

Density and growth rates of spider mites in relation to phenological stages of soybean cultivars in Brazil

Jonas André Arnemann¹^(D) · Rubens Alex Fiorin² · Clérison Régis Perini¹ · Lindolfo Storck¹ · Luis Eduardo Curioletti¹ · Gösta Nachman³ · Jerson Vanderlei Carús Guedes¹

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Abstract The population fluctuations of spider mites on different soybean cultivars were studied in two experiments performed in the municipalities of São Sepé (experiment 1) and Santa Maria (experiment 2) in the state of Rio Grande do Sul, Brazil. The number of cultivars was 20 in São Sepé and 25 in Santa Maria, and at each location leaves were sampled from 15 plants per cultivar every week throughout the entire plant developmental cycle, amounting to 11 and 10 samplings in experiment 1 and 2, respectively. The statistical analysis revealed that mite densities varied significantly with cultivar and time. Besides, the interaction between soybean cultivars and sampling times was significant at both locations. This indicates that the populations did not vary in the same way among cultivars over time, which is attributed to differences among the cultivars with respect to their phenology and response to spider mites. Equations for describing the mite population dynamics were estimated for each cultivar, mostly by fitting cubic equations to the weekly growth rates (per capita changes) in mite densities. We also found that the highest growth rates for mites on soybean cultivars in both locations occurred after the beginning of flowering, when the plants shifted from the vegetative to reproductive stages, about 3 weeks before reaching the peak densities.

Keywords IPM · Population peak · Sampling procedure · Tetranychidae

Jonas André Arnemann jonasarnemann@gmail.com

¹ Department of Plant Protection, Federal University of Santa Maria (UFSM), Santa Maria, RS 97105-900, Brazil

² Product and Market Development Agronomist, Nufarm Indústria Química e Farmacêutica S/A, Maracanaú, CE, Brazil

³ Section of Ecology and Evolution, Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Ø Copenhagen, Denmark

Introduction

The first records of mites in soybeans in the state of Rio Grande do Sul were reported by Flechtmann (1972). Since then, severe occurrences of phytophagous mites, requiring the use of pesticides to control them, have been observed in soybeans during the 2002/2003 and 2003/2004 growing seasons in the state of Rio Grande do Sul, Brazil. The species commonly identified in those infestations were *Mononychellus planki* (McGregor), *Polyphagotarsonemus latus* (Banks), *Tetranychus desertorum* Banks and *Tetranychus gigas* (Pritchard). The predatory mites *Phytoseiulus fragariae* Denmark & Schicha and *Typhlodromalus aripo* De Leon were also identified. *M. planki* was first described in 1950 using specimens collected in Puerto Rico and the species is also present in Argentina, Brazil, Colombia, Trinidad and Venezuela (Bolland et al. 1998). The species *T. desertorum* was described in 1900 using specimens collected in the USA and has a wide distribution in the Americas; it is also found in Australia, China, and Japan (Bolland et al. 1998).

The hypotheses that have been suggested to explain the increased mite abundance in soybeans include the expansion of the area cultivated for soybeans, cropping system modifications introduced with genetically modified cultivars, the increased use of insecticides and fungicides and the occurrence of *veranicos* (dry spells in the rainy season) during the crop season (Guedes et al. 2007).

The mites infesting soybean plants tend to aggregate on the abaxial surface of leaflets where they feed by perforating the cells of the leaf tissue, thus reducing the photosynthetic capacity of the plant. Currently, when the mite population densities increase in soybean crops, chemical control is the primary tactic employed to manage the pest. Increasing concerns regarding the environment and the toxicity of pesticides to humans, coupled with the increased frequency of pest resistance to insecticides, have encouraged the search for alternative control methods.

One of the most important alternative programs for mite management in soybean is the use of genetic resistance (Dehghan et al. 2009). Studies indicate that there are significant differences in the resistance level of soybean cultivars to mites and in the population density of the mites infesting these cultivars (Sedaratian et al. 2009; Dehghan et al. 2009). According to Lara (1991), the following resistance levels can be observed in plants: immunity (the plant suffers no damage under any conditions), high or moderate resistance (the plant suffers little or below average damage compared with the average damage suffered by the varieties in general) and susceptibility (the plant suffers similar damage to the average damage suffered by the varieties in general).

Studies on the population dynamics of spider mites in soybean cultivars were performed by Roggia (2007), who correlated the mite density in a transgenic, glyphosate-tolerant cultivar crop and in a nontransgenic cultivar with different strategies of weed management. The author observed that mite density was more affected by the weed management scheme than by the cultivars used, that the cultivars used had high genetic similarity among themselves, and that the crop cycle and climatic factors influenced the results.

To remedy the lack of information about the vulnerability of the soybean cultivars used in Brazil to spider mites occurrence, we designed and conducted this research. The aim of the present study was to evaluate and model the density, population peaks and population fluctuations of spider mites infesting the commercial soybean cultivars used in the state of Rio Grande do Sul, Brazil.

Materials and methods

Two experiments were conducted, one in the municipality of São Sepé (experiment 1) and another in the municipality of Santa Maria (experiment 2), both in the state of Rio Grande do Sul, Brazil. Experiment 1 was performed from December 2011 through May 2012 in an area located at 30°18′41″S latitude and 53°31′42″W longitude at an elevation of 175 m. Twenty glyphosate-tolerant soybean cultivars (Table 1) were sown in a random block design with four replicates. The seeds were sown on December 06, 2011, using 30 seeds m^{-2} in 5 × 40-m plots. The soybean cultivar Nidera A 6411 RG was sown in the area surrounding the experimental plots.

Experiment 2 was performed from January 2012 through May 2012 in the experimental area of the Federal University of Santa Maria (Universidade Federal de Santa Maria— UFSM), located in the municipality of Santa Maria, state of Rio Grande do Sul, Brazil

Experiment 1 (São Sepé)			Experiment 2 (Santa Maria)		
Soybean cultivar	GF	MG	Soybean cultivar	GF	MG
NA 4990 RG	Indeterminate	4.9	NA 4990 RG	Indeterminate	4.9
NS 4823 RR	Indeterminate	5.0	NS 4823 RR	Indeterminate	5.0
BMX Energia RR	Indeterminate	5.3	BMX Energia RR	Indeterminate	5.3
RoosCamino RR	Indeterminate	5.3	RoosCamino RR	Indeterminate	5.3
Don Mario 5.8i (Apolo RR)	Indeterminate	5.5	Don Mario 5.8i (Apolo RR)	Indeterminate	5.5
BMX Ativa RR	Determinate	5.6	BMX ATIVA RR	Determinate	5.6
NS 5858 RR	Indeterminate	5.8	NS 5858 RR	Indeterminate	5.8
BMX TURBO RR	Indeterminate	5.8	BMX TURBO RR	Indeterminate	5.8
TMG 7161 RR	Indeterminate	5.9	TMG 7161 RR	Determinate	5.9
SYN 1059 RR (V-top RR)	Indeterminate	5.9	SYN 1059 RR (V-top RR)	Indeterminate	5.9
NK 7059 RR (V-max RR)	Indeterminate	6.1	NK 7059 RR (V-max RR)	Indeterminate	6.1
NA 5909 RG	Indeterminate	6.2	NA 5909 RG	Determinate	6.2
FUNDACEP 57 RR	Determinate	6.2	FUNDACEP 57 RR	Indeterminate	6.2
FPS Urano RR	Determinate	6.2	FPS Urano RR	Indeterminate	6.2
SYN 1163 RR	Indeterminate	6.3	SYN 1163 RR	Determinate	6.3
SYN 1161 RR	Indeterminate	6.3	SYN 1161 RR	Indeterminate	6.3
A 6411 RG	Determinate	6.7	A 6411 RG	Indeterminate	6.7
NS 6636 RR	Indeterminate	6.6	NS 6636 RR	Indeterminate	6.6
BMX Potência RR	Indeterminate	6.7	BMX Potência RR	Indeterminate	6.7
A 7321 RG	Indeterminate	7.5	A 7321 RG	Indeterminate	7.5
			SYN 1157 RR	Indeterminate	5.8
			NS 7100 RR	Indeterminate	7.1
			Fundacep 59 RR	Determinate	7.5
			08ca905023 ^a	-	-

 Table 1
 Glyphosate-tolerant soybean cultivars evaluated for the occurrence of spider mites in the municipalities of São Sepé and Santa Maria, RS, Brazil, in 2011/2012

GF growth form, MG maturity group

^a Not registered in the National Cultivar Registration (Registro Nacional de Cultivares—RNC) or with no available information

 $(29^{\circ}42'52''S, 53^{\circ}44'9''W)$, at an elevation of 95 m). Twenty-five glyphosate-tolerant soybean cultivars (Table 1) were sown in a random block design with four replicates on January 16, 2012, using 30 seeds m⁻² in 4.5 × 25-m plots. The soybean cultivar Nidera A 6411 RG was sown in the area surrounding the experimental plots. The cultivars used in both experiments were chosen because of their importance to the soybean producers of southern Brazil, which was judged by the rate at which the producers used the cultivars.

At both locations, 300 kg of nitrogen, phosphorus and potassium (NPK) 0-20-20 fertilizer was applied at sowing, and weed management was performed during presowing and at the V3 stage of the cultivars with the application of 1040 g acid equivalent (a.e.) ha⁻¹ of glyphosate (Crucial, Nufarm, Maracanaú, CE, Brazil). The seeds were treated with 25 g active ingredient (a.i.) ha⁻¹ of fipronil (Belure, Basf, São Paulo, SP, Brazil) and 1.25 + 0.5 g a.i. ha⁻¹ of fludioxonil + metalaxyl-M (Maxim XL, Syngenta Crop Protection, São Paulo, SP, Brazil). For the control of defoliating caterpillars, 10 g a.i. ha⁻¹ of chlorantraniliprole (Premio, DuPont, São Paulo, SP, Brazil) was used at two time points: when most of the cultivars were at the V4 stage (first application) and at the V7 stage (second application). The insecticide imidacloprid (Nuprid, Nufarm, Maracanaú, CE, Brazil) was applied during the R4 and R5.3 stages with the aim of managing insect pests, primarily homopteran and thrips species. In addition, during the reproductive period of the soybean cultivars, a mixture of 60 + 24 g a.i. ha⁻¹ of azoxystrobin + cyproconazole (Priori + Alto 100) (Alamos, Porto Alegre, RS, Brazil) was applied for disease management at three time points, namely, when most cultivars were in the R1, R4 and R5.4 stages.

The phenology of soybean was evaluated according to the scale proposed by Ritchie et al. (1982) and adapted by Yorinori (1996). It divides the soybean stages in two phases: (1) The vegetative phase composed by the sub-stages (VC) from emergence to open cotyledons; (V1) first node, open unifoliate leaves; (V2) second node, first open trifoliate; (Vn) nth (last) node with open trifoliate, before flowering; and (2) the reproductive phase composed by the sub-stages (R1) beginning of bloom stage, up to 50 % of plants with a flower; (R2) full bloom, most racemes with open flowers; (R3) end of bloom stage, pods up to 1.5 cm in length; (R4) most pods, in the upper third, measuring 2–4 cm; (R5.1) seeds perceptible to touch at 10 % seed filling; (R5.2) most pods at 10–25 % seed filling; (R5.3) most pods at 25–50 % seed filling; (R5.4) most pods at 50–75 % seed filling; (R5.5) most pods at 75–100 % of leaves and pods turning yellow; (R7.2) between 50 and 75 % of leaves and pods are yellow; (R7.3) more than 75 % of leaves and pods are yellow; (R8.1) beginning to 50 % defoliation period; (R8.2) more than 50 % defoliation to preharvest; and (R9) harvest maturity point.

Mite infestations of the plants occurred naturally. The population density of mites was estimated by sampling 30 fully expanded leaflets per plot. Thus, at each sampling occasion, 15 plants were selected at random and from each plant one leaf was taken from the middle and one from the top. The plots were sampled weekly from the V5 stage and until the end of the developmental cycle of the cultivars (Tables 2, 3).

The sampled leaflets were placed in paper bags, labelled, stored in a cooler containing ice and transported to the Laboratory of Integrated Pest Management (Laboratório de Manejo Integrado de Pragas—LabMIP-UFSM). In the laboratory, the sampled leaflets were kept in a refrigerator (at approximately 8 °C) until the mites were counted. An area of 20 cm² on each leaflet was inspected (Storck et al. 2012) using a stereomicroscope at 40× magnification. The numbers of eggs, immature, and adult mites were recorded. For species identification, representative samples of mites were counted on microscope slides in Hoyer's medium and analyzed under a phase-contrast light microscope at Empresa Brasileira de Perquisa Agropecuária (Embrapa) Soybean.

Tab 2011	e 2 Soybean cultivars and the /2012 season	dates and re	spective dev	elopmental s	tages at the s	ampling of	soybean leaf	lets in the m	unicipality c	of São Sepé,	RS, Brazil, e	luring the
	Soybean cultivar	01/19/12	01/25/12	02/02/12	02/08/12	02/15/12	02/22/12	02/29/12	03/07/12	03/14/12	03/23/12	04/02/12
1	NA 4990 RG	V6	R1	R2	R2	R4	R5.2	R5.3	R5.4	R6	R7	R8
7	RoosCamino RR	V6	۲V	R1	R2	R3	R4	R5.1	R5.2	R5.4	R7	R8
3	NS 4823 RR	V6	R1	R2	R2	R4	R5.1	R5.3	R5.4	R5.5	R7	R8
4	BMX Energia RR	V6	۲V	R2	R3	R4	R5.1	R5.1	R5.4	R5.4	R7	R9
5	Don Mario 5.8i (Apolo RR)	۲V	V8	R2	R2	R4	R5.1	R5.2	R5.3	R5.4	R6	R8
9	BMX Ativa RR	V6	V6	R1	R2	R3	R4	R5.1	R5.3	R6	R7	R8
7	NS 5858 RR	LΛ	R1	R2	R3	R4	R5.1	R5.2	R5.3	R5.4	R6	R6
×	BMX TURBO RR	V5	V6	R2	R2	R3	R4	R5.1	R5.2	R5.3	R6	R8
6	TMG 7161 RR	LΛ	V8	R2	R2	R4	R5.1	R5.2	R5.3	R5.5	R7	R7
10	SYN 1059 RR (V-top RR)	V5	۲V	R1	R2	R4	R4	R5.1	R5.2	R5.3	R6	R8
11	NK 7059 RR (V-max RR)	V6	V8	V10	R1	R2	R3	R5.1	R5.2	R5.3	R6	R8
12	FPS Urano RR	V6	۲V	R1	R2	R2	R3	R4	R5.1	R5.3	R6	R8
13	NA 5909 RG	V5	۲V	R1	R2	R2	R3	R5.1	R5.3	R5.4	R6	R8
14	FUNDACEP 57 RR	V6	۲V	6 V	V10	R1	R2	R3	R4	R5.2	R5.3	R7
15	SYN 1163 RR	۲V	V8	R1	R2	R2	R4	R5.1	R5.2	R5.2	R5.5	R8
16	SYN 1161 RR	V5	V6	R1	R2	R2	R3	R4	R5.1	R5.2	R5.3	R8
17	A 6411 RG	۲V	V9	6 V	R1	R2	R2	$\mathbb{R}4$	R5.1	R5.2	R5.4	R7
18	NS 6636 RR	۲V	V8	R1	R2	R3	$\mathbf{R4}$	R5.1	R5.2	R5.3	R5.5	R7
19	BMX Potência RR	V6	۲V	6 V	R1	R2	R3	$\mathbb{R}4$	R5.1	R5.2	R5.4	R8
20	A 7321 RG	9A	۲V	R1	R2	R2	R3	R4	R5.1	R5.3	R5.5	R7

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Table 2011/2	3 Soybean cultivars and 012 season	the dates and	respective dev	elopmental sta	iges at the sam	pling of soybe	an leaflets in t	he municipality	<i>y</i> of Santa Maı	ria, RS, Brazil,	during the
	Soybean cultivar	02/17/12	02/24/12	03/02/12	03/09/12	03/16/12	03/24/12	04/03/12	04/11/12	04/20/12	04/27/12
1	SYN 1059 RR	۲V	V8	R2	R2	R3	R5.3	R5.4	R6	R7	I
7	NA 4990 RG	R1	R2	R2	R3	R3	R5.3	R5.4	R7	R8	I
3	BMX Energia RR	V4	RI	R1	R3	R3	R5.4	R5.4	R6	R8	Ι
4	BMX ATIVA RR	LΛ	R1	R1	R2	R5.1	R5.1	R5.4	R6	R7	I
5	A 6411 RG	V5	V8	R1	R2	R2	R5.2	R5.2	R5.5	R6	I
9	NA 5909 RG	V6	V8	R2	R2	R3	R5.3	R5.3	R6	R7	Ι
L	FPS Urano RR	LΛ	LΛ	R1	R3	R5.1	R5.2	R5.5	R6	R7	Ι
8	Don Mario 5.8i	V5	LΛ	R1	R2	R3	R5.2	R5.4	R7	R8	Ι
6	BMX TURBO RR	V6	V8	R2	R3	R3	R5.2	R5.4	R7	R8	I
10	BMX Potência RR	V6	V8	R2	R2	R3	R5.2	R5.3	R6	R8	I
11	NK 7059 RR	V6	LΛ	R1	R2	R3	R4	R5.4	R7	R8	I
12	Fundacep 59 RR	V6	V8	V 9	V10	R2	R4	R5.1	R5.3	R6	R7
13	FUNDACEP 57 RR	V6	V8	V 9	R1	R2	R5.1	R5.1	R5.3	R6	R7
14	A 7321 RG	V6	V8	R1	R2	R3	R5.2	R5.2	R5.5	R6	I
15	SYN 1158 RR	V6	V8	R1	R2	R3	R5.1	R5.3	R7	R8	I
16	08ca905023	9V	V8	R1	R2	R3	R5.2	R5.2	R5.5	R6	I
17	SYN 1157 RR	V5	V8	R1	R2	R3	R5.1	R5.4	R7	R8	I
18	NS 7100 RR	V6	V8	R1	R2	R3	R5.3	R5.4	R7	R8	I
19	SYN 1163 RR	9V	6 V	R2	R2	R3	R5.3	R5.4	R6	R8	I
20	NS 4823 RR	V8	RI	R2	R4	R4	R5.3	R5.5	R8	I	I
21	NS 6636 RR	9V	6 V	R2	R2	R4	R5.1	R5.3	R6	R8	I
22	Roos Camino RR	9A	R1	R2	R2	R4	R5.2	R5.4	R7	R8	I
23	TMG 7161 RR	9V	LΛ	R2	R2	R4	R5.2	R5.4	R7	R8	I
24	NS 5858 RR	R1	R2	R3	R3	R4	R5.3	R5.5	R7	R8	I
25	SYN 1161 RR	76 V6	V8	R1	R1	R3	R5.2	R5.3	R6	R8	Ι

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Analysis of variance was performed using the statistical software Genes (Cruz 2013) for a split-plot randomized block design (samplings over time) with four replicates. The cultivars were randomized among the plots, and the weekly samplings that were performed at 1–11 weeks for experiment 1 (São Sepé) and 1–10 weeks for experiment 2 (Santa Maria) comprised the subplots. Since anovas require that the data (average number of mites per plot) are normally distributed and show variance homogeneity, we applied Taylor's power law (Taylor 1961) to identify the appropriate transformation of the dependent variable (y). As the slopes (b) were found to be 1.56 and 1.66 for São Sepé and Santa Maria, respectively, it indicated that the logarithmic transformation would be the most suitable. Given the presence of the zero values, the ln(y + 1) transformation was applied.

The effect of time on the number of mites was analysed by means of regression analysis. To partially eliminate the effects of autocorrelation between population densities assessed on two consecutive sampling occasions, we introduced a new variable called the net growth rate during the time interval between two successive samplings (R_x), calculated as $R_x = y_{x+1}/y_x$, where y_x is the estimated mite density in a given cultivar at the xth sampling. Values of $y_x^* = \ln R_x = \ln y_{x+1} - \ln y_x$ were plotted against x = 1, 2, 3, ..., n-1 weeks within each cultivar. Because of the presence of zero values, the constant 0.001 was added to all y_x values. Polynomial models up to the third order were fitted to data in order to find the model that best explained the observed growth rates. Higher order terms were only included in the models when these terms improved the fit significantly (P < 0.05) compared with simpler models. The estimated values were back-transformed to the original scale using $R_x = \exp(y^*)$. As $y_{x+1} = R_x y_x$, the population density in week x can be predicted recursively as $\hat{y}_x = R_{x-1}\hat{y}_{x-1} = \hat{y}_1 \prod_{i=1}^{x-1} R_i$, where \hat{y}_1 is the estimated population density at the first sampling where \hat{y} was positive. The cumulated number of mites from the first (x = 1) through the last sampling (x = n) was found as $Y_n = \sum_{x=1}^n \hat{y}_x = \hat{y}_1 \sum_{x=1}^n \prod_{i=1}^{x-1} R_i = \hat{y}_1 M_n$, where $M_n = \sum_{x=1}^n \prod_{i=1}^{x-1} R_i$. Note that M_n is independent of the initial population density (which is likely to be associated with considerable sampling error). This means that M_n for a given number of samplings (n) can be interpreted as a relative measure of how favourable a cultivar is for spider mites.

Regression models given as $\ln R_x = a + bx + cx^2 + dx^3$ were fitted to the empirical growth rates and used to estimate the inflection points, i.e., the values of x where the growth rate changes direction. The inflection points were found by setting the first derivate of $\ln R_x$ equal to 0, i.e., $b + 2cx + 3dx^2 = 0$ and solve the equation with respect to x. For the cubic equation the two inflection points (called CIP1 and CIP2) are found as $\frac{-b'\pm\sqrt{(b')^2-4a'c'}}{2a'}$, where a' = 3d, b' = 2c and c' = b. When a second order polynomial was applied to describe the change in $\ln R_x$, the single inflection point was found as $CIP1 = \frac{-b}{2c}$. The models can be used to standardize population dynamics on the various soybean cultivars by predicting the population density in week x assuming that the initial density on all cultivars was one mite per cm². A population will increase as long as $\ln R_x > 0$ and decrease when $\ln R_x$ becomes negative.

Through the Tocher's optimization method, which takes into account the average immature + adult count and the contribution of each sampling, the soybean cultivars were grouped by similarity in both locations (Rao 1952; Cruz 2013). The method allows for establishing mutually exclusive clusters of objects (cultivars) according to an objective

function that adopts the criterion of optimization, which minimizes the average intracluster distances and maximizes the average inter-cluster distances (Silva and Dias 2013).

Results and discussion

The dominating mite species in the samples were *M. planki*, *T. urticae*, *T. ludeni*, *T. gigas* and *T. desertorum*. These species had previously been observed by Guedes et al. (2007) and Roggia et al. (2008), indicating that they are the most common in soybeans and therefore relevant as subjects for additional studies.

In the present study, predatory mites had little influence on the population dynamics of the pest mites because predators were observed in only a few samples from each location. It is common to observe a low density of predators relative to prey density in soybeans (Roggia 2007). Certain cropping practices used in the current study were potentially unfavourable for the establishment and development of predatory mites, such as the drastic elimination of weeds hosting predators and the use of insecticides having a negative effect on beneficial mites.

The analysis of variance indicated significant differences (P < 0.05) in spider mite density among the cultivars evaluated (Table 4), demonstrating that the cultivars affected development of spider mite populations differently. Thus, the significant effects of cultivars indicate that the cultivars differ with respect to their resistance to mites, whereas the significant interactions among cultivars and samplings indicate that the populations inhabiting the different cultivars develop differently over time, which is attributed to differences among cultivars with respect to phenology. Another possibility is that the cultivars differ with respect to their ability to produce defence compounds serving to protect the plants against pest organisms (induced defence) (War et al. 2012).

Temperature, humidity, rainfall and other climatic factors are considered as the main factors influencing the population dynamics of phytophagous mites (Guedes et al. 2007; Roggia et al. 2008). However, our study indicates that plant characteristics (cultivars) are also important for modulating the population dynamics of phytophagous mites in soybeans. By using the M_n values it was possible to identify the cultivars with the highest and lowest risks of being infected by spider mites.

The weekly variation among the cultivars with respect to the number of eggs, immature mites and adult mites per 20 cm² leaflet⁻¹ did not allow for phenological matching, i.e., the identification of environmental factors responsible for concurrent changes in population densities across the cultivars during the crop cycle.

Because the temporal pattern of mite density differed among cultivars, the dynamics of mites was modelled for each cultivar separately using polynomial regression to find the relationship between sampling occasion (x) and $\ln R_x$. The results of these analyses for the immature + adult mites are shown in Tables 5 and 6 for the two locations.

The coefficients of determination (R^2) , which express the fit of a model to data, were quite low for most of the cultivars, illustrating the difficulty in using a mathematical equation to model population growth of populations subjected to field conditions.

Figure 1a–f shows the predicted growth rate (from week 1 through 11) for 13 cultivars. Most cultivars from the São Sepé field showed the highest growth rates from week 4 through 6, when the cultivars were between R2 (full bloom) and R5.1 (beginning of pod/ seed filling). Figure 2a–f shows the predicted density of mites assuming that the initial density was one mite per cm², showing that the growth rates peak are about 3 weeks before

VS	DF	Mean squares			
		Eggs	Immature	Adult	Immature + adult
São Sepé					
Block (B)	3	85.16**	53.09**	3.32 ^{ns}	81.46**
Cultivar (C)	19	57.94**	81.51**	17.24**	168.25**
Error (a)	57	8.45	3.68	1.67	7.56
Sampling (Sa)	10	206.23**	121.19**	57.27**	335.82**
$C \times Sa$	190	45.63**	52.64**	16.18**	123.51**
$B \times Sa$	30	17.17**	10.17**	1.76 ^{ns}	19.09**
Error (b)	570	9.07	3.75	1.26	7.91
Mean	_	1.39	1.21	0.76	1.97
CV (%)	-	216.94	159.15	147.76	142.40
Santa Maria					
Block (B)	3	642.16**	343.08**	37.21 ^{ns}	587.33**
Cultivar (C)	24	124.35**	88.82**	66.14**	268.05**
Error (a)	72	39.14	37.97	20.54	87.79
Sampling (Sa)	9	3442.43**	3512.11**	2672.36**	12257.85**
$C \times Sa$	216	155.01**	104.16**	59.85**	242.97**
B × Sa	27	106.48**	63.41*	7.00 ^{ns}	97.57 ^{ns}
Error (b)	648	41.70	35.83	21.22	86.26
Mean	-	5.82	5.38	4.34	9.72
CV (%)	_	110.9	111.2	106.2	95.5

Table 4 Variation sources (VS), degrees of freedom (DF) and mean squares for the following dependent variables: number (per 20 cm² leaflet⁻¹) of eggs and immature, adult and immature + adult spider mites in weekly samplings of soybean cultivars from two locations (São Sepé and Santa Maria) in the central region of the state of Rio Grande do Sul during the 2011/2012 season

Overall means were used in the analysis, and CV is the coefficient of variation

Data were ln(y + 1) transformed prior to the analysis

 $*P < 0.05, **P < 0.01, ^{ns}$ not significant

the highest densities are reached (seed filling stages), while Fig. 3a–f shows the cumulated density at week x (M_x).

The soybean cultivars NS 4823 RR, NA 4990 RG, Ross Camino RR, BMX Ativa RR, BMX Turbo RR, TMG 7161 RR, SYN 1059 RR, BMX Potência RR and NS 6636 RR have the highest risk of being infested by spider mites. The values of M_n , i.e., the value of M_x at the last sampling (corresponding to the area under the density curves shown in Fig. 2) are given in Table 5. It is seen that six cultivars had very low densities of mites.

Figure 4a–d shows the predicted growth rate (from week 1 through 10) for 11 cultivars. Most cultivars from Santa Maria field showed the highest growth rates in week 3–4, when the cultivars were between beginning of bloom stage (R1) and end of bloom stage (R3). The highest growth rates for mites on soybean cultivars in both locations occurred after the beginning of flowering, when the plants switched from the vegetative to reproductive stages. Figure 5a–d shows the predicted density of mites assuming that the initial density was 1 mite per cm². We can also see that the peaks in growth rates in São Sepé and Santa Maria coincided with the same phenological stages, i.e., about 3 weeks earlier than the highest densities were achieved.

the 2011/2012 season					
Soybean cultivar	$R_x = \exp(a + bx + cx^2 + dx^3)^*$	R^{2}	M_n	CIP1	CIP2
NA 4990 RG	$R_x = \exp(1.070 - 1.3027x + 0.55609x^2 - 0.050628x^3)$	0.48	25,755.6	1.5	5.9
Roos Camino RR	$R_x = \exp(1.218 - 1.2895x + 0.51867x^2 - 0.046596x^3)$	0.40	12,970.4	1.6	5.8
NS 4823 RR	$R_x = \exp(4.276 - 4.0857x + 1.16382x^2 - 0.087972x^3)$	0.52	55,363.9	2.4	6.4
BMX Energia RR	$R_x = \exp(1.211 - 1.3688x + 0.54097x^2 - 0.047965x^3)$	0.46	10,794.0	1.6	5.9
Don Mario 5.8i	$R_x = \exp(1.517 - 1.6554x + 0.59379x^2 - 0.050490x^3)$	0.48	7260.1	1.8	6.0
BMX Ativa RR	$R_x = \exp(3.708 - 4.5210x + 1.35044x^2 - 0.101932x^3)$	0.76	39,133.7	2.2	6.6
NS 5858 RR	$R_x = \exp(0.556 - 1.1352x + 0.50603x^2 - 0.045603x^3)$	0.72	4170.0	1.4	6.0
BMX TURBO RR	$R_x = \exp(0.279 - 0.9838x + 0.51794x^2 - 0.048957x^3)$	0.70	15,091.1	1.1	5.9
TMG 7161 RR	$R_x = \exp(0.685 - 1.3971x + 0.62873x^2 - 0.056883x^3)$	0.67	28,279.8	1.4	6.0
SYN 1059 RR	$R_x = \exp(-2.548 + 2.1998x - 0.24808x^2)$	0.31	17,193.9	4.4	
NK 7059 RR	$R_x = \exp(6.892 - 5.6878x + 1.38850x^2 - 0.096086x^3)$	0.47	13,903.0	3.0	6.7
FPS Urano RR	$R_x = \exp(0.000)$		11		
NA 5909 RG	$R_x = \exp(0.000)$		11		
FUNDACEP 57 RR	$R_x = \exp(0.000)$		11		
SYN 1163 RR	$R_x = \exp(0.000)$		11		
SYN 1161 RR	$R_x = \exp(0.000)$		11		
A 6411 RG	$R_x = \exp(1.122)$		1,10,725		
NS 6636 RR	$R_x = \exp(3.189 - 2.7070x + 0.81772x^2 - 0.065399x^3)$	0.47	46,555.5	2.3	6.1
BMX Potência RR	$R_x = \exp(4.806 - 3.8347x + 1.00766x^2 - 0.074414x^3)$	0.39	24,691.1	2.7	6.3
A 7321 RG	$R_x = \exp(0.000)$		11		
* Significance $(P < 0.05)$ was	assessed for the higher-level parameter in each equation				

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Soybean Cultivar	$R_x = \exp(a + bx + cx^2 + dx^3)^*$	\mathbb{R}^2	Mn	CIP1	CIP2
SYN 1059 RR	$R_x = \exp(3.440 - 0.8028x)$	40.4	880.0		
NA 4990 RG	$R_x = \exp(-0.552)$		2.3		
BMX ENERGIA RR	$R_x = \exp(-0.743)$		1.9		
BMX ATIVA RR	$R_x = \exp(3.959 - 2.7471x + 0.78920x^2 - 0.06967x^3)$	95.6	2886	2.7	4.8
A 6411 RG	$R_x = \exp(-2.935 + 2.5641x - 0.33344x^2)$	72.6	751.6	3.8	
NA 5909 RG	$R_x = \exp(-2.398 + 2.3491x - 0.31402x^2)$	71.2	1233	3.7	
FPS Urano RR	$R_x = \exp(-2.212 + 2.2727x - 0.30694x^2)$	74.9	1457	3.7	
Don Mario 5.8i	$R_x = \exp(-0.792)$		1.8		
BMX TURBO RR	$R_x = \exp(-0.664)$		2.1		
BMX Potência RR	$R_x = \exp(0.000)$		10		
NK 7059 RR	$R_x = \exp(3.319 - 2.3739x + 0.55941x^2 - 0.039483x^3)$	76.8	215.7	3.2	6.2
Fundacep 59 RR	$R_x = \exp(3.229 - 2.2423x + 0.5071x^2 - 0.033850x^3)$	70.4	326.1	3.3	6.7
FUNDACEP 57 RR	$R_x = \exp(0.901)$		5613		
A 7321 RG	$R_x = \exp(-3.872 + 2.9296x - 0.36228x^2)$	69.8	401.5	4.0	
SYN 1158 RR	$R_x = \exp(6.532 - 5.5540x + 1.49069x^2 - 0.117523x^3)$	91.2	1734	2.8	5.7
08ca905023	$R_x = \exp(-4.046 + 2.9521x - 0.36482x^2)$	72.5	186.7	4.0	
SYN 1157 RR	$R_x = \exp(-0.735)$		1.9		
NS 7100 RR	$R_x = \exp(-0.670)$		2.0		
SYN 1163 RR	$R_x = \exp(-0.574)$		2.3		
NS 4823 RR	$R_x = \exp(0.000)$		10		
NS 6636 RR	$R_x = \exp(-0.400)$		3.0		
Roos Camino RR	$R_x = \exp(-0.400)$		3.0		
TMG 7161 RR	$R_x = \exp(0.000)$		10		
NS 5858 RR	$R_x = \exp(0.000)$		10		
SYN 1161 RR	$R_{v} = \exp(-0.476)$		2.6		



Fig. 1 Predicted net growth rate (week⁻¹) of the number of mites in soybean cultivars during 11 weeks in São Sepé

Figure 6a–d shows the cumulative mites density in week x (M_x). From this figure we can conclude that the soybean cultivars BMX Ativa RR, NA 5909 RG, FPS Urano RR, SYN 1158 RR and 08ca905023 have the highest risk of being infested by spider mites while 14 soybean cultivars seem to be resistant to mites.

Comparing the results from both locations, it is clearly seen that the experiment in São Sepé had higher mite infestations compared with Santa Maria. This indicates that the location of a field also influences the abundance of mites. Siqueira (2011) studied the population fluctuations of mites in soybean cultivars during the 2009/2010 and 2010/2011 growing seasons and observed the highest population densities during the R3–R4 stage of each cultivar. These results differ from those observed in the two experiments of the current study (Figs. 2, 5), in which the highest population size index was found to occur from the middle to the end of R5 stage. By contrast, Roggia (2007) recorded the population peak of *M. planki* and *T. gigas* to coincide with the R4 soybean stage, with a significant influence of the immature mite stages, low precipitation, temperature and low air relative humidity (RH). The mites in the Santa Maria experiment showed more variation with respect to growth rates and densities than the populations in São Sepé.



Fig. 2 Population size index of mites in soybean cultivars during 11 weeks in São Sepé

The equations describing the weekly changes in mite density for each soybean cultivar can be used for predicting the risk that mites occurring early in the season will cause severe damage later on since the predicted damage is assumed to increase monotonically with "mite-days" (modelled as $\hat{y}_1 M_n$), where \hat{y}_1 is the estimated population density at an earlier sampling occasion. Obviously, the error associated with such predictions will be considerable, especially if *n* is large. Nevertheless, the models may serve as guidelines for the producers to design sampling programs and to improve management of the crop during its agricultural cycle.

The two critical inflection points (CIP1 and CIP2 for the cubic equations) delineate the time at which the per capita growth rate changes direction. The CIP1 indicates (in weeks after the sampling started) the beginning of the mite population increase, whereas the CIP2 marks when growth rate peaks. In most of the cultivars planted in the municipality of São Sepé, the mite density increased during the second week of sampling and reached its peak during weeks eight and nine (Figs. 1b, 2b). At Santa Maria, the CIP1 occurred between the first and second week of sampling, and the CIP2 occurred during week seven (Table 6). From an agronomic point of view, the results indicate that producers must be especially



Fig. 3 Cumulated density of mites in soybean cultivars during 11 weeks in São Sepé

alert to the management of spider mites in soybean crops from the full bloom to the set of pod formation, for example by increasing the sampling frequency prior to these stages.

The use of cluster analysis by the Tocher's optimization method allowed for the grouping of the soybean cultivars according to the similarity of their response to mites (Table 7). In São Sepé, the cultivars were classified into two groups, with cultivar BMX ENERGIA RR being the only member of group II while all the other cultivars were clustered in group I. In Santa Maria, the Tocher's optimization method resulted in three groups of soybean cultivars. Most of the evaluated cultivars were classified in group I, whereas cultivars BMX ENERGIA RR and Fundacep 59 RR were classified in group II and III, respectively.

The cluster analysis considered the average number of immature + adult spider mites in each sample and the relative contribution of each sample to the cultivar classification (Table 8). In São Sepé, the sample contributing most to the classification of cultivars was the one from the first week, which contributed 40 %. At this time, the population density of mites was low in all the cultivars. In Santa Maria, sampling 6 (with a population peak for most cultivars on March 24, 2012), sampling 5 (with a population peak for most cultivars on March 16, 2012) and sampling 9 (with a population peak for most cultivars on April 20, 2012) were the ones that contributed most to the formation of the cultivar groups.



Fig. 4 Predicted net growth rate (week⁻¹) of the number of mites in soybean cultivars during 10 weeks in Santa Maria



Fig. 5 Population size index of mites in soybean cultivars in 10 weeks in Santa Maria

The results of the cluster analysis using the Tocher's optimization method can facilitate the understanding of fluctuations in the mite populations infesting soybean cultivars because these results make it possible to consider the performance of spider mite



Fig. 6 Cumulated density of mites in soybean cultivars during 10 weeks in Santa Maria

0 1		
Groups	Number	Cultivars
Santa M	laria	
Ι	23	SYN 1059 RR, NA 4990 RG, BMX ATIVA RR, A 6411 RG, NA 5909 RG, FPS Urano RR, Don Mario 5.8i, BMX TURBO RR, BMX Potência RR, NK 7059 RR, FUNDACEP 57 RR, A 7321 RG, SYN 1158 RR, 08ca9050234, SYN 1157 RR, NS 7100 RR, SYN 1163 RR, NS 4823 RR, NS 6636 RR, RoosCamino RR, TMG 7161 RR, NS 5858 RR, SYN 1161 RR
II	1	BMX ENERGIA RR
III	1	Fundacep 59 RR
São Sep	é	
Ι	19	NS 5858 RR, Ross Camino RR, A 6411 RG, BMX Turbo RR, TMG 7161 RR, FPS Urano RR, NS 6636 RR, NS 4823 RR, BMX Ativa RR, SYN 1163 RR, Don Mario 5.8i (Apolo RR), BMX Potência RR, NA 4990 RG, SYN 1161 RR, A 7321 RG, SYN 1059 RR (V-top RR), NA 5909 RG, NK 7059 RR (V-max RR), FUNDACEP 57 RR
II	1	BMX ENERGIA RR

 Table 7
 Groups of cultivars resulting from the Tocher's optimization method, number of cultivars per group and cultivar names for the municipalities of Santa Maria and São Sepé during the 2011/2012 season

populations to be similar on the cultivars within each group. Each cultivar group can then be monitored in a similar way and treated against mites if needed.

In general, the population densities of spider mites observed on the soybean cultivars from São Sepé were lower than the densities found on the cultivars from Santa Maria. The later sowing in Santa Maria was associated with conditions favourable for mite development, which might have been responsible for the difference between the two sites with

Week of	São Sepé		Santa Maria	
sampling	Contribution (%)	Cumulative relative (%)	Contribution (%)	Cumulative relative (%)
1	40.00	40.00	9.00	9.00
2	5.26	45.26	6.67	15.67
3	5.79	51.05	10.00	25.67
4	8.42	59.47	4.33	30.00
5	0.00	59.47	16.00	46.00
6	8.95	68.42	17.30	63.30
7	1.05	69.47	9.00	72.30
8	3.16	72.63	6.33	78.63
9	3.16	75.79	15.70	94.33
10	22.6	98.39	5.67	100
11	1.58	100		

 Table 8
 Week of sampling, contribution and cumulative relative percentage for each sampling in the cluster analysis performed using Tocher's optimization method for the municipalities of São Sepé and Santa Maria during the 2011/2012 season

respect to density. In experiments performed with the same soybean cultivars during two distinct growing seasons (2009/2010 and 2010/2011), Siqueira (2011) reported significant variation in the densities of *M. planki* and that the population density of the soybean green mite was slightly higher during the 2010/2011 season than the year before.

Low rainfall and air RH are often reported as major abiotic factors that cause an increase in spider mite density (Guedes et al. 2007; Roggia 2007). However, in this study we were unable to link spider mite fluctuations to any abiotic factors. Despite São Sepé suffered from long periods of water stress with low air RH there were no significant effects on mite densities that could be attributed to this circumstance. Sedaratian et al. (2009), who studied the population density of *T. urticae* in soybean cultivars, also observed significant differences in the population density of this pest among soybean cultivars.

Conclusion

The results indicate that spider mites start to infest soybean crops from the phenological stage V5 and to increase in density until the plants reach stage R5 for most cultivars at both locations, as shown by the first inflection point of the equation expressing the mite growth rate as a function of sampling time. The per capita growth rate was found to peak in the reproductive stages (between R2 and R5) while the highest densities were achieved when the crop reached the grain-filling stages. The monitoring of spider mites must encompass the entire cycle of the soybean crop; however, starting with the formation of pods, producers must be especially alert to high densities of spider mites which may require management of this pest in the crop. Based on Tocher's optimization procedure, soybean cultivars NA 4990 RG, Ross Camino RR, BMX Energia RR, BMX Ativa RR, BMX Turbo RR, TMG 7161 RR, NS 6636 RR, SYN 1059 RR, A 6411 RG, NS 6636 RR, NS 4823 RR, BMX Potência RR, NK 7059 RR, NA 5909 RG, FPS Urano RR and SYN 1158 RR were categorized as the most vulnerable to mites infestations.

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