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Research Article

Spatial Modelling and Spread of Corn Stunt and Maize Bushy Stunt in Corn Hybrids (*Zea mays*)

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Abstract

In recent years, there have been growth in number in the outbreaks of Maize Corn Stunt and "Maize Bushy Stunt Phytoplasma" incidence in maize (*Zea mays*) crops, probably due to the increase in the spittle population. The objective was to perform a modelling and evaluate the spatial distribution of corn stunt and "Maize Bushy Stunt Phytoplasma" in different commercial maize hybrids grown in the 2017 harvest. The experiment was carried out with 13 hybrids in the city of Ipameri, Goiás state, Brazil. The experimental area per hybrid showed the dimensions of 4x70 m and 10 m in length, resulting in a population of plants organized in a spacing of 0.5 m and distance between plants of 0.2 m sown in eight growing lines. The lines were referenced in the X and Y axis, with the corn stunt and "Maize Bushy Stunt Phytoplasma" incidence, referenced by Z in a useful area per hybrid of 210 m^2 for modelling and construction of spatial distribution maps. There was a significant difference between the geographic distance and the incidence of both types of corn stunt. The adjustment models recognized for corn stunt and "Maize Bushy Stunt Phytoplasma" by cultivar were Spherical, Exponential, Linear and Gaussian, being the which spherical is the best model to explain the spatial distribution of both fodder was

Keywords: *Dalbulus maidis*; Epidemiology; Phytoplasma; *Spiroplasma kunkelii*; Spread

Introduction

Maize (*Zea mayus)* is a grass of the family Poaceae, Maydeae tribe, genus and specie *Zea mays* L. It is a crop of with international importance, occupying a prominent position among the agricultural species exploited worldwide, as it is a food of high energy value and, in a lower production cost, beyond be used in large numbers of by-products, it has more than 3500 uses directly and indirectly [1].

North America is the greatest producer of maize, but also worthy of mention are West and South America (Center of origin). The EUA, China and Brazil are responsible for 70% of the total volume of production of this crop [2]. In Brazil, it already is one the agricultural species of the greater importance, as relationship of crop area as production [3]. There is a predominance of maize crops in the Midwest and Southern regions of Brazil, which they represent near 68% cultivated area, and this are responsible for 76% of all production in national territory [1].

According Cota., *et al.* [3] among corn plant diseases in Brazil, deserve to be highlighted leaf spot or white spot (*Pantoea ananas*), the grey leaf spot (*Cercospora zea-maydis*), the Helminthosporium leaf spot and blight (*Bipolaris maydis*), the Southern (polysora) rust (*Puccinia polysora*), the Tropical rust (*Physopella zeae*), stalk rots (*Fusarium* spp., *Diplodia* spp., *Colletotrichum* among others), the Corn Stunt Phytoplasma ("Maize Bushy Stunt Phytoplasma") and the Maize Corn Stunt (*Spiroplasma kunkelii*). This last both plant diseases in recent years have caused greater yield losses in the Midwest of Brazil, in both summer and winter crop, It is causing and annual increase in the amount of inoculum in the field, consequently causing the survival of the population of the viruliferous vector leafhopper. Same results have been reported in others countries [4].

The corn leafhopper - *Dalbulus maidis* (DeLong and Wolcott) (Hemiptera: Cicadellidae), It's considered one pest main important of maize in Latin America. It can cause losses of up top to 100% by crop, depending on the infection season and maize hybrid [5]. In

Brazil, the incidence of plant diseases by maize associated on Mollicutes increase considerably in last years, mainly in the southwest and Midwest regions [6]. Flight activity studies and flight behavior of *D. maidis* has indicated high potential of migration and dispersion of this specie by leafhopper [7].

The Maize Corn Stunt is caused by spiroplasma and the Maize Bushy Stunt by phytoplasma, both plant diseases prokaryotes infecting vascular system of plants, and belongs to a class Mollicutes [8]. This plant pathogen change the colors of the leaves also affect the development and production of grains, once the leaves are considered the main sources of photo-assimilates by corn, being the most important part of the plant [9]. The natural host single reported with Maize Corn Stunt (MCS) and Maize Bushy Stunt Phytoplasma (MBSP) are corn this moment and some Teosintes [5], but others grasses are host leafhopper.

It is postulated that these phytoplasmas survive this vector in seasons and that corn aren't cultivated, in others unknown and species leafhopper being be disseminated by long distance in and out of field [10].

The spatial spread of vector insect can be divided to three types: aggregate (or contagious), random or uniform (or regular) [11]. The spatial standard of MCS and MBSP in field generally express the dispersion process of pathogen [12]. Due to difficulty of detection by Mollicutes [13], and studying method of plant diseases, instead of vector, the recognition of spatial spread and temporal of corn stunt aren't describe in literature around the world.

The objective of this work was to perform a modelling and evaluate the spatial spread of corn stunt and choose the best model in different corn hybrids.

Materials and Methods

The experiment was held in the field 2016/2017, it implanted in Experimental Station RC Cruz, farm Esmeralda, address highway BR 050, (coordinates 17°29'31.35'' 48°12'56.93'') height: 908 m, Ipameri, Goiás state, Brazil. The soil was characterized with being red yellow latosol dystrophic [14].

It was adopted no-tillage system being used 13 hybrids (Table 1) hybrids 1©, hybrids 2©, SHS 7920©, SH 7990©, DKB 177©, DKB 290©, ADV 9434©, CD 3612©, CD 3770©, NS 70©, NS 90©, NS 92 PRO© and AG 8070 PRO 3©. The technologies PRO e PRO3 refer transgenic hybrids, presenting resistance à armyworm (*Spodoptera frugiperda*), earworm (*Helicoverpa zea*) and drill-stalk (*Diatraea saccharalis*), and the last protein Bt exclusive by control of rootworm (*Diabrotica speciosa*) [15].

Ord.	Companies	Maize Hybrids	Cycle
1	Unknow	Hybrid1	Unknow
2	Unknow	Hybrid 2	Unknow
3	Santa Helena	SHS 7920 PRO PRO3	Early
4	Santa Helena	SHS 7990 PRO2	Early
5	Dekalb	DKB 177 PRO3	Early
6	Dekalb	DKB 2890 PRO3	Early
7	Advanta	ADV 9434 PRO	Early
8	Coodetec	CD 3612PW	Early
9	Coodetec	CD 3770PW	Early
10	Nidera	NS 70	Medium
11	Nidera	NS 90 PRO PRO2	Medium
12	Nidera	NS 92 PRO PRO2	Medium
13	Sementes Agroceres	AG 8070 PRO 3	Early

Table 1: List of companies, commercial maize and cycle hybrids evaluated in this experiment.

The seeds were planted on 14/11/2016, cultivated in a randomized block design (6 blocks), being each block contain by 13 treatments (hybrids).

The experimental area cultivated with hybrids maize with dimensions of 4x70 m (each block with 10 m of length) resulting one plants bulk organized on line spacing of 0,5 m and 0,2 m column spacing's a total eight crop lines. The sideways lines of each hybrids wasn't evaluable leaving only internal useful area by 6 growing line (30 m2.parcel-1) that they was referenced on three dimensions X (m), Y (m) and Z that is one dependent variable was played by incidence MCS and MBSP, in a useful base area for 180 m^2 producing spatial maps and temporal distribution and identification area in m2 with plant diseases incidence. It was considered symptoms by stunt corn [Maize Corn Stunt (MCS) and Maize Bushy Stunt Phytoplasma (MBSP)], the presence of stretch marks red to purple or yellow at the beginning on base of leaves, or in the leaves border.

It was evaluable the incidence of MCS and MBSP isolated by hybrids (ratio of number of symptoms plants by number of total plants [nt sample = 10 meters]; borders of 2 m of line) by 95 days after planting (DAP) and 109 DAP. The measures of distance X and Y, measured by meter, tt was associated by incidence by area and maize hybrid evaluable with stunt corn was analyzed in software of geostatistical Gamma Design GS+, and the spatial adjustment distributions by models Gaussian, Spherical, Linear and Exponential, creating adjustment model equation and graphics. The software STATISTICA® was utilized to represent for spatial incidence (distributions maps), being put values x, y (spatial positions, m) and z the incidence value of MCS and MBSP at 95 and 109 DAP. The spatial measures for X and Y was given in meters, and sanitary measure was released as a percentage of symptomatic plants, divided by a sample of 10 unit, was inserted by software in Gamma Design GS+®, that allowed to indicate adjusted semivariograms and spatial distributions model adjusted parameters calculated with the residual sum of squares (RSS). About graphics indications the RSS recognized the models, to explain better stunts corn disease in the different hybrids evaluated in the experiment.

Results and Discussion

Symptomatology of maize stunts

It was observed a higher visual incidence and damages of the MCS in the evaluated hybrids. Pereira [16] has pointed out that MCS is more frequent in outcrops and hot regions. Oliveira., *et al.* [6] pointed out a higher incidence of MCS (2-20%) of incidence than MBSP (1-4%) in greenhouse transmission bioassays.

Pereira [16] also highlighted these diseases as being major in the corn crop, pointing out that it is a serious problem in the Brazilian conditions of late planting, started from January. The spreading dynamics of the leafhopper were observed by the higher colonization and frequency of adult insects of the leafhopper on the apical leaves, transmitting the disease in this part, logically distributing systemically to all the organs of the aerial part. Gussie., *et al.* [17] pointed out that when infecting corn seedlings, the Spiroplasma moves first towards the roots and then towards the shoot. There is a greater preference of the insect vector, due to ease of mechanical penetration in the young tissues, and these represent the gateway to colonization in the plant [8].

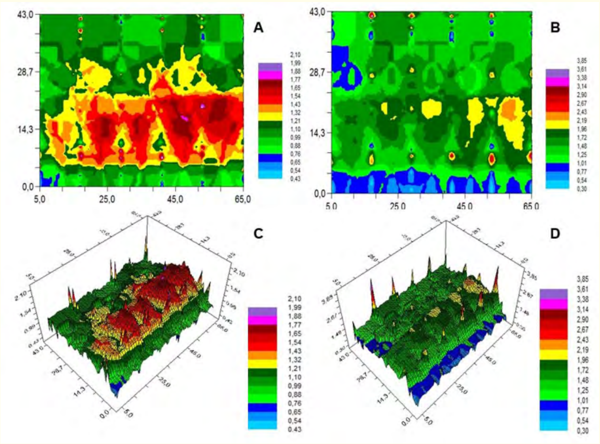

Figure 1: Spatial spread of Maize Corn Stunt (Spiroplasma kunkelii) in maize (Zea mays) at 95 and 109 days after planting (DAP). A. 2D spread at 95 DAP, B. 2D spread at 109 DAP, C. 3D spread at 95 DAP, D. 3D spread at 109 DAP.

Another observed effect, due to the presence of the two maize stunts, is the reduction and failure of ear production, shortening of internodes. Pereira [16] pointed out that plants infected by Phytoplasma may have a higher number of ears that do not produce grains. The plants presented symptoms of shortening of internodes, consequently characterized as dwarfism.

MCS spread

At the 95 DAP level (approximately in R3), the incidence of MCS reached an aggregate pattern (higher incidence) ranging from 0.43% to 2.10% in the evaluation area of the maize hybrids (Figure 1A). At the 109 DAP (approximately R5 cycle), the incidence followed the same pattern in the amplitude, but in a greater amplitude of incidence 0.3-3.85% incidence in the evaluated area (Figure 1B).

MCS spread

Spatially at 95 DAP in the range of incidence of MBSP reached an isolated or aggregate pattern (located at the borders) ranging from 0.02% to 5.16% (range greater than the MCS) of incidence in the area of maize hybrids (Figure 2A). Silva., *et al.* [11], when analyzing the spatial pattern of spreading, not of the disease, but of the leafhopper (*Empoasca kraemeri*) also observed an aggregate pattern in string bean, demonstrating a relationship of the distribution pattern of the disease with the insect vector. At 109 DAP (approximately R5), the incidence followed the same pattern of temporal amplitude of 0.11-4.78% incidence in the evaluated area (Figure 2B).

Hybrids located on the edges of the experimental area had the highest spatial incidences of the disease (except for a few isolated spots) (Figure 2AB).

When comparing the peaks of incidence of MBSP at 95 and 109 DAP, there was an area of greater number of foci of the disease at 95 DAP (Figure 2C) than at 109 DAP (Figure 2D), being located on the edges of the experimental area.

At 95 DAP, the number of peaks with the highest incidence of MBSP, which would represent the sources of onset of the epidemic in the area, was 11 peaks (Figure 2C), while at 109 DAP was eight peaks (Figure 2D).

Adjustment models of MCS and MBSP by cultivar

Four models explained the spatial spread of MCS at 95 and 109 DAP. At 95 DAP were represented by the spherical for Hybrid 1, SH 7990©, ADV 9434© and NS 70©; the Exponential model for Hybrid 2, CD 3770© and NS 92 PRO; the Linear model for the hybrid SH

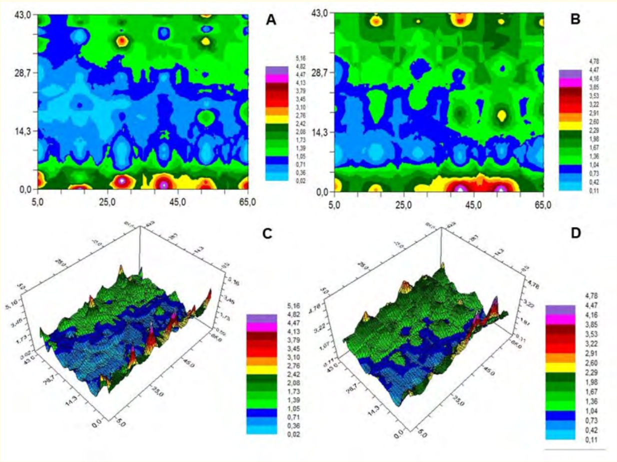

Figure 2: Spatial spread of Maize Bushy Stunt Phytoplasma in maize (Zea mays) at 95 and 109 days after planting (DAP). A. 2D spread at 95 DAP, B. 2D spread at 109 DAP, C. Density spread at 95 DAP, D. Density spread at 109 DAP.

7920©, CD 3612© and AG 8070 PRO 3©; and the Gaussian model for the hybrid DKB 177®, NS 90® and DKB 290® (Table 2). At 109 DAP were represented by spherical SH7920®, DKB 177®, DKB 290®, CD 3770®, ADV 9434® and NS 70®; and the Exponential model for Hybrid 2; the Linear model for SH 7990©, CD 3612©, NS 90© hybrid and AG 8070 PRO 3©; and the Gaussian model for Hybrid 1 and NS 92 PRO (Table 2).

The spatial spread at 95 DAP was better explained for hybrids 1 and DKB 177© and 109 DAP for the hybrid NS 92 PRO©, because they presented more points within the model ("line"). The other hybrids presented one or several discrepant values devaluated by this visual and graphical analysis strategy the best model adjusted and indicated to explain the spatial spread of the mating.

The residual sum of squares (RSS) devalues some better Adjustment models for the MCS at 95 DAP. The three best and worst adjusted hybrids (the incidence of the disease was best explained by the proposed model for the spatial spread), respectively, were

Commercial hybrids	Maize Corn Stunt (Spiroplasma kunkelii)				Maize Bushy Stunt Phytoplasma			
	95 DAP		109 DAP		95 DAP		109 DAP	
	Adjustment model	SRS	Adjustment model	RSS	Adjustment model	RSS	Adjustment model	RSS
ADV 9434©	Spherical	11,9000	Spherical	23,2000	Spherical	0,0014	Linear	0,0174
AG 8070 PRO 3©	Linear	0,2690	Linear	0,0009	Linear	3,7700	Linear	185,0000
CD 3612©	Linear	0,0096	Linear	0,0633	Gaussian	0,0007	Gaussian	0,0007
CD 3770©	Exponential	1,2900	Spherical	1,0300	Linear	0,2100	Exponential	2,4700
DKB 177©	Gaussian	0,1940	Gaussian	0,0007	Spherical	0,0084	Exponential	0,1090
DKB 290©	Gaussian	11,0000	Exponential	0,0219	Gaussian	0,0007	Linear	0,0009
Híbrido 1	Spherical	0,0000	Spherical	0,5080	Gaussian	29,6000	Spherical	87,1000
Híbrido 2	Exponential	0,0166	Spherical	48,0000	Spherical	17,1000	Gaussian	12,7000
NS 70©	Spherical	0,3650	Spherical	4,9700	Gaussian	0,0098	Spherical	0,2840
NS 90©	Gaussian	0,0767	Linear	0,0158	Spherical	2,5300	Spherical	4,1000
NS 92 PRO	Exponential	1,7900	Gaussian	3,2000	Spherical	5,8200	Linear	0,0136
SH 7920©	Linear	0,0336	Spherical	0,0539	Spherical	5,8200	Spherical	2,5100
SH 7990©	Spherical	6,7800	Linear	1,3700	Linear	7,1200	Spherical	4,3500

Table 2: Adjustment model and the residual sum of squares (RSS) for the MCS and MBSP of commercial maize hybrids at 95 days after planting (DAP) and 109 DAP*.

* Parameters marked in bold represent the best models of adjusted semivariograms; parameters marked in underscore represent the worst models of semivariograms adjusted.

hybrid 1 (Spherical), hybrid 2 (Exponential) and CD 3612© (Linear); and SH 7990 (spherical), DKB 290 (Gaussian) and ADV 9434 (Spherical) (Table 2). For the MCS at 109 DAP, the three best and worst adjusted hybrids (the incidence of the disease was best explained by the proposed model for the spatial spread), respectively, were hybrids 1 (Gaussian), AG 8070 PRO 3© (Linear) and NS 90® (Linear); DKB 290® (Spherical), ADV 9434® (Spherical) and NS 70® (Spherical) (Table 2). The most frequent model in corn hybrids evaluated at 95 and 109 DAP was the spherical model, independent of being more adjusted by the parameters (Table 2).

Three models explained the spatial spread of MBSP at 95 DAP being represented by the Spherical for Hybrid 2, SH 7920©, DKB 177©, ADV 9434©, NS 90© and NS 92 PRO; the Linear model for the hybrid SH 7990©, CD 3770© and AG 8070 PRO 3©; and the Gaussian model for Hybrid 1, DKB 290©, CD 3612© and NS 70© (Table 2). Four models explained the spatial spread at 19 DAP of MBSP, being represented by the Spherical for Hybrid 1, SH 7920©, SH 7990©, NS 70© and NS 90©; the Exponential model for DKB 177© and CD 3770©; the Linear model for hybrid DKB 290©, ADV 9434©, NS 92 PRO© and AG 8070 PRO 3©; and the Gaussian model for Hybrid 2, CD3612 (Table 2).

The spatial spread of MBSP at 95 DAP was better explained for the DKB 177© and NS 70© hybrids, and at 109 DAP, for hybrids 2 and DKB 177© (Table 2), because they presented higher number of points within the model ("line"). The other hybrids presented one or several discrepant values devaluated, by this visual and graphical analysis strategy, the best model adjusted and indicated to explain the spatial spread of the maize stunt.

The residual sum of squares (RSS) devalued some better Adjustment models for MBSP at 95 and 109 DAP (Table 2). The three best and worst hybrids adjusted to 95 DAP respectively were hybrids DKB 290 (Gaussian), ADV 9434 (Spherical) and CD 3612 (Gaussian); Hybrid 1 (Gaussian), Hybrid 2 (Spherical) and SH 7990 (Linear) (Table 2).

For the MBSP at 109 DAP the three best and worst adjusted hybrids, respectively, were hybrids CD 3612 (Gaussian), DKB 290 (Linear) and NS 92 PRO (Linear); AG 8070 PRO 3© (Linear), Hybrid 1 (Spherical) and Hybrid 2 (Gaussian) (Table 2).

The most frequent model in corn hybrids evaluated at 95 and 109 DAP was the spherical model, independent of being adjusted for the presence of discrepant value and RSS (Table 2).

Discussion

Symptomatology of maize stunts

Oliveira., *et al.* [6] when studying corn viruses and stunts showed that diseases caused by Mollicutes had more important diseases than corn virus in the PR. Cota., *et al.* [3] pointed out that the late planting conditions increase the amount of inoculum in the field, allowing the survival of the population of *D. maidis* in this period, since it coincides with the longer period of infestation of the leafhopper.

It is recognized that the prokaryotes that were transmitted by the viruliferous leafhoppers (capable of transmitting the phytopathogen) [18] fed the hybrids evaluated, and that from the sucking activity of the leafhopper, allowed the long-distance transmission of these bacteria with the vascular system (xylem and phloem) allowing systemic spreading, with the young leaves being the source of inoculum arrival. The leafhopper has a migratory habit and disseminates the maize crops from diseased maize to young seedlings from newly established crops [10]. There is a preference and migration of leafhoppers from old plants to younger seedlings transmitting pests [16]. The prokaryote ("Maize Bushy Stunt Phytoplasma") as a result of its colonization provokes plastic symptom of anthocyanin production causing purpura of the leaf blade. This MBSP can provoke symptoms in the limb or the veins, in the low leaves and apical leaves, not establishing patterns of appearance of symptoms connected to the age of the organ or plant, as widely cited for white-spot-of-the-corn, diplodia-leaf-spot, helminthosporium-leaf-spot-and-blight by Pereira [16].

MCS spread

The incidence peaks of the disease would be the sources of viruliferous leafhoppers (containing the prokaryotes) arrivals where they produced inoculum sources influenced by varietal susceptibility. The resistance to stunts is determined by the number of infected plants (incidence) and not by the level of resistance of infected plants (severity) [19].

Due to the higher concentrations or peaks of incidence following the hybrid's growth range, the genetic component of response to the disease explained the standardized concentrations, that is, the spreading and dissemination were more dependent on the susceptibility of the cultivar than the infectivity of the insect vector, since the hybrids located on the edges of the experimental area presented the smallest spatial incidences of the disease (except for a few isolated spots) (Figure 1AB). The spread of the disease was linked to the vector from "within" the evaluated area.

Regarding the genetics of mating resistance, Silva., *et al.* [19], in Brazil, showed that they were resistant to genetic diseases as C 333B (single, normal, semi-solid, and semi-hybrid), and P3041 (triple, early cycle, planting late).

MBSP spread

In order to differentiate the two evaluation days (95 and 109 DAP), an increase in the spatial incidence was observed at the largest coordinates of the Y axis towards the smaller coordinates of the X axis (Figure 2CD), where the incidence peaks would be the sources of viruliferous leafhoppers, capable of producing sources of inoculum for dissemination.

Due to the higher concentrations or peaks of incidence being located on the edges of the experimental area, they coincide with the probable arrival and attack sites of the corn leafhopper, not being represented by a genetic component, as was recognized for red nesting (Figure 2).

The genetic component of response to the incidence of the disease did not explain the peaks of red stunting in the marginal regions, that is, the spreading and dissemination depended on the arrival of the vector insects coming from "outside" the plant population.

Nault [5] points out instability in genetic reactions in which the symptomatic variations are dependent on the genetic component (genotype) and climatic conditions (environment) on the hybrids. However, Bustamante [20] selected 2980-93 and 3974 accessions, as presenting slow and late symptoms of "Maize rayado fino virus" - phytopathogen also transmitted by the leafhopper.

Adjustment models of MCS and MBSP by cultivar

The adjustment models presented differential behavior by hybrids, that is, the mathematical modelling to explain the spatial spread of MCS and MBSP at 95 and 109 DAP was different and influenced by discrepant values.

At 95 DAP it became evident that there is no standard model to explain the spatial spread of MCS. At 109 DAP there was a linear model trend for the hybrids best fitted to explain the spatial distribution of MCS.

For MCS there was a trend of the Gaussian model for the hybrids best fitted to the 95 DAP. At 109 DAP there was a trend to the linear model for the best fit hybrids to explain the spatial spread of MBSP.

Even with the best adjustments for each hybrid, a more frequent model was found in all the cultivars for MCS and MBSP, showing that there is no specific model capable of explaining the distribution of the disease in all the scenarios.

Conclusion

The peaks of incidence of the MCS and MBSP presented a system of differentiated dissemination in the culture. It is assumed that the sources of inoculum of the MCS were present within the plot, and were influenced by the genetic component of the hybrid. For the MBSP, the source of inoculum is supposed to have come from outside, influenced by the population of the leafhopper vector in the plot of evaluated hybrids.

Regardless of the best adjustment parameters, the most frequent model that explains the spatial spread of the MCS and MBSP on the evaluated days was the spherical model.

Summary

In recent years, there have been advances in the outbreaks of pale and red stunting in maize (*Zea mays*) crops, probably due to the increase in the spittle population. The objective was to perform a modeling and to evaluate the spatial distribution of the pale and red enfezamiento in different commercial hybrids of corn cultivated in the crop of 2017. The experiment was carried out with 13 hybrids, in the municipality of Ipameri, GO. The experimental area per hybrid showed the dimensions of 4x70 m and 10 m in length, resulting in a population of plants organized in a spacing of 0.5 m and distance between plants of 0.2 m sown in eight growing lines. The lines were referenced in the X and Y axis, with the pale and red enfezamiento incidence, referenced by Z in a useful area per hybrid of 210 m^2 for modeling and construction of spatial distribution maps. There was a significant difference between the geographic distance and the incidence of both types of moths. The adjustment models recognized for the pale and red haunches by cultivar were the Spherical, Exponential, Linear and Gaussian, being the most frequent model to explain the spatial distribution of both enfezamientos was the Spherical.

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