Estimating optimum plot size with radiometer for experiments on soybeans treated with fungicide

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ABSTRACT

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Spectral remote sensing and proximal sensors are important tools for managing the plant-pathogen relationship. The lack of experimental planning and the probability of error in agricultural studies may result in work repetition and, consequently, in financial expenses and costs with human resources. To reduce such problems, determining the optimum size of the experimental plot for treatments is one of the adopted methods. The objective of this study was to estimate the optimum plot size for reflectance in soybeans that were treated with different fungicide levels according to the methods of modified maximum curvature and maximum distance. Reflectance readings were carried out for the soybean crop with a radiometer GreenSeeker®, considering basic units of 0.45 m² in an area of ten rows, 10 m long, for each treatment. Treatments were applied to create a gradient of Asian soybean rust, varying the number of fungicide applications. Data were collected in two phenological stages (R5.5 and R6), obtaining 300 simulations of experimental area for each stage. Based on the results, the use of 5.40 m² plots with a group of three rows, 4 m long, is recommended.

Keywords: NDVI. Maximum distance. Modified maximum curvature.

RESUMO

Michels, R.N.; Canteri, M.G.; Fonseca, I.C.B.; Aguiar e Silva, M.A.; Bertozzi, J.; Tatiane Dal Bosco, C. Estimativa do tamanho ótimo de parcela em experimentos com o radiômetro em soja tratada com fungicida. ***Summa Phytopathologica,*** v.46, n.4, p.308-312, 2020.

O sensoriamento remoto espectral e o sensor proximal são ferramentas importantes para gerenciar a relação planta-patógeno. A falta de planejamento experimental e a probabilidade de erro em estudos agrícolas podem resultar em retrabalho e, consequentemente, despesas financeiras e de recursos humanos. Uma maneira de reduzir esse problema é determinar o tamanho ótimo de parcela experimental para realização dos tratamentos. O objetivo deste estudo foi estimar o tamanho ótimo de parcela para refletância em soja que foi tratada com diferentes doses de fungicida, usando os métodos de curvatura máxima modificada e distância máxima. As leituras de refletância foram realizadas na cultura de soja com o auxílio de um radiômetro GreenSeeker®, com unidades básicas de 0,45 m², em uma área de dez linhas, com 10 metros de comprimento, em cada tratamento. Os tratamentos foram aplicados para criar um gradiente da doença ferrugem asiática da soja, variando o número de aplicações de fungicidas. Os dados foram coletados em dois estágios fenológicos (R5.5 e R6), obtendo-se 300 simulações de áreas experimentais em cada estágio. Com base nos resultados, recomenda-se o uso de parcelas de 5,40 m², com um grupo de três linhas, com 4 m de comprimento.

Palavras-chave: NDVI. Distância máxima. Curvatura máxima modificada.

Using technology to detect phenotypic reactions that occur during the plant-pathogen interaction has become more frequent in recent years (16). Spectral remote sensing and proximal sensing have been widely employed to manage lands and crops (3,17), as well as to quantify damage caused by leaf diseases (4).

GreenSeeker®, produced by Trimble, is a portable device with an active spectral sensor that provides the normalized difference vegetation index (NDVI) via reflectance measurements, i.e., it has a light-emitting diode in the near-infrared (NIR: 770 nm) and red (RED: 650 nm) region and a receiver that absorbs the values reflected in the canopy, rapidly indicating nutritional and physiological conditions, stress, and potential yield by measuring the crop biomass (1, 19, 20, 21). This device accurately reflects the severity of foliar diseases and is a useful tool that precisely traces the level of leaf rust (15)

For a reliable conclusion of proximal sensing application, field experiments should show the least possible experimental errors and meet the statistical parameters (2). Adopting the correct experimental plot size is important to prevent work repetition, financial expenses and human resource losses, keeping experimental accuracy at an acceptable magnitude and maximizing the obtained information (8, 10).

In the study of plant diseases and fungicides, establishing the size and shape of an experimental plot can be empirical, based on the researchers' experience with a specific culture (13); however, there are methodologies to determine the optimum plot size (18).

The modified maximum curvature method, proposed by Lessman & Atkins (7), and the maximum distance method, proposed by Paranaíba (14), are methodologies for determining the optimum plot size, which need experiments with a culture of interest, i.e., without treatment distinction among the analyzed data, followed by the subdivision of the experimental area into small portions — basic experimental units (BEU) - from which data are collected independently, identifying the relative position. After data collection, contiguous plots are set to simulate plots of different sizes and shapes (6).

Thus, the objective of this manuscript was to estimate the optimum plot size for evaluating fungicide treatment on soybeans according to the modified maximum curvature and the maximum distance methods.

MATERIALS AND METHODS

The field experiment was conducted in Londrina, Paraná State, Brazil, located at latitude 23°19'40.92"S and longitude 51°12'19.20"W, altitude of 560 m, during the 2013/14 harvest, with the soybean cultivar 'Monsoy 6410 IPRO'.

Four areas of 12 m length and 12 rows width, 0.45 m between rows, were used; the useful area for data collection was ten rows × 10 m, i.e., 45 m². Each plot was organized to simulate different intensities of Asian soybean rust, which was induced according to the number of scheduled fungicide applications (Table 1).

Table 1. Number and periods of sprays applied in the 2013/14 harvest to induce Asian soybean rust (*P. pachyrhizi*) gradient in the studied areas.

Number of sprays	Spraying (days after germination)
6	30, 45, 60, 75, 90, 105
4	60, 75, 90, 105
3	75, 90, 105
0	-

The fungicide used to induce Asian soybean rust intensity gradient was the commercial mixture of Pyraclostrobin + Epoxiconazole (66.5 + 25 g a.i. ha^{-1}) with spray volume of 200 L.ha^{-1} plus mineral oil as a vehicle, at 500 mL.ha^{-1}. The fungicide was applied with a CO² pressurized backpack sprayer, containing four nozzles adjusted to fully cover the experimental unit, simulating a conventional (vehicular) sprayer.

Data on NDVI were collected in stages R5.5 and R6, between 8:00 a.m. and 8:30 a.m., from the ten central lines for each treatment, at 1-meter intervals, totaling 10 m per row and 100 readings per treatment, per stage. NDVI was measured with GreenSeeker®, model RT100, from Trimble; data were collected at a distance of 0.8 m from the canopy.

To determine the optimum plot size, the modified maximum curvature method (MMC) - Lessman & Atkins (7), was initially used. According to this methodology, the variability given by the coefficient of variation (CV_x) and the size of the plot with X basic experimental units is calculated by $CV_x = aX^{-b}$, where a and b are the parameters to be estimated. The optimum plot size was estimated based on the equation:

$$X_0 = exp\left\{\left[\frac{1}{2b+2}\right] Log\left[\frac{(ab)^2(2b+1)}{b+2}\right]\right\}$$

In this case, X_0 is the abscissa value at the maximum curvature point, which corresponds to the optimum plot size (9).

More than one method is recommended to determine the optimum plot size (13). Thus, the method of maximum distance (MD) was also adopted in our study; its resolution is based on a curve *yc* described by $CV_x = aX^{-b}$ and a line *yr* secant to that curve. The point of curve *yc* was calculated (which was at the longest distance from line *yr*) as the line segment along that distance was perpendicular to line *yr* (6).

The solution method presented by Lorentz (6) proposes that the line perpendicular to line *yr* should be determined to find the requested point of curve *yc*. Such a line perpendicular to line *yr* is called *yp* and is calculated by $y_p = ex + f$. The angular coefficient *c* and the linear coefficient *d*, both of line *yr*, are fixed and can be obtained from two points of line *yr* which are common to the curve *yc*.

The points common to the curve and the line to the left are called X_{CRi} and Y_{CRi}, while the common points to the right are called X_{CRf} and Y_{CRf}. Thus, *c* and *d* are obtained, respectively, by:

$$c = \frac{y_{CRf} - y_{CRi}}{x_{CRf} - x_{CRi}}$$

and

$$d = y_{CRi} - cx_{CRi}$$

or

$$d = y_{CRf} - cx_{CRf}$$

The expressions for *d* are obtained by isolating it in the *yr* equation, substituting the $X_{CRi} + Y_{CRi}$ point or the $X_{CRf} + Y_{CRf}$ point. The angular coefficient *e* of line *yp* is also fixed and can be obtained based on the condition that lines *yr* and *yp* are perpendicular to each other. Therefore:

$$e = \frac{-1}{c}$$

Determining the linear coefficient *f* of line *yp* is part of the iterative method proposed by Lorentz (6) and has the following solution:

$$x_{Rpj} = \frac{f - d}{c - e}$$

The distance between points $X_{Cj} + Y_{Cj}$ and $X_{Rpj} + Y_{Rpj}$ of line *ypj*, which is perpendicular to *yr*, is given by:

$$d_{cr} = \sqrt{\left(y_{Cj} - y_{Rpj}\right)^2 + \left(x_{Cj} - x_{Rpj}\right)^2}$$

The analyses were performed within each treatment and each soybean phenological stage (R5.5 and R6). Thus, according to Lorentz (6), each treatment was considered a blank experiment. Two phenological stages were chosen when significant differences in the NDVI values were found between treatments, i.e., areas with different Asian soybean rust intensities.

To determine the optimum plot size, basic experimental units (BEU) of NDVI data should be grouped. Every possible simulation is shown in Table 2, considering width as meters and length = 0.45 m (distance between rows) for each simulation or unit, relation between length and width (LxW), plot size as m², type of grouping and number of plots. The BEU in this study are considered 0.45 m², i.e., 1 m long and 0.45 m wide.

Table 2. Number of simulations, width (W) and length (L) of simulations, L×W combination, plot size (m²), type of grouping (m) and total number of plots.

Simulation	Width	Length	L×W	Size (m²)	Type of Grouping	Number of plots
1	1	1	1	0.45	0.45 x 1.00	100
2	2	1	2	0.90	0.90 x 1.00	50
3	3	1	3	1.35	1.35 x 1.00	30
4	4	1	4	1.80	1.80 x 1.00	20
5	5	1	5	2.25	2.25 x 1.00	20
6	6	1	6	2.70	2.70 x 1.00	10
7	7	1	7	3.15	3.15 x 1.00	10
8	8	1	8	3.60	3.60 x 1.00	10
9	9	1	9	4.05	4.05 x 1.00	10
10	10	1	10	4.50	4.50 x 1.00	10
11	1	2	2	0.90	0.45 x 2.00	50
12	1	3	3	1.35	0.45 x 3.00	30
13	1	4	4	1.80	0.45 x 4.00	20
14	1	5	5	2.25	0.45 x 5.00	20
15	1	6	6	2.70	0.45 x 6.00	10
16	1	7	7	3.15	0.45 x 7.00	10
17	1	8	8	3.60	0.45 x 8.00	10
18	1	9	9	4.05	0.45 x 9.00	10
19	1	10	10	4.50	0.45 x 10.00	10
20	2	2	4	1.80	0.90 x 2.00	25
21	3	2	6	2.70	1.35 x 2.00	15
22	4	2	8	3.60	1.80 x 2.00	10
23	5	2	10	4.50	2.25 x 2.00	10
24	6	2	12	5.40	2.70 x 2.00	5
25	7	2	14	6.30	3.15 x 2.00	5
26	8	2	16	7.20	3.69 x 2.00	5
27	9	2	18	8.10	4.05 x 2.00	5
28	10	2	20	9.00	4.50 x 2.00	5
29	2	3	6	2.70	0.90 x 3.00	15
30	3	3	9	4.05	1.35 x 3.00	9
31	4	3	12	5.40	1.80 x 3.00	6
32	5	3	15	6.75	2.25 x 3.00	6
33	6	3	18	8.10	2.70 x 3.00	3
34	7	3	21	9.45	3.15 x 3.00	3
35	8	3	24	10.80	3.60 x 3.00	3
36	9	3	27	12.15	4.05 x 3.00	3
37	10	3	30	13.50	4.50 x 3.00	3
38	2	4	8	3.60	0.90 x 4.00	10
39	3	4	12	5.40	1.35 x 4.00	6
40	4	4	16	7.20	1.80 x 4.00	4
41	5	4	20	9.00	2.25 x 4.00	4
42	6	4	24	10.80	2.70 x 4.00	2
43	7	4	28	12.60	3.15 x 4.00	2
44	8	4	32	14.40	3.60 x 4.00	2
45	9	4	36	16.20	4.05 x 4.00	2
46	10	4	40	18.00	4.50 x 4.00	2
47	2	5	10	4.50	0.90 x 5.00	10
48	3	5	15	6.75	1.35 x 5.00	6
49	4	5	20	9.00	1.80 x 5.00	4
50	5	5	25	11.25	2.25 x 5.00	4
51	6	5	30	13.50	2.70 x 5.00	2
52	7	5	35	15.75	3.15 x 5.00	2
53	8	5	40	18.00	3.60 x 5.00	2
54	9	5	45	20.25	4.05 x 5.00	2
55	10	5	50	22.50	4.50 x 5.00	2
56	2	6	12	5.40	0.90 x 6.00	5
57	3	6	18	8.10	1.35 x 6.00	3
58	4	6	24	10.80	1.80 x 6.00	2
59	5	6	30	13.50	2.25 x 6.00	2
60	2	7	14	6.30	0.90 x 7.00	5
61	3	7	21	9.45	1.35 x 7.00	3
62	4	7	28	12.60	1.80 x 7.00	2
63	5	7	35	15.75	2.25 x 7.00	2
64	2	8	16	7.20	0.90 x 8.00	5
65	3	8	24	10.80	1.35 x 8.00	3
66	4	8	32	14.40	1.80 x 8.00	2
67	5	8	40	18.00	2.25 x 8.00	2
68	2	9	18	8.10	0.90 x 9.00	5
69	3	9	27	12.15	1.35 x 9.00	3
70	4	9	36	16.20	1.80 x 9.00	2
71	5	9	45	20.25	2.25 x 9.00	2
72	2	10	20	9.00	0.90 x 10.00	5
73	3	10	30	13.50	1.35 x 10.00	3
74	4	10	40	18.00	1.80 x 10.00	2
75	5	10	50	22.50	2.25 x 10.00	2

To obtain an R^2 (coefficient of determination) of greater significance, all calculations for determining the optimum plot size were made, and the simulations from 1 to 75, 1 to 50 and 1 to 25 (Table 2) were used.

RESULTS AND DISCUSSION

Asian soybean rust is an end-of-cycle disease; therefore, NDVI data were obtained in stages R5.5 and R6, when the disease gradient was greater, as shown in Table 3.

Table 3. Difference in the disease gradient demonstrated by the NDVI values among the treatments with 6, 4, 3 and 0 fungicide sprays in stages R5.5 and R6. The letters show statistical differences according to Scott Knott test at 5% significance.

Number of Sprays	R5.5	R6
6	0.678c	0.319a
4	0.639c	0.346b
3	0.480b	0.354b
0	0.370a	0.288a

To calculate the optimum plot size according to the MMC method, the values *a* and *b* presented by Lessman & Atkins (7) should be estimated, while based on the MD method, the values *c*, *d*, and *e* of the linear and angular coefficients of lines *yr* and *yp* should also be obtained (6). They are represented in Table 4 considering the coefficient of variation obtained according to Table 2.

For the two stages of NDVI data collection (R5.5 and R6), from 75,

Table 4 – Values from the calculation of *a* and *b*, angular coefficient *c* and linear coefficient *d* of line *yr*, angular coefficient *e* of line *yp* and R^2 using the Coefficient of Variation (CV) for treatments with different fungicide sprays in stages R5.5 and R6, within simulations with 75, 50 and 25 possibilities.

Treatment	Stage	Simulation	*a*	*B*	*C*	*d*	E	R^2
6 Sprays	R5.5	75	2.6344	0.522	-0.0481	2.6825	20.790	0.375
		50	2.3295	0.458	-0.0844	2.4139	11.848	0.503
		25	2.0661	0.388	-0.1749	2.3815	5.717	0.617
	R6	75	12.615	0.653	-0.2421	12.857	4.130	0.255
		50	9.511	0.506	-0.3564	9.868	2.805	0.399
		25	8.027	0.400	-0.6491	8.676	1.540	0.404
4 Sprays	R5.5	75	2.151	0.381	-0.0354	2.186	28.248	0.412
		50	2.335	0.428	-0.0893	2.424	11.198	0.541
		25	2.395	0.459	-0.2054	2.601	4.8685	0.744
	R6	75	12.615	0.653	-0.2421	12.857	4.130	0.255
		50	9.512	0.506	-0.3564	9.868	2.805	0.399
		25	8.027	0.400	-0.6491	8.676	1.541	0.404
3 Sprays	R5.5	75	2.528	0.810	-0.0500	2.578	20.000	0.408
		50	1.950	0.664	-0.0785	2.028	12.738	0.565
		25	1.511	0.503	-0.1585	1.669	6.309	0.896
	R6	75	9.960	0.418	-0.1698	10.130	5.889	0.385
		50	8.339	0.321	-0.2593	8.598	3.856	0.371
		25	8.471	0.336	-0.6221	9.092	1.607	0.384
No Sprays	R5.5	75	3.320	0.506	-0.0576	3.377	17.361	0.439
		50	2.678	0.390	-0.0911	2.769	10.976	0.684
		25	2.848	0.430	-0.2884	3.467	3.467	0.754
	R6	75	13.764	0.432	-0.2374	14.001	4.212	0.216
		50	12.031	0.377	-0.3745	11.980	2.670	0.370
		25	13.641	0.473	-1.1476	14.789	0.871	0.440

Table 5 – Combination of length and width (L×W) and optimum plot size (m^2) obtained according to the methods Modified Maximum Curvature (MMC) and Maximum Distance (MD) for Coefficient of Variation (CV) data and simulations with 75, 50 and 25 possibilities in stages R5.5 and R6.

Treatment	**Simulation**	**MMC**	**MD**
6 sprays	75 – R5.5	LxW=1 (0.45m²)	LxW=10 (4.50m²)
	50 – R5.5	LxW=1 (0.45m²)	LxW=4 (1.80m²)
	25 – R5.5	LxW=1 (0.45m²)	LxW=4 (1.80m²)
	75 – R6	LxW=1 (0.45m²)	LxW=8 (3.60m²)
	50 – R6	LxW=1 (0.45m²)	LxW=4 (1.80m²)
	25 – R6	LxW=1 (0.45m²)	LxW=5 (2.25m²)
4 sprays	75 – R5.5	LxW=1 (0.45m²)	LxW=8 (3.60m²)
	50 – R5.5	LxW=1 (0.45m²)	LxW=5 (2.25m²)
	25 – R5.5	LxW=1 (0.45m²)	LxW=4 (1.80m²)
	75 – R6	LxW=1 (0.45m²)	LxW=10 (4.50m²)
	50 – R6	LxW=1 (0.45m²)	LxW=4 (1.80m²)
	25 – R6	LxW=1 (0.45m²)	LxW=4 (1.80m²)
3 sprays	75 – R5.5	LxW=1 (0.45m²)	LxW=9 (4.05m²)
	50 – R5.5	LxW=1 (0.45m²)	LxW=4 (1.80m²)
	25 – R5.5	LxW=1 (0.45m²)	LxW=4 (1.80m²)
	75 – R6	LxW=1 (0.45m²)	LxW=9 (4.05m²)
	50 – R6	LxW=1 (0.45m²)	LxW=4 (1.80m²)
	25 – R6	LxW=1 (0.45m²)	LxW=4 (1.80m²)
No sprays	75 – R5.5	LxW=1 (0.45m²)	LxW=9 (4.05m²)
	50 – R5.5	LxW=1 (0.45m²)	LxW=10 (4.50m²)
	25 – R5.5	LxW=1 (0.45m²)	LxW=4 (1.80m²)
	75 – R6	LxW=1 (0.45m²)	LxW=9 (4.05m²)
	50 – R6	LxW=1 (0.45m²)	LxW=4 (1.80m²)
	25 – R6	LxW=1 (0.45m²)	LxW=4 (1.80m²)

50, and 25 simulation areas according to the MMC method, considering all four treatments and using the coefficient of variation, the optimum plot size was the area of 0.45 m², with length x width relation equal to 1, i.e., an area that is the BEU (Table 5).

Based on the MD method, for treatments with 6, 4 and without fungicide sprays, the optimum plot size was 4.50 m² (L×W = 10), while for the treatment with 3 sprays, it was 4.05 m² (L×W = 9).

According to Paranaíba (13), the modified maximum curvature method can underestimate the plot size due to the low values of the coefficient of variation, which, according to Lorentz (5), influences the optimum plot size calculation.

Moraes (12) stated that, to obtain higher quality data, the largest plot size must be adopted. Thus, the optimum plot size for reflectance studies in soybeans is 4.50 m², with two 5-m rows; adopting immediately higher L×W is also recommended, and in this case, L×W = 12 or 5.40 m², with a group of three 4-m rows. This plot size is the same as that adopted by Michels et al. (11) in their project to examine the effects of different fungicide applications in soybeans; however, their plot size was inferior to the one used by Koga (5), who established a 10m² area to evaluate the fungicide effect on Asian soybean rust development, as well as on control effectiveness and soybean productivity.

CONCLUSION

The maximum distance method allowed estimating the optimum plot size.

Thus, in studies focused on reflectance measurements for the Asian soybean rust pathosystem, the use of 5.40 m² plots is recommended, with groups of three rows of 4 m each.

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