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TRANSLOCATION OF FUNGICIDES IN SOYBEAN PLANTS ASSOCIATED WITH THE COPPER SYSTEMIC CHEMICAL COMPLEX

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ABSTRACT

Soybean is a crop with a high leaf area index, which harms the cover effect of agrochemical sprays. Improvements in the penetration and translocation of active ingredients in plants can optimize the efficacy of systemic fungicides. The Asian soybean rust is a polycyclic disease whose infection begins in the lower leaves of the plants, where fungicides have difficulties to work. The objective of this study was to investigate the translocation of seven fungicidal active ingredients associated with the copper systemic chemical complex. This product combined with different agrochemicals optimizes this physiological process by plants under field conditions. The translocation of the active ingredients sprayed on the upper part of the plants to the lower parts protected from the applications was measured. Two trials were conducted in randomized block design with three fungicides and four replications, in a double factorial scheme. Soybean plants were grown in a greenhouse, performing a single spray of the treatments, with subsequent detection of the compounds by high-performance liquid chromatography. In all treatments were compared, the association and non-association with copper complex and fungicides. As results, combinations with copper systemic chemical complex improved the translocation of sprayed parts to those protected from the sprays of five active ingredients: prothioconazole-desthio, trifloxystrobin, picoxystrobin, tebuconazole and pyraclostrobin. Copper complex did not improve only the translocation of bixafen and fluxapyroxad.

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INTRODUCTION

Some biological targets have acquired resistance very quickly to active ingredients, as ea. *Phakopsora pachyrhizi* (Hess and Nodari, 2022) and (Godoy *et al.*, 2020). The lack of knowledge of basic practices, such as the rotation of chemical groups and modes of action of active ingredients, as well as product combinations and the compatibility of formulations used in tank mixes, are persistent. The reduction in the volumes of sprayed agrochemicals, which aims to optimize the operability of work in

phytosanitary management, also deserves attention.

Regarding disease management in soybean crops, it is necessary to adopt preventive applications with fungicides, alternating and combining different chemical groups and active ingredients (Zuntini *et al.*, 2019) and (Netto *et al.*, 2020). At the same time, soybean farmers have been reducing the rate of spraying in field conditions, a scenario that is challenging for performance of pesticides.

Soybean is a crop with a high LAI (leaf area index),

where values between 6.0 and 6.5 at the peak of the vegetative phase of the plants (where 1 m² of area occupied by plants contains between 6.0 and 6.5 m² of leaf area of these same plants) were those that maximized soybean productivity (Tagliapietra *et al.*, 2018). The uniform maintenance of healthy leaves in these three thirds of the plants is extremely important for good productivity (Boller, Forcelini and Hoffmann, 2007) and (Debortoli *et al.*, 2006).

Assuming that spray volumes of less than 60 L ha⁻¹ are used in field situations, approximately 6 mL m⁻² are applied. Extrapolating this value to the LAI of soybeans, approximately 1 mL m⁻² is applied. This does not consider the umbrella effect caused by higher stand of plants during spraying (Holtz *et al.*, 2014). It is also worth mentioning the eminent losses due to drift and evaporation of the sprays (Maciel *et al.*, 2017).

Studies with fungicides in soybean crops, with an application rate of 100 L ha⁻¹, found coverage between 72.90 and 82.97% (upper third), 31.17 and 36.17% (middle third) and 7.23 and 14.00% (lower third) (Antuniassi *et al.*, 2004). Comparing the middle third with the upper third, there was a 56.70% lower coverage and contrasting the lower third with the middle and upper thirds, there were lower coverages of 68.50% and 86.40% (Antuniassi *et al.*, 2004).

Therefore, there is antagonism. On the one hand, for high productivity in soybeans, a high leaf area index is necessary. On the other hand, there is a latent difficulty for phytosanitary sprays to reach the plant as a whole, with adequate droplet population and deposition uniformity in three thirds of the plants.

Improved translocation of systemic fungicides in soybean plants could optimize this management. However, studies on this topic are still quite limited. Very little is known about the penetration and mobility of active ingredients in plants. There are virtually no measurements of the amount of these compounds that penetrate the leaves, and whether any of them can follow alternative sap flows. Since most of the spray mixture reaches the upper third of the crops, data collection on the protection of the middle and lower thirds with these products is still limited.

In this context, it is necessary to search for new tools that improve the translocation of active ingredients in soybean plants. This study investigated the copper systemic chemical complex (CuSCC), testing the commercial product Fulland® registered as a copper

foliar fertilizer. The product's patent holder explains that its production is done by molecular fusion with several active ingredients and additives. This gives the product high systemicity, allowing it to move upward and downward through the plants, optimizing the combined pulverized active ingredients.

The objectives of this study were: to determine by high-performance liquid chromatography whether the copper systemic chemical complex favors the translocation of seven fungicidal active ingredients in soybean plants. All treatments were sprayed only on the upper part of soybean plants, detecting and measuring the translocation of the sprayed active ingredients to the lower parts protected from the applications, artificially simulating the umbrella effect on fields conditions.

MATERIAL AND METHODS

Two trials (duplicate) were conducted between June and November 2023, in a greenhouse owned by Biosfera Serviços Inteligentes, at Araguari city, Minas Gerais state, Brazil, with geographic coordinates 18°40'9.28" South latitude, 48°9'59.02" West longitude, and an average altitude of 948 m. The local climate is Aw (humid tropical, with hot summers and dry winters). The average annual precipitation is 1,250 mm and the average annual temperature is 22.5°C. Araguari city was chosen because this local adopts high technologies in agriculture and is located in the center of the Brazilian's Cerrado.

Six soybean seeds of the Brasmax Desafio RR 8473RSF variety were sown in flexible pots with a volume of 8.5 liters, using only soil from an agricultural area as substrate. Sprinkler irrigation was performed daily. Thirty days after emergence, the three worst plants were thinned, with only three plants per pot being planted until the end of the work, according to the schedule (Table 1).

The trials were conducted in a randomized block design with four replications, in a double factorial scheme (2 x 3). The first factor was the addition and absence of CuSCC (copper systemic chemical complex) and the second factor, three commercial fungicides: 1) Blindado® + Rumba® (Tepiman + EMOS1); 2) Fox Xpro® + Aureo® (Protribi + EMOS2) and 3) Orkestra® + Assist® (Flupi + OM). These products were chosen because they are mixtures with different modes of fungicidal action. Fox Xpro® being one of the most widely used products on the soybean market, Blindado® being an innovative formulation (oil dispersion) and Orkestra® being a product in declining use (Table 2).

Table 1. Activity schedule.

Activity	Trial 1	Trial 2
Sowing soybeans in pots	07/07/2023	09/20/2023
Soybean emergence	07/16/2023	09/27/2023
Vessel thinning	08/15/2023	10/27/2023
Sprays	09/14/2023	11/23/2023
Sample collection	09/16/2023	11/25/2023
Freezing of samples	09/16/2023	11/25/2023
Sending samples to the Laboratory	12/01/2023	12/01/2023

Table 2. Treatments studied in the trials.

Addition of CuSCC	Rate mL ha ⁻¹	Fungicide	Rate mL ha ⁻¹	Time of single application
No CuSCC	-	Tepiman + EMOS1	2250 + 500	60 DAE*
		Protribi + EMOS2	500 + 375	60 DAE*
		Flupi + OM	350 + 500	60 DAE*
With CuSCC	500	Tepiman + EMOS1	2250 + 500	60 DAE*
		Protribi + EMOS2	500 + 375	60 DAE*
		Flupi + OM	350 + 500	60 DAE*

DAE*: days after emergency. **Key:** Tepiman: tebuconazole + picoxystrobin + mancozeb (PC Blindado®); EMOS1: soybean oil methyl ester (PC Rumba®); Protribi: prothioconazole + trifloxystrobin + bixafen (PC Fox Xpro®); EMOS2: soybean oil methyl ester (PC Aureo®); Flupi: fluxapyroxad + pyraclostrobin (PC Orkestra®); OM: non-ionic mineral oil (PC Assist®).

Each experimental plot consisted of 4 pots with 3 plants, totaling 12 plants per plot. In total, each trial consisted of 24 plots, totaling 96 pots and 288 plants. At 60 days after soybean emergence (between the phenological stages V_N and R₁, approximately half of the cycle), the plants received one single spray of the treatments, with a manual sprayer. The volume of spray used was 160 L ha⁻¹.

¹. The parameterization of the application rate for the experimental plot was: 160 L ha⁻¹ in 360,000 plants ha⁻¹ (soybean variety stand) corresponds to 0.45 mL plant⁻¹. Since each plot had 12 plants (4 pots with 3 plants each), 5.35 mL of total spray was uniformly sprayed in each experimental plot (Figure 1).



Figure 1. Detail of the experimental plots; 1.1) 4 pots that made up one experimental plot, whit three plants for pot, and twelve plants in total plot; 1.2) four pots grouped together (two by two) with plastic bag protection for spraying only the upper part of the plants (artificially simulating the umbrella effect).

The aim of this stage of the work was to detect and quantify the active ingredients of fungicides that translocated to the lower part of the soybean plants. Therefore, the sprayings were carried out in a localized

manner only on the upper part of the plants. The lower parts were protected during spraying with plastic bags (Figure 2).

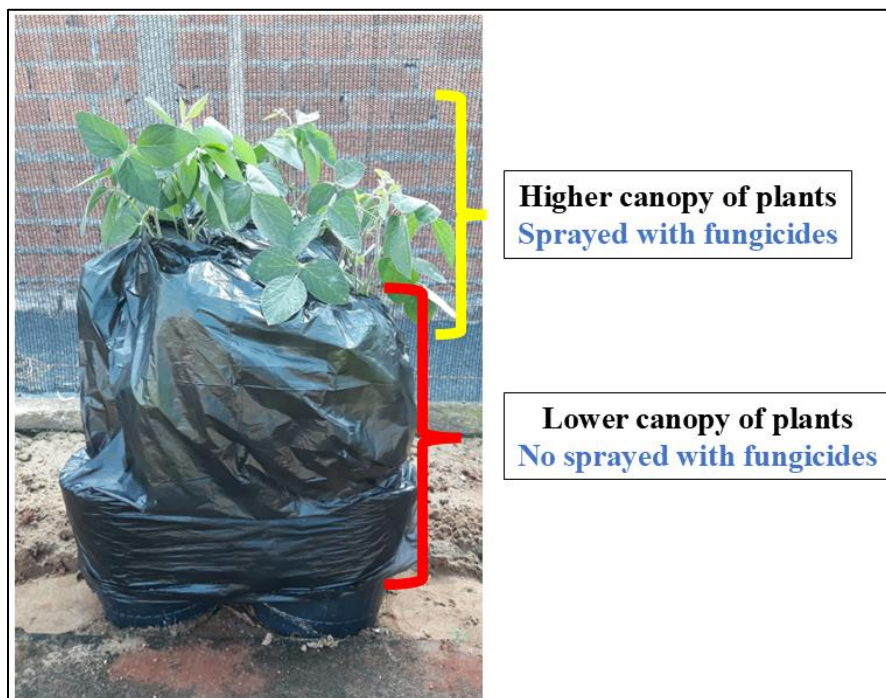


Figure 2. Experimental plot, with the protection of the plastic bag. Highlighted in yellow: higher canopy of plants, sprayed with treatments. Highlighted in red: lower canopy of plants, no sprayed with the treatments (artificially simulating the umbrella effect).

In each experimental plot, the plants were tied with strings, demarcating the sprayed part from the protected part. Spraying was done from 4:00 p.m. onwards, only on the upper leaves of the plants (Figure 3) that were unprotected by the plastic bags. Three hours after the applications, the plastic bags were removed. Exactly 48 hours after spraying, all samples of plant leaves and stems were collected. After spraying, the plants did not receive any treatment, nor did they receive rain or irrigation. The sprayed upper part of the plants (Sample 1) and the unsprayed lower part (Sample 2) were cut. These two distinct samples were stored and identified in zip lock bags, free of residues.

Thirty minutes after collecting and storage in plastic bags, all samples were frozen in a freezer at

temperatures below -15°C . The freezer was specifically designed for storing the samples, remaining sealed until the date they were transported by car to the Center for Advanced Research in Matology (NUPAM), School of Agronomic Sciences (FCA/UNESP), Botucatu city, São Paulo state, Brazil. Thermal boxes with hard artificial ice were used to keep the samples frozen during transportation.

At NUPAM there was quantification of the active ingredients present in the leaves of the upper and lower parts of the soybean canopy, by high performance liquid chromatography (LC-MS/MS). The concentrations and quantities of fungicides applied to the experimental plots are shown in Table 3.

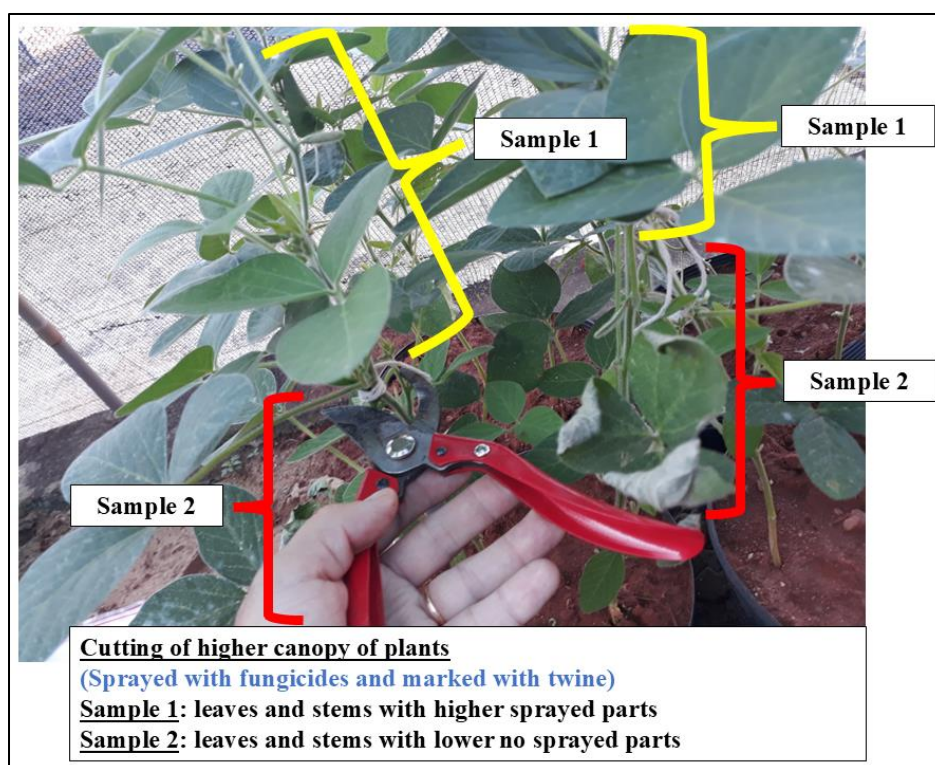


Figure 3. Detail of the collection of samples from the upper and lower parts of the plants. Sample 1, highlighted in yellow: higher canopy of plants, sprayed with treatments. Sample 2, highlighted in red: lower canopy of plants, no sprayed with the treatments (artificially simulating the umbrella effect of spraying).

Table 3. Concentrations of fungicides applied to the experimental plots.

Addition	Rate mL	Fungicide	Active	Conc.	µg
CuSCC	ha ⁻¹		ingredient	g L ⁻¹	plot ⁻¹
No CuSCC	-	Tepiman	tebuconazole	33.33	2.67
			picoxystrobin	26.66	2.14
			mancozeb*	400.00	32.10
		Protribi	prothioconazole	175.00	3.12
			trifloxystrobin	150.00	2.68
			bixafen	125.00	2.23
		Flupi	fluxapyroxad	167.00	2.08
			pyraclostrobin	333.00	4.16
		With CuSCC	500	Tepiman	tebuconazole
picoxystrobin	26.66				2.14
mancozeb*	400.00				32.10
Protribi	prothioconazole			175.00	3.12
	trifloxystrobin			150.00	2.68
	bixafen			125.00	2.23
Flupi	fluxapyroxad			167.00	2.08
	pyraclostrobin			333.00	4.16

mancozeb*: due to the characteristics of the active ingredient and limitations of the laboratory method, it was not possible to detect and quantify this compound. **Key:** µL plot⁻¹ (volume of spray applied per experimental plot); Conc. g L⁻¹ (concentration of the active ingredient in the commercial product); µg plot⁻¹ (amount of active ingredient applied per experimental plot). Acronyms: Tepiman: tebuconazole + picoxystrobin + mancozeb (PC Blindado®); EMOS1: soybean oil methyl ester (PC Rumba®); Protribi: prothioconazole + trifloxystrobin + bixafen (PC Fox Xpro®); EMOS2: soybean oil methyl ester (PC Aureo®); Flupi: fluxapyroxad + pyraclostrobin (PC Orkestra®); OM: non-ionic mineral oil (PC Assist®).

Except for mancozeb, all active ingredients described in Table 3 were detected and quantified through leaf extraction of these compounds. Following the NUPAM laboratory methodology, the soybean leaves were macerated in a mortar with liquid nitrogen, packaged in 15 mL falcon tubes and subjected to the lyophilization process with the Alpha 2-4 LD Plus[®] lyophilizer (Martin Christ, DE) at a temperature of -70°C for 72 hours. After lyophilization, a 100 mg sample was removed and 10 mL of the extraction solution (80:20 phase, methanol:water) was added. The samples were then subjected to an ultrasound bath for 30 minutes and centrifuged at 5,000 rpm for 5 minutes at 20°C. A volume of 1.5 mL of supernatant was collected and filtered through a Millex HV Millipore 0.45 µm filter with a 12 mm durapore membrane and packaged in an amber Analytical vial. 9 mm, for subsequent quantification of

compounds by LC-MS/MS.

The LC-MS/MS system consisted of a Shimadzu[®] Proeminence UFLC High Performance Liquid Chromatograph (HPLC). The chromatograph was equipped with two LC20AD pumps, a SIL-20AC autoinjector, a DGU-20A5 degasser, a CBM20A controller system (which allows fully automated operation) and a CTO-20AC oven (for column temperature control). A 4500 (Applied Biosystems[®]) hybrid triple quadrupole mass spectrometer was coupled to the HPLC.

48 analyses were performed in each trial, totaling 96 analyses. 24 samples from the directly sprayed upper part and 24 samples from the non-sprayed lower part (6 treatments and 4 replicates) were analyzed for each of the two trials. Tables 4 and 5 provide details of the chromatographic method.

Table 4. Chromatographic method used in the leaf extraction of compounds.

Chromatographic Method	
Column	Synergi 2.5µ Hydro-RP 100Å, dimensions 50 x 4.6 mm
Flow	0.6 ml min ⁻¹
Injection	20 µl
Oven	40°C
Mobile Phases	Phase (A) Water with 0.1% acetic acid Phase (B) Methanol with 0.1% acetic acid
Gradient Mode	Initial 95% Phase (A) and 5% Phase (B)
Race time	10 minutes
Running times (min)	Solvent (%)
1 min	30 Phase (B)
2 min	95 Phase (B)
6 min	95 Phase (B)
8 min	30 Phase (B)
10 min	End

Table 5. Masses and fragments used in the chromatographic method.

Active Ingredient	Mass	Fragment	Active Ingredient	Mass	Fragment
picoxystrobin	367.988	204.900	protioconazole-desthio	311.948	124.800
	367.988	145.000		311.948	89.000
	367.988	115.000		311.948	99.100
pyraclostrobin	387.992	193.900	tebuconazole	308.168	69.900
	387.992	163.300		308.168	124.800
	387.992	164.000		308.168	150.900
trifloxystrobin	409.036	185.900	bixafen	413.685	393.900
	409.036	145.000		413.685	266.000
	409.036	206.000		413.685	373.900
prothioconazole	343.935	325.900	fluxapyroxad	381.790	362.000
	343.935	154.000		381.790	341.900
	343.935	124.800		381.790	234.000

Data analysis was with the software R Core Team (2023) using the student's t-test ($p \leq 0.05$) with two-tailed distribution and pairwise data analysis (joint analysis of the two experiments), comparing the average mass of the active ingredients in soybean leaves (ng g⁻¹), whose compounds were detected by LC-MS/MS.

RESULTS AND DISCUSSION

The emergence of less sensitive *Phakopsora pachyrhizi* populations that are less sensitive to strobilurins, triazoles and carboxamides is a major concern (Schmitz *et al.*, 2014) and (Kłosowski *et al.*, 2016). The excessive use of only these chemical groups in recent decades has exposed these active ingredients too much, possibly determining the reduction of their fungitoxicity to this pathogen (Reis, Carregal and Zanata, 2019).

Therefore, the use of only the modes of action (IDM, IQe and ISDH) may incur risks of development of multiple resistance by fungi (Müller, Stammer and May De Mio, 2021). Tests with new generation chemical compounds are needed, seeking new alternative solutions (Hossain *et al.*, 2024). The tests in this study proposed combinations of these fungicidal modes of action with CuSCC, as a way of mitigating these risks.

The chromatographic evaluations for compound extraction

in soybean leaves (non-sprayed lower parts) of the fungicide Flupi + OM did not find differences for the active ingredient fluxapyroxad (with and without CuSCC). This result was also repeated for bixafen in the Protribi + EMOS2 treatment, even though there was a ratio of 6.1. This can be explained by the lower octanol-water partition coefficient (KoW) of carboxamides, when compared to triazoles and strobilurins (Lewis *et al.*, 2016).

Under the experimental conditions, CuSCC favored the translocation of the active ingredients pyraclostrobin, prothioconazole-desthio, trifloxystrobin, picoxystrobin and tebuconazole from the upper to the lower non-sprayed part. These fungicides have higher octanol-water partition coefficient (KoW), when in comparison with the carboxamides (Lewis *et al.*, 2016). For Tepiman, the two compounds evaluated may indicate that the OD formulation (oil dispersion) may have a positive interaction with CuSCC (Table 6).

Table 6. Amount of active ingredient (ng g⁻¹) present in leaves from the lower part of the soybean canopy, according to the presence of CuSCC in the spray (combined analysis: trials 1 and 2).

Product	Active Ingredient	Amount present in lower soybean leaves (ng g ⁻¹)			
		With CuSCC	No CuSCC	Reason	p-value
Flupi	Fluxapyroxad	11.41	10.73	1.06	0.19
	Pyraclostrobin	2.89*	1.66	1.75	0.05
Protribi	Bixafen	4545.82	745.71	6.10	0.11
	prothioconazole-desthio	3249.95*	481.10	6.76	0.04
	Trifloxystrobin	4539.67*	1090.54	4.16	0.02
Tepiman	Picoxystrobin	813.38*	271.31	3.00	0.00
	Tebuconazole	1802.56*	538.10	3.35	0.01

Averages followed by * with and without addition of CuSCC differ from each other, by the student's t-test ($p \leq 0.05$). Key: Tepiman: tebuconazole + picoxystrobin + mancozeb (PC Blindado®); EMOS1: soybean oil methyl ester (PC Rumba®); Protribi: prothioconazole + trifloxystrobin + bixafen (PC Fox Xpro®); EMOS2: soybean oil methyl ester (PC Aureo®); Flupi: fluxapyroxad + pyraclostrobin (PC Orkestra®); OM: non-ionic mineral oil (PC Assist®)

In the analysis of the compounds extracted from the upper parts sprayed with the three fungicides, pyraclostrobin (Flupi + OM) and prothioconazole-desthio and trifloxystrobin (Protribi + EMOS2 treatment) with CuSCC differed among all the compounds evaluated. All the other active ingredients analyzed were the same, with and without the addition of CuSCC (Table 7).

An applicable analysis was to compare the sum of the totals analyzed by LC-MS/MS for all compounds, breaking down these data into percentages quantified in the lower (translocated) and upper (penetrated and/or

deposited) parts. The active fluxapyroxad (Flupi + OM) presented the most balanced percentage, both in the results with the addition of CuSCC (37.2% in the lower part and 62.8% in the upper part), and without CuSCC (35.3% in the lower part and 64.7% in the upper part).

The active ingredient pyraclostrobin also obtained higher percentages (upper and lower parts evaluated), with CuSCC, lower part = 18.5% and upper part = 81.5% and without CuSCC, lower part = 14.0% and upper part = 86.0%). In conclusion, the product Flupi + OM presented a better balance in the tests, when comparing the percentages detected in the lower and upper parts (Table 8).

Table 7. Amount of active ingredient (ng g⁻¹) are present in leaves from the upper part of the soybean canopy, according to the presence of CuSCC in the spray (combined analysis: tests 1 and 2).

Product	Active Ingredient	Amount present in soybean top leaves (ng g ⁻¹)			
		With CuSCC	No CuSCC	Reason	p-value
Flupi	Fluxapyroxad	19.25	19.68	0.98	0.75
	Pyraclostrobin	12.71*	10.14	1.25	0.03
Protribi	Bixafen	160897.03	132490.23	1.21	0.06
	prothioconazole-desthio	99808.14*	43661.91	2.29	0.00
	Trifloxystrobin	118548.20*	94388.83	1.26	0.02
Tepiman	Picoxystrobin	63735.49	59035.26	1.08	0.39
	Tebuconazole	154601.57	141476.64	1.09	0.39

Averages followed by * with and without addition of CuSCC differ from each other, by the student's t-test ($p \leq 0.05$). Key: Tepiman: tebuconazole + picoxystrobin + mancozeb (PC Blindado®); EMOS1: soybean oil methyl ester (PC Rumba®); Protribi: prothioconazole + trifloxystrobin + bixafen (PC Fox Xpro®); EMOS2: soybean oil methyl ester (PC Aureo®); Flupi: fluxapyroxad + pyraclostrobin (PC Orkestra®); OM: non-ionic mineral oil (PC Assist®)

Table 8. Percentage of the total quantity of the different active ingredients present in the lower and upper part of the soybean canopy, according to the presence of CuSCC in the spray (combined analysis: tests 1 and 2).

Product	Active Ingredient	With CuSCC			No CuSCC		
		Total (ng g ⁻¹)	%INF.	% SUP.	Total (ng g ⁻¹)	%INF.	% SUP.
Flupi	Fluxapyroxad	30.65	37.2%	62.8%	30.41	35.3%	64.7%
	Pyraclostrobin	15.60	18.5%	81.5%	11.80	14.0%	86.0%
Protribi	Bixafen	165442.85	2.7%	97.3%	133235.94	0.6%	99.4%
	prothioconazole-desthio	103058.09	3.2%	96.8%	44143.01	1.1%	98.9%
	Trifloxystrobin	123087.88	3.7%	96.3%	95479.36	1.1%	98.9%
Tepiman	Picoxystrobin	64548.88	1.3%	98.7%	59306.57	0.5%	99.5%
	Tebuconazole	156404.12	1.2%	98.8%	142014.73	0.4%	99.6%

Total: Total amount of active ingredient resulting from the sum of the upper and lower parts. %INF.: Percentage of the total sum detected in the non-sprayed lower part; %SUP.: Percentage of the total sum detected in the sprayed upper part. Key: Tepiman: tebuconazole + picoxystrobin + mancozeb (PC Blindado®); EMOS1: soybean oil methyl ester (PC Rumba®); Protribi: prothioconazole + trifloxystrobin + bixafen (PC Fox Xpro®); EMOS2: soybean oil methyl ester (PC Aureo®); Flupi: fluxapyroxad + pyraclostrobin (PC Orkestra®); OM: non-ionic mineral oil (PC Assist®).

For the other compounds, the proportion detected in the upper part was always greater than 96% of the total. The active ingredients picoxystrobin and tebuconazole (Tepiman + EMOS1) always obtained percentages above 98.7% in the comparisons of the upper and lower parts, with and without the addition of CuSCC. The highlight in the tests was Protribi + EMOS2, where the addition of CuSCC seems to have favored a better translocation of the active ingredients. In the lower part of the plants, the quantified percentages, with CuSCC, were: 2.7% for bixafen, 3.2% for prothioconazole-desthio and 3.7% for trifloxystrobin, while without CuSCC they were 0.6%, 1.1% and 1.1%.

These results found on the translocation of fungicides in soybean plants validate the observations for the

management of Asian soybean rust, where the addition of CuSCC reduced the severity of this disease, improving soybean productivity for two consecutive harvests (Lima, 2024).

The active prothioconazole is easily converted to the desthio form, with better fungicidal performance in pathogens, being the main metabolite of the degradation of the original active ingredient (Parker *et al.*, 2013). Translocations of prothioconazole and tebuconazole was detected in wheat plants, with prothioconazole-desthio moved from the treated parts to the untreated parts, and tebuconazole showed greater basipetal translocation (Lehocski-Krsjak *et al.*, 2013). The systemicity of prothioconazole radiolabeled by the xylem in barley plants, finding traces of prothioconazole-desthio in non-

applied parts, however rarely exceeding 5% of basipetal translocation also demonstrated (Hauser-Hahn, Baur and Schmitt, 2004).

The degradation of tebuconazole was estimated as slower than prothioconazole-desthio, a scenario that configures a better fungicidal action of the actives with such characteristic (Lehoczki-Krsjak *et al.*, 2013). A work with absorption, translocation and metabolism of triazoles in wheat crops, reported the transfer of prothioconazole and its metabolite desthio from stem to leaf (Fang *et al.*, 2023). This work, the active tebuconazole, applied with Tepiman, obtained greater translocation to the lower parts of soybean plants, when combined with CuSCC.

Pyraclostrobin is classified as a localized systemic active, with translaminar activity and limited acropetal translocation (Goertz, 2012). This active have relatively low water solubility (unfavorable for long-distance translocation), log KoW = 3.99 (unfavorable for acropetal translocation) and low melting point, favorable for rapid foliar absorption (Goertz, 2012).

Studies with *Alternaria solani* in tomato crops commented that treatments with fluxapyroxad + pyraclostrobin promoted high photochemical yield, greater activity of the nitrate reductase enzyme and increased synthesis and translocation of photoassimilates (Marek *et al.*, 2018). A work evaluating formulations with nickel combined with fluxapyroxad + pyraclostrobin with severity of *Microsphaera diffusa*, etiologic agent for powdery mildew in soybean plants, suggesting greater enzymatic activity, stimulation of antioxidant metabolism and plant defense (Barcelos *et al.*, 2018).

The retention of pyraclostrobin and boscalid in garlic crops was measured, describing residues of these active ingredients in bulbs and green leaves using the solvent extraction method and detection by high-performance liquid chromatography (Parlakidis *et al.*, 2023). These studies found recoveries of 0.02 mg kg⁻¹ of pyraclostrobin and 0.14 mg kg⁻¹ of boscalid, where the combination with the adjuvant Dash CE® (phosphoric acid ester) increased the translocation of the fungicides (Parlakidis *et al.*, 2023). These authors concluded that the translocation of boscalid was greater than that of pyraclostrobin in the experiments conducted.

In spraying of soybean plants with protected parts of the applications, prothioconazole, picoxystrobin and fluxapyroxad were the active ingredients that best

controlled ASR in parts of the leaves not applied with these fungicides (Gotardi, 2022). According to this author, this may evidence the possibility of superficial movement of these compounds from applied to non-applied parts of the leaves.

Fluxapyroxad was one of the compounds studied where there was no difference in the evaluations of the translocation of the active ingredient from the upper to the lower part with and without the addition of CuSCC. However, in percentage terms, it was the active ingredient that translocated the most from the sprayed upper part to the non-sprayed lower part in the tests. Studies cited that fluxapyroxad forms deposits on the soybean leaf, with constant and increasing absorption for up to 72 hours, ensuring continuous penetration of the product into the leaf (Santos, 2016).

This partially contradicts the information for the melting point of fungicidal molecules (Pontzen and Baur, 2011). These authors state that this is an important item to be considered, however, fluxapyroxad is the active ingredient with the highest melting point, compared to all those studied in this research. The melting point of fluxapyroxad is 156.8°C, the higher between the compounds analyzed (Lewis *et al.*, 2016).

Fluxapyroxad and bixafen have full systemic activity, being absorbed by the cuticle and translocated in the plant through the xylem (Suty-Heinze *et al.*, 2011) detail. These authors add that their translocation speed is moderate, being ideal for a uniform distribution of the active ingredient throughout the leaf.

The lethality of some carboxamides also was valued, concluded that the LD50 (median lethal dose) in ASR isolates was on average 12.3 µg ml⁻¹ for bixafen and 8.6 µg ml⁻¹ for benzovindiflupir, demonstrating a lower biological efficacy of the first active ingredient (Müller *et al.*, 2021). Although not without a statistically significant difference, the results of this experiment showed a better translocation of bixafen in association with CuSCC (top to bottom).

Regarding trifloxystrobin, this active moves for a translaminar manner, characterizing it as mesostemic or surface systemic (Vincelli, 2002). The active absorption is done by the cuticle leaves and subsequent penetration into plant tissues (Goertz, 2012).

Picoxystrobin is translaminar activity, but with mobility in the xylem and epi-systemic distribution, having the following characteristics: medium water solubility (favorable for long-distance translocation), medium Log

KoW (favorable for acropetal translocation) and low melting point, favorable for rapid foliar absorption (Goertz, 2012). Picoxystrobin is the active ingredient that presents the greatest systemicity via xylem among all commercial strobilurins (Gotardi, 2022).

Azoxystrobin and picoxystrobin were the most mobile compared to the other strobilurins (Klittich *et al.*, 2008). These authors were able to measure the concentration of the compounds in non-applied areas, reaching percentages of 0.59% (picoxystrobin), 0.25% (azoxystrobin) and 0.021% (trifloxystrobin) of movement. These molecules facilitated better disease control in wheat crops, due to better mobility (Klittich *et al.*, 2008).

The control of powdery mildew in soybean plants (e.a. *Microspheera diffusa*), was investigated (Santos *et al.*, 2018). These authors studied the translocation by xylem of three triazoles (cyproconazole, tebuconazole and flutriafol) and two strobilurins (azoxystrobin and pyraclostrobin). After applying the fungicides only to the first trifoliolate of the plant, they observed the incidence of the disease in the second and third trifoliate, and it was not possible to identify differences between these five fungicides, with differences only for the control without application (Santos *et al.*, 2018).

It is also important to discuss the mechanics of penetration of substances into leaves. Systemic active ingredients move more easily through the phloem, being translocated throughout the plant by symplastic transport (Taiz and Zeiger, 2016). That greater efficiency in this process would allow leaves not affected by agrochemical sprays to benefit (Teicher, 2022). In crops

with high LAI (leaf area index) such as soybeans, this may be relevant, since there are physical barriers imposed by the leaf mass, plant architecture, and high populations used. Another relevant point is linked to the reduction in spray volumes, which makes it difficult for the product to reach the target as a whole.

Regarding the absorption of compounds by plants, it is a passive process driven by concentration gradients, following the principles of Fick's Law of Diffusion (Fick, 1855) and (Stefanello *et al.*, 2017). Some authors partially contradict this, explaining that this Law is not directly related to the penetration of fungicides into the leaves, but it can be an important point in the evaluation of their effectiveness, since fungicides present varying degrees of systemicity in plants (Damicone and Smith, 2009).

One of the factors considered for the systemicity of active ingredients is their lipophilicity. This indicator as the ability of a molecule to dissolve in lipophilic (non-aqueous) environments, so that they permeate through biological membranes (Silva and Fay, 2004). That lipophilicity is measured by the octanol-water partition coefficient (KoW) and is expressed on a logarithmic scale (Log P or Log KoW) (Teicher, 2022). The measurement range of this coefficient is between -2 and 7, and that it is used to measure the speed of translocation and the systemicity of compounds in plants (Silva and Fay, 2004) and (Teicher, 2022).

A simplified graphical representation that can be analyzed is about the mobility of products considering only the Log KoW (Figure 4), following a model proposed (Stefanello, 2018).

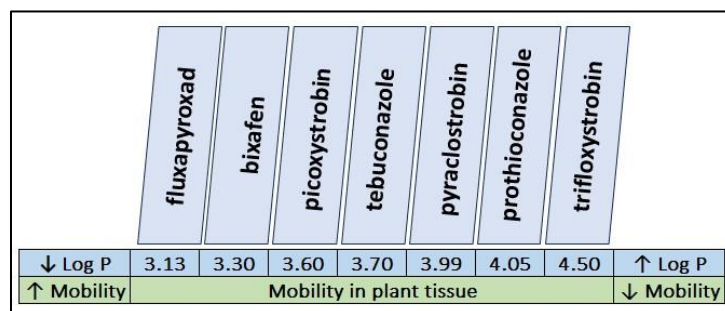


Figure 4. Mobility in plant tissue of the compounds analyzed in this work according to the octanol-water partition coefficients (log P or log KoW) of the fungicides, following the graphical model proposed (Stefanello, 2018). Source of data inserted in the infographic: (Lewis *et al.*, 2016).

CONCLUSIONS

The addition of CuSCC favored the translocation from sprayed to non-sprayed parts in soybean plants, for the

active ingredients: picoxystrobin, tebuconazole, pyraclostrobin, prothioconazole-desthio and trifloxystrobin.

The two carboxamides (fluxapyroxad and bixafen) were the only active ingredients that did not suffer interference with the addition of CuSCC, regarding the translocation from pulverized to non-pulverized parts.

AUTHOR CONTRUBUTIONS

All authors contributed equally to this research.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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