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Sampling Methodology of a Key Pest: Technique and Sampling Unit for Evaluation of Leafhopper *Dalbulus maidis* Populations in Maize Crops

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Abstract: Maize (*Zea mays*) is the most consumed food in the world. The leafhopper, *Dalbulus maidis*, (Hemiptera: Cicadellidae) is one of the most important maize pests, and due to its direct and indirect damage, it can cause losses of up to 100%. Sampling plans are essential components for integrated pest management programs. The first two components to be determined in these plans are the sampling unit and the sampling technique. The sampling unit consists of determining the plant organ to be evaluated in the sampling and the techniques are the devices used to sample populations of pests in crops. Thus, the objective of this work was to select the unit and technique to be used in the sampling of *D. maidis* in maize crops. For this purpose, pest populations in plants at stages V4, V6, V8, and V10 were monitored in 16 commercial crops in the Atlantic Forest and Cerrado biomes over two years. The sample units studied were the leaves at different positions in the canopy of the plants. The techniques studied were direct counting, shaking the apex of the plants in a plastic tray and using a beating cloth. In the process of selecting the unit and technique to be used in sampling populations of pests in crops, adequate criteria must be used. These criteria include precision, representativeness, sampling time and that the use of these components in the sampling plans should not cause damage to the plants. The most suitable sampling unit for evaluating these pest populations was the whorl leaves and the best technique was the direct counting. Therefore, the unit and technique selected in this work can be used in sampling plans for *D. maidis* in maize crops.

Keywords: *Zea mays*; Cicadellidae; direct count; maize whorl; sampling accuracy; sampling time; representative samples

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1. Introduction

Maize (*Zea mays* L.) is the most consumed food in the world. In 2021, 1.21 billion tons of this grain was produced, which was grown on 205.87 million hectares [1]. Maize originates from Central America and is currently grown in 165 countries. It is one of the main sources of human food and animal production, especially cattle, pigs and poultry [2]. Therefore, this cereal is of great importance and has a high potential for contributing to sustaining the world population in this perspective of growth [2]. The world's largest maize

producers are the United States, China, Brazil, Argentina and Ukraine, which produce 76% of the world's production [1].

The leafhopper, *Dalbulus maidis* (DeLong and Wolcott) (Hemiptera: Cicadellidae), is one of the main pests that attack maize crops (DeLong and Wolcott) (Hemiptera: Cicadellidae) [3,4]. This insect is present in South America, Central America and the south of the United States and it is a quarantine pest in other regions of the world [5]. Adults of *D. maidis* are about 4 mm long and their color is yellowish with two black spots on the head. Females lay their eggs inside the leaves' tissue. Their nymphs are yellowish and they go through five instars [4,6]. *D. maidis* nymphs and adults suck phloem sap [4]. It is a monophagous species that can only complete its life cycle when its feeds on plants of the genus *Zea* [3,4,7]. Due to its direct and indirect damage, *D. maidis* can cause losses of up to 100% [3,4,8]. The direct damage is due to the nymphs' and adults' feeding because, when sap-sucking, they inject toxins into the vascular system of the plants [3,4,8]. In addition, opportunistic fungi develop on the honeydew excreted by the nymphs and adults and this causes the formation of sooty mold that covers the leaf surface, reducing plant photosynthesis [8,9]. The indirect damage is due to the transmission of other microorganisms during feeding, such as pale stunt (*Maize Stunt Spiroplasma*-CSS) caused by the spiroplasma *Spiroplasma kunkelii*, red stunt (*Maize Bushy Stunt Phytoplasma-MBSP*) caused by phytoplasma and the fine streak virus (*MRFV*) [3,4,8–10]. The critical period for carrying out the control of this pest is the vegetative stage of the plants. In this stage, *D. maidis* nymphs and adults are more likely to cause direct and indirect damage to maize plants [11].

Sampling plans are essential components of integrated pest management programs [12–15]. These plans are used for making decisions about pest control in crops. In addition, the use of sampling plans to monitor pest populations in crops makes it possible to determine whether the applied control methods are efficient or not [13,16–18]. The first two components to be determined for establishing sampling plans are the selection of the sampling unit and the technique to be used for pest attack intensity assessments [13,19]. The sampling unit consists of the plant organ to be evaluated in the sampling [15,20,21]. Adults and the nymphs of *D. maidis* attack maize leaves, so it is necessary to determine which one is more suitable for sampling this pest [4,19]. Finally, the techniques are the devices used to sample the populations of pests in crops [20,21].

In the process of selecting the unit and technique to be used in sampling the populations of pests in crops, adequate criteria must be used. The components of precise sampling plans make it possible to determine insect pest densities with low relative variances (less than 25%) [22,23]. These make it possible to use fewer samples in sampling plans. As for the criterion of representativeness, components of the sampling plans that determine the relative variances of the pests are selected and they relate directly to the absolute densities of these organisms in the crops. Thus, when crop densities increase, there is also a similar increase in relative densities assessed by the sampling plans. The same thing happens when insect pest densities decrease in crops [13,20,24]. The use of components that are quick to execute and that take less time to perform makes it possible to make decisions to control pests before they cause economic damage. This ensures that high yields are obtained in the crops. In addition, this reduces the cost of carrying out sampling, because labor is the main cost component of sampling plans.

Despite the importance of *D. maidis* as a pest in maize crops, sampling plans to be used in the evaluation of attack intensity of this pest in crops have not yet been determined. Thus, the objective of this work was to select the unit and technique to be used in the sampling of *D. maidis* in maize crops.

2. Materials and Methods

2.1. Experimental Characteristics

This research was conducted in sixteen commercial maize crops in Atlantic Forest and Cerrado biomes during 2022 and 2023. The crops in the Atlantic Forest biome were located in Viçosa (20°45′17″ S, 42°52′57″ W), state of Minas Gerais, Brazil, with an altitude of 648 m

and a tropical climate. The crops in the Cerrado biome were located in Gurupi (11°45′19″ S, 48°50′29″ W), state of Tocantins, Brazil, with an altitude of 265 m and a tropical climate with a dry winter. These sites were selected because they have different environmental characteristics and because these biomes are the main maize planting sites in Brazil [25]. Fertilization was carried out following chemical analysis of the soils and the crops were not irrigated; additionally, insecticides and fungicides were not used and weed control was carried out using herbicides [26].

This research was divided into two parts. In the first one, the sampling unit to be used in the evaluation of *D. maidis* populations was selected. In the second part, the most appropriate technique for evaluating pest densities was selected. Data analyses were performed using R-4.1.3 and R Studio software [27]. For this purpose, populations of this insect were monitored over two years in sixteen commercial corn crops in two biomes.

2.2. Sampling Unit Selection

This part of the research was carried out in eight maize crops. Four crops were in the Atlantic Forest biome and four in the Cerrado biome. In each crop, the plants were in stages V4, V6, V8 and V10, which were plants with four, six, eight and ten fully expanded leaves, respectively. These are the critical stages of attack from *D. maidis* on maize plants [11]. In each field, 20 plants were randomly selected. The leaves of each selected plant were numbered from the apex to the base. Leaves that were not fully expanded (whorl leaves) were numbered 0; the first, most apical, fully expanded leaf was numbered 1, and so on (Figure 1A). For each leaf, the density of *D. maidis* was assessed using the direct count sampling technique. This was chosen because the direct counting technique is the most used in the evaluation of pest insect populations in crops [13,28–31].

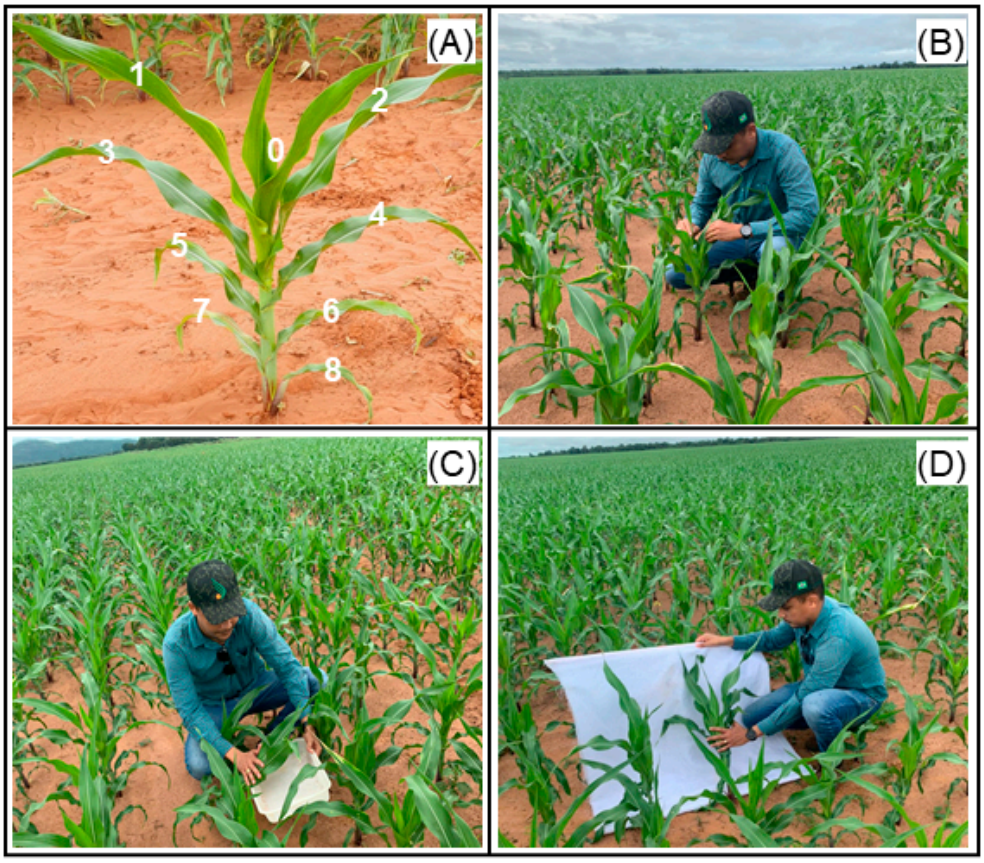

Figure 1. (**A**) Position of leaves in the canopy of plants (0 = whorl leaves; 1, 2, 3, 4, 5, 6, 7 and 8 = 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th apical leaf of the plant canopy, respectively); density sampling of *Dalbulus maidis* using the techniques of (**B**) direct counting, (**C**) plastic tray and (**D**) beating cloth.

For selecting the most appropriate sampling unit to evaluate *D. maidis* populations at each phenological stage of the plants (V4, V6, V8 and V10), precision and representativeness criteria were used [21,23,32]. The relative variance of *D. maidis* densities on each maize leaf was used to select the sampling unit through the precision criterion. Relative variances were calculated using Equation (1):

$$RV = 100 \times (SE//\bar{x}) \quad (1)$$

where RV is the relative variance (%), SE is the standard error of *D. maidis* densities and $\bar{x}$ is the average insect density. The selected sampling units were those with relative variance below 25% [22,23].

2.3. Sampling Technique Selection

In this part of the work, eight maize crops were evaluated. Four of these crops were in the Atlantic Forest biome and the other four in the Cerrado biome. In each of the crops, the plants were in the stages V4, V6, V8 and V10. The techniques used were direct counting (Figure 1B), shaking the plants in a plastic tray (Figure 1C) and using a beating cloth (Figure 1D). These techniques were chosen because they are the main ones used in the evaluation of populations of sucking insects in crops [20,30,33]. In each crop, 20 groups of three plants were randomly selected. The density of *D. maidis* was evaluated using one of the sampling techniques for each of the plants in these groups.

The technique of shaking the apical part of the plants in a plastic tray consisted of shaking the apex of the plant in a white plastic tray (40 × 25 × 3 cm), then counting the number of nymphs and adults of *D. maidis* on the tray (Figure 1C). In the beating cloth technique, a white cloth 1.0 m long was opened on the ground between two rows of maize. Then, a plant in one row was shaken on the cloth and the number of nymphs and adults *D. maidis* that fell on the cloth was counted (Figure 1D). In each repetition, the time spent sampling during each of the three techniques was recorded. In addition, it was recorded whether the use of the technique caused damage to the plant.

The relative variances of the densities evaluated using each technique in each plant stage were calculated using Equation (1). As per the precision criterion, the technique whose densities presented a relative variance of less than 25% was selected [22,23].

2.4. Statistical Analysis

For the sampling unit selection, relative densities (number of insects/leaf) and absolute densities (number of insects/plant) were calculated. Relative density and absolute density data were submitted to Pearson's correlation analysis. Sampling units that showed positive and significant correlations ($p < 0.05$) between relative densities and absolute densities were selected. When more than one sample showed positive and significant correlations, simple linear regression analysis of relative densities in these samples as a function of absolute densities at $p < 0.05$ was performed. The slopes of these regressions were compared using their 95% confidence intervals. The samples with the highest angular coefficients were selected [20,22,33,34].

For the sampling technique selection, was tested whether the data errors of *D. maidis* densities and sampling times had a normal probability distribution and whether the variances of different factors in studies were homogeneous. The normal distribution of errors was tested using the Lilliefors test, whereas the existence of homogeneity of error variances was tested using the Cochran test. Mean *D. maidis* densities and sampling times for each plant stage and biome were compared using the non-parametric Kruskal–Wallis test at $p < 0.05$. This was used because the errors of these variables did not present a normal probability distribution and their variances were not homogeneous.

3. Results

3.1. Sampling Unit Selection

The *D. maidis* densities varied depending on the biome in which the crop was grown, the plant stages and the leaf position in the canopy. In crops grown in the Cerrado biome, *D. maidis* densities were higher than in those conducted in the Atlantic Forest biome (Tables 1 and 2). In both biomes and in plants at different stages, the highest densities of *D. maidis* occurred in the whorl followed by the densities of this insect in the first, most apical leaf of the canopy. In the leaves positioned below the canopy of the plants, the insect densities were lower (Tables 1 and 2).

Table 1. Sample selection to be used in the evaluation of *Dalbulus maidis* densities in maize crops in the Atlantic forest biome: relative density (mean ± standard error), relative variance (RV), Pearson correlation (r) between relative density (insects.leaf^{-1}) and absolute density (insects.plant^{-1}) and slope (r) of the simple linear regression of relative density as a function of absolute density in leaves at different positions in the canopy.

Leaf Position in the Canopy §	Relative Density	Characteristics Used in Sample Selection		
		RV (%)	R	b ± SE
	Plants in the V4 stage			
0	1.07 ± 0.11	9.98	0.82 *	0.97 ± 0.09
1	0.24 ± 0.04	18.50	−0.01	
2	0.09 ± 0.03	31.04	0.09	
3	0.00 ± 0.00			
4	0.00 ± 0.00			
	Plants in the V6 stage			
0	1.70 ± 0.14	8.34	0.85 *	0.74 ± 0.06
1	0.32 ± 0.07	21.29	0.49 *	0.22 ± 0.05
2	0.07 ± 0.02	36.42	0.21	-
3	0.03 ± 0.02	51.98	0.08	-
4	0.00 ± 0.00	-	-	-
5	0.00 ± 0.00	-	-	-
6	0.00 ± 0.00	-	-	-
	Plants in the V8 stage			
0	1.77 ± 0.18	9.99	0.91 *	0.88 ± 0.05
1	0.47 ± 0.06	13.74	0.11	-
2	0.18 ± 0.04	23.27	0.31 *	0.08 ± 0.03
3	0.05 ± 0.02	42.25	0.08	-
	Plants in the V10 stage			
0	1.97 ± 0.16	7.94	0.89 *	0.79 ± 0.05
1	0.33 ± 0.05	15.90	0.37 *	0.12 ± 0.04
2	0.15 ± 0.04	23.70	0.41 *	0.10 ± 0.03

§: 0 = whorl; 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 = 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th apical leaf of the plant canopy, respectively (Figure 1). For plants in the V8 stage and leaf positions 4 up to 8 the *D. maidis* densities were zero. For plants in the V10 stage, leaf positions 3 up to 10 the *D. maidis* densities were zero. * Significant correlations according to the *t* test at $p < 0.05$.

In all plant stages and in both biomes, *D. maidis* densities in the whorl showed relative variances smaller than 25%. In the first most apical leaf of the plants' canopies in all stages in the Atlantic Forest biome and in stages V6 and V10 in the Cerrado biome, the densities of *D. maidis* also showed relative variances smaller than 25%. Furthermore, in the second most apical leaf of the plants' canopies at stages V8 and V10 in the Atlantic Forest biome, the densities of *D. maidis* showed relative variances lower than 25% (Tables 1 and 2).

Table 2. Sample selection to be used in the evaluation of *Dalbulus maidis* densities in maize crops in the Cerrado biome: relative density (mean ± standard error), relative variance (RV), Pearson correlation (r) between relative density (insects.leaf^{-1}) and absolute density (insects.plant^{-1}), and slope (r) of the simple linear regression of relative density as a function of absolute density in leaves at different positions in the canopy.

Leaf Position in the Canopy §	Relative Density	Characteristics Used in Sample Selection		
		RV (%)	R	b ± SE
	Plants in the V4 stage			
0	2.05 ± 0.17	8.28	0.65 *	0.61 ± 0.17
1	0.00 ± 0.00	-	0.00	-
2	0.05 ± 0.05	100.00	0.19	-
3	0.25 ± 0.12	49.20	0.50 *	0.33 ± 0.13
4	0.00 ± 0.00	-	0.00	-
	Plants in the V6 stage			
0	10.50 ± 1.32	12.60	0.84 *	0.55 ± 0.08
1	5.55 ± 0.51	9.19	−0.11	-
2	0.30 ± 0.13	42.58	0.63 *	0.16 ± 0.05
3	0.60 ± 0.20	32.89	0.33	-
4	0.60 ± 0.23	38.99	0.29	-
5	0.60 ± 0.18	30.59	0.37	-
6	0.45 ± 0.14	30.05	0.52 *	0.08 ± 0.03
	Plants in the V8 stage			
0	3.35 ± 0.21	6.23	0.70 *	0.37 ± 0.09
1	0.20 ± 0.12	58.49	0.34	-
2	0.20 ± 0.12	58.49	0.22	-
3	0.20 ± 0.12	58.49	0.39 *	0.12 ± 0.07
4	0.20 ± 0.09	45.88	0.50 *	0.12 ± 0.05
5	0.10 ± 0.07	68.82	−0.06	-
6	0.10 ± 0.07	68.82	0.34	-
7	0.30 ± 0.15	48.97	0.19	-
8	0.15 ± 0.11	72.95	0.35	-
	Plants in the V10 stage			
0	5.65 ± 0.44	7.73	0.94 *	0.40 ± 0.03
1	1.55 ± 0.20	12.80	0.28	-
2	0.55 ± 0.20	36.06	0.63 *	0.12 ± 0.04
3	0.70 ± 0.19	27.62	0.34	-
4	0.45 ± 0.18	41.02	0.16	-
5	0.70 ± 0.21	29.50	0.17	-
6	0.20 ± 0.09	45.88	−0.01	-
7	0.55 ± 0.25	44.68	0.53 *	0.12 ± 0.05
8	0.50 ± 0.21	42.30	0.40 *	0.08 ± 0.04
9	0.30 ± 0.13	42.58	0.40 *	0.05 ± 0.03
10	0.20 ± 0.14	68.82	0.31	-

§: 0 = whorl; 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 = 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th apical leaf of the plant canopy, respectively (Figure 1). * Significant correlations according to the *t* test at $p < 0.05$.

In all plant stages and in both biomes, the relative densities (insects/sample) of *D. maidis* showed positive and significant correlations ($p < 0.05$) with the absolute densities (insects/plant). In the first most apical leaf of the plants' canopies in stages V6 and V10 in the Atlantic Forest biome, in the second most apical leaf in plants in stages V8 and V10 in the Atlantic Forest biome and in stages V6 and V10 in the Cerrado biome, the relative densities (insects/sample) of *D. maidis* also showed positive and significant correlations ($p < 0.05$) with absolute densities. Furthermore, the relative densities of the insect on the third and fourth most apical leaves in the plants at stage V8 and the seventh, eighth and ninth most apical leaves on plants at stage V10 in the Cerrado biome showed positive and significant correlations ($p < 0.05$) with the absolute densities (Tables 1 and 2).

In both biomes and all plant stages, the curves of the relative densities (insects/sample) of *D. maidis* in the whorl as a function of the absolute densities (insects/plant) showed the highest inclinations (Tables 1 and 2). Therefore, based on the criteria of the precision (relative variance < 25%) and representativeness of the absolute density (positive and significant correlations and greater slopes of the density curves), the best sample for evaluating the populations of *D. maidis* in maize crops in different stages for both biomes was the whorl of the plants.

3.2. Sampling Technique Selection

In both biomes and in all stages of the plants, higher densities of *D. maidis* were detected when the direct counting technique was used than when the techniques of shaking the apex of the plants in a plastic tray and the beating cloth were used (Figure 2A). Kruskal-Wallis test values for pest densities are described in Table 3. In both biomes and in all plant stages, it was verified that the densities of *D. maidis* evaluated using the direct counting technique showed relative variances smaller than 25%. For crops conducted in the Cerrado biome in plants at stages V8 and V10, the densities of *D. maidis* evaluated using a beating cloth also showed relative variances lower than 25% (Figure 2B).

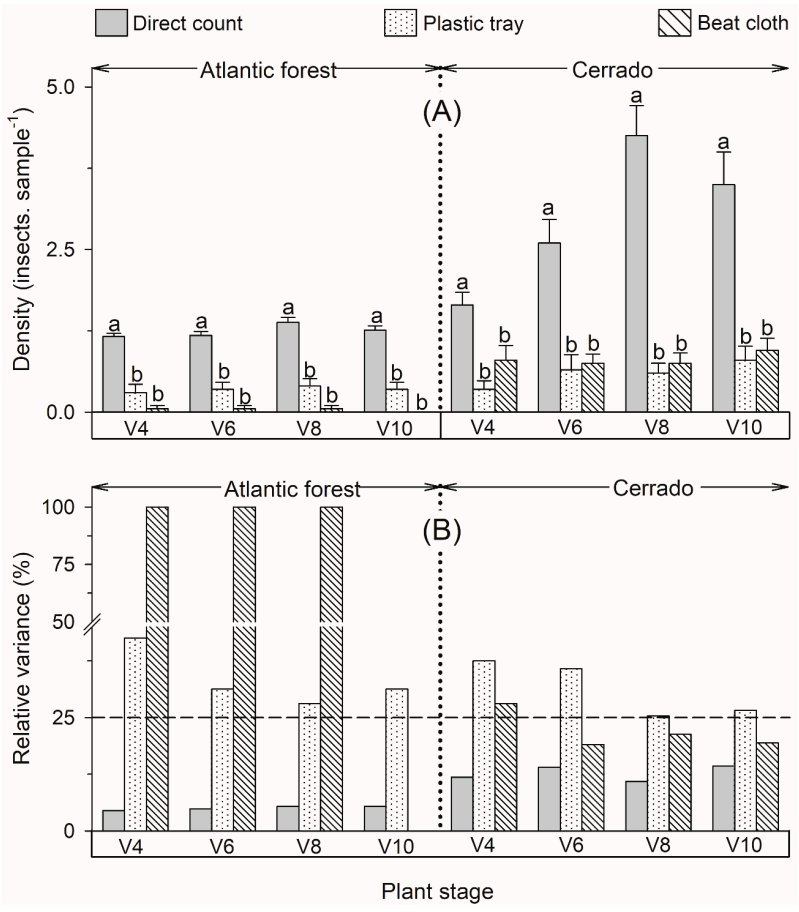

Figure 2. Selection of the technique to be used in sampling *Dalbulus maidis* in maize crops conducted in the Atlantic Forest and Cerrado biomes with plants in stages V4, V6, V8 and V10: (**A**) insect density (mean ± standard error) and (**B**) relative variance (RV). In a plant stage and location, histograms followed by the same lowercase letter do not differ from each other according to the non-parametric Kruskal–Wallis test at $p < 0.05$. Techniques with VR ≥ 25% are not suitable for use in sampling.

Table 3. Kruskal–Wallis test values for pest densities.

Plant Stage	Atlantic Forest			Cerrado		
	DF	χ^2	*p*	DF	χ^2	*p*
V4	2	29.08	<0.0001	2	19.58	<0.0001
V6	2	29.50	<0.0001	2	22.62	<0.0001
V8	2	32.15	<0.0001	2	33.51	<0.0001
V10	2	32.25	<0.0001	2	20.27	<0.0001

For the crops studied in the Atlantic Forest biome and in all stages of the plants, the evaluations of *D. maidis* populations using the direct counting technique showed the shortest sampling times, while use of the beating cloth was the technique that presented the longest sampling time. In general, the sampling time for the direct counting technique was less than half of the time spent using the beating cloth technique. On the other hand, the sampling time for the technique of shaking the plants apex in a plastic tray was intermediate to these two extremes (Figure 3A). The same sequence of sampling times observed in the crops grown in the Atlantic Forest was observed in samplings carried out in crops grown in the Cerrado biome when the plants were in stages V6 and V10. In crops with plants in stages V4 and V8 conducted in the Cerrado biome, the evaluations of *D. maidis* populations using the direct counting technique showed shorter sampling times than when using the techniques of shaking the plants' apex in a plastic tray and beating cloth (Figure 3A). Kruskal-Wallis test values for pest densities are described in Table 4. Therefore, in all stages and biomes, the evaluations of *D. maidis* populations using the direct counting technique were the ones that presented the shortest sampling time.

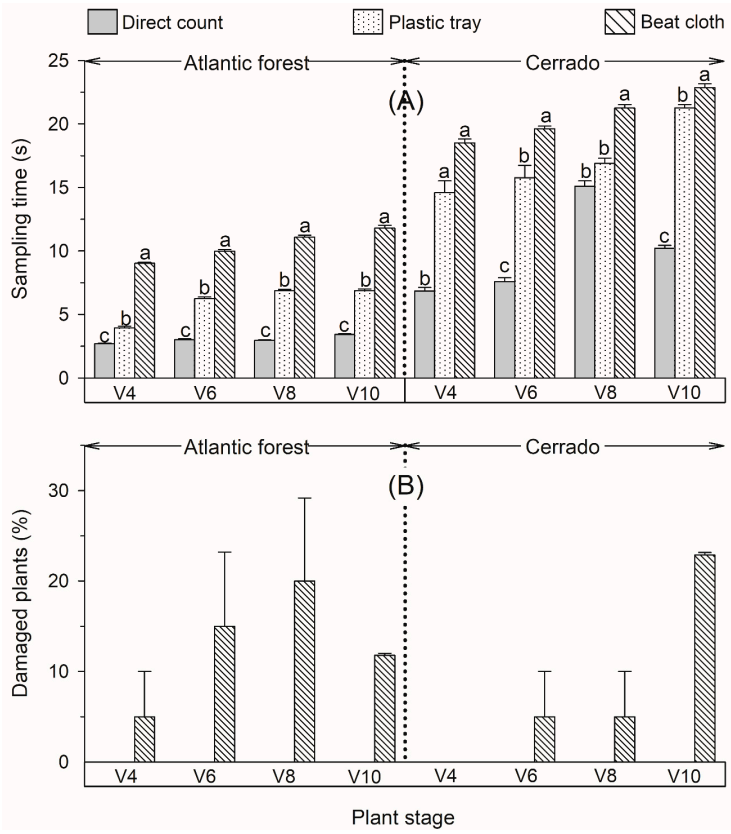

Figure 3. Selection of the technique to be used in sampling *Dalbulus maidis* in maize crops conducted in the Atlantic Forest and Cerrado biomes with plants in stages V4, V6, V8 and V10: (**A**) sampling time (mean ± standard error) and (**B**) percentage of plants damaged by the technique. In a plant stage and location, histograms followed by the same lowercase letter do not differ from each other according to the non-parametric Kruskal–Wallis test at $p < 0.05$.

Table 4. Kruskal–Wallis test values for pest densities.

Plant Stage	Atlantic Forest			Cerrado		
	DF	χ^2	*p*	DF	χ^2	*p*
V4	2	50.56	<0.0001	2	51.81	<0.0001
V6	2	52.46	<0.0001	2	52.33	<0.0001
V8	2	52.46	<0.0001	2	44.63	<0.0001
V10	2	52.46	<0.0001	2	52.46	<0.0001

The use of the beating cloth technique caused damage to the plants at all stages in the crops conducted in the Atlantic Forest and in stages V6, V8 and V10 in the Cerrado biome. In these damaged plants the leaves were torn and the stems were injured. On the other hand, the use of direct counting techniques and the agitation of the plants' apex in a plastic tray did not cause damage to the maize plants (Figure 3B).

4. Discussion

The fact that we found higher densities of *D. maidis* in maize crops cultivated in the Cerrado biome than in the Atlantic Forest biome must be related to differences in the environment and in the maize crop cultivation systems in these two biomes. Corroborating the results obtained in this work, Santana Jr et al. [5] verified that areas of the Cerrado biome are more favorable to *D. maidis* than areas of the Atlantic Forest biome. The landform in the Cerrado biome is flat while the Atlantic Forest biome is mountainous. This flat landform allows the winds to propagate over greater distances, and in the Cerrado biome, the winds have a predominant direction from east to west. The flat landform and the winds contribute to the dispersion of insects over greater distances [35–37].

According to Davis [6], Madden et al. [38] and Waquil et al. [4], the optimum temperature range for *D. maidis* is 26 to 29 °C. The occurrence of higher air temperatures in the Cerrado biome possibly favored the occurrence of larger populations of *D. maidis* in the maize crops in this biome. According to Santana Jr. [5], populations of *D. maidis* are favored by environments with lower humidity. It has been reported that the intense rains that occur in tropical climate regions cause negative impacts on insect populations that live in the canopy of plants [39–41]. These negative effects are caused by the mechanical impact of raindrops on insects that live in the canopy of plants, causing mortality [41–43]. In addition, the rain occurrence can disturb mating and the dispersal of insects [41]. Finally, the humid environment favors the mortality of insects by entomopathogenic fungi [3,44,45].

The fact that the plant whorl was the most suitable unit for evaluating the densities of *D. maidis* in maize crops is related to the characteristics of the leaves of this part of the plants. Furthermore, it was observed that the highest densities of *D. maidis* occurred in the maize whorl leaves. Generally, the ideal locations for sampling insect pests are those where the highest densities of these organisms occur [15,46]. The leaves of the maize whorl are young and have not yet fully expanded [47]. Younger leaves generally have a higher concentration of nutrients, especially nitrogen, and lower defenses against herbivorous insects [48,49]. In addition, the arrangement of the leaves in the maize whorl possibly provides greater protection to insects against meteorological inclement weather.

The maize whorl was the best sampling unit for carrying out the evaluations of *D. maidis* populations, because the densities of this insect in this part of the plant are more accurate and representative of the absolute density. Sampling units in which pest densities are more accurate make it possible to determine better practicable sampling plans because they have a smaller number of samples and, consequently, less time and cost for their execution [33]. The selection of sampling units with relative densities of pests that are representative of their absolute densities in plants is important for decision-making systems, because it indicates the control of these organisms at the correct time. In the sampling units where pest populations have relative densities that are representative of their absolute densities, these two variables show proportional variation. Thus, when the pest density

increases in the plant, it also increases by a similar proportion in the selected sampling unit [13,20,24,33].

Direct counting was the most appropriate technique for evaluating the densities of *D. maidis* in maize crops due to its greater accuracy, speed of execution and the fact that it does not damage the plants. Thus, the evaluation of *D. maidis* densities when using the direct counting technique should make it possible to determine practicable sampling plans with a small number of samples and a low execution time and cost [24,33,50]. The fact that the use of the direct counting technique has a shorter execution time than the techniques of agitating the plant apex in a plastic tray and the beating cloth is important. As labor is the main cost component of pest sampling plans in crops [19,22,51], the selection of the technique with the shortest execution time is important. It was observed that the use of the beating cloth technique in sampling *D. maidis* in maize crops caused damage to some plants. This probably happen because of the agitation of the plants to count the fall insects onto the beating cloth that was on the ground. Crop pest sampling plans must be practicable so they are more likely to be adopted by farmers. Practicable sampling plans should be quick and simple to execute. In this context, the sampling unit and technique selected in this work presented short execution times (up to 3.25 s), which indicates that its use is promising in determining practicable sampling plans. The use of the sampling unit and technique selected in this work to evaluate densities in maize crops is simple. This simplicity is due to the fact that the direct counting technique does not use any apparatus [22,52]. In addition, the sampling unit and technique selected in this work were the most appropriate in different biomes in which maize crops were grown, in the different phenological stages of the plants and in the evaluation of different insect densities. Therefore, due to its efficiency in these different situations, the evaluation of *D. maidis* densities in the plant whorl by direct counting proved to be promising for use in sampling plans for this pest in maize crops. In addition, the use of an adequate methodology for monitoring populations of *D. maidis* in maize crops will enable the control of this insect pest that causes important economic damage to this culture. This is important for making sure these insects are controlled before they transmit one of the three diseases transmitted by *D. maidis* to many plants in crops [3,4,8,9].

5. Conclusions

In conclusion, the evaluation of *D. maidis* densities by directly counting insects in the plant whorl can be used in sampling plans for this pest in maize crops. This sampling unit and technique make it possible to assess the densities of this pest in a precise, representative, quick, and practicable way.

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