SHORT COMMUNICATION

SAMPLE SIZE FOR QUANTIFICATION OF CERCOSPORA LEAF SPOT IN SWEET PEPPER

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SUMMARY

Cercospora leaf spot of sweet pepper (*Capsicum annuum*) caused by *Cercospora capsici* is an important disease occurring in the tropics. Due to the lack of standard methods to quantify this disease in field conditions, the objective of this study was to determine the ideal sample size for Cercospora leaf spot quantification in the field. Disease severity was determined in eight commercial sweet pepper (cv. All Big) fields located in the Agreste region of Pernambuco state (northeast Brazil). In each field, two diagonals (X) were established in a 0.5 ha area. Twenty-five plants were evaluated along each diagonal with the aid of an empiric scale to consider different sampling rates (3, 6, 9, 12 leaves/plant). The spatial pattern of the disease was estimated using Lloyd's Index of Patchiness (LIP) and ideal sample sizes were calculated based on degrees of acceptable error (5, 10 and 20%). The spatial pattern was predominantly random and there was no correlation ($P = 0.44$) between the LIP values and sample size. Considering a sample of 3 leaves/plant with 10 and 20% rate of acceptable error, the mean ideal sample size was 103 and 26 plants, respectively, for each 0.5 ha of cultivated field.

Key words: Cercospora capsici, *Capsicum annuum*, sampling, epidemiology.

Sweet pepper (*Capsicum annuum* L.), one of the most valuable vegetables grown in Brazil, is grown throughout the country. Cercospora leaf spot, an important disease of sweet pepper, is caused by the fungus *Cercospora capsici* Heald et Wolf, that affects the entire plant canopy, especially the leaves. The main symptoms of Cercospora leaf spot are circular, brown, and necrotic lesions exceeding 1 cm in diameter, with a light gray central portion. Similar lesions, but of smaller size, are found on the stalk, branches and leaf stems (Lopes and Ávila, 2003). The occurrence and development of leaf spot is favored at temperatures above 25°C and at a relative humidity greater than 90%, which are common growing conditions in warm regions or during summer. Leaf spot causes a considerable reduction in the photosynthetic area due to the loss of tissue caused either by the increase in the size and/or number of lesions or by leaf shedding during the development of the disease. Under these conditions, pepper fruits are small, twisted, sun-burned and unfit for marketing (Monteiro *et al*., 2000).

Surveys are fundamental for drafting, planning, implementing and evaluating plant disease management strategies (Campbell and Madden, 1990; Holderness, 2002). Through such surveys, it is possible to determine the relative importance of diseases, monitor fluctuations in intensity throughout the growing season and determine the efficiency and acceptance of recommended control measures (Holderness, 2002). However, surveys are only reliable when the methods employed in the quantification of disease severity and sampling are standardized. In other words, methods should be previously established for assessing severity, number of samples and collection method (Campbell and Madden, 1990; Holderness, 2002). For the quantification of Cercospora leaf spot in the sweet pepper, a diagrammatic scale was designed that offers a good degree of accuracy in estimating disease severity (Michereff *et al.*, 2006). However, there is not an established method for field sampling.

Sample size should optimize the balance between the number of samples and reliability of the data. Although faster and easier to perform, small sample sizes can provide unreliable data, whereas a very high number of samples may contribute little to reliability when compared with the confidence level and precision of an intermediate number of samples (Campbell and Madden, 1990).

There are at least three methods to estimate sample size, which depend on the operational definition of the reliability and on the costs of sample collection: (i) reliability is defined by the coefficient of variation of the mean or standard error; (ii) equations of probability define reliability; (iii) components of variance and cost functions are used to optimize sample number, consid-

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Table 1. Number of sweet pepper plants to be sampled in 0.5 ha surface for quantification of severity of Cercospora leaf spot (*Cercospora capsici*) infections based on four numbers of leaves sampled per plant and reliability defined by the degree of acceptable error.

Field	Localization (City)[1]	Number of leaves/plant	Severity (%)[2]	LIP[3]	Sample size[4] Error/Number of plants		
					5%	10%	20%
A	BEZ	3	2.5 ± 7.3	1.8	467	117	29
		6	2.4 ± 4.4	1.3	306	77	19
		9	2.2 ±2.3	1.0	186	46	12
		12	2.5 ± 2.0	0.9	125	31	8
B	CGR	3	3.1 ± 13.0	2.0*	539	135	34
		6	2.9 ± 6.8	1.5	322	80	20
		9	2.2 ± 3.6	1.3	298	75	19
		12	2.7 ± 4.8	1.3	266	66	17
C	BEZ	3	5.4 ± 38.4	2.1*	527	132	33
		6	5.9 ± 32.5	1.8	373	93	23
		9	5.6 ± 16.0	1.3	204	51	13
		12	6.1 ± 18.5	1.3	199	50	12
D	CSF	3	7.3 ± 57.8	1.9*	434	108	27
		6	8.2 ± 70.6	1.9*	420	105	26
		9	7.1 ± 41.0	1.7	325	81	20
		12	7.9 ± 34.8	1.4	223	56	14
E	CGR	3	10.1 ± 81.0	1.7	318	79	20
		6	10.5 ± 84.6	1.7	307	77	19
		9	8.9 ± 49.0	1.5	247	62	15
		12	9.5 ± 53.3	1.5	236	59	15
F	BEZ	3	9.5 ± 96.0	2.0*	426	106	27
		6	10.2 ± 90.3	1.7	347	87	22
		9	9.7 ± 65.6	1.6	279	70	17
		12	10.3 ± 51.8	1.4	195	49	12
G	CSF	3	11.1 ± 94.1	1.7	305	76	19
		6	11.8 ± 90.3	1.6	259	65	16
		9	12.4 ± 77.4	1.4	201	50	13
		12	12.6 ± 65.6	1.3	165	41	10
H	CSF	3	13.5 ± 130.0	1.6	285	71	18
		6	13.9 ± 125.4	1.6	260	65	16
		9	14.7 ± 96.0	1.4	178	44	11
		12	13.1 ± 59.3	1.3	138	35	9
Mean		3	7.8 ± 64.7	-	413 a[5]	103 a	26 a
		6	8.2 ± 63.1	-	324 ab	81 ab	20 ab
		9	7.9 ± 43.9	-	240 bc	60 bc	15 bc
		12	8.1 ± 36.3	-	193 c	48 c	12 c

[1] BEZ = Bezerros, CGR = Chã Grande, CSF = Camocim de São Félix.
[2] Estimated with empirical scale (Michereff *et al.*, 2006). Mean ± variance of 50 plants evaluated per field.
[3] Lloyd's Index of Patchiness. Values marked with an asterisk are significantly greater than 1.0 (P=0.05) and indicate a patchy spatial pattern (Campbell and Madden, 1990).
[4] Calculated using a random pattern of diseased plants based on mean severity, variance and level of acceptable error (Campbell and Madden, 1990).
[5] Means followed by the same letter in a column do not differ from each other, according to Kruskal-Wallis test (P=0.05).

ering that each sample type has an associated cost (Campbell and Madden, 1990). Considering that the reliability of disease estimate is directly related to sample size and spatial heterogeneity of disease, all above methods can be associated to distributions, which represent different models of spatial distribution of the disease in the field (Perry, 1994).

Despite the importance of Cercospora leaf spot in sweet pepper, no studies have been made to establish the number of plants to be sampled for disease severity quantification. Thus, the aim of the present study was to determine the ideal sample size for the quantification of leaf spot in different growing areas with varying levels of disease severity.

Pilot samplings were carried out to assess the severity of leaf spot in eight commercial fields of cv. All Big located in the Agreste region of the state of Pernambuco (Brazil) (Table 1). The minimal distance between fields was 3.5 km. The trench system was employed in all fields, with flood irrigation and a spacing of 1.0x0.80 m between rows and plants, respectively. All plants were vegetating when evaluated.

On each field, a 0.5 ha area was chosen, consisting of 50 rows and 125 plants/row, for a total of 6,250 plants. Two diagonals (X) were established in each area and 25 plants were evaluated along each diagonal, with the first plant located approximately 5 m from the beginning of the diagonal and the remaining plants spaced at distances of approximately 5 m. With the aid of the empiric scale, which includes levels from 0 to 50% of damaged leaf area (Michereff *et al.*, 2006), the leaf damage by Cercospora leaf spot was estimated using 50 selected plants, with further selection of 3, 6, 9 and 12 leaves/plant. In the three-leaf sample, one leaf from each third of the plant (lower, middle and upper) was evaluated. In the 6-, 9- and 12-leaf samples, two, three and four leaves were evaluated in each third of the plant, respectively.

The Cercospora leaf spot data obtained from the pilot samplings were used in the determination of the ideal sample sizes based on the spatial pattern of the diseased plants. The spatial pattern was evaluated for each field area using Lloyd's Index of Patchiness (LIP) through the equation:

$$\text{LIP} = [(\bar{x} + (S^2/\bar{x}) - 1)]/\bar{x}$$

in which $\bar{x}$ is the mean severity of the disease from 50 plants, using 3, 6, 9 and 12 leaves/plant, and S^2 is the sample variance. Values of LIP lower than, equal to or greater than 1.0 indicate regular, random or patchy spatial patterns, respectively (Campbell and Madden, 1990). The significance ($P = 0.05$) of the values was tested using the chi-square test (X^2) with (n-1) degrees of freedom.

Ideal sample sizes (n) were estimated for each crop area based on the coefficient of variation of the mean (CV_X) and random pattern of diseased plants, considering sample of 3, 6, 9 and 12 leaves/plant by the equation:

$$n = S^2/(x^2 \cdot CV_x^2)$$

with pre-established acceptable errors of 5, 10 and 20% (CV_X= 0.05, 0.1 and 0.2) (Campbell and Madden, 1990). Using the data obtained from each field, the mean ideal sample size was calculated when 3, 6, 9 and 12 leaves/plant were assessed. Mean sample sizes within each category of acceptable error were compared with the Kruskal-Wallis test ($P = 0.05$). Pearson's correlation analysis ($P = 0.05$) was used to determine a possible influence from the degree of disease severity on the intensity of the patchiness (LIP) among the diseased plants in the different sample sizes.

The severity of Cercospora leaf spot on sweet pepper from the different fields ranged from 2.2 to 13.9% (Table 1). Because the ideal sample size can vary depending on the spatial pattern of the disease in the field (Kranz, 1988; Perry, 1994), the Cercospora leaf spot pattern was estimated using LIP. The pattern did not differ from 1 ($P > 0.05$) in 84% of the situations evaluated (Table 1), indicating that the Cercospora leaf spot pattern in the field is predominantly random. This type of pattern was expected for this disease, as wind is the principal dispersion agent (Monteiro *et al.*, 2000). Therefore, diseased plants that are relatively distant from a set of healthy plants can serve as a source of inoculum (Burdon, 1987). The different samplings of 3, 6, 9 and 12 leaves/plant did not affect the LIP estimates, a result that was confirmed by the lack of a significant correlation (r = 0.14; $P = 0.44$) between the degree of disease severity and intensity of patchiness within samplings with different numbers of leaves. This conclusion supports the hypothesis that the origin of the inoculum is an exogenous source.

Sample size for quantification of Cercospora leaf spot on sweet pepper was estimated based on a random pattern of diseased plants. No significant correlation (r = -0.42; $P = 0.28$) was found between the degree of disease severity and the number of plants sampled.

In terms of mean among fields, there was no significant difference in sample size for the quantification of leaf spot when 3 or 6 leaves per plant were assessed; however, with 12 leaves there was a significantly different sample size (Table 1). The number of plants to be sampled (regardless of the number of leaves evaluated) was reduced when the degree of acceptable error was increased (Table 1). The choice of the degree of acceptable error depends on the purpose of the sampling (Kranz, 1988). For regional phytopathological surveys, which are often faced with financial restrictions, a 20% error can be used (sampling with 80% accuracy). Considering this degree of error and the mean value obtained, sampling 26 plants with 3 leaves/plant is recommended for each 0.5 ha of cultivated field (Table 1) in surveys for assessement of Cercospora leaf spot severity

in sweet pepper. If there are no limitations to the survey execution, a 10% error is considered ideal for field surveys (Southwood, 1978), in which case, the sampling would require 103 plants with 3 leaves/plant for each 0.5 ha of cultivated field (Table 1). Either way, 3-leaf samples were chosen with a greater number of plants, to increase the precision of sampling.

When using different methods for estimating sample size, the data from the locations analyzed should be representative of what could occur in other fields, an assumption whose validity varies between pathosystems (Campbell and Madden, 1990). Thus, sample size for quantification of Cercospora leaf spot severity on sweet pepper derived from this study are applicable across areas where this crop is grown, because the data came from fields with different conditions and were estimated based on increasing need for precision.

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