Decontamination Procedure for Sorghum and Coffee Leaves Sprayed With Zinc and a Surfactant

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Summary

Decontaminating leaf samples from crops sprayed with pesticides and nutrient solutions is important for foliar analysis. This study evaluated the effect of different washing methods in coffee and sorghum foliage that had been sprayed with zinc (with or without surfactant). The plants were sprayed with a 3 g L^{-1} zinc sulfate solution, with and without surfactant. Seven days later, leaves were collected and washed. The experiment was completely randomized in a 2 x 2 x 3 + 2 factorial, with three replications. The first factor represents foliar zinc applications with or without surfactant, the second represents the number of washes (1 or 2) and the third represents the concentration of the wash solution (detergent + hydrochloric acid) at (0 + 0 mL L^{-1}; 1.0 + 3.5 mL L^{-1} and 2.0 + 7.0 mL L^{-1}). The last one represents two additional treatments without washing (zinc sprayed with and without surfactant). Surfactant strengthens contact between zinc and foliage and enhances absorption. Washing is an indispensable pretreatment for leaf analysis and our study showed that a single wash with detergent + hydrochloric acid (1.0 + 3.5 mL L^{-1}) was the most effective washing method for coffee and sorghum.

Keywords: foliar fertilizer, leaf washing methods, sample decontamination, foliar analysis

Resumen

Procedimiento de descontaminación de hojas de sorgo y de café pulverizadas con zinc y un surfactante

La descontaminación de las muestras de hojas de cultivos pulverizados con pesticidas y soluciones nutritivas es importante para el análisis foliar. Este estudio evaluó el efecto de diferentes métodos de lavado en hojas de café y de sorgo que habían sido pulverizadas con zinc, con y sin surfactante. Siete días más tarde, las hojas se recogieron y se lavaron. El diseño experimental fue completamente aleatorizado, en esquema factorial 2 x 2 x 3 + 2, con tres repeticiones. El primer factor representa aplicaciones foliares de zinc con o sin surfactante; el segundo representa el número de lavados (1 ó 2), y el tercero la concentración de la solución de lavado (detergente + ácido clorhídrico) en (0 + 0 mL L^{-1}; 1,0 + 3,5 mL L^{-1} y 2,0 + 7,0 mL L^{-1}). El último factor representa dos tratamientos adicionales sin lavar (zinc rociado con y sin surfactante). El surfactante refuerza el contacto entre el zinc y el follaje y mejora la absorción. El lavado es un tratamiento previo indispensable para el análisis foliar, y nuestro estudio mostró que un solo lavado con detergente + ácido clorhídrico (1,0 + 3,5 mL L^{-1}) fue el método de lavado más eficaz para el café y el sorgo.

Palabras clave: fertilizante foliar, métodos de lavado de hoja, descontaminación de muestras, análisis foliar

Introduction

Brazilian soils generally lack zinc and therefore require applications of this mineral to maximize productivity (Pozza *et al*., 2009). Zinc activates approximately 100 enzymes in different plants and is essential for the synthesis of DNA and RNA and the metabolism of carbohydrates, fats, proteins, and alcohols (Malakouti, 2008). Despite zinc's importance in plant metabolism, studies that monitor this nutrient are still scarce.

Foliar application is the most common application method for crops deficient in zinc because it allows more efficient uptake (Mann *et al*., 2002; Orioli Júnior *et al*., 2008). Soil applications are less efficient because zinc is easily chelated by organic compounds and precipitated by iron oxides (Pokrovsky *et al*., 2005) and alkaline precipitates (Malakouti, 2008) making it unavailable for crops.

Foliar fertilization is possible because plants are capable of absorbing nutrient solutions directly from the leaf surface, and is an effective and economical way to deal with micronutrient deficiencies in coffee and sorghum (Volkweiss, 1991; Boaretto and Rosolem, 1989). Surfactants and other adherents are added to the spray solution to increase the efficiency of foliar applications of zinc and other nutrients. This practice maximizes leaf wetting and minimizes runoff (Prado *et al*., 2003; Martins *et al*., 2010).

However, applications of foliar zinc and other nutrients can produce erroneous leaf analyses because some of the applied nutrients are not absorbed by the plant, but are adsorbed into the leaf surface. Thus, Vale *et al*. (2008) point out that mango trees sprayed with zinc can be decontaminated using deionized water, detergent and an acid solution. Faifer *et al*. (2012) determined that adding surfactant to zinc spray led to higher levels of the micronutrient in corn and that decontamination of leaf samples from these plants was more efficient when they were washed twice by immersion in detergent and hydrochloric acid.

The detergent solution is used primarily to eliminate dust and contaminants whereas the acidic solution (hydrochloric acid) removes metals such as those applied during foliar applications and adsorbed to the leaf surfaces (Prado, 2008). Thus, acid and detergent solutions are highly efficient at removing iron, manganese, copper and zinc (Alvarez-Fernandes *et al*., 2001).

Washing leaf samples prior to analysis is important to reliably measure nutrient concentrations (Martins and Reissmann, 2007) because it reduces contamination from foliar applications, fertigaton, fungicides, herbicides and insecticides. This is especially important for perennial crops. Washing leads to more accurate foliar diagnoses that allow corrections and adjustments to the nutritional state of the plants. This, according to Souza *et al*. (2010), provides changes to management of present and future crops. Martins and Reissmann (2007) highlight the relevance of washing duration to the reliability of foliar diagnoses of various nutrients.

It is worth noting that the efficiency of a washing method may be affected by leaf morphology and anatomy (Faquin, 2005). Plants with smooth, rough or waxy leaves can offer different levels of resistance to washing. There are few studies evaluating different decontamination procedures for leaf samples from different crops. The paucity of these publications and limited access to this information for users and operators have impacted foliar-based diagnoses and affected nutritional management of annual and perennial crops.

Thus, we evaluated foliar levels of zinc in coffee and sorghum crops due to washing methods of leaf samples that had been sprayed with zinc, with and without surfactant.

Materials and Methods

The study was conducted at the Educational Farm, Research and Extension (FEPE) at the School of Agriculture and Veterinary Sciences (FCAV/UNESP), Jaboticabal Campus, located in Jaboticabal, Brazil.

Sorghum and coffee were used due to the distinct morphological and anatomical structures of their leaves and because these crops typically receive foliar zinc applications. The crops were sprayed with a solution containing 3 g of zinc sulfate per liter of water (Raij *et al.*, 1997), with and without surfactant. Surfactant Gotafix® (Monil phenol polyethylene glycol ether, 125 g L^{-1}), a non-ionic, concentrated aqueous solution, was used as a dispersant at the manufacturer's recommended rate (60 ml per 100 liters of water).

Samples of 10 leaves per parcel were collected seven days after spraying. Once collected, the leaves were immediately sent to the laboratory and subjected to treatments. The experiment was completely randomized in 2 x 2 x 3 + 2 factors and three repetitions. The treatments were: (2) leaves sprayed with zinc in the presence and absence of surfactant, (2) one and two washes and (3) concentrations of detergent + hydrochloric acid (0 + 0 mL L^{-1}; 1.0 + 3.5 mL L^{-1} and 2.0 + 7.0 mL L^{-1}), and two additional treatments without rinsing (zinc spray with and without surfactant).

Prado (2008) recommends a standard concentration of detergent + HCl at 1.0 + 3.5 mL L^{-1}. Thus, in this experiment the wash concentrations of detergent + HCl used were:

deionized water with neither detergent nor HCl, a standard concentration (1.0 mL L^{-1} of detergent and 3.5 mL L^{-1} of HCl) and double the standard concentration (2.0 mL L^{-1} of detergent with 7.0 mL L^{-1} of HCl).

All of the samples were immersed and washed for 30 seconds. Washing duration was standardized in order to eliminate time as a variable.

After collecting the leaves, the samples were prepared as previously described. After washing, the samples were dried in a forced air oven (65 to 70 ºC) until reaching a constant weight. The dried samples were then ground and zinc levels were determined by the methodology described by Bataglia *et al*. (1983).

The resulting data were analyzed by analysis of variance and averages were compared by the Tukey test (5 %). These calculations were made with the statistical program AgroEstat using the proposed experimental design.

Results and Discussion

There was no significant interaction between any of the factors for any of the crops (P>0.05). However, there was an isolated effect from the surfactant for coffee leaves and for detergent + HCl for both species (P<0.01). There was also a significant effect between the controls (P<0.01) and between the average of the factorial versus the average of the control treatments (P<0.01). The number of washings was not significant (P>0.05) (Table 1).

Applications of foliar zinc with surfactant produced higher levels of zinc in the leaf samples from coffee, but the same result was not observed for sorghum (Table 2). Higher levels of foliar zinc in coffee can be attributed to greater absorption due to surfactant and the zinc that was not removed by washing and remained on the leaf surface. Spray runoff is greater with coffee because its leaves are smoother and waxier than those of sorghum. Thus, the surfactant caused the spray droplets to spread out more, which maximized leaf wetting, minimized runoff, as highlighted by Prado *et al*. (2003) and Martins *et al*. (2010), and decreased losses. This result shows that leaf structure interferes with absorption.

Velini *et al*. (2000) and Mann *et al*. (2002) recommend adding dispersants and adhesive agents to the spray in order to improve absorption. However, using these additives necessitates additional sample pre-treatments to decrease or dilute spray residue. This in turn, could interfere with nutritional analysis because part of the nutrients could be absorbed into the surfaces of the leaves.

Faifer *et al*. (2012) observed the effect of surfactant on foliar zinc retention in greenhouse-grown corn plants. The authors noted that adding surfactant to the zinc spray resulted in higher micronutrient levels in plant tissue regardless of washing method. They also noted that the most effective wash consisted of two immersions in the same standard concentration of detergent and hydrochloric acid used in the present study.

Faquin (2002) emphasized the importance of physical and chemical treatment to remove contaminants in samples from sprayed crops. The same author noted that the efficiency

Table 1. Summary of the analysis of variance for zinc levels in coffee and sorghum plants due to washing methods after applications of foliar zinc with and without surfactant.

Source of variation	GL	Coffee	Sorghum
		-------- F test --------	
Surfactant (A)	1	10.85**	1.70^{NS}
Number of washes (B)	1	1.71^{NS}	0.20^{NS}
Detergent + HCl (C)	2	5.20**	12.43**
A x B	1	0.28^{NS}	0.27^{NS}
A x C	2	0.30^{NS}	0.31^{NS}
B x C	2	0.13^{NS}	0.53^{NS}
A x B x C	2	0.22^{NS}	0.47^{NS}
Controls	1	14.87**	10.13**
Factorial x Control	1	175.27**	190.0**
Residue	42	-	-
CV (%)	-	17.9	12.4

** and ^{NS}: Significant at 1% probability for the F test and not significant.

Table 2. Foliar zinc levels in coffee and sorghum plants due to leaf washing methods after zinc sulfate spraying, with and without surfactant.

Surfactant	Coffee	Sorghum
	--- Zn (mg kg^{-1}) ---	
With	31.8 a	384.3
Without	26.2 b	364.9
Number of washes		
One	30.1	378.0
Two	27.9	371.2
Detergent + HCl (mL L^{-1})		
0.0 + 0.0	32.7 a	425.7 a
1.0 + 3.5	28.3 ab	358.6 b
2.0 + 7.0	26.0 b	339.4 b
Controls		
With surfactant	67.2 a	703.2 a
Without surfactant	51.0 b	587.5 b
Means		
Factorial	29.0 b	374.6 b
Controls	59.1 a	645.4 a

Means followed by same letters in columns do not differ significantly by the Tukey test at 5% probability.

of washing methods can vary depending on leaf morphology and anatomy (Faquin, 2005).

The wash consisting of only deionized water produced the highest levels of zinc in both crops and did not effectively remove surface zinc because it lacked detergent and HCl (0 + 0 mL L^{-1}). This result agrees with Chamel *et al*. (1982) who reported that using detergent and acid in sample washing removed large quantities of zinc from the leaf surfaces that had been sprayed with zinc. This also reinforces the idea that HCl in the wash solution removes metals like zinc that had been adsorbed into leaf surfaces (Álvarez-Fernándes *et al*., 2001; Prado, 2008).

Using twice the standard concentration of detergent + HCl had no effect compared to the standard concentration. Thus, increasing the concentration of detergent + HCl in the wash is unnecessary. It should be noted that detergent and hydrochloric acid in the wash solution at the standard concentration (1.0 + 3.5 mL L^{-1}) decreased zinc content by 13.5 % for coffee and 15.8 % for sorghum compared to washing only with deionized water (0 + 0 mL L^{-1}).

Finally, the importance of leaf washing can be seen in the average zinc levels, which were 104 % (coffee) and 72 % (sorghum) higher than those in the treatments with leaf washing. Within the control treatments, average foliar zinc was 32 % (coffee) and 20 % (sorghum) higher when zinc was applied with surfactant than when surfactant was not used.

Therefore, higher foliar zinc levels in both crops can be attributed to the absence of washing. This increase was enhanced by surfactant, which may have caused greater foliar absorption and/or adhesion of zinc.

Vale *et al*. (2008) found that washing Mango leaves twice with deionized water followed by a single wash with the standard solution used in the present study, maximized the decontamination of leaf samples that had been sprayed with zinc. The authors further stated that the addition of acid reduced zinc content by about 30 %. Peryea (2005) also found that washing with dilute hydrochloric acid after washing with detergent effectively removed surface zinc. Nevertheless, before extrapolating these results, it is necessary to consider that the heterogeneity of plant morphology, anatomy, and phyllotaxy, can mask results, and make it difficult to define an optimal decontamination protocol (Faquin, 2005).

The number of washes in this study did not influence zinc levels. Therefore, one wash is recommended, because it will increase efficiency, decrease labor and reduce the consumption of washing solution. In addition, Martins and Reissmann (2007) indicate the need to consider immersion and washing time to maximize cleaning and decontamination. In the present study, this variable was standardized at 30 seconds.

The average zinc levels obtained in this study for coffee are above those considered adequate by Malavolta (1992). The author classifies <5 mg kg^{-1} as low, 5 to 10 mg kg^{-1} as average and 11-20 mg kg^{-1} as adequate. Zinc levels for sorghum in this study are very high compared to those considered adequate (20 mg kg^{-1} on average) by Malavolta *et al.* (1997).

Conclusions

Surfactant enhances the contact and absorption of foliar zinc applications.

Washing is indispensable as a pretreatment for foliar analysis.

A single wash with detergent + hydrochloric acid at a concentration of 1.0 + 3.5 mL L^{-1}, was the most suitable method for both species.

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