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SAMPLE SIZE FOR ASSESS THE LEAF BLAST SEVERITY IN EXPERIMENTS WITH IRRIGATED RICE[1]

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ABSTRACT - The aim of this study was to determine the sample size needed to assess the severity of leaf blast in rice in experiments with different fungicide treatments. The severity and the area under the disease progress curve data of three chemical disease control treatments carried out in Rio Grande do Sul, were used in the study. Analysis of variance was performed to verify whether the severity of the disease differed between treatments. The spread of disease was was also found to be different between treatments and assessments, using the variance/mean ratio and Morisita index. The spatial distribution of the disease among the treatments and during the evaluations is important for the choice of the equation used to calculate the sample size. The spatial distribution of the disease was not the same across the experiments, and it varied between treatments and evaluations. Thus, we decided to use a formula that was not associated with distributions to indicate the spatial distribution (negative binomial or Poisson) of the disease in the field. The sample size to estimate the average of rice leaf blast severity varied between treatments and evaluations. The area under the disease progress curve is necessary to be determined to reduce the number of samples needed. Thus, it is recommended to assess 293 sheets to estimate severity, and 63 to estimate AUDPC at 20% error.

Keywords: *Oryza sativa*. *Pyricularia grisea*. Experimental precision. Sampling.

TAMANHO DE AMOSTRA PARA AVALIAR A SEVERIDADE DE BRUSONE DA FOLHA EM EXPERIMENTOS COM ARROZ IRRIGADO

RESUMO - O objetivo deste trabalho foi determinar o tamanho de amostra necessário para avaliar a severidade da brusone da folha no arroz irrigado em experimentos com diferentes tratamentos fungicidas. Foram utilizados dados de severidade da brusone e área abaixo da curva de progresso da doença de três experimentos utilizando o controle químico realizados no Rio Grande do Sul. Foi realizada a análise de variância, para verificar se a severidade da doença foi diferenciada entre os tratamentos. Também foi verificado se a dispersão da doença foi diferenciada entre os tratamentos e as avaliações, através da razão variância/média e do índice de Morisita. A dispersão da doença entre os tratamentos e ao longo das avaliações é importante para a escolha da fórmula utilizada no cálculo do tamanho da amostra. A dispersão da doença não foi a mesma ao longo dos experimentos, variando entre tratamentos e avaliações. Diante deste comportamento, optou-se por utilizar uma fórmula de cálculo que não estivesse associado a distribuições que indicassem a distribuição espacial da doença no campo (binomial negativa ou Poisson). O tamanho de amostra para a estimação da severidade média da brusone do arroz variou entre os tratamentos e as avaliações. Para avaliar a área abaixo a curva de progresso da doença é necessário avaliar menos folhas. Recomenda-se a avaliação de 293 folhas para estimar a severidade, e 63 para estimar a AUDPC, com 20% de erro.

Palavras-chave: *Oryza sativa*. *Pyricularia grisea*. Precisão experimental. Amostragem.

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INTRODUCTION

The leaf blast caused by the fungus *Pyricularia grisea* (Cooke) Sacc. (=*Pyricularia oryzae* Cavara) is a disease commonly found in irrigated rice. The characteristic symptoms of the disease on the leaves are elliptical lesions with a gray center and reddish brown edges, with the reproductive structures (conidia) of the pathogen in the necrotic center (BEDENDO, 1997). The disease occurs in all rice-producing areas and results in yield losses that can reach 100% (FILIPPI et al., 2007). Owing to the high potential for damage caused by leaf blast on rice, research on fungicide efficiency is critical for proper disease management, as well as to find an alternative way to chemical control, which is one of the main methods to control rice leaf disease (CELMER et al., 2007; SANTOS et al., 2008).

In agricultural experiments, the quality of the results obtained depends on experimental precision. Therefore, the experimental error corresponding to the variation between repetitions of the same treatment must be minimized so that the effect of the treatments is reliably estimated (CATAPATTI et al., 2008). Experimental precision can be improved by the proper sizing of the number of repetitions and choice of experimental design (STORCK et al., 2006; CATAPATTI et al., 2008). However, many variables must be obtained by sampling experimental plots (KRAUSE et al., 2013), since the entire population cannot be sampled due to the excessive demand for labor, time, and financial resources. Sampling within the plot also generates a new variance within the plot, and this should be minimized by an appropriate sample size (CARGNELUTTI FILHO et al., 2009).

Sample size is influenced by the variability of the data, which is affected by genetic and environmental factors (MARTIN et al., 2005; CARGNELUTTI FILHO et al., 2008),the application of treatments (TOEBE et al., 2011),and, in case of pests and diseases, by their spatial distribution in the field (LÚCIO et al., 2009; MICHEREFF et al., 2011). The distribution of the disease in the field influences the choice of methodology to calculate sample size. For randomly distributed diseases, the Poisson distribution is used for sample calculation, whereas in the case of aggregated distribution, the *k* parameter of the negative binomial distribution is the most informative (MICHEREFF et al., 2008; MICHEREFF et al., 2011).

Plant disease sampling has been widely studied, including the determination of sample size for the quantification of water-stain (*Acidovorax avenae subsp.* citrulli) in melon (SILVA et al., 2003), soft rot (*Pectobacterium carotovorum* subsp. C*arotovorum* Jones) in lettuce and Chinese cabbage (SILVA et al., 2008), leaf blight (*Curvularia eragrostidis* P. Henn. Meyer) in yam (MICHEREFF et al., 2008) and cercospora spot (*Cercospora capsici*) in chili (MICHEREFF et al., 2011). However, no published studies have estimated the sample size for the quantification of leaf blast of irrigated rice.

The purpose of this study was to determine the sample size, i.e., the number of leaves needed to assess the severity of leaf blast on irrigated rice, in experiments with different fungicide treatments.

MATERIAL AND METHODS

All data used in this study are from three chemical control experiments of the blast in irrigated rice, one conducted in agricultural harvest 2009/2010 and two in agricultural harvest of 2010/2011. All field experiments were performed in an experimental area in the Santa Maria-RS, with an altitude of 95 m, latitude 29°43′43.2″S, and longitude 53°33′43.9″W. In the agricultural harvest of 2009/2010, sowing was carried out on 01/06/10, while in the agricultural harvest 2010/2011, it was carried out on 12/23/2010. Late sowing was conducted with the aim to enhance the severity of the blast, subjecting the rice plants to conditions favorable for the development of the disease. Seeding rate, fertilization, weed and pest control followed the technical recommendations for the culture (SOSBAI, 2007).

A randomized block design, with four repetitions, was used in all experiments. The experimental plots were 2 m wide and 5 m long. The treatments and cultivars of each experiment are described in detail in Table 1. Fungicides were applied with the aid of a precision backpack sprayer pressurized with carbon dioxide, consisting of a bar with four nozzles, spaced 0.5 m from each other. The spray tip used was type XR 110015, and spraying was calibrated to an application volume of 150 L ha^{-1}. In all experiments, two fungicide applications were performed, with the first being carried out during the phenological stage of anthesis (COUNCE et al., 2000) and the second 14 days thereafter.

The variables studied were the severity of rice blast and the area under the disease progress curve (AUDPC). Severity assessments of the disease were carried out 7, 14 and 21 days after fungicide application. For each replication (plot), 10 flag sheets were randomly assigned (for a total of 40 sheets per treatment), and a value corresponding to the percentage of leaf area with disease symptoms was assigned to each one. To assign blast severity values to leaves, a diagrammatic scale proposed by the International Research Institute of Rice (IRRI, 2002) was used.

Table 1.Year of performance, cultivars and doses of fungicides used in the three experiments.

Treat.[1]	Cultivar (Year)	Fungicides
----------	----------	Experiment 1 ----------
T1	IRGA 422 CL (2009/2010)	Aproach Prima (0.3L ha^{-1}) + Nitro LL (2L ha^{-1})
T2		Aproach Prima (0.3L ha^{-1}) + Nitro LL (4L ha^{-1})
T3		Aproach Prima (0.3L ha^{-1}) + Nimbus (0.75L ha^{-1})
T4		Brio (0.75L ha^{-1}) + Nitro LL (2L ha^{-1})
T5		Brio (0.75L ha^{-1}) + Nitro LL (4L ha^{-1})
T6		Brio (0.75L ha^{-1}) + Assist (0.75L ha^{-1})
T7		Nativo (0.75L ha^{-1}) + Aureo (0.375L ha^{-1})
T8		Control (without application)
----------	----------	Experiment 2 ----------
T1	INIA Olimar (2010/11)	Kasumin (1L ha^{-1}) + Eminent (0,5L ha^{-1})
T2		Kasumin (1L ha^{-1}) + Eminent (0.5L ha^{-1}) + K-tionic (0.2L ha^{-1})
T3		Kasumin (1L ha^{-1}) + Eminent (0.5L ha^{-1}) + K-tionic (0.3L ha^{-1})
T4		Kasumin (1L ha^{-1}) + Eminent (0.5L ha^{-1}) + K-tionic (0.4L ha^{-1})
T5		Nativo (0.75L ha^{-1})
T6		Brio (0,75L ha^{-1})
T7		Priori (0.4L ha^{-1}) + Score (0.2L ha^{-1})
T8		Folicur (0.75L ha^{-1}) + Bim (250g ha^{-1})
T9		Control (without application)
----------	----------	Experiment 3 ----------
T1	INIA Olimar (2010/11)	Aproach Prima (0.3L ha^{-1})
T2		Aproach Prima (0.3L ha^{-1}) + NitroLL (4L ha^{-1})
T3		Aproach Prima (0.3L ha^{-1}) + Mo (17.7g ha^{-1})
T4		Aproach Prima (0.3L ha^{-1}) + Zn (186g ha^{-1})
T5		Aproach Prima (0.3L ha^{-1}) + Mo (17.7g ha^{-1}) + Zn (186g ha^{-1})
T6		Aproach Prima (0.3L ha^{-1}) + Mo (17.7g ha^{-1}) + NitroLL (4L ha^{-1})
T7		Aproach Prima (0.3L ha^{-1}) + Zn (186g ha^{-1}) + NitroLL (4L ha^{-1})
T8		Aproach Prima (0.3L ha^{-1}) + Mo (17.7g ha^{-1}) + Zn (186g ha^{-1}) + NitroLL (4L ha^{-1})
T9		Nativo (750 ml ha^{-1})
T10		Brio (750 ml ha^{-1})
T11		Priori Xtra (300 ml ha^{-1})
T12		Control (without application)

[1] Treatments.

The area under the disease progress curve (AUDPC) was subsequently calculated using the severity values obtained in the three assessments. The AUDPC for each treatment was calculated by the equation:

$$\text{AUDPC} = \sum \left[\frac{(Y_i + Y_{i+1})}{2} \times (T_{i+1} - T_i) \right]$$

where i is the number of days after the application of fungicides, Y is the percentage of leaf area affected by the blast at observation i, T_i is the time of evaluation, and T_{i+1} is the evaluation time i+1.

Disease severity data (percentage of leaf area attacked by the pathogen) and AUDPC were subjected to the Shapiro-Wilk and Levene tests, to assess normality and homogeneity of errors, respectively. When these assumptions were violated, the variables were transformed using the Box-Cox methodology. In cases where assumptions continued to be violated even after transformation, the nonparametric Friedman test was used to detect differences between treatments (STORCK et al., 2006). The difference in severity was examined to assess whether the 40 leaves evaluated in each treatment should or should not be considered a specific sample.

Then, considering the 40 leaves evaluated in each treatment, the following statistics were calculated: minimum, maximum, mean, standard deviation, variance, and average coefficient of variation. To determine whether the 40 leaves should or should not be considered as a specific sample, a Levene test was again applied to verify the homogeneity of the variances, referring to the data for the severity of the blast in the following situations: between treatments in each experiment and between evaluations in each treatment. As for the AUDPC variable, the homogeneity of variances between treatments was verified.

For the severity variable in the three assessments, the variance/mean ratio (R) and Morisita Index (I_δ) were also calculated. Then, the difference from randomness was calculated using the chi-square test (χ^2) with n-1 degrees of freedom. The purpose of these tests was to determine the field distribution of the disease spread, i.e., whether the distribution was aggregated or random; this information is important when determining the appropriate formula to calculate the sample size (CAMPBELL; MADDEN, 1990).

The sample size (number of flagged leaves) required to estimate the severity of leaf blast was determined for each treatment in each evaluation, using the following equation (CAMPBELL; MADDEN, 1990):

$$n = \frac{z^2_{(\alpha/2)}\, s^2}{CV^2_{\bar{x}}\, \bar{x}^2}$$

where $z_{\alpha/2}$ is the critical value of the distribution z (α = 0,05); s^2 is the sample variance; $\bar{x}$ corresponds to the average severity of the disease in the 40 leaves evaluated for a given treatment; and $CV_{\bar{x}}$ corresponds to a pre-established acceptable error of 5%, 10%, 15%, 20%, 25%, or 30%.

The sample size (number of flag leaves) required to estimate the severity of AUDPC was determined for each treatment in each evaluation, using the following equation:

$$n = \frac{t^2_{(\alpha/2)}\, s^2}{CV^2_{\bar{x}}\, \bar{x}^2}$$

where $t_{\alpha/2}$ is the critical value of the Student's *t* distribution (α = 0,05); s^2 is the sample variance; $\bar{x}$ corresponds to the average severity of the disease in 40 leaves evaluated per treatment; and $CV_{\bar{x}}$ corresponds to the pre-established acceptable errors of 5%, 10%, 15%, 20, 25%, and 30%.

It is noteworthy that the theoretical distributions used in the two equations above are related to the nature of the study variables. The severity is proportional and follows a standard normal distribution when the sample is large (greater than 30); AUDPC is a continuous variable presenting a normal distribution (confirmed by the Shapiro-Wilk test), and it can be represented by the Student's *t* distribution.

All statistical analyses were performed at α = 5%, using the R software (R DEVELOPMENT CORE TEAM, 2012) and Microsoft Excel®.

RESULTS AND DISCUSSION

The experiments demonstrated that the treatments and the evaluations within each treatment in severity (Table 2, 3 and 4). The values of disease severity observed in the samples (ranging from 0% to 40,75%) and the average severity for the treatments (ranging between 0.52% and 27,80%) show that the experiments encompassed some extreme situations, which is very important in this type of study. These extreme situations can be better realized when it is considered that 59,6% loss in productivity can occur when the severity values of the blast on the leaf and panicle are 33,6% and 49,9%, respectively (PRABHU et al., 2003).

Table 2. Minimum and maximum values, mean, standard deviation (SD), variance, coefficient of variation (CV) variance, mean ratio (R), and Morisita index (I_δ) of blast's severity, and area under the disease progress curve, in experiment 1.

Treat.[1]	Minimum	Maximum	Mean	SD	Variance	CV (%)	R	I_δ
---------- Severity (%) of 1st evaluation ----------								
T1	0.000	12.156	5.101	3.629	13.171	71.145	2.582*	1.303*
T2	0.000	11.731	5.364	3.504	12.282	65.336	2.289*	1.235*
T3	0.000	14.487	7.444	3.811	14.526	51.196	1.951*	1.125*
T4	0.000	11.731	4.50	3.716	13.814	82.550	3.068*	1.450*
T5	0.000	14.487	5.632	5.598	31.338	99.386	5.563*	1.298*
T6	0.000	14.487	6.789	4.378	19.170	64.486	2.823*	1.262*
T7	0.000	10.625	2.941	3.294	10.854	112.001	3.689*	1.899*
T8	0.000	11.731	5.582	3.267	10.675	58.531	1.912*	1.160*
---------- Severity (%) of 2st evaluation ----------								
T1	0.000	7.578	1.688	2.327	5.415	137.843	3.207*	2.294*
T2	0.000	10.059	2.152	2.404	5.781	111.694	2.685*	1.772*
T3	0.000	10.059	2.017	2.399	5.758	118.962	2.854*	1.907*
T4	0.000	6.296	0.973	1.702	2.898	174.810	2.975*	3.030*
T5	0.000	4.296	1.098	1.293	1.672	117.682	1.521*	1.473*
T6	0.000	9.578	1.848	2.364	5.592	127.915	3.025*	2.082*
T7	0.000	4.578	1.071	1.514	2.292	141.359	2.140*	2.062*
T8	0.000	6.296	2.431	2.319	5.377	95.3678	2.211*	1.141ns
---------- Severity (%) of 3st evaluation ----------								
T1	0.000	11.648	3.362	4.004	16.033	119.082	4.768*	1.366*
T2	0.000	7.648	3.190	2.215	4.908	69.434	1.538*	1.165*
T3	0.000	6.135	2.649	2.140	4.581	80.778	1728*	1.270*
T4	0.000	5.904	1.867	2.620	6.865	140.319	3.676*	1.508*
T5	0.000	6.135	1.990	2.503	6.266	125.753	3.148*	1.355*
T6	0.000	5.904	1.939	1.736	3.015	89.521	1.554*	1.282*
T7	0.000	5.904	1.948	1.603	2.570	82.266	1.318 ns	1.161ns
T8	0.000	10.135	3.490	3.062	9.379	87.728	2.686*	1.474*

* Randomness rejected at 5% probability of error. ns Randomness is not rejected. [1]Treatments described in details in Table 1. –Values not calculated.

Table 2. Continuation.

Treat.[1]	Minimum	Maximum	Mean	SD	Variance	CV (%)	R	I_δ
----------				AUDPC			----------	
T1	0.000	92.022	40.836	24.734	611.792	60.568	-	-
T2	10.691	108.691	44.450	21.891	479.240	49.249	-	-
T3	0.672	105.191	49.000	26.107	681.597	53.280	-	-
T4	0.000	85.613	28.411	22.397	501.650	78.831	-	-
T5	0.000	71.613	33.705	19.414	376.926	57.600	-	-
T6	0.000	99.022	42.849	25.410	645.715	59.302	-	-
T7	0.000	60.172	24.273	16.699	278.886	68.797	-	-
T8	0.673	106.613	48.387	26.948	726.238	55.693	-	-

*Randomness rejected at 5% probability of error. [ns] Randomness is not rejected. [1]Treatments described in details in Table 1. –Values not calculated.

Table 3. Minimum and maximum values, mean, standard deviation (SD), variance, coefficient of variation (CV) variance, mean ratio (R), and Morisita index (I_δ) of blast's severity, and area under the disease progress curve, in experiment 2.

Treat.[1]	Minimum	Maximum	Mean	SD	Variance	CV (%)	R	I_δ
----------				Severity (%) of 1st evaluation			----------	
T1	1.619	13.752	5.575	2.416	5.838	43.341	1.047[ns]	1.008[ns]
T2	1.752	14.275	7.425	2.812	7.910	37.880	1.065[ns]	1.008[ns]
T3	0.752	9.352	5.525	2.122	4.507	38.424	1.033[ns]	0.967[ns]
T4	8.710	12.845	10.300	3.673	13.491	55.442	2.036*	1.153*
T5	0.000	16.352	6.699	3.927	15.426	58.627	2.302*	1.190*
T6	2.275	16.752	7.250	3.637	13.229	50.168	1.824*	1.111*
T7	2.752	11.352	6.800	2.402	5.769	35.324	0.848[ns]	0.978[ns]
T8	1.752	12.619	7.400	2.687	7.224	36.321	0.976[ns]	0.996[ns]
T9	3.352	19.275	11.025	4.962	24.622	45.007	1.825*	1.073*
----------				Severity (%) of 2st evaluation			----------	
T1	8.710	13.314	10.360	1.134	1.287	10.951	0.124[ns]	0.917[ns]
T2	8.710	13.314	10.390	1.528	2.336	14.707	0.224[ns]	0.919[ns]
T3	9.130	12.710	10.275	0.864	0.746	8.409	0.072[ns]	0.911[ns]
T4	8.710	12.845	10.300	0.959	0.921	9.318	0.089[ns]	0.913[ns]
T5	8.814	14.130	10.705	1.278	1.634	11.943	0.152[ns]	0.922[ns]
T6	8.710	12.314	10.275	0.981	0.963	9.552	0.093[ns]	0.913[ns]
T7	8.710	13.814	11.070	1.215	1.476	10.976	0.133[ns]	0.923[ns]
T8	9.130	14.130	10.787	0.960	0.922	8.902	0.085[ns]	0.917[ns]
T9	9.210	16.710	12.162	1.717	2.950	14.122	0.503[ns]	0.939[ns]
----------				Severity (%) of 3st evaluation			----------	
T1	2.515	21.715	10.013	4.901	24.024	239.913	1.767*	1.074*
T2	2.715	32.515	13.150	7.620	58.075	57.952	4.416*	1.253*
T3	3.248	34.715	14.575	7.876	62.044	54.043	4.256*	1.178*
T4	1.715	22.515	10.362	4.790	22.944	46.224	2.214*	1.114*
T5	3.515	35.748	15.675	10.475	109.733	66.828	7.001*	1.302*
T6	0.000	35.748	13.187	8.090	65.464	61.355	4.964*	1.293*
T7	2.748	29.715	14.350	6.889	47.461	48.008	3.307*	1.157*
T8	14.715	40.748	27.800	7.860	61.787	28.275	2.222*	1.042*
T9	9.515	32.515	17.050	7.457	55.611	43.737	1.450*	1.025*
----------				AUDPC			----------	
T1	135.978	183.881	178.186	29.490	869.712	16.038	-	-
T2	144.557	207.121	195.046	38.569	1487.607	18.621	-	-
T3	140.621	197.575	199.394	36.307	1318.212	18.376	-	-
T4	118.894	190.793	185.946	28.835	831.502	15.113	-	-
T5	131.144	213.815	208.957	54.738	2996.340	25.601	-	-
T6	134.228	204.750	206.100	31.372	984.219	15.322	-	-
T7	136.421	214.060	215.517	35.049	1228.475	16.373	-	-
T8	202.894	262.368	257.564	32.947	1085.551	12.557	-	-
T9	179.728	264.556	256.061	51.658	2668.594	19.526	-	-

* Randomness rejected at 5% probability of error. [ns] Randomness is not rejected. [1]Treatments described in details in Table 1. –Values not calculated.

Table 4. Minimum and maximum values, mean, standard deviation (SD), variance, coefficient of variation (CV) variance, mean ratio (R), and Morisita index (I_δ) of blast's severity, and area under the disease progress curve, in experiment 3.

Treat.[1]	Minimum	Maximum	Mean	SD	Variance	CV (%)	R	I_δ
----------Severity(%) of 1st evaluation----------								
T1	0.098	0.902	0.964	0.429	0.184	47.541	0.203^{ns}	0.115^{ns}
T2	0.000	0.659	0.525	0.489	0239	74.184	0.362^{ns}	0.021^{ns}
T3	0.000	0.811	0.754	0.705	0.497	86.935	0.613^{ns}	0.190^{ns}
T4	0.000	0.658	0.525	0.492	0.242	74.817	0.368^{ns}	0.028^{ns}
T5	0.000	0.979	0.964	0.546	0.299	55.839	0.305^{ns}	0.290^{ns}
T6	0.000	0.643	0.525	0.501	0.251	77.888	0.390^{ns}	0.038^{ns}
T7	0.000	0.796	0.598	0.621	0.386	78.03	0.485^{ns}	0.349^{ns}
T8	0.000	0.835	0.525	0.805	0.648	96.365	0.775^{ns}	0.730^{ns}
T9	0.000	0.860	0.754	0.601	0.361	69.866	0.419^{ns}	0.322^{ns}
T10	0.000	1.022	1.025	0.717	0.514	70.115	0.502^{ns}	0.514^{ns}
T11	0.000	1.288	1.218	0.826	0.683	64.182	0.530^{ns}	0.637^{ns}
T12	0.964	2.962	2.525	2.226	4.955	75.143	1.672*	1.092^{ns}
---------- Severity (%) of 2st evaluation ----------								
T1	0.405	5.405	2.025	1.233	1.522	60.936	0.751^{ns}	0.879^{ns}
T2	0.000	7.000	2.326	2.404	5.780	103.36	1.085^{ns}	1.036^{ns}
T3	0.000	2.740	0.976	0.696	0.484	71.317	0.496^{ns}	0.484^{ns}
T4	0.000	5.405	1.641	1.144	1.308	69.707	0.797^{ns}	0.877^{ns}
T5	0.000	4.000	1.610	1.182	1.397	73.432	0.868^{ns}	0.918^{ns}
T6	0.000	2.240	0.916	0.618	0.382	67.456	0.417^{ns}	0.362^{ns}
T7	0.000	1.905	0.767	0.609	0.371	79.383	0.483^{ns}	0.322^{ns}
T8	0.000	3.405	1.046	0.965	0.931	92.251	0.890^{ns}	0.895^{ns}
T9	0.000	2.854	0.787	0.704	0.495	89.398	0.629^{ns}	0.526^{ns}
T10	0.000	4.405	1.731	1.131	1.281	65.368	0.739^{ns}	0.851^{ns}
T11	0.905	5.854	2.775	1.238	1.533	44.620	0.552^{ns}	0.841^{ns}
T12	4.405	17.74	9.750	4.325	18.711	44.365	1.919*	1.023^{ns}
---------- Severity (%) of 3st evaluation ----------								
T1	0.000	16.521	5.295	4.103	16.838	77.495	3.179*	1.403*
T2	0.000	4.898	2.465	1.401	1.963	56.840	1.395^{ns}	0.918^{ns}
T3	0.521	8.840	2.287	1.552	2.409	67.854	1.053^{ns}	1.022^{ns}
T4	0.439	3.898	2.045	0.910	0.829	44.546	0.405^{ns}	0.713^{ns}
T5	0.021	7.521	2.477	1.388	1.927	56.039	0.778^{ns}	0.911^{ns}
T6	0.439	6.898	2.125	1.235	1.525	58.131	0.718^{ns}	0.869^{ns}
T7	0.439	10.240	3.002	3.043	9.261	101.36	3.084*	1.682*
T8	0.000	4.440	1.648	1.125	1.265	68.232	0.767^{ns}	0.860^{ns}
T9	0.000	11.521	2.034	2.185	4.775	107.41	2.347*	1.653*
T10	0.521	11.240	4.410	3.091	9.556	70.097	2.166*	1.259*
T11	0.521	19.521	5.965	4.160	17312	69.753	2.902*	1.312*
T12	0.021	10.339	4.800	3.949	15.6	82.285	3.250*	1.144*
----------AUDPC----------								
T1	19.721	46.086	40.806	21.234	450.898	46.075	-	-
T2	2.241	37.572	33.568	29.516	871.252	78.56	-	-
T3	5.416	23.668	22.729	8.850	78.332	37.394	-	-
T4	3.291	28.9012	29.420	12.811	164.143	44.33	-	-
T5	7.471	32.296	32.541	14.170	200.815	43.878	-	-
T6	3.666	21.385	19.568	14.072	198.041	65.807	-	-
T7	1.216	23.738	21.471	13.675	187.020	57.609	-	-
T8	5.721	22.295	17.070	14.540	211.440	65.221	-	-
T9	0.000	21.074	15.554	11.755	138.199	55.782	-	-
T10	11.820	40.657	40.381	20.195	407.853	49.672	-	-
T11	29.320	59.027	57.531	17.447	304.400	29.558	-	-
T12	82.416	139.912	136.631	34.990	1224.316	25.009	-	-

*Randomness rejected at 5% probability of error. ^{ns} Randomness is not rejected. [1]Treatments described in details in Table 1. –Values not calculated.

The assumptions of the mathematical model were not met in case of the disease severity variable and therefore the non-parametric Friedman test was carried out to compare the severity of the treatments for the three evaluations. In the case of the AUDPC variable, the assumptions have been met, since the transformation of the variable in experiments 2 and 3 was required. The Friedman test showed that the severity of the disease was not the same in all treatments (p-value <0,05). Moreover, the difference in AUDPC values emphasized the difference in the progress of the disease among the fungicide treatments (Table 5).

Table 5. Summary of the analysis of variance (ANOVA) of the three experiments for the area under the disease progress curve (AUDPC).

SV[2]	DF	MS	DF	MS	DF	MS
----------AUDPC----------						
	------ Experiments 1------		----- Experiments 2-----		----- Experiments 3-----	
Block (B)	3	1336.45^{ns}	3	0.08*	3	12.17*
Treatments (A)	7	3662.83*	8	0.69*	11	114.68*
Experimental error	21	990.41*	24	0.22*	33	1.03^{ns}
Amostral error	288	516.07	324	0.02	432	1.77
CVe (%)		81.89		8.68		15.61
CVa (%)		59.11		2.69		20.48
SW (*p*-valor)		0.21		0.91		0.22
Levene (*p*-valor)		0.05		0.11		0.06

*Significant effect by the F test at 5% error probability; :^{ns}Not significant effect according to the F test at 5% error probability. [1]Experiments described in details in Table 1; [2]SV = Sources of variation; DF = degrees of freedom; MS = Mean square; CVa = coefficient of variation of the amostral error; CVe = coefficient of variation of the experimental error; SW (*p*-value) = *p*value of the Shapiro-Wilk normality test; Levene (*p*-value) = *p*value of the homogeneity test of Levene variances.

In experiments 1 and 2, the residual and sample variances were heterogeneous between each other and the variability between plots (residual) was greater than the variability within plots (sample), indicating that the experimental error was greater than the sampling error (Table 5). The values of the coefficient of variation of higher sampling errors reinforced that the variability between plots was greater than within plots, and reveal the need to resize the experiments in terms of the number of repetitions. However, the high value of the coefficient of variation by analysis of variance of experimental and amostral error, especially in experiment 1, indicates low precision and reinforces the need for resizing the experiments by increasing the number of repetitions and sample size (CARGNELUTTI FILHO et al., 2008; CARGNELUTTI FILHO et al., 2009; KRAUSE et al., 2013).

The high experimental error leads to an increased mean square of the experimental error, hindering the H_0 rejection of the hypothesis and raising possibility of Type II error (STORCK et al., 2006). Thus, the number of repetitions must be increased along with proper sizing of the sample size, which could contribute to reduced experimental error and thus obtain more precise conclusions (CARGNELUTTI FILHO et al., 2008; CARGNELUTTI FILHO et al.,2009; KRAUSE et al, 2013).

Variances of the data observed were heterogeneous between treatments, both for severity and AUDPC variable, in all experiments. The heterogeneity of variances of the data observed between assessments was observed in 79,41% of the treatments. The difference in blast's severity, disease progress (determined by AUDPC) between treatments, heterogeneity of variances of the data observed between treatments and evaluations showed that the 40 leaves evaluated in each treatment should be considered as an independent sample. Therefore, we chose to determine the sample size required to evaluate the severity of the blast and AUDPC for each treatment and assessment, separately.

The variability observed in field experiments is usually attributed to environmental factors, soil variability, or genetic factors (MARTIN et al., 2005; CARGNELUTTI FILHO et al., 2009; LÚCIO et al., 2009). However, in the case of diseases, factors such as the spread of the disease on the field must also be taken into consideration (MICHEREFF et al., 2011). To obtain an optimal sample size, different conditions are desirable so that the recommendation is not so limited. In case of diseases, sampling practices should be considered among the different field conditions, since disease spread and, therefore, sample size may vary according to the year, sowing time, cultivation site, and time of evaluation (SILVA et al., 2008; MICHEREFF et al., 2008; MICHEREFF et al., 2011).

The interpretation of the values referring to the average coefficient of variation of blast severity and area under the disease progress curve variables (Table 2, 3 and 4) is indicative of the variation between the leaves evaluated and, thus, the sample size (STURMER et al., 2013). The larger the coefficient of variation, the greater the dispersion of the observations around the average, suggesting the need for a larger number of samples (number of flagged leaves) for more accurate estimation. Based on these values, the tendency of the sample size to be higher for the estimation of blast's severity in relation to AUDPC was observed.

From blast disease severity data, the distribution of the disease in each treatment and evaluation was determined. Variance/mean ratio and Morisita index values greater than 1 indicate an aggregated dispersion of the disease, whereas values less than or equal to 1 indicate a random dispersion (Table 2, 3 and 4). The type of distribution of the disease is associated with the statistics used to estimate the sample size. According to Campbell and Madden (1990), if the distribution of the disease is

aggregated, the size of the sample is calculated by the equation $n = \frac{(k+\bar{x})z^2_{\alpha/2}}{\bar{x}\, k\, CV^2_{\bar{x}}}$, where k is a parameter associated with the negative binomial distribution, describing the aggregated arrangement of infected plants. According to the same authors, infected plants spread randomly in the field follow the Poisson distribution, which is characterized by $\bar{x} = s^2$. In this case, the equation used to calculate the sample size is $n = \frac{z^2_{\alpha/2}}{\bar{x}\, CV^2_{\bar{x}}}$. Finally, when the dispersion of the disease is not the same over time or between treatments, as in this study, the recommended formula to determine the sample size is $n = \frac{z^2_{(\alpha/2)}\, s^2}{\bar{x}^2 CV^2_{\bar{x}}}$, which many authors refer to as undetermined distribution. In our experiments, the dispersion of leaf blast was dependent on the treatment and the time of evaluation. The type of distribution of the disease was not the same throughout the experiment, indicating that the method used to calculate the sample size in this study was suitable.

The sample size for the average estimation of blast's severity was different among treatments and evaluations. This behavior was expected, since the difference observed in the average severity of treatments and evaluations, as well as the heterogeneity of variances between treatments and evaluations in each treatment, leads to changes in the ratio between variance and variables average values. This ratio is indicative of disease spread in the field, which affects the sample size, and this relationship was included in the methodology used. Thus, three sample sizes per treatment (one evaluation) were estimated for this variable, with only the largest presented in Table 6.

In case of AUDPC, only one sample size was determined per treatment. It was observed that the tendency to reduce the number of flagged leaves evaluated when using the AUDPC variable in relation to severity variable. AUDPC is widely used in phytopathological studies, as it characterizes the interaction between the pathogen, the environment and the host, in addition to being used as a form of evaluation of control strategies (BERGAMIN FILHO, 1997).

Table 6. Sample size, given as expressed as number of flagged leaves per plot, to estimate the average severity and area under the disease progress curve (AUDPC) of the blast in the three experiments analyzed.

Treat.[1]	Severity						AUDPC					
	5%	10%	15%	20%	25%	30%	5%	10%	15%	20%	25%	30%
	Experiment 1											
T1	2920	730	324	182	117	81	599	150	67	37	24	17
T2	1917	479	213	120	77	53	396	99	44	25	16	11
T3	2175	544	242	136	87	60	463	116	51	29	19	13
T4	4696	1174	522	293	188	130	1014	254	113	63	41	28
T5	2128	532	236	133	85	59	542	135	60	34	22	15
T6	2514	629	279	157	101	70	542	135	60	34	22	15
T7	3071	768	341	192	123	85	773	193	86	48	31	21
T8	1398	349	155	87	56	39	506	127	56	32	20	14
	Experiment 2											
T1	289	72	32	18	12	8	42	10	5	3	2	1
T2	516	129	57	32	21	14	57	14	6	4	2	2
T3	449	112	50	28	18	12	55	14	6	3	2	2
T4	472	118	52	30	19	13	37	9	4	2	1	1
T5	686	172	76	43	27	19	107	27	12	7	4	3
T6	578	145	64	36	23	16	69	17	8	4	3	2
T7	354	89	39	22	14	10	44	11	5	3	2	1
T8	203	51	23	13	8	6	26	6	3	2	1	1
T9	254	64	28	16	10	7	34	8	4	2	1	1
	Experiment 3											
T1	923	231	103	58	37	26	346	87	38	22	14	10
T2	870	217	97	54	35	24	477	119	53	30	19	13
T3	1161	290	129	73	46	32	228	57	25	14	9	6
T4	860	215	96	54	34	24	321	80	36	20	13	9
T5	829	207	92	52	33	23	314	79	35	20	13	9
T6	932	233	104	58	37	26	283	71	31	18	11	8
T7	1579	395	175	99	63	44	542	135	60	34	22	15
T8	1427	357	159	89	57	40	694	174	77	43	28	19
T9	1773	443	197	111	71	49	694	174	77	43	28	19
T10	755	189	84	47	30	21	403	101	45	25	16	11
T11	748	187	83	47	30	21	143	36	16	9	6	4
T12	1040	260	116	65	42	29	102	26	11	6	4	3

[1]Treatments described in details in Table 1.

It is observed that the number of leaves to be evaluated in order to estimate the severity (percentage of the attacked tissue) of the blast is greater than the number used to estimate AUDPC. The smaller number of leaves required to estimate AUDPC is mainly related to the reduction of the effect of null values (zeros) in the database. Inflation of zeros in the database is the result of the absence of symptoms in some leaves that were sampled while others present symptoms. The presence of null values increases the variability of the data, requiring more extensive sampling to estimate the average severity of the disease.

For an acceptable error of 5%, the sample size required to estimate the average severity of blast is of 4696 flag leaves. As for AUDPC variable, the number of leaves that needs to be evaluated is 1014 (Table 6). The evaluation of this number of leaves becomes impractical, due to the amount of labor and time required. Therefore, it is recommended to use larger sample sizes with greater pre-established errors. Furthermore, the use of the variable AUDPC is recommended whenever possible, as a means of comparison between treatments. Therefore, for an error of 20% and confidence level of 95%, the evaluation of 63 flag leaves is necessary to estimate AUDPC, a more common form of quantification of the disease in works in the plant pathology field. If an experiment is formed of 4 repetitions, it is necessary to evaluate 16 leaves per plot.

The greater the precision required the more the leaves that should be evaluated. The accuracy of the estimate and, subsequently, the sample dimension should be left to the researcher, as the ideal sample size will depend on the minimum acceptable error in every situation (type of study) as well as the labor and resources available to each researcher (MICHEREFF et al., 2008; MICHEREFF et al., 2011; TOEBE et al., 2011; STÜRMER et al., 2013).

CONCLUSION

A variability of the sample size was observed in the evaluation of leaf blast, according to the treatments used and type of evaluations carried out over time. The sample size required to estimate the average area under the blast progress curve is smaller than that to assess severity. For an acceptable error of 20%, the sample size per plot required to estimate the average severity of the blast is 293 flag leaves and for the variable AUDPC, the number of flag leaves to be evaluated is 63.

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