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CROP PROTECTION

Buffer Zones for 2,4-D Applications Nearby Tobacco Fields

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ABSTRACT: *An increase in 2,4-D use is expected as tolerant crops have been approved to use in Brazil, which may negatively affect important crops such as tobacco. Our objective was to determine safe distances between 2,4-D applications and tobacco fields considering herbicide contamination to the harvested product. A field experiment was conducted, consisting of a 2,4-D application done perpendicularly to the wind direction, using a tractor sprayer. Drifted herbicide was collected using tobacco plants placed at various points (-50 up to 400 meters from application zone), following three schemes: a) 0 to 0.5 hours after application (HAT); b) 0 to 24 HAT; and c) 0.5 to 24 HAT. Environmental conditions were recorded. Herbicide in tobacco leaves was quantified. Drift was detected up to 200 m in both years. Vapor movement of 2,4-D was detected up to 400 m from the application strip in 2016, on plants taken to the field after herbicide application. Environmental conditions in 2015 favored off-target movement (higher wind speed and air temperature and lower humidity); although, in 2016 the herbicide traveled further due to wet deposition. These results indicated that a 100-meter buffer zone is enough to significantly decrease chances of tobacco contamination above the tolerated threshold, and highlighted the importance of environmental conditions in the transport processes for 2,4-D under field conditions.*
Key words: *herbicide residue, synthetic auxin, drift, volatility, environmental conditions.*

Zonas de exclusão para aplicações de 2,4-D próximas a campos de tabaco

RESUMO: *O aumento no uso do 2,4-D é esperado, uma vez que as culturas tolerantes foram aprovadas para uso no Brasil, o que pode afetar negativamente culturas importantes, como o tabaco. Nosso objetivo foi determinar a distância segura entre aplicações de 2,4-D e lavouras de tabaco, considerando a contaminação do herbicida no produto colhido. Um experimento de campo foi realizado, utilizando aplicação de 2,4-D perpendicularmente à direção do vento, com pulverizador tratorizado. A deriva do herbicida foi coletada plantas de tabaco colocadas em vários pontos (-50 até 400 metros da zona de aplicação), seguindo três esquemas: a) 0 a 0,5 horas após a aplicação (HAT); b) 0 a 24 HAT; e c) 0,5 a 24 HAT. As condições ambientais foram registradas. Foi quantificado o herbicida em folhas de tabaco. A deriva foi detectada até 200 m em 2015 e até 150 m em 2016. A volatilização ocorreu em ambos os anos, pois o 2,4-D foi detectado em plantas transportadas para o campo após a aplicação. As condições ambientais em 2015 favoreceram o movimento fora do alvo (maior velocidade do vento e temperatura do ar e menor umidade). Esses resultados indicam que uma zona de exclusão de 100 metros é suficiente para diminuir significativamente as chances de contaminação do tabaco acima do limite tolerado e, destaca a importância das condições ambientais nos processos de transporte do 2,4-D em condições de campo.*
Palavras-chave: *resíduo de herbicida; auxina sintética; deriva; volatilidade; condições ambientais.*

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is one of the most important crops in Brazil. In 2019, almost 300,000 ha of tobacco were grown, resulting in 664 tons of tobacco leaves (AFUBRA, 2020), and placing Brazil as the second largest producer worldwide. Furthermore, Brazilian tobacco has high quality and competitive prices, which helped Brazil to become the largest tobacco exporter in the world (BARRETO

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& NOVAIS, 2016). Tobacco production in Brazil is concentrated in the southern region, where 97% of the nation's tobacco is produced (AFUBRA, 2020). Most of the tobacco production occurs in small farms (less than 15 ha) and requires family labor. Often, tobacco production represents the main source of income to these families (AREND, 2014).

Tobacco is not a food crop; therefore, guidance residue levels (GRL) and preharvest interval (PHI) of pesticides have not been stablished. Pesticide residues in tobacco leaves may interfere with organoleptic properties of tobacco products and result in contamination of consumers and other people exposed to the smoke of cigarettes (RAHMAN et al., 2012). Therefore, tobacco companies along with the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA, 2020) established guidance residue level (GRL) for 106 compounds reported in tobacco leaves (CORESTA, 2020). Many of these compounds are not utilized in tobacco production but may be transported from other locations through off-target movement (OTM) processes such as pesticide drift, volatilization, and/or tank contamination.

According to the United States Environmental Protection Agency (EPA, 2019a), pesticide drift may be defined as "the movement of pesticide dust or droplets through the air at the time of application or soon after, to any site other than the area intended". Pesticide volatilization is defined as "the movement of pesticide vapors through the air" (EPA, 2019b), and occurs especially with pesticide molecules with high vapor pressure. Herbicide OTM can be mitigated by observing appropriate environmental conditions during application as well as selecting appropriate spray nozzles (type and orifice size), carrier volume, and speed of application (CONTIERO et al., 2016; KALSING et al., 2018). Additionally, the use of buffer zones between target fields to be sprayed and sensitive crops is another tool for mitigating herbicide OTM (UCAR & HALL, 2001; HILL et al., 2002; BURN, 2003).

The herbicide 2,4-D is prone to OTM and may cause phytotoxicity and yield loss in sensitive crops, as well as herbicide injury to native vegetation. However, 2,4-D also has several desirable features, including low toxicity to mammals, low cost, broad-spectrum control of many broadleaf weeds, especially herbicide-resistant and/or tolerant weeds to glyphosate, and may show synergistic (ISAACS et al., 2006) or additive effects with other herbicides (GANIE & JHALA, 2017) when tank-mixed. Due to these features, 2,4-D is widely used worldwide for the selective control of broadleaf weed species in cereal crops such as wheat, oats, corn, rice, and pastures, as well as burndown applications to soybeans and cotton.

The widespread use of 2,4-D in cereal crops in southern Brazil plus the recent approval of the Enlist™ technology raised concerns within the tobacco industry over the impact of 2,4-D potential OTM to tobacco fields. The Enlist technology will allow farmers to spray 2,4-D to greater acreage during a much wider application window. The potential impact of this technology to sensitive crops in this geography is still unknown. Therefore, the objective of this study was to determine appropriate buffer zones between tobacco fields and 2,4-D treated fields.

MATERIALS AND METHODS

Field experiments were conducted in Santa Cruz do Sul, Rio Grande do Sul, Brazil (29.814090 S, 52.324708 W), to evaluate OTM of a 2,4-D application using a tractor mounted boom sprayer. The first experiment was established on February 22nd, 2015 and replicated on February 23rd, 2016. Total trial area measured 50 m wide by 500 m in length and was arranged in a manner that the predominant wind direction was parallel to the trial length. The area was predominantly covered by native grass species and was mowed prior to the test conduction. A 2,4-D application strip (10 m wide by 50 m in length) was then marked perpendicularly to the predominant wind direction. Sample collection points were marked/flagged perpendicularly to the application strip both upwind (-50 m) and downwind (0; 12.5; 25; 50; 75; 100; 150; 200; 300 and 400 m). Environmental conditions during the execution of the experiment were monitored using a weather station located *in situ*.

A tractor mounted sprayer boom measuring 10 m wide was used for the 2,4-D application. The sprayer boom was equipped with TeeJet XR 11002 nozzles (TeeJet Technologies, 200 W. North Avenue, Glendale Heights, IL 60139, USA) spaced 50 cm apart travelling at speed of 4.1 km h^{-1} and calibrated to deliver 150 L ha^{-1} of spray solution at 150 kPa operating pressure. The distance between nozzles and the target vegetation was set at 50 cm. The 2,4-D formulation used consisted of DMA® 806 BR (The Dow Chemical Company) applied at a rate of 1005 g ae ha^{-1} plus mineral oil at 0.5% v/v (Assist®, BASF S.S).

Tobacco plants (hybrid Virgínia PVH2254) were grown individually in the greenhouse in 40 L, 45 cm tall plastic pots filled with potting mix, until they reached approximately 1m in height, at the elongation and rapid growth stage (ORLANDO et al., 2011). Three tobacco plants (three replications) were placed at each sample collection point during three time periods [group A = 0 to 0.5 hours after treatment (HAT); group B = 0 to 24 HAT; and group C = 0.5 to 24 HAT] (Figure 1). Only plants from group C were placed at the 0m sampling point. According to their time scheme, three leaves were collected from each tobacco plant (one leaf from the upper 1/3 portion, one leaf from the middle, and one leaf from the bottom 1/3 of the plant), placed into paper bags, and oven dried at 40 C until constant weight was achieved to simulate the curing process of commercial tobacco. Samples were sent for analysis as soon as the drying process was over.

Pesticide residue analysis for 2,4-D in tobacco leaves was conducted by Eurofins Analytik (Neuländer Kamp 1, Hamburg 21079, Germany). The extraction of 2,4-D from tobacco leaves was done as follow: a) 2 g of ground tobacco was weighted into a 50mL plastic extraction vial; b) 10 mL of demineralized water was added and let soaked in for 30 minutes; c) 10 mL acetonitrile was added and thoroughly shaken for 5 min; d) a salt mixture (4g MgSO4 + 1 g NaCl + 1 g $C6H5Na3O_7$. $2H_2O$ + 0,5 g $C_{12}H_{18}Na_4O_{17}$) was added and shaken for 5 min; and subsequently, centrifuged at 4000 rpm for 5 min; and e) an aliquot of the upper acetonitrile phase was transferred into a vial and is further analyzed for 2,4-D by LC-MS/MS. Extracts were analyzed using an HPLC (Agilent 1260, Santa Clara, CA) equipped with a tandem mass spectrometric detector (Sciex API 5500, Concord, ON). Chromatographic separation was achieved using a Phenomenex Luna C18(2) analytical column (5 µm, 150 mm × 2 mm) fitted with a Phenomenex C18 Security Guard cartridge (4× 2 mm), with a mobile phase flow rate of 0.4 mL min^{-1}. The mobile phase contained 0,05% formic acid in water (A) and 0,05% formic acid in methanol (B). Gradient elution was used with a starting composition of 10% B, rising linearly to 90% B over 3 min. The composition was held at 90% B for a further 9 min before returning to the initial conditions over 2 min. The column was re-equilibrated for 2 min at the initial mobile phase composition. The injection volume was 20 µL in total composed of 2 µL sample extract plus 18 µL 0,05% formic acid in water using the autosampler dilution functionality. The retention time for 2,4-D was 8.82 ± 0.1 min. Samples were analyzed in negative electrospray ionization mode using 2 mass transitions 219->161 amu and 221->163 amu. Calibration standards were prepared in acetonitrile at 0.5, 1, 2.5, 5, 10, 25 and 50 ng mL^{-1}.These concentrations encompass the expected range of responses in the final sample aliquots. The R2 for the calibration curves prepared during the study was above 0.999. The limit of detection and limit of quantification were 0.01 and 0.02 mg Kg^{-1}, respectively.

Bootstrapping technique was used to compare 2,4-D concentrations in tobacco leaves across treatments and to generate confidence

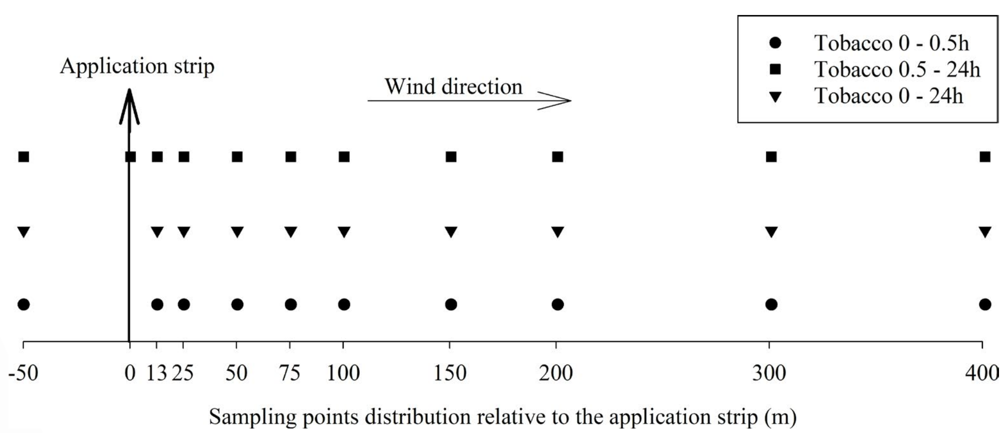

Figure 1 - Schematic representation of the experiment layout in the field showing tobacco plants downwind of the application strip. Plants from different timing schemes and replications were distributed randomly across each sampling point. A 3-meter gap was allowed between plants from a same sampling point.

intervals (EFRON & TIBSHIRANI, 1986). Bias-corrected and accelerated (BCa) method was used, which is recommended for dealing with small data sets that deviate from the normality assumption (MANGIAFICO, 2016). Confidence intervals were calculated for 500 resamplings at 95% confidence level using the Rcompanion package (version 1.10.1) in R software (R CORE TEAM, 2019). The overlap of confidence intervals between treatments indicated the lack of statistical significance.

RESULTS AND DISCUSSION

Herbicide concentration levels decreased as the distance from the herbicide application strip increased (Figure 2). The data also suggested that drift and volatilization levels were lower in 2016. Concentration of 2,4-D in tobacco leaves only reached 0.2 ppm (guidance residue limit established by CORESTA, 2020) or higher for sampling points less than 50 m from application strip in 2016, while it reached 0.2 ppm for all sampling points less than 100 m from the application strip in 2015. Furthermore, 2,4-D concentration showed a sharp increase at 200 m downwind from the application strip in 2016, which could not be explained and is likely due to human error. The lack of 2,4-D at the upwind sample collection point (-50 m) suggested that there were no significant changes in wind direction within the 0 to 0.5 HAT period (Figure 2).

Similarly, to the previous set of plants mentioned, 2,4-D concentration in leaves of plants that stayed in the field from 0 to 24 HAT decreased as the distance from the application strip increased (Figure 3). The minimal buffer required to satisfy the GRL established by CORESTA (0.2 ppm) remained the same (less than 50 m) for 2016 and it was increased to around 75 m for group B in 2015.

Although, tobacco plants from group B (0 – 24HAT) remained in the field longer than group A (0 – 0.5 HAT) and were subjected to volatilization from the application strip, the concentration of 2,4-D in the leaves of those plants was generally lower, against of what one could expect. The processes that may have led to these results includes: 1) volatilization of 2,4-D from the surface of tobacco leaves on plants that remained in the field for 24 HAT; 2) small size of application strip, resulting in small amount of 2,4-D volatilized to the atmosphere and low atmospheric loading with the herbicide; 3) herbicide translocation and/or metabolism; 4) light rain event at 2 HAT in 2016 (1.2 mm of precipitation).

In general, volatilization of pesticides from plant foliage is higher than the observed from soil,

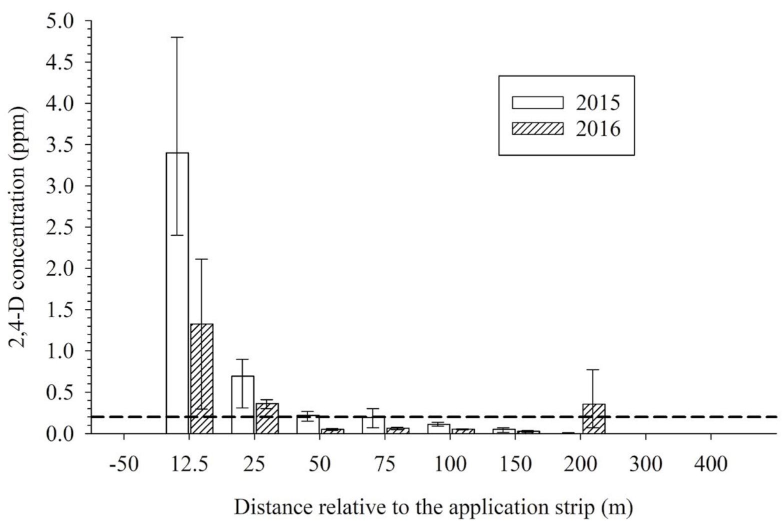

Figure 2 - Concentration of 2,4-D in tobacco leaves as a function of the distance from the application strip for plants left in the field from 0 to 0.5 hours after treatment. Dotted line represents the guidance residue levels (GRL) for 2,4-D established by CORESTA (2020).

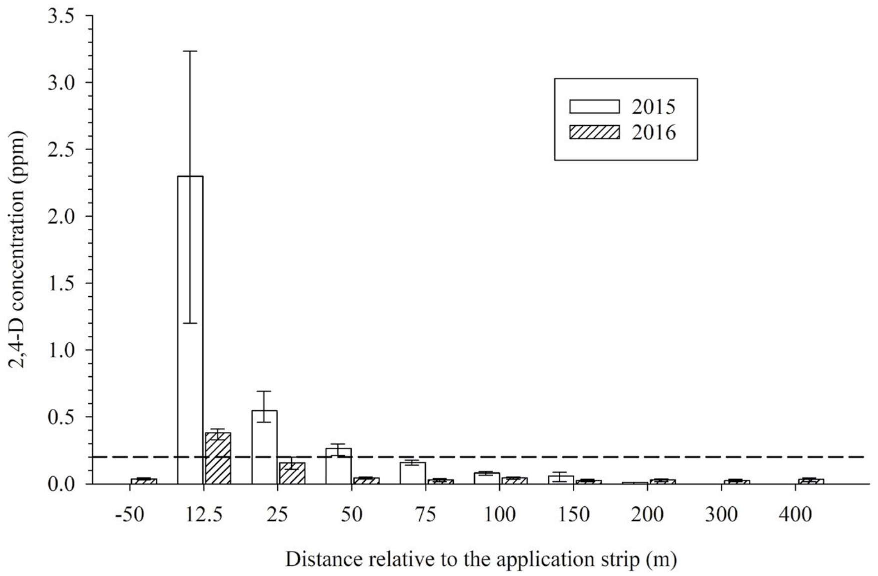

Figure 3 - Concentration of 2,4-D in tobacco leaves as a function of the distance from the application strip for plants left in the field from 0 to 24 hours after treatment. The dotted line represents the guidance residue levels (GRL) for 2,4-D established by CORESTA (2020).

due to the greater air flow and turbulence through the plant canopy, the greater water evaporation from leaf surfaces compared to soil surface and the lower affinity with the leaf surface compared to soil particles (BEDOS et al., 2002; SOSNOSKIE et al., 2015). WOLTERS et al. (2004) indicated that volatilization from soil is comparatively small after applications to soil-plant systems, reinforcing the relevance of this phenomena. Therefore, volatilization of 2,4-D from tobacco leaves may have contributed to reduce the concentration of the herbicide from plants of group B (0 to 24h) compared to group A (0 to 0.5h). Additionally, movement of the absorbed 2,4-D to other plants parts will reduce herbicide concentration by dilution (JACOBSEN et al., 2015). This might be especially relevant to 2,4-D, considering its great mobility in plants as a result of its intermediate lipophilicity and weak acid nature (PETERSON et al., 2016).

A light rainfall event in 2016 may have influenced the results in two ways: 1) washing out part of the herbicide deposited on tobacco leaves due to primary drift; 2) wet deposition of 2,4-D in suspension in the atmosphere onto tobacco leaves (GOEL et al., 2005; WAITE et al., 2005). Wet deposition would explain the presence of 2,4-D at all group B sampling distances in 2016. Even though these two processes are contrary to each other, both will occur simultaneously if rain happens after an herbicide application.

Finally, 2,4-D was detected on plants that were moved to the field after 0.5 hours from herbicide application (Figure 4). The herbicide detected on these samples was deposited via wet (caused by rain) or dry (caused by wind) deposition of the herbicide in suspension in the atmosphere. Both processes have been extensively reported for innumerous pesticides and contaminants (WAITE et al., 2005; SAURET et al., 2009; OWENS, 2021). It is interesting to observe the magnitude differences between plants that were taken to the field prior and after herbicide application. At 12.5m, 2,4-D concentration was almost 10x higher in plants from the 0 to 24h group compared to the group taken to the field after 30 minutes from herbicide application, indicating that drift contributed to a higher extent to 2,4-D contamination than volatilization.

The fate of a given molecule in the environment will be dictated by, among other things, its physicochemical properties and the environmental conditions in which the molecule lies. In the present study, higher wind speeds and temperatures as well as lower relative humidity

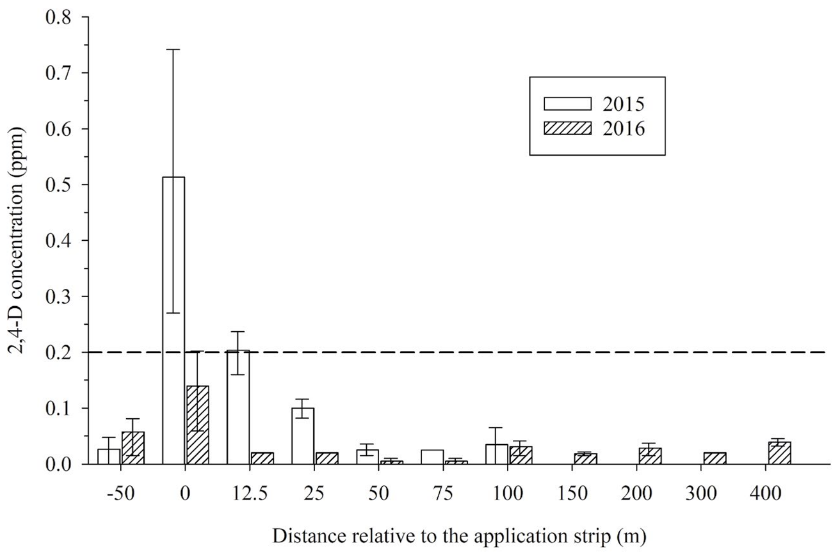

Figure 4 - Concentration of 2,4-D in tobacco leaves as a function of the distance from the application strip for plants left in the field from 0.5 to 24 hours after treatment. The dotted line represents the guidance residue levels (GRL) for 2,4-D established by CORESTA (2020).

occurred in 2015 when compared to 2016 (Table 1). These conditions are known to favor herbicide drift and volatilization (ARVIDSSON et al., 2011; MUELLER & STECKEL, 2019; STRIEGEL et al., 2021). OSELAND et al. (2020) identified wind speeds following application as key factor affecting off-target movement of dicamba, another auxin-like herbicide molecule. High temperatures are favorable for herbicide volatility because vapor pressure, the main physicochemical property related to volatility, is dependent on temperature (BEDOS et al., 2002). Therefore, considering the information presented above and that the experiment's methodology was not modified in any way between 2015 and 2016, we conclude that the differences in 2,4-D concentration between years in tobacco leaves are due to different environmental conditions, despite the fact they were within the recommended parameters for application.

Wet deposition occurred in 2016 and the results indicated that this process may transport 2,4-D to distance as much as 400 m from the source. Additional sampling points would be necessary to evaluate the extent to which this process may influence long distance OTM of herbicides such as 2,4-D under our experiment's conditions. This phenomenon has been demonstrated by HILL et al. (2002) in rural and urban areas of Alberta, Canada.

Finally, it can be argued that the nozzles used in this experiment are not suitable for 2,4-D application. According to a recent survey by BUTTS et al. (2021), only 28% of herbicide applicators in Arkansas were able to provide information on specific nozzle type used across ground and aerial spray equipment. This demonstrated that farmers, in many occasions, are unaware of the importance of nozzle selection in regards to weed management and mitigation of OTM of pesticides. Despite of that, nozzles selection is important to prevent drift. In this study, nozzles were selected based on worst-case scenario from nozzles frequently used by farmers. It was not the goal of the study to test new formulations and/or application technologies tools. For example, the use of hooded sprayers considerably reduced spray drift and can be added as a tool for the mitigation of OTM of pesticides (FOSTER et al., 2018).

CONCLUSION

Herbicide applications of 2,4-D under environmental conditions similar to the observed in our experiments would require a 100 m buffer zone

Table 1 - Average environmental conditions during 2,4-D application and after 24 hours for 2015 and 2016.

Variable	--------------During application--------------		-----------------24 hours after application-----------------	
	2015	2016	2015	2016
Wind speed ($km.h^{-1}$)	9.72	5.4	7.04	4.10
Air temperature (C)	29.5	28.8	26.10	28.10
Air humidity (%)	68.0	75.1	77.7	79.0

between target fields and tobacco fields to maintain 2,4-D residue concentrations below GRLs established by CORESTA (2020). The herbicide 2,4-D has the potential to volatilize and contaminate surrounding farms. In addition, wet deposition of suspended herbicide particles can play an important role in the amount of herbicide residues found in agricultural products.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

MMN, LAA and ERC conceived and designed experiments. MMN, LAA, MZ, RB, KE and ERC performed the experiments, MMN carried out the lab analyses. LAA and ERC supervised and coordinated the experiments. MMN performed statistical analyses of experimental data. MMN and MZ prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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