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Research Note

Recovery of metallic markers sprayed on soybean plants

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Abstract

To understand aspects related to the application of plant protection products, the analysis of spray deposits using metallic markers is a simple and low cost alternative. However, choose the appropriate marker is a challenge. Here, we investigated the recovery of metallic ions used as markers sprayed on soybean plants. The experiment was carried out with soybean plants grown in vases from April to June 2012. The experimental design was completely randomized with 5 treatments, each one composed by the metallic markers: manganese sulfate ($MnSO_4$), zinc sulfate ($ZnSO_4$), copper sulfate ($CuSO_4$), copper hydroxide ($Cu(OH)_2$) and copper oxychloryde ($CuCl_2.3Cu(OH)_2$). Each marker was sprayed on soybean leaves in 5 dosages and 2 replications (plants at phenological stages R1 and R2). Furthermore, the metallic markers were applied over the glass laminae due to their inability to absorb. Later on, the markers were washed from soybean leaves and the glass laminae. In this process, samples were submitted to the acid solution (0.2 mol L^{-1}HCl) for 60 minutes and the quantification of the recovered concentration of each ion was accomplished by means of an atomic absorption spectrophotometer. Hence, standard curves of the cations Cu^{2+}, Mn^{2+}, and Zn^{2+} with concentrations of 0.25, 0.5, 1.0, 2.0 were used. The values were submitted to the statistical analysis of regression. Considering the extraction method used and the rate of recovery, the markers copper hydroxide and copper oxychloride are not recommended for such studies, since these products either on soybean leaves or in a glass laminae showed lower recovery (lower than 97%). Otherwise, the manganese, copper and zinc sulfates (above than 98%) are suitable for studying spray deposits. Appropriated metallic markers to study deposits are discussed.

Keywords: analytical methods; application efficiency; *Glycine max* L.; spectrophotometry.
Abbreviations: PPP_plant protection product; µg_microgram; DAE_days after seedling emergence; nm_nanometer.

Introduction

Soybean [*Glycine max* (L) Merril] is one of the most important crops in the world. As a crop, it is faced with countless challenges if higher grain quality and production are searched. Among those difficulties, efficaciously controlling pests and plant diseases, mainly leaf diseases, are the biggest (Cunha et al., 2011). Among research works concerning technologies for the application of plant protection products (PPP), those in which deposits resulting from the application are measured are important to evaluate the application efficacy in controlling pests, diseases, and weeds, as well as to reduce control flaws and environmental contamination (Yu et al., 2009). The most representative analysis of deposits to understand aspects related to the application of PPP is based on the detection and recovery of chemical elements or substances on the plants surface, artificial targets or specific sampling equipment (Palladini et al., 2005). Each target type shows advantages and disadvantages, though the natural surfaces are the best since they represent more precisely the real conditions of a field application (Miller et al., 1993). Studies of spray deposits may be carried out with PPP or markers (Travis et al., 1985). The disadvantage of using PPP is the difficulty in getting reproducible methods (Marchi et al., 2005) and also the high costs in recovery process of these products by chromatography. Otherwise, markers are such a low cost alternative, easily visible and removable from plant leaves or collecting targets by atomic absorption spectrophotometry. Nevertheless, data resulting from recovery process of markers are not so accurate comparing with those resulting from PPP (Hewitt, 2010; Souza et al., 2007). Several papers show the option of researchers for markers such as metallic ions (Christovam et al., 2010; Oliveira and Machado-Neto, 2003; Ramos et al., 2007), fluorescent pigments and food coloring material (Costa et al., 2012; Pinto et al., 2007; Solimões et al., 2009) to study spray deposits. The main advantage of metallic ions in comparison with others is their stability, avoiding the degradation by sun light (Hermosilla et al., 2008). In addition to that, they are easily detectable by atomic absorption spectrophotometry, giving analyses more credibility (Zabkiewicz et al., 2008). However, if ions are absorbed by the leaves this may cause problems for their analytical quantification. That is the reason why choosing the metallic marker is very important (Murray et al., 2000). So, the objective of this research was to determine the recovery of metallic ions used as markers viewing to adequate them to studies of spray deposits in soybean plants.

Table 1. Regression equation, coefficient of determination (R^2), F test (F) and coefficient of variation (CV), obtained in the study of the recovery of markers applied in soybean leaves.

Markers	Evaluation		Equation	R^2	F	CV (%)
Manganese sulfate	50 DAE[1]	1º	$\hat{y}$ = 1.033 x	0.998	17,099.42**	2.71
	70 DAE	2°	$\hat{y}$ = 1.066 x	0.998	14,555.78**	2.96
Zinc sulfate	50 DAE	1º	$\hat{y}$ = 0.986 x	0.994	3,991.23**	5.65
	70 DAE	2°	$\hat{y}$ = 1.007 x	0.968	865.86**	12.02
Copper sulfate	50 DAE	1º	$\hat{y}$ = 1.010 x	0.993	3,523.77**	5.89
	70 DAE	2°	$\hat{y}$ = 1.004 x	0.992	5,858.65**	4.46
Copper hydroxide	50 DAE	1º	$\hat{y}$ = 0.919 x	0.987	2,733.85**	7.08
	70 DAE	2°	$\hat{y}$ = 0.978 x	0.996	6,802.01**	4.33
Copper oxychloryde	50 DAE	1º	$\hat{y}$ = 0.941 x	0.993	4,664.23**	5.33
	70 DAE	2°	$\hat{y}$ = 0.982 x	0.993	3,910.97**	5.63

[1]DAE - days after emergence of the plants. 1º - First evaluation. 2°- Second evaluation. According to the F test: ** significant at the 1% level of probability.

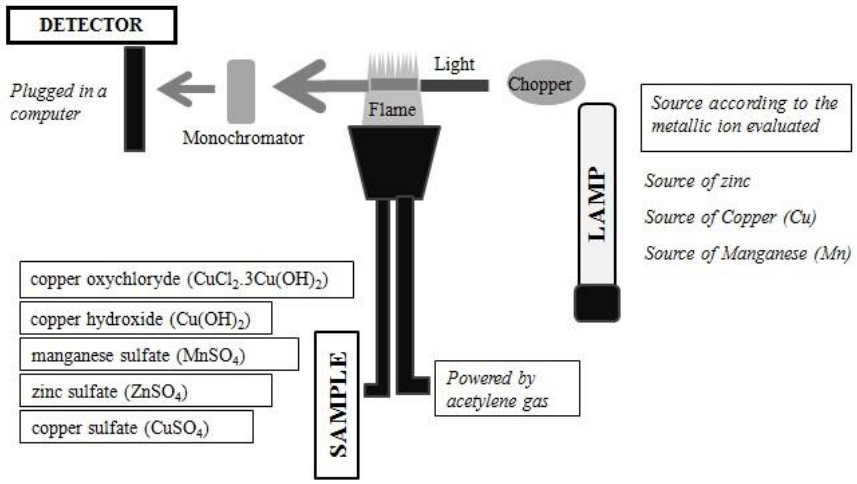

Fig 1. Atomic spectrophotometry process: a roadmap for the determine of ions concentration by atomic spectrophotometer.

Table 2. Regression equation, coefficient of determination (R^2), F test (F) and coefficient of variation (CV), obtained in the study of the recovery of markers applied in glass laminae.

Markers	Evaluation	Equation	R^2	F	CV (%)
Manganese sulfate	1º	$\hat{y}$ = 0.998 x	0.999	30,850.47**	2.01
	2°	$\hat{y}$ = 1.052 x	0.999	29,241.00**	2.09
Zinc sulfate	1º	$\hat{y}$ = 1.036 x	0.999	26,862.87**	2.18
	2°	$\hat{y}$ = 1.057 x	0.997	10,544.24**	3.40
Copper sulfate	1º	$\hat{y}$ = 0.997 x	0.990	4,430.57**	5.43
	2°	$\hat{y}$ = 1.063 x	0.999	30,617.77**	2.01
Copper hydroxide	1º	$\hat{y}$ = 0.887 x	0.994	6,025.95**	4.69
	2°	$\hat{y}$ = 0.973 x	0.993	4,586.73**	5.38
Copper oxychloryde	1º	$\hat{y}$ = 0.860 x	0.979	1,091.72**	10.95
	2°	$\hat{y}$ = 0.985 x	0.994	3,911.81**	5.69

1º - First evaluation. 2°- Second evaluation. According to the F test: ** significant at the 1% level of probability.

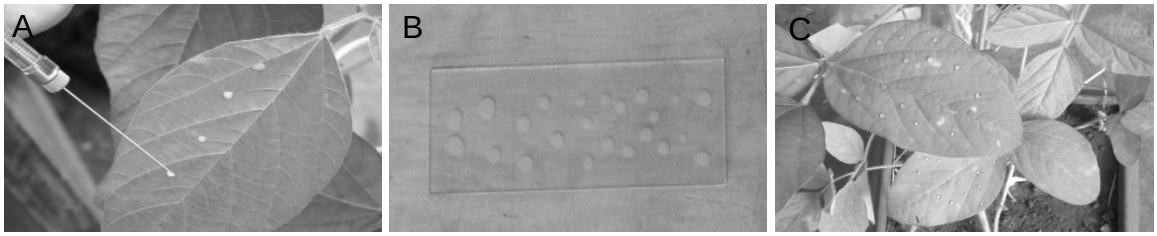

Fig 2. Application of markers on soybean leaves (A) and on glass laminae (B). Droplet size and distribution of the deposit of spray applied by micro syringe (C).

Results and Discussion

Regression analysis and descriptive statistics parameters

The data concerning recovered concentration by atomic spectrophotometer (Fig. 1), when compared with the predetermined concentration of markers, were submitted to regression analysis and showed an adjustment to the linear model with equations which allowed to estimate the recovery of the markers applied both to soybean leaves and glass laminae (Tables 1 and 2). In the regression equation, "ŷ" corresponds to the recovered concentration (µg mL^{-1} or mg. L^{-1}) and "x" to the predetermined concentration (µg mL^{-1} or mg L^{-1}). The markers recovery from soybean leaves and glass laminae showed determination coefficients above 0.96 on both evaluations, thus indicating that more than 96% of the observed values are included in the recovery estimation of markers indicated by the equations (Tables 1 and 2).

Analysis the recovery of metallic markers

Manganese and copper sulfates

From soybean leaves a 100% recovery was verified for Mn and Cu when applied as sulfate, on both evaluations (Table 1). Similar results were found by Zabkiewicz et al. (2008) in an experiment with metallic cations used as markers. Though these authors observed that field spray deposition results from the Mn and Cu spray were anomalous with a 101% and 131,4% recovery, respectively. According to the authors, "the source of the irregular cooper contamination was not identified unambiguously". However, possible reasons are that errors which were at analytical limits of detection slightly above (for Mn) or very above (for Cu) may occur due to the tank spray solution carryover, weighing markers wrong and even by the complexity in reading elements by atomic absorption spectrofotometry. Otherwise, there is a possibility of absorbing the metallic markers by leaves. In this case, the recovery from spray solution results rates below. Therefore, in this study we compare the recovery rate in leaves with glass laminae, thus giving results more credibility. Despite the complexity involved in the process of recovery markers, coherent results has been successfully reported using markers formulated from a base of Mn and Cu (Costa et al., 2013; Bauer et al., 2003). Hence, we consider that the recovery rates verified to manganese and copper sulfates are reliable.

Zinc sulfate

Zinc recovery was of 98.2 and 100.0% in the first and second evaluations, respectively (Table 1). Xu et al. (2006) used zinc chelate to quantify the amount of deposit resulting from the application of PPP in apple fruits. They reported a zinc recovery rate larger than 92%, a value approximate to those found in this research work. In other hand, the authors carried out this study under field conditions. In these cases, potential differences in canopy structure may be an important contributing factor to decrease the recovery rates of markers, like many studies have shown that initial spray deposit and retention are critically influenced by canopy structure in tree crops (Cross et al., 2001; Travis, 1987; Smith et al., 1984).
The usefulness of recovery of metallic markers in studying PPP deposition on soybean, as well as others crops, depends upon the permanence of the marker on required targets. Thus, we expect real values of zinc recovery, once application were realized under controlled conditions.

Copper hydroxide and copper oxychloride

Regarding to the recovery of copper applied as copper hydroxide and oxychloryde, were found 91.9 and 94.1%, respectively, in the first evaluation (Table 1). Despite comparatively low values, they are above the minimum (80% of recovery) for the validation of analytical procedures established by the National Agency for Sanitary Surveillance (ANVISA, 2002). Copper recovery in the second evaluation both when applied as copper hydroxide and oxychloryde was above 97% (Table 1). These are results close to those reported by Prado et al. (2010). These authors, in order to measure the deposits resulting from the PPP applied in soybean crop, made use of a cupric fungicide based on copper oxychloryde (Cobox 50%) and found mean copper recovery values above 99.0%. Copper both as hydroxide and oxychloride applied to soybean leaves and glass laminae was less recoverable than the other markers. In addition to that, the differences between recovery values from the first to the second evaluation were more variable than those verified for the other markers (Table 1 and 2). These results are not ascribable to the soybean leaves age or to the possibility of the markers having been absorbed by the plants since similar results were found to the glass laminae. Nevertheless, we observed that copper when used in the form of hydroxide or oxychloryde did not result in homogeneous solutions, such the way of other markers did. Therefore, when applied to the soybean leaves or glass laminae in a suspension form, variations in the recovery rate were expected. The present study clearly shows that the recovery rate values are variable, but was demonstrated that the spray deposits of PPP can be better undertaken when choosing the suitable metallic markers. Overall, more investigation using different markers on different crops would be interesting to improve the field application. Also, we suggest researches about the interaction between markers and PPP, regarding the efficiency of those products.

Materials and Methods

Experimental details

The experiment was conducted from April to June 2012 in the Jaboticabal municipality, state of Sao Paulo (SP), Brazil (21°15'22" S latitude, 48°19'20" W longitude), with an average altitude of 575 m, and Aw climate according to the Köppen climate classification. The coordinates were recorded in the UTM (Universal Transverse Mercator) coordinate system. Were used soybean cultivar plants "BRS Valiosa RR". This cultivar was chosen all because it is amply adopted by Brazilian farmers. Soybean plants grown in five-liter-plastic vases were carried out under greenhouse conditions. Until the end of this study, plants shown great development, no damage by insects or diseases was reported.

Metallic markers: Dosages and formulations

The treatments consisted in the adding of one of the following metallic markers to the distilled water to be applied to soybean plants: manganese sulfate (10 g L^{-1} - $MnSO_4.H_2O$, 31% Mn^{2+}), zinc sulfate (13 g L^{-1} - $ZnSO_4.7\ H_2O$, 23% Zn^{2+}), copper sulfate (12 g L^{-1} - $CuSO_4.5\ H_2O$, 25% Cu^{2+}), copper hydroxide (9 mL L^{-1} - $Cu(OH)_2$, 35% Cu^{2+}) and copper oxychloride (6 g L^{-1} - $CuCl_2.3Cu(OH)_2$, 50% Cu^{2+}). The solutions were prepared aimed to approximately 3.000 mg L^{-1} of the respective metallic ion. Copper hydroxide and oxychloride used in this experiment are usually found in the

commerce as cupric fungicide formulations by the names Supera™ and Cuprogarb™, respectively. Both are made by Oxiquimica Agrociência LTDA. Manganese, copper, and zinc sulfates were of the type "pure for analysis" (p.a.).

Application of treatments

The metallic markers were applied with the help of a micro syringe (Hamilton, DS500/GT) at the following amounts: 0.025, 0.050, 0.075, and 0.100 mL of each solution/suspension prepared with each product. We used five doses from each marker to allow a regression curve. For each marker, the amounts mentioned were distributed over the upper surface of five leaves randomly chosen in soybean plants (Fig. 2 A) and over five samples of glass laminae (Fig. 2 B), being 5 repetitions for each volume. A check treatment (without application) was also considered. The rate of recovery of metallic ions was also evaluated by means of glass laminae. Since, according to Iost and Raetano (2010), the glass laminae are a standard hydrophilic surface to which comparisons are made. The applications were made when the plants were at the phenological stages R1 (50 DAE) and R4 (70 DAE) (Ritchie et al., 1982), in order to evaluate the repeatability of results considering that those are the stages when PPP applications are usually applied. The use of micro syringe to apply of the solutions was a simulation of the application in the field, although the size and distribution of droplets were probably more uniform than those verified under field conditions (Fig. 2 C). This aspect though is not considered as having a significant influence on the results since the quantification is based on the absolute total applied and is directly related with the solution concentration.
The solutions were applied during the morning period (between 10h and 12h) inside the greenhouse, with mean temperatures and relative humidity of 28 and 25°C and 68 and 61%, respectively at 50 and 70 DAE.

Collecting samples and quantification of metallic markers

After applied, we waited for the droplets deposited on leaves and glass laminae to dry (it took around 120 min.). Regarding to studies of spray deposits, samples are usually removed as soon as possible after the application of solution (Costa et al., 2013). Once, possible losses promoted by runoff, rain, insects attack, among others, could influence the recovery rations of metallic markers. However, the adopted waiting time was considered necessary to depositing and spreading of solutions in the surfaces. The leaves and glass laminae were characterized as samples, they were manually placed inside the polyethylene bags to which 100 mL of a 0.2 mol L^{-1} hydrochloric acid solution were added. Thus, this being followed by a repose of 60 minutes aim to extracting the markers, following procedures described in Oliveira and Machado-Neto (2003). According to the authors, with 0.2 mol L^{-1} hydrochloric acid the recovery of metallic markers hits 100%. The markers concentrations on soybean leaves and glass laminae were determined by means of an atomic absorption spectrophotometer (iCE 3000) with a multi-element hollow cathode lamp with specific wave lengths of 324.8, 279.5, and 213.9 nm for the detection of, respectively, Cu^{2+}, Mn^{2+}, and Zn^{2+}. In the standard curves of the cations Cu^{2+}, Mn^{2+}, and Zn^{2+} standards concentrations of 0.25, 0.5, 1.0, 2.0, and 4.0 mg L^{-1} were used. The coefficients of determination of the curves were above 0.99. With the help of the equation resulting from the calibration curve, the absorbance values were transformed in concentration (mg L^{-1} or µg mL^{-1}). Using the solution concentration and the dilution volume of the samples, the recovered amount of each marker was determined.

Statistical analysis

The experimental design was completely randomized with a total of 125 treatments (5 markers X 5 dosages with 5 repetitions) and 2 replications (plants at 50 and 70 DAE). The recovered concentration of each marker was compared with that determined by the statistical analysis of regression. After that, the linear regression equations passing by the origin (a = 0, so, $\hat{y}$= bx) were determined.

Conclusions

Based on the preceding results and taking in consideration the extraction method used as well as the recovery rate, the markers manganese, copper, and zinc sulfates were considered adequate for studies of the deposits resulting from the application of PPP to soybean. However, the calculated recovery values should be similar to those resulting from measurements. Copper hydroxide or as oxychloryde are not recommended as markers in studies of deposits since they showed lower recovery values. Moreover, the recovery values for those markers showed larger variations than those shown by the other markers.

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