

# Spatio-temporal distribution of *Anthonomus grandis grandis* Boh. in tropical cotton fields

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## Abstract

**BACKGROUND:** Knowledge of the spatio-temporal distribution of pests is important for the development of accurate management approaches. The boll weevil, *Anthonomus grandis grandis* Boh., is a deleterious cotton, *Gossypium hirsutum* L., pest in the western hemisphere. The spread of boll weevils across cotton fields remains poorly understood. We assessed the dispersal pattern of adult weevils through cotton fields cultivated in a tropical area during dry and wet seasons using geostatistics for the number of adults and infested reproductive structures (buds, bolls and total).

**RESULTS:** Adult weevils and infested reproductive structures increased across both seasons despite the prevailing climatic variables. In both seasons, boll weevil adults and infested reproductive structures followed an aggregated distribution. The distances over which samples maintained spatial dependence varied from 0.7 to 43.4 m in the dry season and from 6.0 to 614.4 m in the wet season. Boll weevil infestations started at field borders and the infested reproductive structures (oviposition and/or feeding punctured) were greater than the adults regardless of cotton growth stage.

**CONCLUSION:** Sampling for boll weevils in cotton fields should start at the field borders and focus on total infested reproductive structures (buds + bolls) and as cotton plants develop, sampling should focus on the field as a whole. Distances among samples will vary from 6 to 470 m. Thus, despite the cotton phenological stage or growing season, monitoring of boll weevil should be done by sampling total infested reproductive structures with a minimum distance of 6 m among samples.

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Supporting information may be found in the online version of this article.

**Keywords:** geostatistics; *Gossypium hirsutum*; economic entomology; sample units

## 1 INTRODUCTION

The boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), is considered to be the most harmful cotton, *Gossypium hirsutum* L., pest throughout subtropical, and tropical regions of the western hemisphere.<sup>1,2</sup> Boll weevil damages cotton by feeding upon and laying eggs inside its reproductive structures, where hatched larvae feed and pupate,<sup>3,4</sup> causing abscission or reduction of fiber quality,<sup>3,5,6</sup> and economic losses of up to US\$74 million per year.<sup>7</sup> Boll weevil chemical control interventions are based on economic thresholds obtained by sampling the plants and for adult boll weevils captured in pheromone-baited traps.<sup>3,8,9</sup> In order to develop an accurate monitoring and management program, estimates of population density are essential.<sup>10,11</sup>

Biotic and abiotic factors affect dynamics and within-field distribution (aggregated, random or uniform patterns) of insect populations.<sup>10,12</sup> Knowing a pest's distribution within a field can help to: (1) develop site-specific sampling and control efforts; (2) predict pest movement; (3) improve insecticide-resistance management; (4) conserve biological control agents by precision targeting sprays for the infested areas; and (5) reduce the economic, social and environmental costs associated with pest control.<sup>10,11,13</sup> The spatial distribution of boll weevils has been investigated using mean-variance

relationships<sup>4,14</sup> without considering within-field spatial density distribution, or has been based on pheromone-baited trap captures.<sup>15</sup> The most accurate approach is the use of geostatistics because the position of the samples in space is accounted for.<sup>16</sup> Recent work reported that geostatistics is of particular interest for pest management because it allows inferences about the minimum inter-sample

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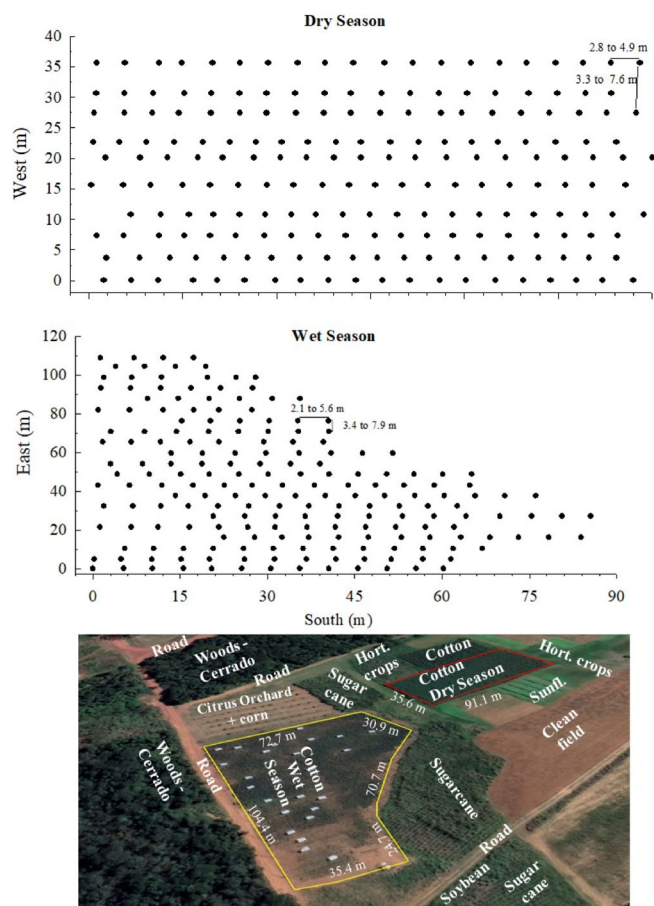
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**FIGURE 1.** Sampling points for boll weevils in two cotton fields (dry and wet season) and an aerial photograph of the areas and their surroundings. Hort. Crops, horticultural crops, that is, broccoli, cabbage, carrot, cassava, kale, garlic, tomato and sweet potato; Sunfl., sunflower.

distance needed to obtain independent estimations and indicates patterns of distribution and colonization of an organism, all of which are crucial for the development of effective sampling programs.<sup>11,13</sup> The purpose of this study was to investigate the spatial dynamics of *A. grandis grandis* on cotton by determining within-field distribution of adults and infested reproductive structures (having feeding and/or oviposition punctures).

## 2 MATERIAL AND METHODS

### 2.1 Trials and crop management

The trials were conducted at the experimental station of the University of Brasília (UnB) called Fazenda Água Limpa, Vargem Bonita Co., Brasília, Brazil (15°56'57.48"S, 47°56'06.4"W). The climate is tropical with an average annual precipitation of 1400 mm. Trials were run in two seasons: dry, with sowing on 28 August 2020 (15°57'01.33"S, 47°56'03.54"W); and wet, with sowing on 9 November 2020 (15°57'05.09"S, 47°56'06.33"W) (Figure 1). The cultivated areas surveyed during the dry and wet seasons were 91.1 × 35.6 m (44 rows, maximum length 91.1 m, minimum length 84.3 m) and 107.4 × 86.1 m (141 rows, maximum length 86.1 m, minimum length 22 m), respectively. Plants were located within 10 rows during the dry season and 21 rows during the wet season. The total area were approximately 3243.2 and 6368 m<sup>2</sup> for the dry and wet seasons, respectively. Row spacing was 0.7 m and the linear density of plants was six per meter. Initial soil fertilization, at sowing, used 4–30–16 N–P–K and approximately 40 days after planting (DAP) plants were fertilized with N using urea (Table 1). During the dry season, plants were also sprayed with an amino acid formulation (Table 1) at 42 DAP.

At 15 days before sowing, mechanical soil cultivation involved two plowings with a heavy 28-disc model harrow plow NVCR (Baldan) followed by leveling the soil with a 12-disc harrow plow (GAM Tatu Marchesan) both coupled to a TL 85E (New Holland) tractor. Sowing was accomplished using a 3060 PD (Jumil) planter

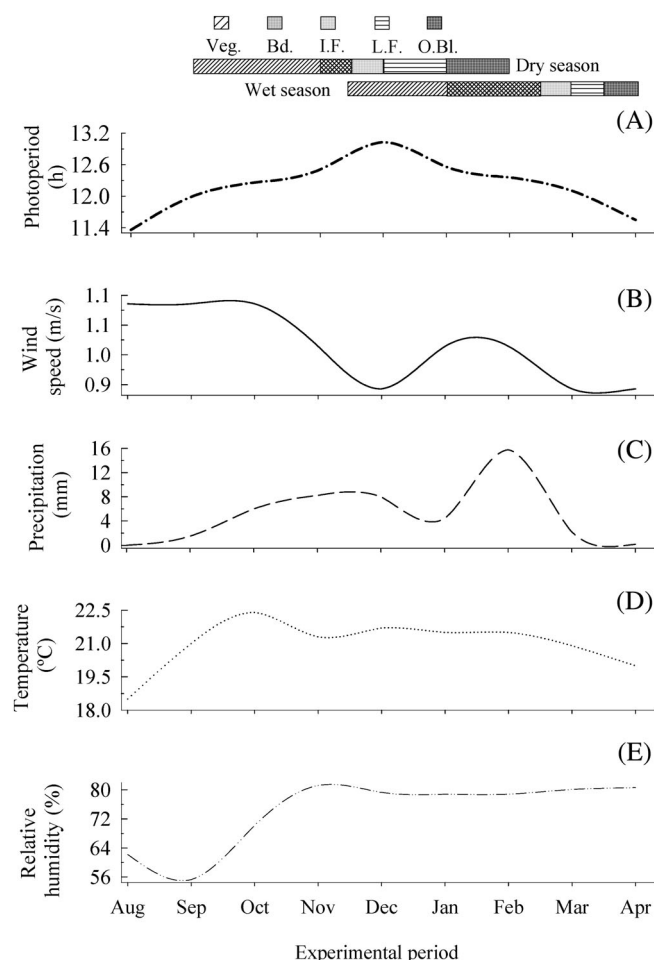
**TABLE 1.** Fertilizers, pesticides and additional products used in the cotton fields during the trials

Treatment type	Commercial name	a.i.	Concentration (g L <sup>-1</sup> )	Dose C.P.	Manufacturer	Place
Foliar spray	ROUNDUP ORIGINAL MAIS®	Glyphosate	577	5 L ha <sup>-1</sup>	Monsanto	São Paulo, SP
		Acid equivalent	480			
	Volcane®	MSMA	790	3 L ha <sup>-1</sup>	Corteva	Paulínia, SP
	Assist®	Mineral oil	756	0.1 L ha <sup>-1</sup>	Basf	São Paulo, SP
	Naturamin® WSP	Free aminoácidos	80% w/w	0.5 kg ha <sup>-1</sup>	Daymsa	Zaragoza, Spain
		Total nitrogen	12.8% w/w			
		Organic nitrogen	12.8% w/w			
Seed treatment	Sponsor®	Mepiquat chloride	250	0.2 L	FMC	Campinas, SP
	Terra Forte®	Fipronil	250	0.3 L 100 kg sementes <sup>-1</sup>	Ouro Fino	Uberaba, MG
		Derosal Plus®	Carbendazim	150	0.6 L 100 kg de sementes <sup>-1</sup>	Bayer
	Thiram	350				
Fertilization at sowing	4–30–16 (N–P–K)	Total nitrogen	4%	217 kg ha <sup>-1</sup>	Rifertil	Rio Verde, GO
		P <sub>2</sub> O <sub>5</sub>	30%			
		K <sub>2</sub> O	16%			
		SO <sub>4</sub>	2.1%			
Side-dressing fertilization	Uréia Nitro Fértil®	Ca	4%	120 kg ha <sup>-1</sup>	Rifertil	Rio Verde, GO
		Total nitrogen	45%			

Abbreviations: C.P. - commercial product; w/w - weight/weight.

and fertilization (Table 1), containing seven lines and coupled to a New Holland TL 85E tractor. Herbicides (Table 1) were sprayed using a 20-L backpack sprayer (model P1B, Jacto) equipped with a fan-type beak and drift guard, calibrated for an application rate of 200 L ha<sup>-1</sup>. This spray technology was chosen because herbicides in commercial cotton areas are sprayed through terrestrial applications. Before sowing, the experimental areas were treated with glyphosate + MSMA (Table 1) for weed control using an M12775 (Jacto) sprayer coupled to the TL 85E tractor. After cotton emergence, weeds were controlled over the whole area with glyphosate (Table 1) using a 20-L backpack sprayer model P1B.

Cotton cultivars used in the dry and wet seasons were BRS 371 and BRS 368 (Embrapa), respectively, both susceptible to boll weevils and glyphosate tolerant. Cotton seeds were chemically delinted and treated with fipronil and carbendazim (Table 1) because the areas had a history of infestation by *Atta* spp. (Hymenoptera: Formicidae) and the fungal plant pathogen *Ramularia areola* (Mycosphaerellaceae), and damping off (caused by *Colletotrichum*, *Fusarium*, *Pythium* and *Rhizoctonia*). A plant growth regulator was used during the wet season, because in this season plants reached the vegetative growth stage when its use is recommended (Table 1).



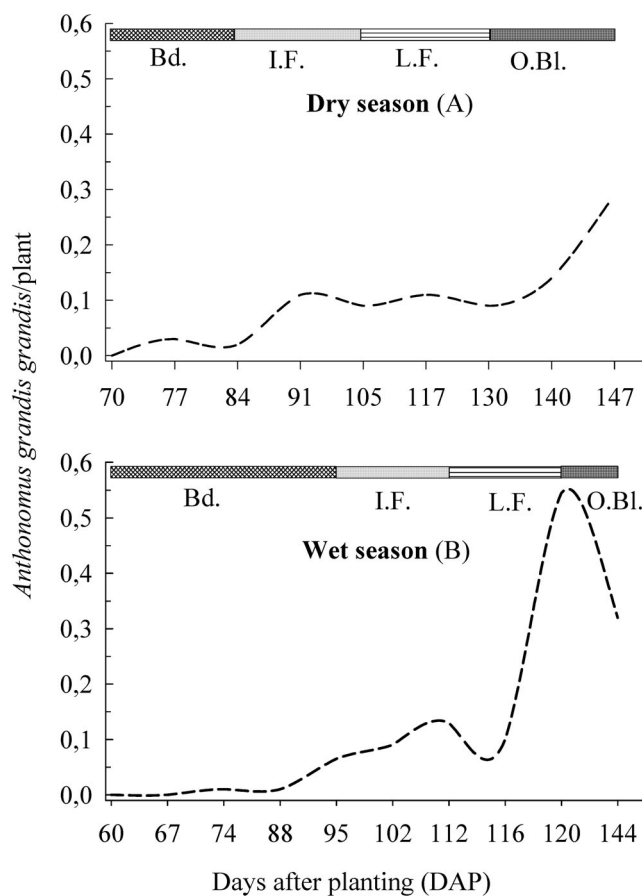
**FIGURE 2.** Climatic variables registered during two cotton-growing seasons: dry and wet seasons. (A) Photoperiod, (B) wind speed, (C) precipitation, (D) temperature and (E) relative humidity. Veg., vegetative stage; Bd., buds; I.F., initial flowering; L.F., late flowering; O.Bl., open bolls.

## 2.2 Sampling

In each field for each season, 200 sampled plants, with each plant representing an experimental replicate, were georeferenced, tagged and numbered. Plants were distributed across the areas keeping the following distances: (1) dry season—distances among subjacent plants in the horizontal plane were 2.8–4.9 m and in the vertical plane were 3.3–7.6 m; (2) wet season—distances among subjacent plants in the horizontal plane were 2.1–5.6 m and in the vertical plane were 3.4–7.9 m (Figure 1). Plants were sampled weekly throughout both seasons. Sampling was initiated when plants began producing flower buds at 70 and 59 DAP during the dry and wet seasons, respectively, and was terminated when bolls opened (147 and 144 DAP, dry and wet seasons, respectively). Whole-plant inspections were conducted and the numbers of boll weevil adults, punctured (feeding and oviposition) and non-damaged reproductive structures (buds, flowers and bolls) were recorded. Data on photoperiod, wind speed and direction, precipitation, temperature and relative humidity (RH) were collected using a thermohygrometer model HMP155 (Vaisala), anemometer model 051103 (R.M. Young), pluviometer model TB4 (HyQuest) and pyranometer model SP Lite 2 (Kipp and Zonnen), respectively. Climatic data was used to draw figures representing mean values for the month.

## 2.3 Data analyses

Percentages of infested buds, bolls and total reproductive structures (buds + bolls + flowers) were calculated by dividing the



**FIGURE 3.** Seasonal variation in the mean density of boll weevils per cotton plant during (A) dry and (B) wet seasons. Bd., buds; I.F., initial flowering; L.F., late flowering; O.Bl., open bolls.

number of damaged buds, bolls or total reproductive structures by the total number of reproductive structures (damaged + non-damaged).

Data on the density of adult boll weevil and the percentages of infested buds, bolls and total reproductive structures per plant were used to calculate the mean and the standard errors (SEM), and were used together with the climatic variables to show fluctuations throughout the experimentation period.

Geostatistical interpolation was performed using the ordinary kriging technique, which considers the distance and the degree of variation between points and uses spatial dependence between samples to estimate unknown values of unsampled points.<sup>17,18</sup> To estimate the unsampled values within the evaluated cotton fields, semivariograms were modeled for all sampling dates. For this purpose, the semivariance was first calculated using the equation:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [Z(x_i) - Z(x_i + h)]^2$$

where  $\gamma(h)$  is the semivariance,  $Z(x_i)$  is the value that variable  $x$  assumes at position  $i$ ,  $Z(x_i + h)$  is the value that variable  $x$  assumes at position  $i + h$ , and  $N(h)$  is the number of measured pairs in the lag distance  $h$ .<sup>19</sup> Data from all the sampling dates were then adjusted to the linear, Gaussian, spherical and exponential

semivariogram models and tested for the existence of spatial autocorrelation.<sup>20–22</sup> Spherical models are described by the formula<sup>20–22</sup>:

$$\gamma(h) = \begin{cases} 0, & h = 0 \\ C_0 + C \left( \frac{3h}{2a} - \frac{1h^3}{2a^3} \right), & 0 < h \leq a \\ C_0 + C, & h > a \end{cases}$$

Where  $C_0$  is the nugget constant,  $C_0 + C$  is the sill,  $C$  is the structure variance and  $a$  is the effective range. The model shape follows a linear growth close to the origin (short distances between two points) and it plateaus above the range value.<sup>20–22</sup>

Omnidirectional models, having the highest regression coefficients and lowest residual sum of squares together with the linear regression models parameters, were used for cross-validation of the selected models, that is, those having: (1) determination coefficients ( $R^2$ ) close to one; (2) intercepts ( $\beta_0$ ) close to zero; and (3) slopes ( $\beta_1$ ) close to one.<sup>16,21,23,24</sup>

After adjusting the models, the parameters nugget effect ( $C_0$ ), sill ( $C_0 + C$ ), and range ( $R$ , in m) were obtained and used to calculate the spatial dependence ( $SD$ ) as the nugget ( $C_0$ ) to sill ( $C_0 + C$ ) ratios:

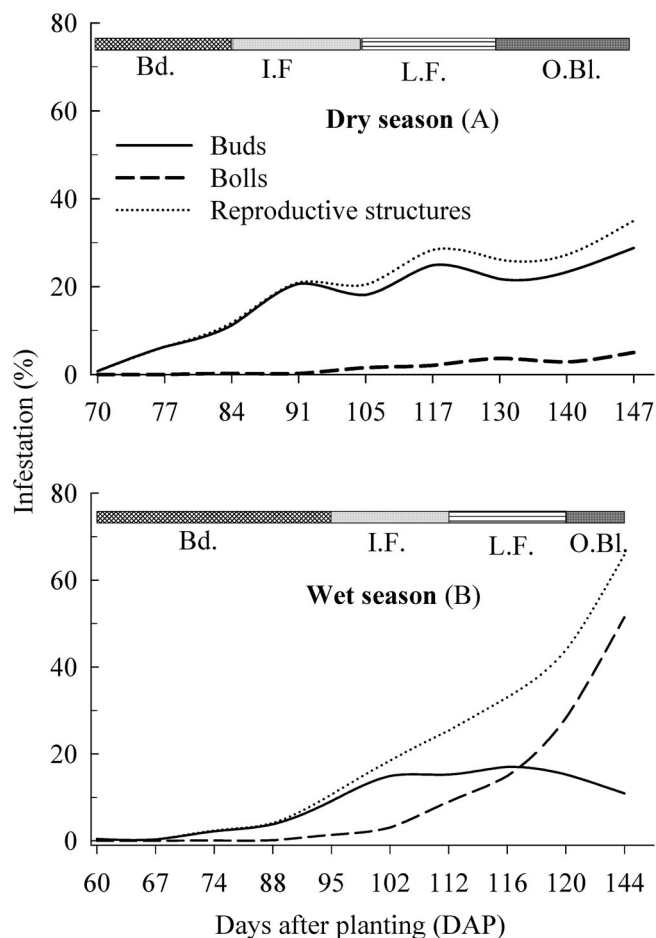
$$SD = \frac{C_0}{(C_0 + C)} \times 100$$

$SD$  varies between strong ( $SD \leq 25\%$ ), moderate ( $25\% < SD \leq 75\%$ ) and weak ( $SD > 75\%$ ) aggregations as suggested by Cambardella *et al.*<sup>25</sup> Whereas  $SD$  is observed (or samples are autocorrelated) over distances closer than the estimated range ( $R$ ), the sill ( $C_0 + C$ ) indicates where the semivariogram models plateau and the nugget effect ( $C_0$ ) corresponds to the value where the semivariogram models intercepts the y-axis.<sup>16,26</sup> Linear variogram models indicate random distribution, whereas exponential, spherical or Gaussian models indicate aggregation or spatial dependence.<sup>11,13</sup> Therefore, following selection of the curvilinear models (exponential, spherical or Gaussian),  $SD$  was ensured, and digitalized maps containing interpolated data were built using ordinary kriging interpolation. Maps allowed to keep track of boll weevil infestation across seasons.<sup>20,23,24</sup> The program Gs+ version 7 was used for all of the spatial analyses as recommended by Vieira *et al.*<sup>20,27</sup>

### 3 RESULTS

Photoperiod varied from 11.4 to 13.0 h of light (Figure 2A), wind speed from 0.9 to 1.1  $\text{m s}^{-1}$  (Figure 2B), precipitation from 0 to 15.8 mm (Figure 2C) and temperature from 18.5 to 22.4°C (Figure 2D), whereas RH varied from 55.3% to 81.2% (Figure 2E). Density of adult boll weevils increased as the cotton plants developed, particularly in response to the production of reproductive structures (Figure 3). At the end of the dry season, boll weevil densities continued to increase (Figure 3A) in contrast to a decline observed at the end of the wet season (Figure 3B). Mean ( $\pm$  SEM) numbers of boll weevils per plant were similar across seasons:  $0.1 \pm 0.4$  and  $0.1 \pm 0.5$  adults for the dry and wet season, respectively. In each season, the greatest densities occurred when the bolls were opening (Figure 3).

Percentage of infested cotton reproductive structures generally increased across both seasons, exhibiting relatively small variations (Figure 4). Infestations of buds contributed the most to the proportion of total infested reproductive structures until the beginning of the initial flowering stage in both seasons when



**FIGURE 4.** Seasonal variation in the mean infestation percentage of buds, bolls and total (buds + bolls) reproductive structures by boll weevils per cotton plant during (A) dry and (B) wet seasons. Bd., buds; I.F., initial flowering; L.F., late flowering; O.Bl., open bolls.



**Table 2.** Parameters and theoretical models adjusted to the spatial distribution pattern of *Anthonomus grandis grandis* during dry season

Phenological stage	Variables	Characteristics of the spatial distribution models							Cross-validation		
	Model	$C_0$	$C_0 + C$	$R$	$SS$	$R^2$	$SD$	$\beta_0$	$\beta_1$	$R^2$	
Buds	BW	–	–	–	–	–	–	–	–	–	
	I.Bd.	Gaussian	0.01	21.31	6.51	168	0.718	0.0005	0.82	–0.07	0.003
	I.BI.	–	–	–	–	–	–	–	–	–	
	I.R.S.	Gaussian	0.01	21.31	6.51	168	0.718	0.0005	0.82	–0.07	0.003
Initial flowering	BW	Exponential	0.05	0.18	43.44	$8.61 \times 10^{-3}$	0.650	0.2560	0.04	0.61	0.092
	I.Bd.	Gaussian	1.00	420.10	7.00	36.657	0.792	0.0024	10.21	0.09	0.004
	I.BI.	Gaussian	0.00	2.95	7.38	3.92	0.665	0.0003	0.23	–0.03	0.001
	I.R.S.	Gaussian	1.00	437.20	6.89	39.566	0.795	0.0023	10.99	0.06	0.002
Late flowering	BW	Exponential	0.03	0.15	7.50	$2.65 \times 10^{-3}$	0.345	0.2310	0.01	0.81	0.088
	I.Bd.	Exponential	59.60	144.50	16.92	718	0.820	0.4125	4.71	0.81	0.182
	I.BI.	Gaussian	0.71	14.58	4.87	15.50	0.726	0.0487	2.18	–0.11	0.002
	I.R.S.	Exponential	89.00	580.50	12.12	19.230	0.791	0.1533	7.10	0.67	0.110
Open bolls	BW	Gaussian	0.02	0.40	5.42	0.02	0.725	0.0532	0.23	0.23	0.011
	I.Bd.	Gaussian	42.60	333.30	0.69	15.682	0.000	0.1278	7.14	0.77	0.097
	I.BI.	Gaussian	0.10	86.20	6.10	2.432	0.674	0.0012	2.96	0.16	0.009
	I.R.S.	Gaussian	53.00	455.00	5.92	5.548	0.915	0.1165	11.79	0.54	0.080

Abbreviations: BW, boll weevil mean density;  $C_0$ , nugget effect;  $C_0 + C$ , sill; I.Bd., infested buds (%); I.BI., infested bolls (%); I.R.S., total infested reproductive structures (%);  $R$ , range;  $R^2$ , determination coefficient;  $SD$ , spatial dependence;  $SS$ , residual sum of square;  $\beta_0$ , intercept of the curve;  $\beta_1$ , slope of the curve. (–) Occasions with the absence of either the insect or structure, restraining the adjustment of a model.

**TABLE 3.** Parameters and theoretical models adjusted to the spatial distribution pattern of *Anthonomus grandis grandis* during wet season

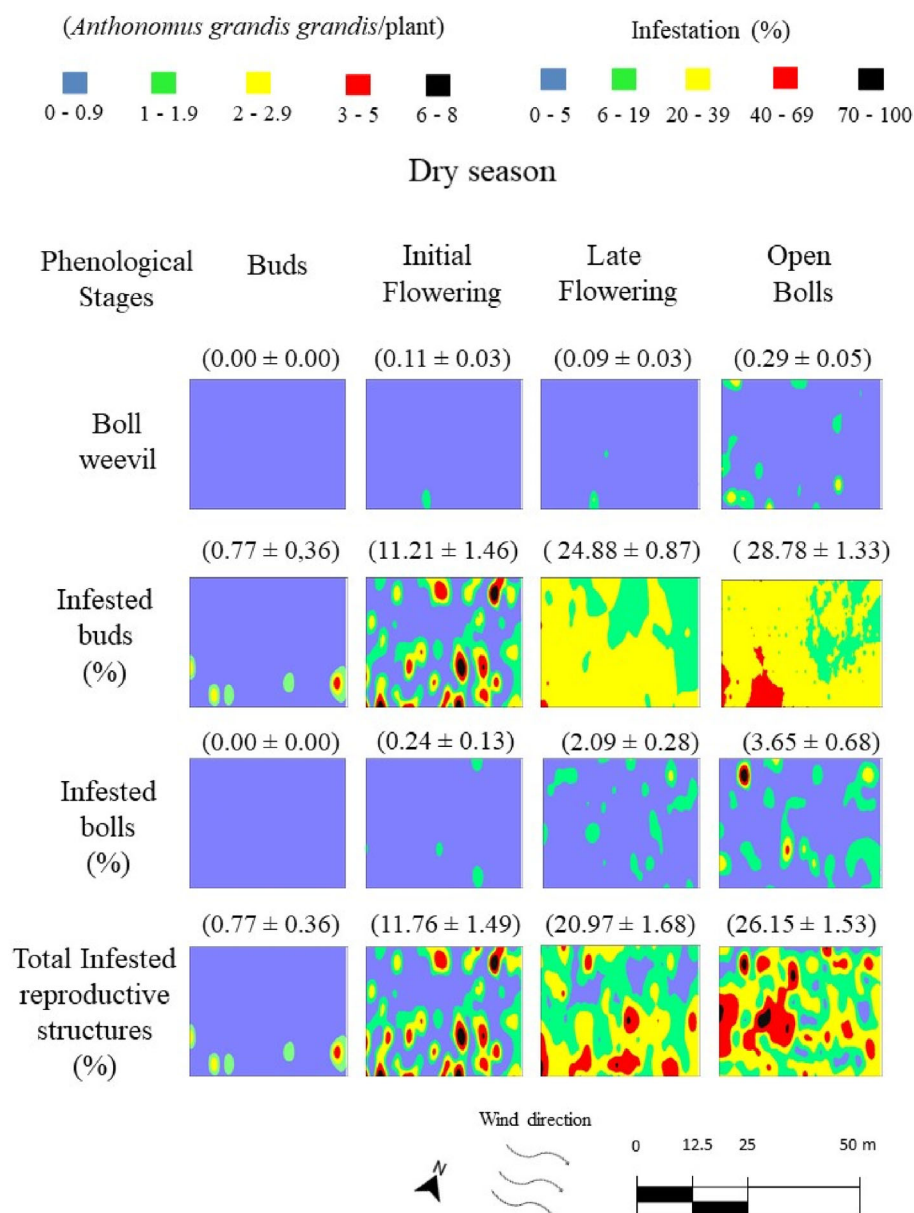
Phenological stage	Variables	Characteristics of the spatial distribution models							Cross-validation		
	Model	$C_0$	$C_0 + C$	$R$	$SS$	$R^2$	$SD$	$\beta_0$	$\beta_1$	$R^2$	
Buds	BW	–	–	–	–	–	–	–	–	–	
	I.Bd.	Exponential	3.59	7.47	259.20	6.31	0.361	0.4805	0.20	0.38	0.013
	I.BI.	–	–	–	–	–	–	–	–	–	
	I.R.S.	Exponential	3.59	7.47	259.20	6.31	0.361	0.4805	0.20	0.38	0.013
Initial flowering	BW	Exponential	0.11	0.42	614.40	$1.39 \times 10^{-3}$	0.813	0.2632	0.12	0.05	0.000
	I.Bd.	Exponential	67.30	224.80	450.00	485	0.846	0.2994	0.68	0.94	0.180
	I.BI.	Exponential	2.66	25.36	12.60	34.90	0.371	0.1049	1.49	0.48	0.054
	I.R.S.	Exponential	83.60	281.30	470.10	749	0.840	0.2972	0.36	0.99	0.196
Late flowering	BW	Exponential	0.09	1.06	9.00	0.05	0.230	0.0870	0.25	0.55	0.039
	I.Bd.	Exponential	8.60	162.40	7.20	2.526	0.027	0.0530	7.32	0.56	0.057
	I.BI.	Spherical	1.80	164.70	8.10	851	0.174	0.0109	8.23	0.44	0.036
	I.R.S.	Exponential	172.40	447.60	434.70	3.663	0.710	0.3852	2.34	0.91	0.176
Open bolls	BW	Exponential	0.52	1.04	44.70	0.131	0.407	0.4995	0.11	0.679	0.066
	I.Bd.	Exponential	7.80	237.10	7.20	2086	0.076	0.0329	8.78	0.424	0.028
	I.BI.	Spherical	1.00	769.80	6.00	29 933	0.000	0.0013	22.24	0.559	0.028
	I.R.S.	Exponential	17.0	497.60	6.00	8406	0.031	0.0342	21.11	0.520	0.028

Abbreviations: BW, boll weevil mean density;  $C_0$ , nugget effect;  $C_0 + C$ , sill; I.Bd., infested buds (%); I.BI., infested bolls (%); I.R.S., total infested reproductive structures (%);  $R$ , range;  $R^2$ , determination coefficient;  $SD$ , spatial dependence;  $SS$ , residual sum of square;  $\beta_0$ , intercept of the curve;  $\beta_1$ , slope of the curve. (–) Occasions with the absence of either the insect or structure, restraining the adjustment of a model.

infestation of total reproductive structures was a result of infestations from both buds and bolls (Figure 4). Dry season infestations of buds, bolls and total reproductive structures were greatest at the boll opening stage and were  $28.8\% \pm 1.3\%$ ,  $5.0\% \pm 0.6\%$  and  $34.9 \pm 1.4\%$ , respectively. During the wet season at the same cotton stage, infestation of bolls and total reproductive structures reached  $51.4\% \pm 1.1\%$  and  $65.8\% \pm 1.9\%$ , respectively, although maximum infestation of buds,  $17.0\% \pm 0.9\%$ , occurred at late flowering stage. The average rate of infested structures to boll

weevil adults (that is, the average cumulative numbers of infested reproductive structures divided by the average cumulative numbers of adults per plant across each season for each plant stage) observed in our study was 30.5, 10.3 and 52.7 during initial flowering, late flowering and open bolls for the dry season, and 31.4, 37.9 and 7.3 during initial flowering, late flowering and open bolls for the wet season.

From the 69 (33 for the dry season and 36 for the wet season) variogram models adjusted to the data obtained from the



**FIGURE 5.** Spatial distribution maps over time of the densities (*Anthonomus grandis grandis*/plant) and infestation (%) of cotton structures by boll weevils across cotton phenological stages during the dry season. Numbers in parentheses above each map represent the mean  $\pm$  SEM of all evaluated plants ( $n = 200$ ) in that stage. Different colors in the maps indicate different ranges for boll weevil density or infestation percentages as described in the legends above the maps.

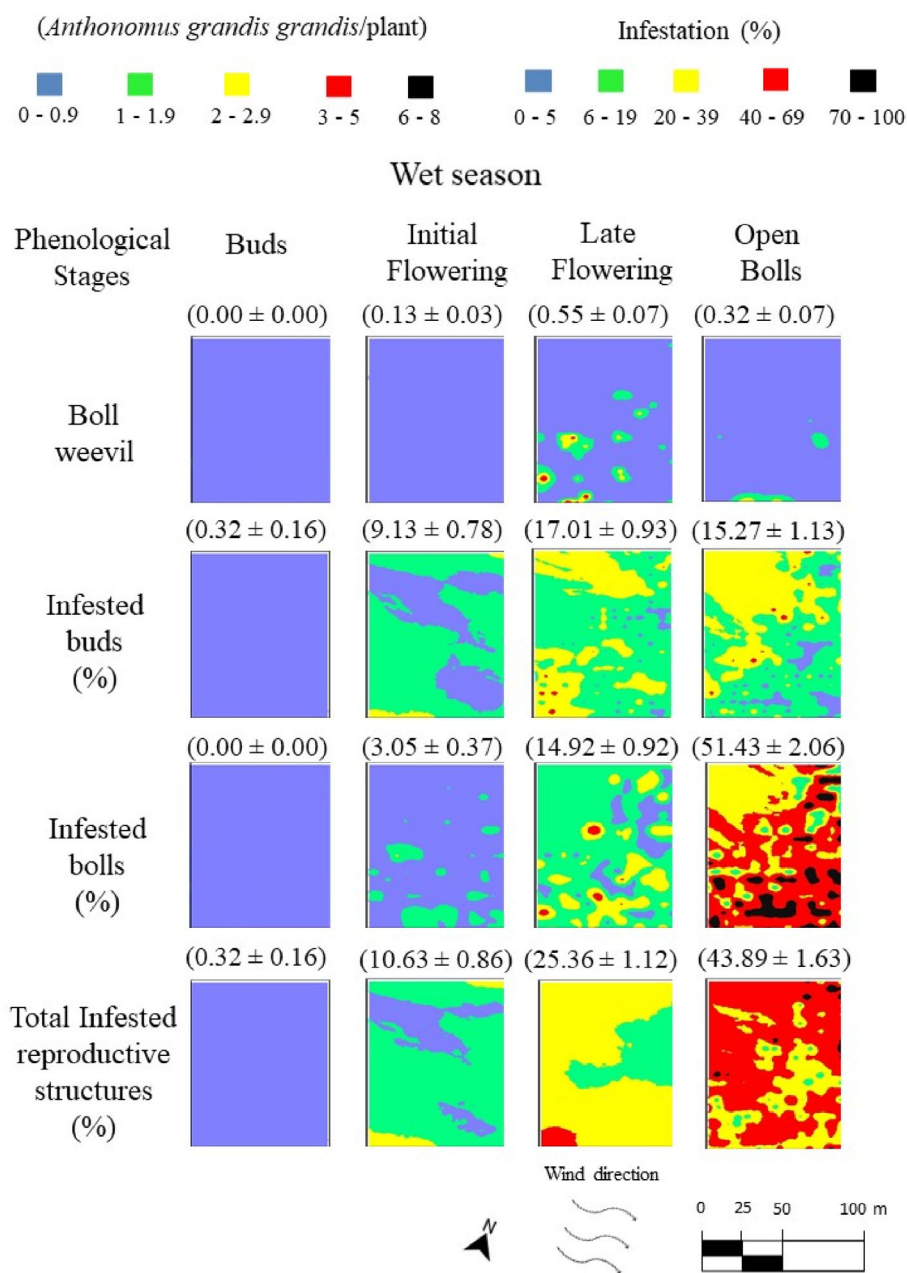
combination of four variables (boll weevil density, infested buds, infested bolls and total infested reproductive structures) and nine (dry season) and 10 (wet season) dates of evaluation, 14 were selected for each season based on  $\beta_0$  values close to zero, and  $\beta_1$  and  $R^2$  values close to one. All models were isotropic exhibiting the same spatial autocorrelation in all the directions. For the dry season and among the 14 selected models, 10 were Gaussian and 4 were exponential (Table 2), whereas for the wet season, 12 models were exponential and 2 were spherical (Table 3). The 41 remaining models, which were not selected, are included in Tables S1 and S2.

Spatial dependence among sampled plants was detected and varied from strong in 19 models ( $SD < 0.25$ ) to moderate in 9 models ( $0.25 < SD < 0.75$ ) (Tables 2 and 3), and confirmed an

aggregated distribution of the boll weevil population in both seasons. Spatial dependence varied from 0.03% to 41.2% in the dry season (Table 2), and from 0.1% to 49.9% in the wet season (Table 3).

Range varied from 0.7 to 43.4 m during the dry season (Table 2) and from 6.0 to 614.4 m during the wet season (Table 3). Maximum range values were 6.5, 43.4, 16.9 and 6.1 m for buds, initial flowering, late flowering and open boll stages, respectively, for cotton cultivated in the dry season (Table 2), and 259.2, 614.4, 434.7 and 44.7 m in the wet season (Table 3).

The spatial distributions of boll weevils and infested reproductive structures were determined using maps generated through ordinary kriging from 19 sampling dates. Each map was generated for the combination of each sampling date (nine or ten)



**FIGURE 6.** Spatial distribution maps over time of the densities (*Anthonomus grandis grandis*/plant) and infestation (%) of cotton structures by boll weevils across cotton phenological stages during the wet season. Numbers in parentheses above each map represent mean ± SEM of all evaluated plants ( $n = 200$ ) in that stage. Different colors in the maps indicate different ranges for boll weevil density or infestation percentages as described in the legends above the maps.

and variable (four), giving 36 maps for the dry season and 40 maps for the wet season. Of these, four maps only were chosen for each variable to show infestation evolution across cotton phenological stages, that is, buds, initial flowering, late flowering and open bolls (Figures 5 and 6). The remaining generated maps are included in Figures S1 and S2.

Adult boll weevils were not detected on cotton plants when they had buds during both seasons (Figures 5 and 6). At the initial flowering stage and later, the insect was observed on cotton plants, always with an aggregated distribution pattern (Figures 5 and 6). Mean boll weevil densities of 1–1.9 adults per plant were observed across all cotton phenological stages during both seasons (Figures 5 and 6), except during the initial flowering stage

of the wet season when adults of boll weevil did not occur (Figure 6). The maximum density ranges of 2–2.9 and 3–5 adults per plant for dry and wet seasons (Figures 5 and 6), respectively, were observed close to harvest, at the late flowering and open boll stage, in some parts of the fields. Mean adult boll weevil densities varied from 0 to  $0.3 \pm 0.05$  per plant (Figure 5) and from 0 to  $0.5 \pm 0.07$  (Figure 6) in the dry and wet seasons, respectively.

Infested reproductive structures, even at the early cotton stages, were as high as 6%–19% for both seasons (Figures 5 and 6), or up to 40%–69% at certain locations in the cotton field during the dry season (Figure 6). Infestations were aggregated, reaching 70%–100% in some locations by the open boll stage, regardless of season (Figures 5 and 6). Based on the colors of the maps and on the

mean values, buds, when available, were preferred over bolls for infestation (Figures 5 and 6). The only exception occurred during open bolls stage of cotton plants growing in the wet season (Figure 6).

## 4 DISCUSSION

In spite of the presence of boll weevils being a serious pest of cotton in the Americas for more than a century,<sup>28</sup> there is a lack of knowledge on the dynamics of infestation across cotton fields. Data on the prevailing climatic variables in the trials are within the ranges that allow proper boll weevil development and population increase.<sup>29,30</sup> Adult densities increased by the end of the dry season and decreased by the end of the wet season. Overlapping generations within fields occur when infested fallen fruiting structures are not removed<sup>31,32</sup> and when intensive (every 2–3 days) insecticide applications are used to suppress the population. Previous work reported that increases in infested reproductive structures are expected until 'cut-out' (end of square production) because, after that, square production declines rapidly and, therefore, boll weevil populations generally plateau.<sup>33,34</sup> This pattern was observed late in the wet season, when the boll weevil population declined. In the dry season, the boll weevil population was still increasing at the end of the season, probably because a plant growth regulator was not used, continuing the production of flower buds.

Because boll weevils prefer to infest buds,<sup>35–38</sup> sampling should start when plants begin producing buds and continue until bolls open. Boll weevil adults are able to detect small quantitative differences in the concentration of plant attractive compounds such as the acyclic homoterpenes (tetranorterpene) (*E*)-4,8-dimethyl-1,3,7-nonatriene and (*E,E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene,<sup>39</sup> and free amino acids<sup>40</sup> and, consequently, differentiate between vegetative and reproductive cotton stages. Monitoring should also focus on infested reproductive structures rather than on free-living adults, which were less numerous, only observed later (initial flowering stage) in the growing season, not representative of crop injury and inaccurate because most of the boll weevil population is inside fruiting structures.

Previous research on adult boll weevil in-field distributions used whole-plant sampling<sup>14</sup> and pheromone-based traps both possessing different dimensions for analyzing dispersion compared with the current approach.<sup>8,15,41</sup> Alternatively, application of kriging and other techniques to characterize in-field cotton pest distributions were used to describe spatial distribution of pests such as the pink bollworm,<sup>42</sup> cotton bollworm,<sup>43,44</sup> pentatomid stink bugs<sup>45–48</sup> and thrips.<sup>49</sup> Our study is the first to describe spatial distribution of the boll weevil with ordinary kriging.

Similar to other studies on curculionid spatial distributions,<sup>11,50</sup> the boll weevil exhibited a moderate to strong aggregated pattern for adults and for infested reproductive structures. This suggests relatively slow in-field dispersal of adults when plants offer plenty of structures for food and as oviposition sites;<sup>51</sup> hence, there is no impetus to move farther away. Aggregation in boll weevil populations can result from the release of aggregation pheromone,<sup>52</sup> and the prevalence of preferred reproductive structures.<sup>36–38</sup> Although in-field infestations were widespread at advanced phenological cotton stages (late flowering and open bolls), aggregation patterns remained because buds declined and increasing percentages of already-infested reproductive structures increased.

In our study, infestations were initiated at the field edges and spread in all directions into the field (Figures 5 and 6). In tropical and subtropical areas boll weevils are able to feed on a variety

of host plants,<sup>3,53–56</sup> but in the absence of cotton (and a few other host plants), viable egg production does not occur.<sup>57–59</sup> Colonization of a field then occurs from surrounding cotton fields or other host plants, used mostly for food, located nearby.<sup>3,54</sup> Field borders are, therefore, important places to commence sampling when plants begin producing buds (Figures 5 and 6).

Range values are the minimum distances over which the dependence among samples is maintained.<sup>13,60</sup> For sampling purposes, to determine infestation levels for triggering insecticide treatments, the minimum distance adopted to monitor the boll weevil population should be higher than the average range value of the variograms.<sup>11,13</sup> The range values in our study varied according to the cotton's phenological stage; the minimum estimated distance between samples should be more than 0.7 m and the maximum distance should be approximately 600 m. This recommendation should be incorporated in the future development of sampling plans for the pest and contribute to the reduction in the sampling effort while monitoring the pest.

Such variations in range (maximum distance that samples show spatial dependence) are common.<sup>13,24</sup> When numbers of infested reproductive structures were low, plants had more variable infestation levels between them, and shorter distances were recommended to better characterize variation. Conversely, when infestation spread and levels were high, most places in the field had similar infestation levels; hence, larger sampling distances (ranges) could be adopted without compromising sampling precision, because in those cases moderate spatial dependence was also noticed.

We recommend that at the bud stage, when infestations begin, sampling for boll weevils in cotton fields should start at the field borders, focusing on infested reproductive structures using a distance of 7–260 m between samples. Later, as cotton plants develop and in-field infestations spread, sampling of infested reproductive structures should focus on the field as a whole, using among-sample distances of 7–470, 12–435 and 6 m when the plants are at the initial flowering, late flowering and open bolls stage, respectively. Selection of the sampling distance to be adopted, considering an aggregated distribution of the boll weevil, will ultimately be dependent on the growth stage of the cotton plant which, in turn, influences the variability in infestation levels between plants and the spatial dependence among samples. Closer distances between plants should be used when infestations are relatively low due to heightened variability, and strong spatial dependence is noticed. Wider distances are recommended when infestation levels between plants are less variable, usually associated with relatively high numbers of infested reproductive structures, and spatial dependence is moderate. Based on the fact that most growers sample infested reproductive structures while monitoring the boll weevil,<sup>61</sup> no matter the cotton growth season or phenological stage, high precision can be assured by adopting a conservative approach that includes using the narrowest range found for sampling infested reproductive structures. Therefore, as a general recommendation, the minimum distance that should be adopted between samples to monitor boll weevil-infested reproductive structures across all phenological stages is 6 m. The recommendation found herein is valid for any other area cultivating cotton under tropical/subtropical conditions, which prevail in most cotton-producing regions across the globe.<sup>62</sup>

## ACKNOWLEDGMENTS

The authors are grateful for Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) for the financial scholarship to the first author.



## CONFLICT OF INTEREST

The authors declare they have no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

## REFERENCES

- Scataglini MA, Lanteri AA and Confalonieri VA, Diversity of boll weevil populations in South America: a phylogeographic approach. *Genetica* **126**:353–368 (2006).
- Showler AT, Roles of host plants in boll weevil range expansion beyond tropical mesoamerica. *Am Entomol* **55**:234–243 (2009).
- Showler AT, Subtropical boll weevil ecology. *Am Entomol* **53**:240–249 (2007).
- Grigolli JFJ, Souza LA, Fernandes MG and Busoli AC, Spatial distribution of adult *A. grandis* Boheman (Coleoptera: Curculionidae) and damage to cotton flower buds due to feeding and oviposition. *Neotrop Entomol* **46**:442–451 (2017).
- Showler AT, Greenberg SM, Scott AW Jr and Robinson JEC, Effects of planting dates on boll weevils (coleoptera: curculionidae) and cotton fruit in the subtropics. *J Econ Entomol* **98**:796–804 (2006).
- Paim EA, Dias AM, Showler AT, Campos KL, Oliveira AAS, Grillo PPC *et al.*, Cotton row spacing for boll weevil management in low-input production systems. *Crop Prot* **145**:105614 (2021).
- Oliveira CM, Auad AM, Mendes SM and Frizzas MR, Economic impact of exotic insect pests in Brazilian agriculture. *J Appl Entomol* **137**:1–15 (2013).
- Showler AT, Effects of routine late season field operations on numbers of boll weevils (Coleoptera: Curculionidae) captured in large-capacity pheromone traps. *J Econ Entomol* **96**:680–689 (2003).
- Azambuja R and DeGrande PE, Trinta anos do bicudo-do-algodoeiro no Brasil. *Arq Inst Biol* **81**:377–410 (2014).
- Rosado JF, Picanço MC, Sarmiento RA, Pereira RM, Pedro-Neto M, Galdino TV *et al.*, Geostatistics as a tool to study mite dispersion in physic nut plantations. *Bull Entomol Res* **105**:381–389 (2015).
- Shrestha G, Rijal JP and Reddy GVP, Characterization of the spatial distribution of alfalfa weevil, *Hypera postica*, and its natural enemies, using geospatial models. *Pest Manag Sci* **77**:906–918 (2021).
- Sciaretta A and Trematerra P, Geostatistical tools for the study of insect spatial distribution: practical implications in the integrated management of orchard and vineyard pests. *Plant Protect Sci* **50**:97–110 (2014).
- Ribeiro AV, Ramos RS, Araújo TA, Soares JRS, Paes J d S, VCR de A *et al.*, Spatial distribution and colonization pattern of *Bemisia tabaci* in tropical tomato crops. *Pest Manag Sci* **77**:2087–2096 (2021).
- Fonseca PRB, Fernandes MG, DeGrande PE, Mota TA and Kassab SO, Spatial distribution of adult *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) and buds with feeding punctures on conventional and Bt cotton. *Semina: Ciênc Agrár* **34**:1129–1138 (2013).
- Arruda LS, Rolim GG, Barros EM, Pereira FF and Torres JB, Colonization and spatial distribution of boll weevil in commercial cotton fields. *Neotrop Entomol* **49**:901–915 (2020).
- Liebhold AM, Rossi RE and Kemp WP, Geostatistics and geographic information systems in applied insect ecology. *Annu Rev Entomol* **38**:303–327 (1993).
- Shi G, Chapter 8 - Kriging, in *Data Mining and Knowledge Discovery for Geoscientists*, ed. by Shi G. Elsevier Science Publishers, London, pp. 238–274 (2014).
- Pasini MPB, Engel E, Dal'Col Lúcio A and Bortolotto RP, Semivariogram models for rice stem bug population densities estimated by ordinary kriging. *Acta Sci Agron* **43**:e4831 (2021).
- Webster R and Oliver MA, *Geostatistics for Environmental Scientists*. John Wiley & Sons, Chichester, Chichester, UK, p. 336 (2007).
- Vieira SR, Hatfield JL, Nielsen DR and Biggar JW, Geostatistical theory and application to variability of some agronomical properties. *Hilgardia* **51**:1–75 (1983).
- Isaaks EH and Srivastava RM, *An Introduction to Applied Geostatistics*. Oxford University, New York, p. 561 (1989).
- Galdino TV, Ferreira DO, Santana PA Jr, Arcanjo LP, Queiroz EA, Sarmiento RA *et al.*, The role of the beetle *Hypocryphalus mangiferae* (Coleoptera: Curculionidae) in the spatiotemporal dynamics of mango wilt. *J Econ Entomol* **110**:865–874 (2017).
- Lima CH, Sarmiento RA, Galdino TV, Pereira PS, Silva J, Souza DJ *et al.*, Spatiotemporal dynamics of whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in commercial watermelon crops. *J Econ Entomol* **111**:1895–1903 (2018).
- Martins JC, Picanço MC, Silva RS, Gonring AH, Galdino TV and Guedes RN, Assessing the spatial distribution of *Tuta absoluta* (Lepidoptera: Gelechiidae) eggs in open-field tomato cultivation through geostatistical analysis. *Pest Manag Sci* **74**:30–36 (2018).
- Cambardella CA, Moorman TB, Novak JM, Parkin TB, Karlen DL, Turco RF *et al.*, Field-scale variability of soil properties in Central Iowa soils. *Soil Sci Soc Am J* **58**:1501–1511 (1994).
- Liebhold AM, Zhang XU, Hohn ME, Elkinton JS, Ticehurst M, Benzon GL *et al.*, Geostatistical analysis of gypsy moth (Lepidoptera: Lymantriidae) egg mass populations. *Environ Entomol* **20**:1407–1417 (1991).
- Gamma Design Software – vs 9.0, GS+ Geostatistics for the environmental sciences, Michigan (2004).
- Lange F, Olmstead AL and Rhode PW, The impact of the boll weevil, 1892–1932. *J Econ Hist* **69**:685–718 (2009).
- Spurgeon DW and Raulston JR, Boll weevil (Coleoptera: Curculionidae) reproductive development as a function of temperature. *Environ Entomol* **27**:675–681 (1998).
- Greenberg SM, Sappington TW, Adamczyk JJ, Liu T-X and Setamou M, Effects of photoperiod on boll weevil (Coleoptera: Curculionidae) development, survival, and reproduction. *Environ Entomol* **37**:1396–1402 (2008).
- Neves RCS, Colares F, Torres JB, Santos RL and Bastos CS, Rational practices to manage boll weevils colonization and population growth on family farms in the Semiárido region of Brazil. *Insects* **5**:818–831 (2014).
- CAD da S, Duarte M d MF, Gonçalves SG and Vasconcelos ED, Spatial and temporal distribution of cotton squares and small cotton bolls fallen on ground after damage by boll weevil and the efficiency of the equipment used to collect them. *Ciênc Rural* **47**:e20160653 (2017).
- Showler AT, Greenberg SM, Scott AW Jr and Robinson JRC, Effects of planting dates on boll weevils (Coleoptera: Curculionidae) and cotton fruit in the subtropics. *J Econ Entomol* **98**:796–804 (2005).
- Showler AT, Boll weevil (Coleoptera: Curculionidae) damage to cotton bolls under standard and proactive spraying. *J Econ Entomol* **99**:1251–1257 (2006).
- Busoli AC, Pereira FF, Lopéz VAG, Soares JJ, Almeida CA *et al.*, Preferência alimentar do bicudo-do-algodoeiro por frutos de diferentes cultivares e idades. *Pesq Agropec Bras* **39**:101–104 (2004).
- Showler AT, Influence of cotton fruit stages as food sources on boll weevil (Coleoptera: Curculionidae) fecundity and oviposition. *J Econ Entomol* **97**:1330–1334 (2004).
- Greenberg SM, Spurgeon DW, Sappington TW and Sétamou M, Size-dependent feeding and reproduction by boll weevil (Coleoptera: Curculionidae). *J Econ Entomol* **98**:749–756 (2005).
- Showler AT, Relationships of different cotton square sizes to boll weevil (Coleoptera: Curculionidae) feeding and oviposition in field conditions. *J Econ Entomol* **98**:572–579 (2005).
- Magalhães DM, Borges M, Laumann RA, Woodcock CM, Pickett JA, Birkett MA *et al.*, Influence of two acyclic homoterpenes (tetranorterpenes) on the foraging behavior of *Anthonomus grandis* Boh. *J Chem Ecol* **42**:305–313 (2016).
- Showler AT, Free amino acid profiles in reproductive and rind portions of cotton fruiting bodies. *Subtrop Plant Sci* **61**:37–48 (2009).
- Showler AT, Robacker DC, Salgado E and Cantú RV, Grandlure dosage and attraction of boll weevils (Coleoptera: Curculionidae). *J Econ Entomol* **99**:1675–1681 (2006).
- Borth PW and Huber RT, *Modeling Pink Bollworm Establishment and Dispersion in Cotton with the Kriging Technique 1987 Proceedings of the Beltwide Cotton Production Research Conference; 1987 Jan 4–8*. Memphis, National Cotton Council of America, Dallas, TX, pp. 267–274 (1987).
- Shao-Kui G, Carruthers R, Zu-Fei M, Guang-Xue Z and Dian-Mo L, Spatial heterogeneity and population risk analysis of cotton bollworm, *Helicoverpa armigera*. *China Insect Sci* **12**:255–262 (2005).

- 44 Qing-Nian L, Jun-Zhan L, Jing-Tao L, Lu-Sheng Z and Jian-Qing L, Geostatistical analysis on population dynamics of second generation cotton bollworm. *Chin J Ecol* **26**:378–382 (2007).
- 45 Tillman PG, Northfield TD, Mizell RF and Riddle TC, Spatiotemporal patterns and dispersal of stink bugs (Heteroptera: Pentatomidae) in peanut-cotton farmscapes. *Environ Entomol* **38**:1038–1052 (2009).
- 46 Reay-Jones FPF, Toews MD, Greene JK and Reeves RB, Spatial dynamics of stink bugs (Hemiptera: Pentatomidae) and associated boll injury in southeastern cotton fields. *Environ Entomol* **39**:956–969 (2010).
- 47 Pulakkatu-Thodi I, Reisig DD, Greene JK, Reay-Jones FPF and Toews MD, Within-field spatial distribution of stink bug (Hemiptera: Pentatomidae)-induced boll injury in commercial cotton fields of the southeastern United States. *Environ Entomol* **43**:744–752 (2014).
- 48 Reay-Jones FPF, Greene JK and Bauer PJ, Stability of spatial distributions of stink bugs, boll injury, and NDVI in cotton. *Environ Entomol* **45**:1243–1254 (2016).
- 49 Reay-Jones FPF, Greene JK and Bauer PJ, Spatial distributions of thrips (Thysanoptera: Thripidae) in cotton. *J Insect Sci* **19**:1–12 (2019).
- 50 Gireesh M, Rijal JP and Joseph SV, Spatial distribution of hunting billbugs (Coleoptera: Curculionidae) in sod farms. *Insects* **12**:402 (2021).
- 51 Arruda LS, Torres JB, Rolim GG and Silva-Torres CSA, Dispersal of boll weevil toward and within the cotton plant and implications for insecticide exposure. *Pest Manag Sci* **77**:1339–1347 (2021).
- 52 Tumlinson JH, Hardee DD, Guedner AC, Thompson AC, Hedin PA and Minyard JP, Sex pheromones produced by the male boll weevil: isolation, identification and synthesis. *Science* **166**:1010–1012 (1969).
- 53 Cuadrado G, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) em la zona central y sur oeste de Misiones, Argentina: pólen como fuente alimenticia y su relation com el estado fisiológico en insectos adultos. *Neotrop Entomol* **31**:121–132 (2002).
- 54 Showler AT and Abrigo V, Common subtropical and tropical nonpollen food sources of the boll weevil (Coleoptera: Curculionidae). *Environ Entomol* **36**:99–104 (2007).
- 55 Ribeiro PA, Sujii ER, Diniz IR, Medeiros MA, Salgado-Labouriau ML, Branco MC *et al.*, Alternative food sources and overwintering feeding behavior of the boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) under the tropical conditions of Central Brazil. *Neotrop Entomol* **39**:28–34 (2010).
- 56 Macêdo JA, Castellani MA, Santos F d AR d, Oliveira PP and Maluf RP, Fontes alternativas de pólen utilizadas pelo bicudo-do-algodoeiro em duas regiões produtoras de algodão na Bahia. *Rev Caatinga* **28**:255–262 (2015).
- 57 Showler AT, Three boll weevil diapause myths in perspective. *Am Entomol* **55**:40–48 (2009).
- 58 Showler AT, The conundrum of chemical boll weevil control in subtropical regions, in *Insecticides*, ed. by Perveen F. InTech Publishing, Rijeka, Croatia, pp. 437–448 (2012).
- 59 Paula DP, Claudino D, Timbó RV, Miranda JE, Bemquerer MP, Ribeiro ACJ *et al.*, Reproductive dormancy in boll-weevil from populations of the Midwest of Brazil. *J Econ Entomol* **106**:86–96 (2013).
- 60 Carvalho SC, Santana PA Jr, Pereira PS, Sarmiento RA, Farias ES, Lima CHO *et al.*, Spatial distribution of *Frankliniella schultzei* (Thysanoptera: Thripidae) in open-field yellow melon, with emphasis on the role of surrounding vegetation as a source of initial infestation. *J Econ Entomol* **113**:2997–3003 (2020).
- 61 Miranda JE and Rodrigues SMM, *Manejo do bicudo-do-algodoeiro em áreas de agricultura intensiva*. Circular Técnica, Embrapa Algodão, p. 18 (2016).
- 62 Azhar MT, Wani SH, Chaudhary MT, Jameel T, Kaur P and Du X, Heat tolerance in cotton, in *Heat Stress Tolerance in Plants: Physiological, Molecular and Genetic Perspectives*, ed. by Wani SH and Kumar V. John Wiley & Sons Inc., pp. 1–22 (2020).