differ from the treatments applied by CS.

The interaction between soybean canopy sections (top, middle, and bottom) and spray techniques (CS and AAS) on soybean spray deposits are summarized in Table 1. A significant difference was detected only for spray deposits from the top canopy for the techniques used. The CS provided greater spray deposits compared to AAS on the top canopy level of the plant.

Soybean rust assessment

Differences in application volumes were detected in AUDPC (F = 8.7, $P< 0.01$) and TSW (F = 4.9, $P < 0.05$) only in the 2010/11 crop season. No differences in application technique and interaction spray volume with application technique were reported for AUDPC, TSW, and soybean yield in any crop season, indicating the independence of the variables.

The mean AUDPC, TSW, and yield values in 2009/10 and 2010/11 are presented in Table 2. The AUDPC values of all treatments that received fungicide application, regardless of the application technique and spray volume adopted, differed significantly from the control treatment in both crop seasons. In 2009/10, the AUDPC detected in the treatment of 210 L ha^{-1} using CS was significantly lower compared to that in the treatment of 110 L ha^{-1} using a CS. The AUDPC values were greater in the treatment of 210 L ha^{-1} using CS compared to the treatments applied at 110 L ha^{-1} using CS and AAS and the treatment of 160 L ha^{-1} using CS in 2010/11 (Table 2).

The treatment applied with a spray volume of 210 L ha^{-1} presented the lowest AUDPC mean values, differing to the treatments applied at 110 L ha^{-1} in the 2010/11 when data was combined with application technique. The spray volume of 160 L ha^{-1} did not differ from the other treatments (Fig. 2).

All treatments that received fungicide application showed TSW values significantly greater than the control (Table 2). In the 2009/10 crop season, the treatment of 210 L ha^{-1} using CS presented greater TSW values compared to the treatment applied at 110 L ha^{-1} using CS. The TSW values were greater in the treatments of 160 L ha^{-1} using AAS and 210 L ha^{-1} using CS compared to the treatments of 110 L ha^{-1} using CS and AAS in 2010/11 (Table 2). When data of application techniques were combined during 2010/11, the TSW was greater with the spray volumes of 160 and 210 L ha^{-1} when compared to the volume of 110 L ha^{-1} (data not shown).

The mean values of soybean yields in 2009/10 and 2010/11 are presented in Table 2. All treatments that received fungicide application, independent of the adopted application technique, differed significantly from the control treatment. No significant difference was detected between treatments that received fungicide application in both crop seasons (Table 2). The control showed yield reductions of 27 and 31% in the 2009/10 and 2010/11 crop seasons, respectively, compared to the average of treatments that received fungicide application.

Significant negative correlations between yield and disease severity (AUDPC) occurred in the two crop seasons ($P < 0.0001$) (Fig. 3). A greater negative correlation in the 2009/10 (r = -0.72; F = 28.4) and a moderate negative correlation in the 2010/11 crop season (r = -0.59; F = 13.7) were detected, indicating that treatments with better disease control (lower AUDPC) also had greater yield values.

The 2009/10 crop season had greater values of AUDPC compared to the 2010/11 season. Although a greater disease severity was presented in the 2009/10 crop season compared to the 2010/11 crop season, the soybean yield was not proportional to the disease severity.

Discussion

Based on the results presented in the current study and on the results reported in the literature, increasing spray volumes do not necessarily imply a significant increase in spray deposit concentrations on soybean leaves. The lack of spray deposit differences with increasing spray volume could be due to the different characteristics of the soybean varieties (leaf area index, plant architecture, growth habits, leaf morphologies, application stage, and row spacing) or the spray volume variation used in these studies may not be sufficient to show significant spray deposit differences. It should be noted that the studies published in the literature assessing spray deposits on soybean plants with a small range of spray volumes (usually ranging from 60 to 300 L ha^{-1}) and likely using a greater or lower spray volume may appear to have significant differences on spray deposits. Derksen et al. (2008) also reported that AAS treatments did not necessarily perform better in the soybean canopies than traditional boom sprayer treatments.

Similar to these results, Prado et al. (2010) reported greater spray deposits on the top canopy of soybean plants with CS and lower spray deposits when using AAS. A possible explanation for the smaller spray deposits on the top canopy with AAS may be related to the airflow generated by the sprayer fan carrying the spray droplets towards the interior of the soybean canopy, although no significant increase in spray deposits was detected for the middle and bottom canopy when using this technique.

In general, spraying performed with AAS did not differ significantly from spraying via CS, regardless of the crop season, contradicting the results obtained by Zhu et al. (2008) and Prado et al. (2010), who obtained better spraying deposit results on the leaves from the middle and bottom soybean canopy when AAS was used. However, the results obtained in this study regarding the use of AAS corroborate the results obtained by Derksen et al. (2008), who did not observe differences in fungicide residues detected on soybean leaves between AAS with CS in two crop seasons. Christovam et al. (2010), studying different application techniques, did not report significant differences in spray deposits in the bottom canopy of soybean plants when spraying with AAS compared to CS.

The different results obtained by the studies associated with the efficacy of AAS on spray deposits could be related to (1) different canopy structures and characteristics of soybean varieties, such as plant height, leaf area index, and seeding rates; (2) characteristics inherent to spraying, such as nozzle type, nozzle pressure, spray quality, sprayer travel speed, sprayer model pressure, air speed at the outlet of the sleeve, and (3) meteorological conditions at the time of application, making it difficult to successfully compare results from different studies.

Based on the data presented in this study and in other papers (Cunha et al., 2006; Zhu et al., 2008b; Christovam et al., 2010; Prado et el., 2015), it is clear that spray deposits do not reach the middle and bottom canopy of soybean plants at a high rate, even when using AAS. The need for techniques that provide increased spray deposits in the lower canopy is fundamental, as SBR infection initiates in this plant portion.

The efficacy of the treatments including fungicide application on reducing disease severity was expressed as the response ratio Rs [disease severity of the treatment represented by the AUDPC divided by the AUDPC of the corresponding treatment control (Scherm et al., 2009)]. The lower the Rs, the greater the difference between treatments that received fungicide compared to treatments without fungicide, and consequently, the greater the effectiveness of disease control means. Thus, the Rs value was superior in 2009/10 (average of all treatments that received fungicide application) compared to the 2010/11 (0.45 vs. 0.56), corresponding to a disease reduction of 55 and 44%.

Significant differences between application volumes of 115 and 160 L ha^{-1} in the severity of SBR, represented by AUDPC, values were not detected by Cunha et al. (2006). It is possible that spray volume variations of 50 L ha^{-1} are not sufficient to infer significant differences in SBR severity. Based on previously reported results, there are tendencies of a reduction of AUDPC when the spray volume is increased from 110 to 210 L ha^{-1} (Fig. 2).

It was clear that SBR interfered with the soybean seed weights, and the plots treated with fungicides had greater values of TSW. Prado et al. (2010; 2015) and Christovam et al. (2010) reported greater TSW average values in treatments that received a fungicide application compared to treatments without a fungicide application. The greater SBR severity in the treatments when applied the spray volume of 110 L ha^{-1} (Fig. 2) probably contributed to reduce seed weight. The SBR disease reduces soybean yield in Brazil and other countries around the world (Scherm et al., 2009; Costa et al., 2015; Murithi et al. 2016). A study published by Scherm et al. (2009), showing a quantitative review of 71 fungicide efficacy trials conducted in Brazil from 2003/2004 to 2006/7, verified a yield reduction average of approximately 22% relative to the treatment without fungicide application. In Brazilian trials, Prado et al. (2010) reported soybean yield reductions of 37%, Christovam et al. (2010) of 49%, and Prado et al. (2015) of 24%.

In both crop seasons studied, spray volumes of 110, 160, and 210 L ha^{-1}, either applied by AAS or CS, did not significantly differ in terms of soybean yield, which may be associated with favorable environmental conditions: air relative humidity of 73 ± 5%; air temperature of 25 ± 6°C, and wind speed ranging between 3.7 and 7.0 km h^{-1} [average values of five sprays of fungicide] at the time of fungicide application.

Table 1. Mean values of spray deposits (nL cm^{-2}) for the interaction between soybean canopy sections (top, middle, and bottom) and techniques (conventional sprayer and air-assisted sprayer) in the 2009/10 crop season.

Canopy sections	Application techniques	
	Conventional sprayer	Air-assisted sprayer
	nL cm^{-2}	
Top	486 A a	384 A b
Middle	138 B a	148 B a
Bottom	47 C a	65 C a

Means followed by the same letter are not significantly different at *P* ≤ 0.05 according to an LSD test. Lowercase letters compare application technique deposits and uppercase letters compare canopy sections deposits.

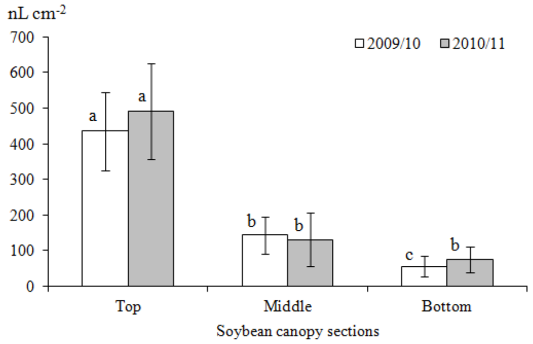

Fig 1. Mean spray deposits (nL cm^{-2}) on top, middle, and bottom soybean canopy sections. Standard deviations are represented by vertical bars. Means followed by the same letter are not significantly different at *P* ≤ 0.05 according to an LSD test; statistical significance is only valid within each crop season.

Table 2. Mean (± SD) severity of Soybean rust represented by the area under the disease progression curve (AUDPC), thousand seed weights (TSWs), and soybean yield (kg ha^{-1}) after applying fungicide by different spray volumes and techniques.

	AUDPC		TSW (g)		Yield (kg ha^{-1})	
	Crop season					
Treatments	2009/10	2010/11	2009/10	2010/11	2009/10	2010/11
Control	2,896 (86) a	1,592 (69) a	124.4 (1.4) c	132.8 (11.2) c	2,770 (414) b	1,890 (157) b
110 CS	1,484 (179) b	1,077 (252) b	145.3 (8.0) b	144.1 (2.6) b	3789 (468) a	2,590 (309) a
110 AAS	1,315 (132) bc	1,088 (166) b	147.0 (6.4) ab	143.2 (4.3) b	3,822 (498) a	2,713 (422) a
160 CS	1,381 (325) bc	921 (159) bc	150.3 (5.3) ab	149.4 (4.7) ab	3,730 (154) a	2,656 (180) a
160 AAS	1,291 (56) bc	839 (176) bcd	150.7 (6.7) ab	154.4 (8.3) a	3,652 (216) a	2,951 (334) a
210 CS	1,141 (153) c	637 (274) d	152.5 (4.4) a	155.1 (3.0) a	3,828 (534) a	2,758 (214) a
210 AAS	1,275 (158) bc	769 (169) cd	151.0 (6.3) ab	149.7 (2.7) ab	3,881 (77) a	2,807 (183) a
P	< 0.0001	< 0.0001	< 0.0001	0.0013	0.0143	0.0010
F	45.5	11.8	18.9	6.1	3.7	6.4
LSD	267	270	6.7	9.3	603	404

Values followed by the same letter in a column are not significantly different at *P* ≤ 0.05 according to an LSD test. 110 CS: Conventional sprayer applying 110 L ha^{-1}; 110 ASS: Air-assisted sprayer applying 110 L ha^{-1}; 210: 210 L ha^{-1}; 160 CS: Conventional sprayer applying 160 L ha^{-1}; 160 ASS: Air assisted sprayer applying 160 L ha^{-1}; 210 CS: Conventional sprayer applying 210 L ha^{-1}; 210 ASS: Air-assisted sprayer applying 210 L ha^{-1} and control (no fungicide application).

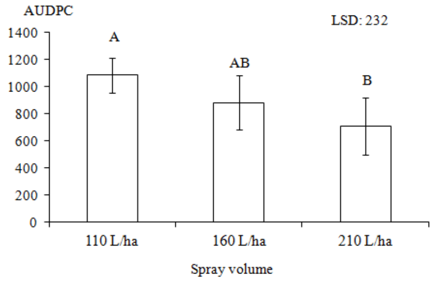

Fig 2. Effect of spray volume on disease severity represented by the area under disease progression curve (AUDPC) in the 2010/11 crop season. Standard deviations are represented by vertical bars. Means followed by the same letter are not significantly different at *P* ≤ 0.05 according to an LSD test.

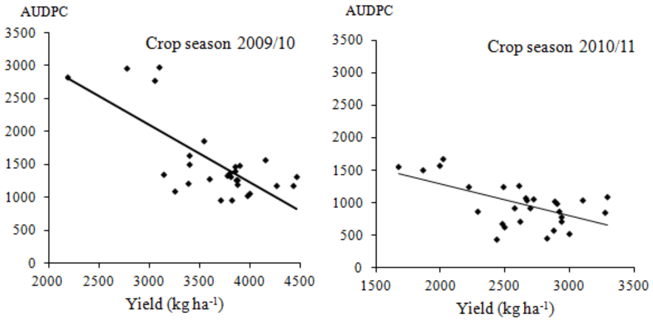

Fig 3. Correlation coefficients between Soybean rust severity (AUDPC) and soybean yield.

The advantages of AAS may be more evident when fungicide applications are made under unfavorable environmental conditions, such as the presence of winds over 10 km h^{-1} or in the total absence of wind (thermal inversion), situations that did not occur in these two crop seasons.

Greater yield values were observed in the 2009/10 crop season compared to the 2010/11 crop season (Table 2). This implies that the tolerance potential against SBR disease severity in the specific soybean cultivars must be considered. Differences in soybean cultivar susceptibility, variable disease pressure, and year-to-year environmental variations may be considerations for the yield differences between the two crop seasons studied.

The lower AUDPC values seen in the 2010/11 crop season (Table 2) when fungicide spray application was performed in the treatments with 210 L ha^{-1} spray volume were not sufficient to provide soybean yield compared to the treatments applied with a spray volume of 110 L ha^{-1}, regardless of the techniques (ASS or CS). The intensity of disease severity between the treatments that received fungicide applications was likely too small to be reflected in the yield difference (Table 2).

A reduction in the applied spray volume may provide an increase in the sprayer operational capacity by reducing the number of refilling stops (Costa et al., 2015), allowing to spray a larger crop area (Berger-Neto et al., 2017) and consequently resulting in a reduction of plant protection costs. However, ground applications with lower spray volume may be economically worthwhile, since the biological efficiency of pesticides does not appear to be compromised by volume reductions. When the biological treatment efficacy does not provide desirable disease control, yield losses will occur due to the disease severity, and other fungicide applications will be necessary in many cases to ensure satisfactory yield.

In this study, fungicide applications with spray volumes of 110, 160, and 210 L ha^{-1} did not significantly influence soybean yield (Table 2), and given the advantages mentioned before, spray application using a volume of 110 L ha^{-1} may be preferred. Although spray volumes tested in the current study did not influence the soybean yield, regardless of the crop season, a tendency towards greater AUDPC and lower TSW values was observed when fungicide applications were performed with a volume of 110 L ha^{-1} (Table 2).

Lower values of soybean yield when fungicide application was performed with spray volumes of 60 and 110 L ha^{-1} compared to 160 L ha^{-1} have been reported by Prado et al. (2015); similar results have been published by Costa et al. (2015). These authors reported greater soybean yields when fungicide was sprayed to control SBR with a hydraulic spray nozzle at 150 L ha^{-1}, differing significantly from the results obtained with spray volumes of 50 and 100 L ha^{-1}. The choice of a desirable fungicide spray volume application for SBR control may be detrimental to providing satisfactory control. Thus, we believe that fungicide application with a spray volume lower than 160 L ha^{-1} to reduce SBR severity and to avoid yield loss may be not provided by a ground sprayer equipped with hydraulic nozzles.

Materials and Methods

Site descriptions

The experiments were conducted in the south-west state of São Paulo, Brazil (22°48'S and 48°25'W and 724 m a.s.l.), during the 2009/2010 and 2010/2011 crop seasons. The soil at this location is classified as Rhodic Ferralsol (FAO, 2006) [kaolinitic, thermic Typic Haplorthox (Soil Survey Staff, 2014) with sandy loam texture] and had been managed since 2008 in a no-tillage system, always growing soybean in the summer and black oat in the fall. The climate, according to Köeppen's classification, is Cwa (tropical, with a dry winter and a hot, rainy summer).

Soybean sowing and cultivars

Soybeans were sown on November 24, 2009 and November 05, 2010, using the cultivars FTS Campo Mourão RR and BRS Valiosa RR (determinate growth habits), respectively. Row spacing was 0.45 m, with approximately 17 seeds per meter. Seed were treated with carboxin [5,6-dihydro-2-methyl-1,4-oxathi-ine-3-carboxanilide] + thiram [tetramethylthiuram disulfide] 50 + 50 g a.i. ha^{-1} for 100 kg of seeds (Vitavax Thiram 200 mL for 100 kg of seeds) and an inoculant (*Bradyrhizobium japonicum*). The cultivars FTS Campo Mourão RR and BRS Valiosa RR were 1.0 m and 1.3 m tall, respectively, at the R2 reproductive stage (Fehr et al., 1971). Soybean plant emergence was established on December 05, 2009 and on November 22, 2010. The base fertilization consisted of 310 kg ha^{-1} 04-20-20 N-P-K in 2009 and 320 kg ha^{-1} 00-20-20 N-P-K in 2010, applied at planting. In both crop seasons, the following insecticides were applied: spinosad at 14.4 g a.i ha^{-1} (Tracer 30 mL ha^{-1}) at the V8 vegetative stage for caterpillar control and thiamethoxam + lambda-cyhalothrin 26.5 + 35.25 g a.i. ha^{-1} (Engeo Pleno 250 mL ha^{-1}) at the R3 and R5 for stink bug control. Herbicides were applied 1 week before sowing herbicides - glyphosate 1.585 g a.i. ha^{-1} (Roundup WG 2 kg ha^{-1}) plus carfentrazone-ethyl 30 g.a.i. ha^{-1} (Aurora 400 EC 75 mL ha^{-1})] and approximately 3 weeks after soybean emergence glyphosate 791 g a.i. ha^{-1} (Round WG 1 kg ha^{-1}). Insect and weed control were performed based on soybean recommendations and requirements for all treatments, including the control (no fungicide application), to isolate the effects of insects and weeds in the experiments. Wheat (*Triticum* spp.) and black oat (*Avena strigosa*) were sown as a fall crop in April 2009 and June 2010. Soybeans were harvested on April 1 and 28 for 2010 and 2011, respectively.

Experimental design and spray deposit quantifications

The experimental design was a randomized block with four replications in a tri-factorial scheme (3 × 2 × 3). The treatments imposed included the following: three spray volumes (110, 160, and 210 L ha^{-1}), two application techniques (AAS and CS), and three canopy sections (top, middle, and bottom). The dimensions of each plot were 8 m wide by 9 m long (72 m^2). Spray treatments were as follows: spray volume of 110 L ha^{-1} (TeeJet XR 110015 at 300 kPa - 0.61 L min^{-1}) using CS; spray volume of 110 L ha^{-1} (TeeJet XR 110015 at 300 kPa - 0.61 L min^{-1}) using AAS; spray volume of 160 L ha^{-1} (TeeJet XR 11002 at 400 kPa - 0.89 L min^{-1}) using CS; spray volume of 160 L ha^{-1} (TeeJet XR 11002 at 400 kPa - 0.89 L min^{-1}) using AAS; spray volume of 210 L ha^{-1} (TeeJet XR 11003 at 300 kPa - 1.17 L min^{-1}) using CS; spray volume of 210 L ha^{-1} (TeeJet XR 11003 at 300 kPa - 1.17 L min^{-1}) using AAS. All treatments were applied with fine droplets according to the ASABE Standard S572.

The AAS was an Advance 2000 AM18 Vortex pull-type sprayer manufactured by Jacto, Inc. (Pompéia - Brazil). The sprayer has an 18-m long air-bag along the entire length of the boom, equipped with 37 nozzles spaced 0.5 m apart. Nozzles were located ahead of the narrow air outlet, which ran the entire length of the boom. The average air speed measured by a digital anemometer (ITTAD 500) positioned 0.5 m from the outlet of the sleeve was 9.8 ± 1 m s^{-1}. The air jet was delivered vertically toward the soybean canopy. Nozzles were positioned 0.5 m above the top canopy, with a travel speed of 6.7 km h^{-1}, and spraying from one side of the spray boom to apply the treatments to the plots. The CS was the same application equipment (AAS) with air assistance turned off and adjusted at the same calibration.

A spray mixture containing water and Brilliant Blue dye (FD&C Blue n.1) at 1.5 g L^{-1} was used to determine spray deposit on soybean leaves. When quantifying spray deposits, no fungicide was used in the mix. Meteorological conditions were monitored during all applications by a portable digital thermo-hygrometer and an anemometer (Lutron, HT-3003 and AM-4201); data were recorded at 0.5 m from the top of the soybean canopy. In 2009/10, the temperature was 31 ± 2°C, air relative humidity (RH) was 60 ± 5%, and wind speed was less than 4.4 km h^{-1}. In 2010/11, the temperature was 30 ± 2°C, with an RH of 65 ± 5% and a wind speed of less than 4.8 km h^{-1}. Both applications were performed at the R2 growth stage.

Few minutes after application, 10 leaflets from the top canopy [top leaflets ~1.0 m (2009/10 crop season) and 1.3 m (2010/11 crop season) from the ground], middle [intermediate canopy leaflets ~0.6 m (2009/10), and 0.8 m (2010/11) from the ground] and bottom (last leaflets of the bottom portion ~0.2 m (2009/10) and 0.3 m (2010/11) from the ground) were collected from each plot to determine droplet penetration. Leaflets were placed in a plastic bag and carried to the laboratory. In the laboratory, 30 mL of distilled water were added to each plastic bag, and the content was stirred for approximately 15 seconds to remove the dye. The solution resulting from washing was quantified in a spectrophotometer (Shimadzu UV 1601 PC) at a wavelength of 630 nm. After dye extraction, leaflets were dried and individually measured with an area meter (LI-COR 3100, Lincoln, Neb.). With the dye concentrations of 10.0, 5.0, 2.5, 1.25, 0.625, and 0.15625 mg L^{-1}, a standard curve was generated, which allowed the transformation of the values of absorbance into dye concentration (mg L^{-1}). With the values of dye concentration in the mixture, dye concentration in the leaflets, and the dilution volume of the sample, it was possible to establish the volume captured by the target through Equation 1:

$$\mathrm{V_i} = (\mathrm{C_f} \times \mathrm{V_f})/\mathrm{C_i},$$
(1)

where Vi = volume captured by the target (mL), $\mathrm{C_f}$ = dye concentration detected in the spectrophotometer (mg L^{-1}), $\mathrm{V_f}$ = dilution volume of the sample (30 mL), and $\mathrm{C_i}$ = dye concentration in the spray mixture (1,500 mg L^{-1}).

For better data presentation, the spray volume retained on each leaflet in mL was transformed into nL and divided by its respective foliar area, thus obtaining the quantity in nL cm^{-2}. For comparison purposes, deposit data for different spray volumes were normalized to 110 L ha^{-1}.

Experimental design and Soybean rust assessments

The experimental design to compare SBR control was a randomized block with four replications in a factorial scheme (3 × 2) + 1 with three spray volumes (110, 160, and 210 L ha^{-1}), two application techniques (AAS and CS), plus a non fungicide application treatment (control). The application equipment and calibrations (treatments) as well as the size of the plots were the same as described above.

Weekly monitoring for SBR started at the V8 growth. Disease assessments were performed on leaflets of the bottom canopy, and when the first symptoms in the experimental field were observed [55 days after emergence (DAE) in 2009/10 and 53 DAE in the 2010/11 crop seasons], a fungicide application of pyraclostrobin plus epoxiconazol at 133 + 50 g a.i. ha^{-1} (Opera, BASF S.A. Brazil Inc.) was performed. The other fungicide applications were sprayed using the same fungicide at the same rate. The ranges of meteorological conditions at the moment of fungicide applications were: RH of 65 ± 5%, 29 ± 2°C, and winds less than 2.0 km h^{-1} (first application at R2 - 2009/10); RH of 85 ± 5%, 24 ± 2°C, and winds less than 6.2 km h^{-1} (second application at R3 - 2009/10); RH of 75 ± 5%, 23 ± 3°C, and winds less than 10 km h^{-1} (third application at R5 - 2009/10); RH of 68 ± 5%, 31 ± 2°C ,and winds less than 8.5 km h^{-1} (first application at R2 - 2010/11); and RH of 70 ± 5%, 29 ± 2°C, and winds less than 6.3 km h^{-1} (second application at R4 - 2010/11).

The severity of SBR was assessed on 15 leaflets arbitrarily selected from the middle and bottom sections of the center plot, using a scale from 1 to 6, based on the percentage of disease leaf area (DLA), where 1 = 0.6% DLA, 2 = 2% DLA, 3 = 7% DLA, 4 = 18% DLA, 5 = 42% DLA and 6 = 78.5% DLA (Godoy et al. 2006). Leaves from the top canopy showed no symptoms of SBR and were not considered. The average DLA values (120 leaflets per treatments) were used to plot disease progression curves. The area under the disease progress curve (AUDPC) was calculated for treatments to express the amount of disease over time (Campbell and Madden, 1990), as described in Equation 2:

$$\text{AUDPC} = \sum_{i=1}^{n-1}[(x_{i+1}+x_i)/2]\ \times(t_{i+1}-t_i),$$
(2)

Where; x_i = disease percentage severity at the i^{th} observation, t_i = time (days), and n = total number of observations (six observations in both crop seasons).

At the end of the crop season, the center three soybean rows, at a length of 5 m (6.75 m^2), of each plot were harvested mechanically (Wintersteiger Nursery Master Elite A- 4910 Ried/Austria). Seed moisture was measured, and the seed weight and yield were adjusted to 13% moisture. Yields were calculated in kg ha^{-1} and thousand seed weights (TSWs), determined via an analytical balance (Marte AY 220, São Paulo - SP, Brazil).

Statistical analysis

When necessary, spray deposit, AUDPC, TSW, and yield data were previously transformed to assess the assumptions of normality and homogeneity of the variances considered normal by the Kolmogorov-Smirnov test at 5% probability, keeping the original means. The significance of the factors was determined by analysis of variance, and means were compared using the least significant difference (LSD) test at 5%. Statistical analysis was also performed to compare the means between treatments for AUDPC, TSW, and yield and compared by the LSD test at 5%. Pearson's correlation coefficient was used to evaluate correlations among AUDPC and yield. All statistical analyses were conducted using the statistical software package SISVAR (UFLA 208 - Lavras, Minas Gerais, Brazil) (Ferreira, 2011).

Conclusions

The application spray volume did not have any influence on the amount of spray deposits as well as the use of AAS. As expected, greater values of spray deposits were achieved on leaves from the top soybean canopy compared to the middle and bottom canopies. No spraying technique could improve spray droplet penetration through the soybean canopy.

All treatments that received fungicide application, regardless of the technique and spray volume, showed reduced SBR disease severity compared to the unsprayed control. The fungicide applications using 210 L ha^{-1} provided lower AUDPC values compared to the spray volume of 110 L ha^{-1}. Reducing the spray volume from 210 to 110 L ha^{-1} decreased fungicide efficacy against SBR and TSW, although no significantly soybean yield difference between treatments that received fungicide application was detected.

The choice of the fungicide spray volume application for SBR control may be detrimental to providing satisfactory control. Thus, we believe that fungicide application with a spray volume lower than 160 L ha^{-1} to reduce SBR severity and to avoid yield loss may be not provided by a ground sprayer equipped with hydraulic nozzles.

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