Research Article

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Soybean seed treatment: how do fungicides translocate in plants?

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Abstract

BACKGROUND: Soybean seed treatment with fungicides is a well-established disease management strategy. However, the movement of these fungicides within seedlings is not always well characterized. Thus, the objectives of this study were to determine the pattern of translocation of three fungicides with different modes of action applied as a seed treatment, and the effect of soil type on translocation.

RESULTS: Most of the absorbed radioactivity was concentrated in the cotyledons and the maximum sum of the rates of absorption by roots, stems, and leaves of the plants was 15%. In most cases, absorption by roots, stems, and leaves were lower than 5% for ^{14}C-pyraclostrobin and ^{14}C-metalaxyl, and 1.6% for ^{14}C-carbendazim. Fungicides absorbed by the roots and the whole seedlings were higher when plants were grown in soil with lower organic matter content. Fungicides in the cotyledons are unlikely to be redistributed and are lost when cotyledons fall off the plants.

CONCLUSION: Cotyledons are the part of the plant where fungicides are most absorbed, regardless of the fungicide. Soil type affects the absorption of fungicides, and in this study it was most likely caused by soil organic matter. These data improve knowledge of the movement of seed treatment fungicides in soybean seedlings and may help the development of seed treatment chemistry to manage seed and soilborne pathogens.
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Keywords: radiolabeled; *Glycine max*; disease management; translocation; K_{ow}

1 INTRODUCTION

Fungicide seed treatment is a disease management practice used to protect germinating seeds from seedborne and soilborne pathogens. The first systemic fungicide seed treatment, carboxim, was introduced in United States only in the 1970s.[1] Fungicide seed treatment reduces infection of both seed borne and soil borne pathogens of seed and seedlings, including *Diaporthe* sp., *Fusarium* sp., *Rhizoctonia* sp., and *Phytophthora* sp. and thereby mitigates stand loss and protects the health of seedlings.[2–8] Phytophthora root rot, for example, was estimated to cause more than two million tons in yield losses in the eight top soybean producing countries around the globe, while damping-off caused by *Rhizoctonia* sp. was estimated at over one and a half million tons.[9]

Currently, many different groups of fungicides have been used in soybean seed treatments, to control both seed and soilborne pathogens. Among these, benzimidazoles (which affects β-tubulin assembly, and affects mitosis and cell division), strobilurins [which inhibits complex III in the mitochondrion (bcl complex), and affects respiration], and phenylamides (which inhibits RNA synthesis, and affects protein production)[10] represent important molecules for fungicide seed treatment. Benzimidazoles, for example, thiabendazole, carbendazim, and thiophanate-methyl, are effective against a wide range of ascomycetes and basidiomycetes, but not against oomycetes. While, phenylamides (e.g. metalaxyl and mefenoxam) are effective exclusively against oomycetes, strobilurins (e.g. pyraclostrobin, azoxystrobin, and trifloxystrobin) are effective against some members of ascomycetes, basidiomycetes and oomycetes.[10] In addition to the mode of action of each active ingredient, other physiochemical characteristics may contribute to the classification of fungicides, such as the log K_{ow} (as known as log P), which indicates the partition

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coefficient between *n*-octanol and water and measures hydrophobicity/hydrophilicity of the molecule.[10]

Although seed treatments have increased in use in the past decade,[11–13] especially with adoption of newer and more accurate seed treatment application machinery, there is limited information on how the fungicides applied to the seed translocate in the plants, the same is also true for how fungicides may interact with the soil. The soil component, such as organic matter and clay minerals, may directly affect the amount of pesticides absorbed by plants.[14–18] Notably, most of research to date has been focused on herbicides, which directly impacts the recommendation of which active ingredient to use based not only in weed control, but also on molecule characteristics (e.g. K_{ow}), soil properties and precipitation.[19] Such information could lead to a better understanding of how seed treatments reduce infection from soil borne pathogens and allow for targeted applications in a sustainable manner.

The translocation of pesticides in plants can be measured directly, the amount of active ingredient; or indirectly, through spraying the molecule on one part of the plant and assessing the development of pathogen in another part of the same plant.[20] Direct methods involve the extraction of the active ingredient from plant tissues and quantification through spectrometry, which can be time consuming.[21] Direct measures of the translocation of fungicides in plants can also be made using the radiolabeled technique.[22–24] This method consists of an active ingredient in which one of the carbons (usually the most stable one) is the radioactive isotope carbon-14 (^{14}C).

The objectives of the present study were to determine (i) the pattern of translocation of three fungicides, carbendazim, pyraclostrobin and metalaxyl, in soybean seedlings when applied as a seed treatment; and (ii) if soil organic matter can limit the absorption and movement of these fungicides.

2 MATERIAL AND METHODS

In order to assess the pattern of translocation of fungicides applied as seed treatment in soybean seedlings, three experiments using radiolabeled molecules were performed at the Center for Nuclear Energy in Agriculture (Cena), University of São Paulo (USP), Piracicaba, São Paulo State (SP), Brazil.

2.1 Experiment I – movement of pyraclostrobin in a commercial formulation

In the first experiment, soybean seeds treated with 1667 Bq of ^{14}C-pyraclostrobin each were planted into sandy soil (10 g dm^{-3} of organic matter). The radiolabeled molecule of pyraclostrobin, diluted in methanol and radioactivity purity equal to 95%, was added to the commercial product Standak Top® (pyraclostrobin + thiophanate-methyl + fipronil) plus colorant polymer (Polyplus®). The mix of ^{14}C-pyraclostrobin + Standak Top® + Polyplus® was applied to 50 soybean (cv. TMG 7062 RR2 IPRO) seeds placed in a 250 mL high density polyethylene flask by gently mixing until all seeds were uniformly covered with the treatment.

A day after the treatment, one seed was planted into a per 500 mL pot for a total of ten pots and placed in a glasshouse exclusively dedicated for radioactive materials, with sprinkler irrigation with deionized water. To determine the maximum amount of radiation that each plant could absorb, the radioactivity of seven seeds was measured using the method described later. Additionally, the testa and embryo from another seven seeds was evaluated separated so radioactivity could be determined in both parts. Fourteen days after planting (DAP), three plants were dug up, roots were washed, and dried and then placed in a continuous hot and dry air flow chamber for 72 h at 45°C. The radioactivity from roots, hypocotyl + cotyledons, epicotyl and leaves of each plant was determined after burning them in a biological oxidizer (OX 600, Harvey Instruments Crop., Hillsdale, NJ, USA), which traps ^{14}C from the plants into a scintillation solution vial. The vials were then placed in a scintillation counter (Packard 1900 TR, Meriden, CT, USA) and values were expressed in Becquerel (Bq).

2.2 Experiment II – comparison of fungicides

A second experiment compared the translocation of the three fungicide molecules used in seed treatment. 166 700 Bq of ^{14}C-pyraclostrobin (K_{ow} = 3.99; water solubility = 1.9 mg L^{-1}; radiochemical purity = 95%) diluted in methanol, 166 700 Bq ^{14}C-carbendazim (K_{ow} = 1.52; water solubility = 8 mg L^{-1}; radiochemical purity = 94%) diluted in methanol, and 166 700 Bq of ^{14}C-metalaxyl (K_{ow} = 1.65; water solubility = 8400 mg L^{-1}; radiochemical purity = 94%) diluted in acetonitrile, were added to commercial products for seed treatments (Standak Top®: pyraclostrobin + thiophanate-methyl + fipronil; Maxim Advanced®: thiabendazole + fludioxonil + metalaxyl; Derosal Plus®: carbendazim + thiram, respectively) and colorant polymer (Poliplus®). In this manner, each ^{14}C-fungicide was mixed to its correspondent commercial seed treatment. The mix of ^{14}C-fungicides + commercial seed treatment + Polyplus was applied to a 100 soybean (cv. TMG 7062 RR2 IPRO) seeds placed in a 250 mL high density polyethylene flask by gently mixing until all seeds were uniformly covered with the treatment.

A day after treatment, for each radiolabeled fungicide, 32 treated seeds were planted in 32 150 mL pots (one seed per pot) filled with an organic substrate (71 g dm^{-3} of organic matter) and grown in a glasshouse exclusively dedicated for radioactive materials all at the same time. The pots were placed in containers with deionized water, so that the water demand was supplemented according to the evapotranspiration of each pot. Pots were organized in a completely randomized design. For each radiolabeled fungicide, ten seeds were evaluated for the maximum amount of radioactivity as described earlier.

For each radiolabeled molecule, five treated plants were removed from the pots and the roots were washed 16 DAP, and dried in a continuous hot and dry air flow chamber for 72 h at 45 °C. The radioactivity from roots, cotyledons, unifoliate leaves, trifoliate leaves, and stem of each plant was determined after burning them in a biological oxidizer (OX 600, Harvey Instruments Crop.), which traps ^{14}C in plants into a scintillation solution vial. The vials were then placed in a scintillation counter (Packard 1900 TR) and values were expressed in Bq.

2.3 Experiment III – understanding the relationships between soil organic matter and fungicides

The third experiment evaluated if two different soils with different levels of organic matter could affect the distribution of two fungicides with different K_{ow}. Soybean (cv. TMG 7062 RR2 IPRO) seeds from experiment II and treated with ^{14}C-pyraclostrobin and ^{14}C-metalaxyl were planted in 500 mL styrofoam cups (one seed per pot) filled with organic substrate (71 g dm^{-3} of organic matter) or sand (5 g dm^{-3} of organic matter), and placed in a glasshouse exclusively dedicated for radioactive materials, with sprinkler irrigation with deionized water. The experiment followed a 2 × 2 factorial scheme with two fungicides and two types of substrate, with seven replications in a completely randomized design.

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For each combination of fungicide *versus* substrate, seven treated plants were removed from the cups and roots were washed at 18 DAP, and dried in a continuous hot and dry air flow chamber for 72 h at 45 °C. The radioactivity from roots, cotyledons, unifoliate leaves, trifoliate leaves, and stem of each plant was determined as described in experiment II, with values expressed in Bq.

2.4 Data analysis

Data from experiment II and III were analyzed using the free software R Studio (citation).[25] Values of Bq where transformed as percentage based on the seed radioactivity for each fungicide, and then transformed using the function *a*sin(√(*x*/100)). Generalized linear mixed model was used through the *lme4* package.[26] When differences inside or between factors were significant, results were analyzed by the least-squares differences using the *emmeans* package.[27,28]

3 RESULTS AND DISCUSSION

The first experiment evaluated the effect of one fungicide pyraclostrobin in a commercial formulation planted into sandy soil. The amount of ^{14}C-pyraclostrobin added was enough to result in a total of 1667 Bq per seed. However, after treating the seeds inside the high-density polyethylene flask, the mean value from seven seeds was 596 Bq per seed. The seed treatment was concentrated on seed testa (seed coat), and only insignificant values of radiation was recovered from seed embryo (cotyledons + embryonic axis) (Table 1). The results from the 14 DAP plants on experiment I indicated that most of the radiation recovered was concentrated in the cotyledons and less than 50% of the radiation was recovered from the whole plant (Table 1).

In the second experiment, the translocation between three fungicides (carbendazim, pyraclostrobin and metalaxyl) was compared. All parts of the plants showed significant differences among the fungicides, except the roots (Table 2). As in the first experiment, cotyledons had the highest levels of radiation. Similarly, less than 50% of the absorbed radiation was detected in the plant when compared to initial values from the seed. The amount of ^{14}C-pyraclostrobin fungicide mixture absorbed in the whole plant was higher than ^{14}C-metalaxyl and ^{14}C-carbendazim.

The last experiment compared how soil types may influence the amount of fungicide absorbed by the plants, using pyraclostrobin and metalaxyl. An increase in soil organic matter affected fungicide absorption for all parts of the plants but the first trifoliate leaf (Table 3). Roots of plants grown in sand absorbed more ^{14}C-pyraclostrobin than ^{14}C-metalaxyl, but there was no difference for those grown in organic substrate with a higher content of soil organic matter. Similar to both previous experiments, cotyledons had higher levels of fungicide compared to other parts of the plant. Nevertheless, pyraclostrobin had a reduction in the amount of radiation in the cotyledons when soil organic matter content increased. Unifoliate leaves of plants grown in sand had a greater amount of radiation regardless of the fungicide. The first true leaves also had a higher value of ^{14}C-metalaxyl than ^{14}C-pyraclostrobin, which differs from the result shown for the first trifoliate leaf. Similar to roots, stem + petioles of plants grown in sand absorbed more ^{14}C-pyraclostrobin than ^{14}C-metalaxyl, but no difference was found when plants were grown in an organic substrate with higher soil organic matter content. Finally, for the whole plant, ^{14}C-pyraclostrobin had higher rates than ^{14}C-metalaxyl regardless of the soil type. Moreover, soil type affected the amount of ^{14}C-pyraclostrobin absorbed by the whole plants, but did not affect ^{14}C-metalaxyl (Table 3).

These results reveal some of the characteristics related to absorption of active ingredients during the application of fungicide seed treatment products to soybean seeds. The majority of the radioactivity absorbed by the plants for all of the molecules evaluated was concentrated in the cotyledons, which was possibly absorbed at the time of seed imbibition (Tables 1–3). These results also agree with Gupta *et al.*[29] as they reported that most part of ^{14}C-metalaxyl was retained by the soybean cotyledons when applied as a seed treatment. In that same study, soil drench and seed treatment application were compared and although soil drench allowed the roots to absorb higher percentages of ^{14}C-metalaxyl, the total amount of radiation recovered by the plants was lower than those with seed treatment. Another fungicide for oomycete disease management as a soybean seed treatment, ethaboxam (K_{ow} = 2.89; water solubility = 12.4 mg L^{-1}) also has the same pattern of translocation as the other fungicides used in this study, with most of the active ingredient concentrated in the cotyledons of soybean plants 14 DAP (McDuffee D, personal communication). This is of concern for two reasons. Firstly, the root tissues are the primary target for soilborne pathogens thus the fungicide is not predominantly in the tissues that need protection. In most cases, less than 2% of the fungicides were found in the roots (Tables 1–3). Secondly, the cotyledons senesce from the seedlings very early in their growth, soon after the first true leaves emerge and thus the fungicide would be lost.

All fungicides in this study had absorption values by the plants lower than 50% in most cases (Tables 1–3). Thus, it is expected that some of the products would have remained in the soil close to the seed and also in the testa. In experiment I, we were able to recover testa fragments from the soil where up to 29.2% of ^{14}C-pyraclostrobin was found, while in experiment III, pieces of testa with up to 8.5% of ^{14}C-pyraclostrobin was found.

Moreover, the distribution of ^{14}C-labeled fungicides in soybean roots was different than expected, since the fungicides were concentrated in the cotyledons and hypocotyl regions. This suggests that protection of root tissues may not be as efficient as previously

Table 1. Mean percentage of radiation (100% = 596 Bq) from ^{14}C-pyraclostrobin[7] recovered from seeds (mean of seven seeds) immediately following treatment and plants [mean of three plants at 14 days after planting (DAP) in sandy soil] on experiment I

Seed[a]		Plant				
Test	Embryo	Roots	Hypocotyl + cotyledons	Epicotyl	Leaves	Total
96.9%	3.1%	1.5%	44.9%	0.4%	0.7%	47.5%

[a] ^{14}C-pyraclostrobin + Standak Top® + Polyplus®.

Table 2. Mean percentage of radiation of [14]C-carbendazim (100% = 832 Bq), [14]C-metalaxyl (100% = 1417 Bq) and [14]C-pyraclostrobin (100% = 689 Bq) recovered from plants [mean of five plants at 16 days after planting (DAP) in organic substrate]

Fungicide	Root[1]	Stem + petioles[2]	Cotyledon[3]	Unifoliate leaves[4]	First trifoliate leaf[5]	Total[6]
carbendazim	0.4%a	0.7%b	10.0%b	0.4%c	0.1%b	11.6%b
metalaxyl	0.5%a	0.7%b	11.2%b	1.2%a	0.2%a	13.8%b
pyraclostrobin	0.9%a	1.3%a	29.5%a	0.8%b	0.2%a	32.6%a

[1]$P = 0.1298$.
[2]$P = 0.0003504$.
[3]$P = 0.0003433$.
[4]$P = 0.0003902$.
[5]$P = 0.0221$.
[6]$P = 0.0003018$.
Values followed by the same letter were not statistically different by the Tukey test ($\alpha = 0.1$).

Table 3. Percentage values of radiation of [14]C-metalaxyl (100% = 1417 Bq) and [14]C-pyraclostrobin (100% = 689 Bq) recovered from plants [mean of seven plants at 18 days after planting (DAP)] on Experiment III

	Sand	Substrate			Sand	Substrate	
	Roots[1,8]				Cotyledons[2,8]		
Metalaxyl	1.4%bA	0.9%aB		Metalaxyl	15.7%bA	14.8%bA	
Pyraclostrobin	7.0%aA	0.9%aB		Pyraclostrobin	48.8%aA	32.8%aB	
	First trifoliate leaf[3,7]				Unifoliate leaves[4,7]		
Metalaxyl	0.2%	0.2%	**0.2%b**	Metalaxyl	1.8%	1.1%	**1.5%a**
Pyraclostrobin	0.5%	0.4%	**0.4%a**	Pyraclostrobin	0.9%	0.5%	**0.7%b**
	0.4%ns	**0.3%**			**1.4%a**	**0.8%b**	
	Stem + petioles[5,8]				Total[6,8]		
Metalaxyl	1.1%bA	1.3%aA		Metalaxyl	20.3%bA	18.4%bA	
Pyraclostrobin	3.3%aA	1.5%aB		Pyraclostrobin	60.5%aA	36.0%aB	

[1]Molecule ($P = 1.819e^{-06}$); soil ($P = 3.639e^{-08}$); molecule × soil ($P = 2.001e^{-06}$).
[2]Molecule ($P = 1.873e^{-07}$); soil ($P = 0.02788$); molecule × soil ($P = 0.03816$).
[3]Molecule ($P = 0.001323$); soil ($P = 0.459234$); molecule × soil ($P = 0.375040$).
[4]Molecule ($P = 3.403e^{-07}$); soil ($P = 1.371e^{-05}$); molecule × soil ($P = 0.9521$).
[5]Molecule ($P = 0.0003237$); soil ($P = 0.0458875$); molecule × soil ($P = 0.0060907$).
[6]Molecule ($P = 3.111e\text{-}07$); soil ($P = 0.003622$); molecule × soil ($P = 0.010052$).
[7]Numbers in bold are the mean of each level in each factor. Values followed by the same letter were not statistically different by the Tukey test ($\alpha = 0.1$).
[8]Values followed by the same lowercase letter were not statistically different in the columns by the Tukey test ($\alpha = 0.1$); values followed by the same uppercase letter were not statistically different in the lines by the Tukey test ($\alpha = 0.1$).
ns, no significant difference.

thought. Radiation present in the roots did not come systemically from the cotyledon, and we suspect that part of it came from the soil and another part of it was absorbed during the emergence of the radicle during the germination. Additionally, a higher soil organic matter content may decrease the amount of fungicides on the roots, with higher reduction rates for fungicides with higher K_{ow} values (Table 3).

The difference in absorption of fungicides by the roots in the two different soils suggests that a better understanding about the interactions of these products with the soil is definitely an important step to improve seed treatment. In this study, we believe that a difference in soil organic matter content (5 g dm^{-3} in sand to 71 g dm^{-3} in organic substrate) may be contributing to the differences found between the two soils for both fungicides tested. Considering the interaction of these molecules with other components of the soil, few studies showing interactions of fungicide seed treatments with clay minerals are available.[24] This knowledge could improve the control of soilborne pathogens.

When soil organic matter content was low (sand: 5 g dm^{-3} of organic matter), pyraclostrobin was higher in the roots compared to metalaxyl. The later active ingredient is known for increasing its mobility in soils with low organic matter content.[30] Therefore, metalaxyl was probably leached from the root zone, differently from pyraclostrobin, which has a higher K_{ow} and bounds stronger to soil organic matter than metalaxyl, and thus have a higher soil adsorption.[31,32]

In conclusion, this study was able to show that the total amounts of fungicides absorbed by seedlings are frequently less than the half of what is present on the seed, and most of the amount absorbed was concentrated in the cotyledons, not in seedling roots. Moreover, soil type affects the amount of fungicide absorbed by the plants for both lipophilic and hydrophilic molecule, and this effect includes the amount of fungicides in the roots.

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