# News Soybean Sowing Arrangements on Spray and Control of Asian Rust

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Received: December 18, 2018	Accepted: February 5, 2019	Online Published: April 15, 2019
doi:10.5539/jas.v11n5p206	URL: https://doi.org/10.5539/jas.v11n5p206	

# Abstract

Asian soybean rust (ASR) is one of the most severe diseases of the soybean crop, and the use of fungicides is the main form of control. Among the updates of soybean production system is adoption of new sowing arrangements. However are still incipient the studies to combine soybean sowing arrangements and pesticide spraying techniques on phytosanitary treatments on this crop. Therefore, the study aimed to evaluate the impact of different soybean sowing arrangements on spraying, ASR control, agronomic parameters and yield. The experimental design was randomized blocks in the subdivided plots scheme, with five sowing arrangements and two application techniques (with and without adjuvant). The spray deposition and coverage levels, the vertical distribution of leaves in the plants on different sowing arrangements. The adjuvant increased spray coverage. The vertical distribution of the leaves and number of branches in the soybean plants is affected by the sowing arrangement. The disease severity and yield were not affected by sowing arrangements.

Keywords: spatial distribution of plants, Application technology, Phakopsora pachyrhizi

## 1. Introduction

The soybean crop is the main commodity of the Brazil and the soybean complex represents 48% of Brazilian agribusiness exports (CONAB, 2018). The growing demand for soybeans requires constant technological upgrades. In this context, recent studies seek to elucidate the benefits of new sowing arrangements in increasing soybean productivity (Heiffig, Câmara, Marques, Pedroso, & Piedade 2006; Modolo et al., 2016). One of the challenges for changing sowing arrangements is the impact on the interception of light and pesticides, as well as the occurrence of pathogens. The Asian rust (*Phakopsora pachyrhizi*), for example, is highly dependent on leaf wetting to develop the epidemic (Alves et al., 2007; W. T. Igarashi, Silva, S. Igarashi, Saab, & França, 2014). Thus, spraying strategies should be increased to the new sowing arrangements in order to reduce the severity of Asian soybean rust (ASR) during the crop cycle.

The addition of adjuvant to fungicide spray may be a differential in phytosanitary treatment. The adjuvants for agricultural use, besides modifying the physicochemical properties of the spray, can alter the size of the spray droplets and the spreading of the spray on the vegetal surface. Such modifications can reduce losses and favor greater deposition of the pesticide at the desired target (Nascimento et al., 2012; Prado, Raetano, Dal Pogetto, Costa, & Christovam, 2015; Baio, Gabriel, & Camolese, 2015). The change in soybean sowing arrangements provides modifications in the architecture and may also increase the deposition levels of the pesticide in certain parts of the plant (Madalosso, Domingues, Debortoli, Lenz, & Balardin, 2010). Although changes in sowing arrangements on the occurrence of ASR are still incipient, as well as the joint adoption with other spraying techniques, such as the addition of adjuvant to the fungicide spray. Thus, the study aims to evaluate the impact of different sowing arrangements on spraying, ASR control, agronomic parameters and soybean yield.

## 2. Method

## 2.1 Installation and Management

The experiment was conducted at the São Paulo State University (UNESP), the School of Agriculture in Botucatu, in an area belonging to the Teaching, Research and Extension Farms-FEPE.

The sowing was carried out on November 19, 2015, using a seed cultivator (Semeato<sup>®</sup>, model SHM 15/17) and soybean cultivar 5D 634 (Dow AgroSciences<sup>®</sup>) of undetermined growth. Fertilization was carried out at the time of sowing with 200 kg of commercial formulation 04-20-20 (N-P-K) for all treatments, depending on soil chemical analysis.

Five sowing arrangements were installed: (1) spacing of 0.40 m between rows, denominated conventional (CO); (2) perpendicular spacing (90° angle) of 0.40 m between rows, of CO, denominated crossed row (CR); (3) spacing with two soybean rows spaced apart with an "internal" spacing of 0.20 m and an "external" spacing of 0.40 m between rows, denominated double row (DR,  $20 \times 40$ ); (4) spacing with two soybean rows spaced apart with an "internal" spacing of 0.60 m between rows, denominated double row (DR,  $20 \times 40$ ); (4) spacing with two soybean rows spaced apart with an "internal" spacing of 0.60 m between rows, denominated double row (DR,  $20 \times 40$ ); (2) spacing of 0.20 m and "external" spacing of 0.60 m between rows, denominated double row (DR,  $20 \times 60$ ) and (5) spacing of 0.20 m between lines, denominated narrow row (NR).

The emergence of the plants occurred five days after sowing. The population of soybean plants was maintained at 400 thousand plants per hectare, after thinning at 21 days after emergence (DAE).

To follow the development of the culture and to indicate the moment of the management a scale of phenological stage was used (Fehr & Caviness, 1977).

Weed control was performed with a spray of glyphosate herbicide (Roundup WG<sup>®</sup>), at the dosage of 1.5 kg ha<sup>-1</sup>, at the vegetative stage V3. During the conduction of the crop, weekly insect-pest monitoring was carried out using the vertical beating cloth method.

#### 2.2 Application and Evaluations

In order to elucidate the effects of the soybean sowing arrangements on the spraying, the levels of spray deposit in the leaflets, the spray coverage in artificial targets and the vertical distribution of the leaves in the soybean plants were evaluated.

The applications were performed with a spray (Jacto<sup>®</sup>, model Condor 600 AM 12), mounted on tractor (New Holand<sup>®</sup>, model 3030), in 3<sup>rd</sup> gear reduced, at 1800 rpm on the engine to get 540 rpm on PTO, and speed of the tractor-spray assembly of 5.14 km h<sup>-1</sup>, pressure of 172.5 kPa and spray nozzles (Jacto<sup>®</sup>, model AXI 11002). The calibration of the sprayer resulted in a spray volume of 150 ha<sup>-1</sup>.

According to the variables involved in this study (presence or absence of adjuvant), different solutions were prepared, using the addition of the fungicide trifloxystrobin associated with prothioconazole (Fox<sup>®</sup>) and the bright blue marker, at the doses of 0.4 L ha<sup>-1</sup> and 1.5 g L<sup>-1</sup>, respectively.

For the preparation of the second solution, after the first application, the tank was depleted and cleaned. Then the same components were added and in the same amounts, in addition to the soybean oil methyl ester (Aureo<sup>®</sup>) at the doses of 0.25 L in 100 L<sup>-1</sup>. At the end of each spray, a sample of the spray mixture was collected for further dilution and the calibration curve was elaborated.

After the spraying, 20 leaflets were collected, individually, in plastic bags, by subplot. From these leaflets, ten came from the upper third of the plants, collecting the central leaflet most exposed to the application, with fully developed leaf and ten leaflets of the lower third of the plant, opting to highlight the central leaflet coming from the leaf of the second node from bottom to top.

The leaves samples were sent to the Laboratory of Technology Pesticides Application (LTPA), belonging to the Department of Plant Protection, School of Agriculture, Botucatu (FCA/UNESP). The leaves were washed with 40 mL of distilled water and shaken manually for removal of the marker dye. The resulting solution was placed in plastic bottles and kept under refrigeration ( $8\pm3$  °C) for 12 hours. After this period, the samples were analyzed in spectrophotometer (Shimadzu UV 1601 PC) to read the absorbance at the wavelength of 630 nm. After the extraction of the marker the area of the leaflets was measured with foliar bench area meter (LICOR, model LI-3100).

The samples collected in the field with the presence of the marker dye were diluted in known concentrations. Based on the spectrophotometer reading, it was possible to construct a calibration curve and calculate the concentration of the dye in each of the samples (mg  $L^{-1}$ ). Finally, using the equation:

$$C_1 V_1 = C_2 V_2 \tag{1}$$

it was possible to establish the volume captured by the target (mL). For better exposure, the data were transformed to microliter ( $\mu$ L) and then divided by the respective leaflet area. Results were obtained in the volume of spray per leaf area ( $\mu$ L cm<sup>-2</sup>) (Bauer & Raetano, 2000; Palladini, Raetano, & Velini, 2005).

In order to compare the spray deposits in the upper and lower parts, the ratio between both (ratio = lower/upper) was calculated. That is, the closer to 1 is the ratio, the more similar are the deposits between the parts of the plants.

For the evaluation of the spraying coverage, horizontally arranged, water-sensitive papers were distributed on a wooden stake. The stem was adjusted so that the top card approached the top of the plant and the bottom card approached the bottom of the plant. After the treatments were applied, the samples were collected, identified and transported to the LTPA. The images of the paper cards were scanned on a desktop scanner with the resolution of 600 dpi. Then the images were analyzed by the software Gotas<sup>®</sup>, from Embrapa (Chaim, Camargo Neto, & Visoli, 2012).

The vertical distribution of leaf area in soybean plants was evaluated at the reproductive phenological stage R2, close to fungicide applications. For this, ten plants of each subplot were collected and sent to the LTPA. The height was measured from the base to the apex of the plants, with the aid of a ruler. The number of branches was counted and the plants were divided into three equal parts (upper, middle and lower). The leaflets of the respective parts were highlighted. With the aid of the bench leaf area meter it was possible to measure the total area of the leaflets in each of the plant parts.

To clarify the effect of soybean sowing arrangements on ASR severity, the disease was monitored with the help of a diagrammatic scale (Godoy, Koga, & Canteri, 2006). For this, ten leaflets were collected weekly from the bottom of the plants of each subplot, beginning after flowering.

The productivity of each sowing arrangement was calculated after manual harvesting. Made in two samples of 1.0 m<sup>2</sup>, with the aid of a wooden frame, in the central lines of the plots. The soybean was threshed with a forklift attached to the power take-off of the tractor. After collection, the masses of the samples were measured with a digital scale (Toledo<sup>®</sup>, 0.002 kg accuracy) and humidity by the benchtop meter (Gehaka<sup>®</sup> AGRI, model G929). The productivity data were adjusted to a moisture content of 13%.

## 2.3 Experimental Design and Analysis

The experiment was installed and conducted in the experimental design of randomized blocks in the subdivided plots scheme. The factors analyzed were adequate according to the variables of interest, so the deposition and spray coverage levels were composed of 5 sowing arrangements  $\times$  2 application techniques (fungicide spray with and without adjuvant), in 4 replicates. For the analysis of ASR severity and crop productivity, 5 sowing arrangements  $\times$  3 application techniques (with air assistance in the bar, without air assistance and control without application) were considered in 4 replications. Finally, the analysis of the vertical distribution of leaf area in soybean plants considered the 5 sowing arrangements, in 4 replicates.

The data were submitted to analysis of variance by the F test at 5% probability, in case of significant difference, the means were compared by the Tukey test at 5% probability. The variables of deposit and spray coverage, disease severity and vertical distribution of leaf area in soybean plants were transformed into  $\sqrt{x + 1}$ . Statistical analysis of the data was performed using the statistical software SISVAR<sup>®</sup>, version 5.3 (Ferreira, 2011).

## 3. Results and Discussion

The isolated analysis of the factors considered in the experiment demonstrates that there was no influence of the soybean sowing arrangement on the deposition of the spray in the upper and lower parts of the soybean plants (P > 0.05).

The deposit levels in the leaflets of the upper part of the plants were not influenced by the sowing arrangement, probably due to the similarity between the heights of the plants in the different arrangements and proximity of the spray tip. The descriptive analysis of plant height data at the moment of spraying shows a coefficient of variation of 4.3% and a mean of 89.2 cm, showing the similarity of this parameter (Data not shown).

In addition, there was also no difference in the amount of spray deposited in the leaflets present in the lower part of the plant (P > 0.05). The amount of spray recovered in the lower part of the plant is often reported as lower in relation to the other parts of the plant and, given this effect, the physical barrier of leaves to the spraying transposition (Bauer & Raetano, 2000; Cunha, Reis, & Santos, 2006; Cunha & Peres, 2010; Prado et al., 2015). This natural feature makes it impossible for the homogenous vertical distribution of the spray throughout the plant. This fact is reinforced by the analysis of the ratio between the deposit levels of the lower and upper parts.

Regardless of the arrangement used, the average deposit at the bottom of the canopy does not reach 30% of the deposit found at the top of the crop (Table 1).

The adjuvant added to the spray provided an increase in the deposit levels in the leaflets of the lower part of the soybean plants, according to Table 1 (P < 0.05). The alteration on physical and chemical properties of the spray mixture by the adjuvant may have influenced the deposition levels obtained in the lower part of the plant. The addition of the soybean oil methyl-ester adjuvant to the spray may reduce the volumetric median diameter of the spray droplets relative (Sasaki et al., 2015). The reduction of the diameter of the droplets in turn provides greater penetration of the spray droplets in the canopy of the soybean crop (Hanna, Robertson, Carlton, & Wolf, 2009).

The increment of the deposit at the bottom, provided by the adjuvant, can also be verified by calculating the ratio between the bottom and top. The presence of adjuvant in the spray resulted in a 61.5% higher ratio to the spray without adjuvant, thus representing a less heterogeneous distribution.

Table 1. Average values of the spray deposits ( $\mu$ L/cm<sup>2</sup>) in different sowing arrangements [without (SA) and with adjuvant (CA)<sup>1</sup>] and parts of the soybean plants [upper and lower], phenological stage R3. Botucatu-SP, January 29, 2016

Sowing arrangement	Upper	Lower	Ratio	
1. Conventional	0.375 a	0.061 a	0.16	
2. Crossed row	0.419 a	0.105 a	0.25	
3. Double row $(20 \times 40)$	0.374 a	0.087 a	0.23	
4. Double row $(20 \times 60)$	0.384 a	0.057 a	0.15	
5. Narrow row	0.350 a	0.075 a	0.21	
Adjuvant	Upper	Lower	Ratio	
Without adjuvant (SA)	0.414 a	0.065 b	0.16	
With adjuvant (CA)	0.346 a	0.089 a	0.26	
CV 1 (%)	2.71	1.56	-	
CV 2 (%)	3.88	1.07	-	

*Note.* <sup>1</sup> Fungicide spray (triflostrobin + prothioconazole), without and with the presence of adjuvant (methyl ester of soybean oil).

Means followed by the same letters in the column do not differ by the Tukey test (P < 0.05).

The spray coverage in the artificial targets was not influenced by the interaction between the variables: sowing arrangement and adjuvant (P > 0.05). The different sowing arrangements of the soybean did not provide increases in the average spray coverage, regardless of the part of the evaluated plant (P > 0.05), although the samples of the water-sensitive papers were distributed between the lines of the soybean plants. At the time of application the crop was in the phenological stage R3, probably the closing between the lines may have mitigated the effect of the sowing arrangements in the spray coverage of these artificial targets. Other studies have already reported the difficulty of penetration of the spray in the soybean canopy in a conventional sowing arrangement (lines spaced 0.45 m) at the reproductive development stages (Bauer & Raetano, 2000; Cunha et al., 2006).

On the other hand, the presence of the adjuvant was sufficient to provide greater coverage of the targets in the middle and lower parts of the canopy. The variation in the volumetric median diameter of the droplets, probably provided by the adjuvant, may reflect higher spray penetration in the canopy of the soybean crop (Nascimento et al., 2012; Sasaki et al., 2015).

However, the reduction in spray droplet spectra may lead to greater penetration of the droplets in the canopy and consequently increase the coverage of the artificial targets (Oliveira, Balan, Foncesa, & Saab, 2012). Thus, the use of the soybean methyl-ester adjuvant in the fungicide spray proved to be efficient in increasing the spray coverage on the target in the middle and lower parts of the plant (Table 2).

It should also be considered that the spray tip (Cunha & Peres, 2010) can also influence the droplet spectrum resulting from the addition of the adjuvant and increasing the number of droplets smaller than 100  $\mu$ m can increase the losses in the application (Nuyttens etal., 2009).

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Sowing arrangement	Upper	Lower	Ratio
1. Conventional	45.3 a	19.5 a	0.43
2. Crossed row	42.0 a	17.4 a	0.41
3. Double row (20x40)	34.5 a	27.3 a	0.79
4. Double row (20x60)	40.9 a	23.9 a	0.58
5. Narrow row	37.9 a	18.7 a	0.49
Adjuvant	Upper	Lower	Ratio
Without adjuvant (SA)	38.9 a	17.2 b	0.44
With adjuvant (CA)	41.3 a	25.5 a	0.62
CV 1 (%)	13.55	22.55	-
CV 2 (%)	12.70	16.60	-

Table 2. Average values of spray coverage (%), in different sowing arrangements, without (SA) and with adjuvant  $(CA)^1$ , and parts of soybean plants (upper, middle and lower), phenological stage R3. Botucatu-SP, January 29, 2016

*Note.*<sup>1</sup> Fungicide spray (triflostrobin + prothioconazole), without and with the presence of adjuvant (soybean oil methyl ester) in the spray.

Means followed by the same letters in the column do not differ by the Tukey test (P < 0.05).

The leaf area of the soybean plants was influenced by the sowing arrangement, when compared inside the upper and lower parts (P > 0.05). However, it is possible to observe that more than half of the leaflets are present in the upper third of the soybean plants, regardless of the sowing arrangement used (Table 3).

By the levels of the spray deposits in the different parts of the soybean plant, in this and other works, it is evident that the upper part receives larger quantities of spray when compared to the other parts of the plant. Considering that different sowing arrangements may interfere with the distribution of leaves in the plants (Table 3), the amount of intercepted spray may be different in the upper, middle and lower parts of the plant. Thus, the upper part of the canopy may be benefited by larger amounts of deposit of the spray, either by the absence of physical barrier or by the greater proximity of the spray jet, especially to the arrangement which provides greater amount of leaves in that part of the plant.

When finding a larger leaf area in the upper third of the plants distributed in the conventional sowing arrangement, it can be inferred that this arrangement provides conditions to obtain higher levels of spray deposits in the leaves than the other arrangements. In this way, the lower leaves of the plants, more prone to *P. pachyrhizi* attack, lose importance relative to the values of the deposits due to the sowing arrangement.

On the other hand, the arrangement of double row  $(20 \times 40)$  sowing showed lower leaf area in the upper part, and higher in the lower part of the plant, resulting in a less heterogeneous distribution of leaves in the plant (Table 3). The distribution of leaves in this arrangement may be less favorable to the control of pathogens present in the lower part of the plants in relation to the other sowing arrangements. However, it should be noted that this arrangement may provide a different response in the plant morphology when the cultivar is altered (Balbinot, Procópio, Debiasi, & Franchini Jr., 2014; Procópio, Balbinot Junior, Debiasi, Franchini, & Panison, 2014).

In the narrow row arrangement the leaf area was smaller in the lower part of the plants. The reduction of the leaf area in this sector of the plants of greater difficulty of deposition of the spray can make a strategy to increase the protection of the plants by means of spraying. However, the leaf arrangement may interfere with the interception of sunlight and consequently the production (Heiffig et al., 2006; Madalosso et al., 2010).

The sowing of soybean in the conventional arrangement provided a higher average number of branches per plant (Table 3). The number of branches per plant, as well as number of pods and number of grains, is considered a production component and in the absence of other variables is usually proportional to productivity.

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Sowing arrangement	Upper	Middle	Lower	Total	Branches
1. Conventional	791.7 a	462.8 a	36.2 ab	1,291 a	11.8 a
2. Crossed row	518.6 ab	291.4 a	36.2 ab	846 a	8.3 ab
3. Double row $(20 \times 40)$	477.2 b	336.1 a	76.8 a	890 a	8.9 ab
4. Double row $(20 \times 60)$	597.1 ab	286.2 a	54.9 ab	888 a	7.0 b
5. Narrow row	622.6 ab	235.5 a	4.7 b	863 a	7.3 ab
CV (%)	22.73	36.24	93.23	21.02	15.32

Table 3. Average values of leaf area (cm<sup>2</sup>) and number of branches, in different sowing arrangements and parts of soybean plants (upper, middle and lower), phenological stage R2. Botucatu-SP, January 18, 2016

*Note.* Means followed by the same letters in the column do not differ by the Tukey test (P < 0.05).

There was no impact of the sowing arrangements or the addition of adjuvant to the spray in the ASR control (Table 4). On the other hand, the absence of fungicide application resulted in a greater severity of the disease in the phenological stage R5.3.

The onset of the epidemic occurred only at the stage of grain filling. This fact may have contributed to attenuate the effects of sowing arrangements on the progress of ASR disease (Roese, Melo, & Goulart, 2012).

Although some planting arrangements increase the period of leaf wetting, it does not necessarily result in greater disease intensity or anticipation of symptoms (Igarashi et al., 2014). This result indicates that the intensity of the Asian rust epidemic is not affected by sowing arrangements. Therefore, the occurrence of ARS is not a limiting factor for the selection of soybean sowing arrangements.

Table 4. Mean values of disease severity (%), in different sowing arrangements, without (SA), with adjuvant (CA) and without application (T) <sup>1</sup>, phenological stage R5.3. Botucatu-SP, January 26, 2016

Sowing arrangement	Severidade (%)
1. Conventional	8.9 a
2. Crossed row	10.8 a
3. Double row $(20 \times 40)$	12.1 a
4. Double row $(20 \times 60)$	8.3 a
5. Narrow row	9.5 a
Adjuvant	Severidade (%)
Without adjuvant (SA)	0.5 b
With adjuvant (CA)	0.4 b
Without application (T)	28.9 a
CV 1 (%)	31.9
CV 2 (%)	33.2

*Note.* <sup>1</sup>Fungicide spray (triflostrobin + prothioconazole), without, with adjuvant (methyl-ester of soybean oil) in the spray and without application

Means followed by the same letters do not differ by the Tukey test (P < 0.05).

There was no interaction effect of sowing arrangements and adjuvant use (P > 0.05) on soybean yield (Table 5). The highest productivity was obtained with the conventional sowing arrangement and the lowest one in the double row arrangement ( $20 \times 60$ ), evidencing a relation with the number of branches per plant in the different sowing arrangements.

In a subdivided plots scheme, the error associated with the main plots, in this case, the sowing arrangements, is larger compared to the error associated with the subplots (Gomes, 1987). This fact may explain that despite remarkable differences between productivities, they were not significant according to the F test (P > 0.05). However, in the literature, soybean cultivars exhibit high plasticity and even in different arrangements, the productivities are similar (Heiffig, 2006; Peixoto et al., 2000).

There was no difference in productivity when the adjuvant was added to the spray liquor. Despite the increases obtained in the deposition and spray coverage levels, the addition of the adjuvant to the spray did not contribute to increase productivity.

Table 5. Average productivity (soybean bag/hectare), in different sowing arrangements, without (SA), with adjuvant (CA) and without application (T). Botucatu-SP, March 21, 2016

Sowing arrangement	Yield (soybean bag/hectare)
1. Conventional	70.6 a
2. Crossed row	62.7 a
3. Double row $(20 \times 40)$	60.2 a
4. Double row $(20 \times 60)$	46.2 a
5. Narrow row	54.4 a
Adjuvant	Yield (soybean bag/hectare)
Without adjuvant (SA)	62.9 a
With adjuvant (CA)	58.2 a
Without application (T)	55.4 b
CV 1 (%)	31.6
CV 2 (%)	13.6

*Note.* Means followed by the same letters do not differ by the scott-knott test (P < 0.05).

## 4. Conclusion

The soybean sowing arrangement does not influence spray deposits and cover;

Addition of adjuvant to the spray did not influence deposit levels, but increased spray coverage;

The soybean sowing arrangement affects the vertical distribution of the leaves and number of branches;

The leaf area in the upper part of the plants and the number of branches are larger in the plants arranged in the conventional sowing arrangement in relation to the others;

The severity of the Asian soybean rust disease (ASR) was not influenced by the sowing arrangement either by the addition of adjuvant to the fungicide spray;

The soybean yield was not affected by the sowing arrangement.

#### Acknowledgements

The authors are grateful to the institutions of the São Paulo State University (Unesp), School of Agriculture (FCA/UNESP): Laboratory of Technology Pesticides Application (LTPA) for the support in the evaluations and conduction of the experiment; (FEPE), for the support in the installation and conduction of the experiment and the Research Group on Integrated Management of Pests in Agriculture (AGRIMIP), for the support in the evaluations.

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