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Development and validation of mathematical model for population estimation of the two-spotted spider mite in soybeans

Ezequiel G. Souza, Antonio J. Steidle-Neto, Daniela C. Lopes, Priscilla T. N. Maia, Marcos A. M. Fadini

Universidade Federal de São João del-Rei, Sete Lagoas, MG, Brazil.
Corresponding author: priscillatavares16@gmail.com

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Abstract. The two-spotted spider mite *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) causes bronzing and leaf drop in soybeans, reducing grain production and quality. Using colorimetric measurements in soybean-producing areas can be an alternative for monitoring the mite, aiding in the estimation of field populations. The objective of this study was to estimate the quantity of the two-spotted spider mite in soybeans through colorimetric measurements using the CIE-L*a*b* color space. Soybean plants at the V3 stage were used with 4 treatments (Control=0; T1=30; T2=60; and T3=100 adult female *T. urticae* per plant), 4 repetitions, and 170 measurements. Regression and variance analyses were performed using Origin Pro 2015 software. Prediction equations were calibrated. Our results show that there was a significant difference for the *L**, *a**, and Δ*E** variables. Regression analyses presented determination coefficient values of 0.87, 0.93, and 0.55 for *L**, *a**, and *b**, respectively. The use of the (*a**) variable in the CIE-L*a*b* color space proved effective in distinguishing between infestation levels. The model created to estimate the quantity of mites on soybean leaves has the potential to be applied in soybean production areas with infestations of the two-spotted spider mite.

Keywords: Sampling, *Tetranychus urticae*, *Glycine max*, colorimetry.

Different groups of insects stand out as pests in soybeans; however, in recent years, the two-spotted spider mite *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) has shown potential to cause injuries and damage, significantly reducing crop yields (Roggia et al. 2008; Padilha et al. 2020). Currently, the control of pest mites is mainly based on the use of organosynthetic acaricides (De Araújo et al. 2022). Spraying should be carried out based on the economic damage level of the plants (Jakubowska et al. 2022). However, due to the difficulties in finding adequate sampling techniques for large territorial areas, sampling becomes labor-intensive and costly.

Integrated pest management considers sampling as one of the main steps for decision-making. Through sampling, pest populations in the field are estimated, and based on these estimates, control decisions are made. Inaccurate estimates can lead to incorrect decisions, which most often result in the unnecessary use of agricultural pesticides (Pedigo & Rice 2009; Nansen 2016).

The injuries caused by mites, such as chlorosis, are easily identifiable by color changes in the leaves. Thus, the use of colorimetric techniques can facilitate sampling by processing the injuries on the leaves.

The CIE-L*a*b* color space is used to detect color differences in various materials, wood (De Paula et al. 2016), soils (Vodyanitskii & Kirillova 2016), and fruits (Cáceres 2016). The color changes are similar to the changes perceived by the human eye.

In the CIE 1986 (L*, a*, b*) color space, the lightness coefficient L* ranges from black = 0 to white = 100. The a* and b* coordinates are related to chromaticity, with the a* coordinate expressing the variation between red and green, and the b* coordinate expressing the variation between blue and yellow, located perpendicular to the L* axis (McGuire 1992). As the values of a* and b* increase, the point moves away from the center, and the color saturation increases. Additionally, a* and b* are coordinates that indirectly reflect hue and chroma, but are difficult to interpret separately and are not independent variables (Francis 1980).

Therefore, considering the real need to develop fast, accurate, and cost-effective sampling methodologies, the present study aimed to estimate the number of two-spotted spider mites *T. urticae* in soybeans through CIE-L*a*b* measurements.

Soybean plants of the BRS 7780 IPRO cultivar were maintained in a greenhouse in plastic pots until the V3 stage, when infestations were carried out. The technical aspects of planting and the nutritional needs of the plants were rigorously monitored.

The infestation levels established were 0, 30, 60, and 100 adult female *T. urticae* per plant, with 4 repetitions. Ten days after the initial infestation, chromatic measurements were taken.

To obtain the leaf readings, a portable spectrophotometer (Minolta CM 700d) was used, in the CIE-*L*a*b** color space (CIE 1986). In addition to the *L**, *a**, and *b** variables, the color difference Δ*E** was calculated, as described by Vilhalva et al. (2012). The formula is used to express the color difference in the CIE-*L*a*b** color space, as it represents the three parameters of the CIE-*L*a*b** color space, facilitating comparisons between samples.

During the evaluations, a five-minute time frame was considered for the proper calibration of the equipment. This calibration corresponded to the standard maintained by the equipment. Since the soybean leaf is thin, a matte black surface was used to prevent the contribution of the light reflection transmitting through the leaf. During the measurements, the soybean leaf was positioned between the matte black surface and the measurement orifice of the equipment. The measurements were taken by positioning the equipment on the adaxial side of the leaf, avoiding the central vein and edges. For better classification, the total number of measurements was previously established. 20 measurements were taken on each sample, using the average value of the colorimetric measurements to create the model.

After the measurements were taken, the number of mobile forms of the two-spotted spider mite on the leaves was counted using an optical stereo microscope. The mites were individually counted and removed with the aid of a brush, so that there was no interference in the readings.

The analyses were conducted using R software version 4.2.3 (R Core Team 2008). For regression and correlation analyses, the *ERA* and *hnp* packages were used. For comparison between treatments, the data were subjected to One-Way ANOVA. A model contrast test (post-hoc) was performed to compare means, applying the *mcp* function on the model from the *multcomp* package, which considers differences between the studied factors. Data clustering was performed using the K-means clustering method. For all analyses, a 5% significance level was adopted (P=0.05).

The leaf color distribution graphs were determined and plotted with the *L**, *a**, and *b** coordinates using Origin Pro 2015 software. For model calibration, 70% of the data was used, and for model validation, 30% of the data was used.

The lightness (*L**) ($F_{3,8}$=30.0; P<0.05), red-green (*a**) ($F_{3,8}$=41.9; P<0.05), and color difference (*ΔE**) ($F_{3,8}$=27.1; P<0.05) variables were significant. The yellow-blue variable (*b**) ($F_{3,8}$=2.54; P=0.1301) was not significant (Tab. 1).

Table 1. Means of color parameters in CIE-L*a*b* space and color differences (ΔE*) between infestation levels of Two-spotted Spider Mite.

Initial Infestation levels	Variables			
	*L**	*a**	*b**	*ΔE**
0	36.48a	-9.20a	18.03a	0a
30	37.95a	-8.52b	18.98a	4.25ab
60	44.13b	-7.59c	22.05a	8.97bc
100	48.88c	-6.59c	24.35a	14.30c
Standard deviation	**5.41**	**1.06**	**3.75**	**5.84**
CV (%)	**12.92**	**13.30**	**17.99**	**84.83**

* Averages followed by the same letter vertically do not differ statistically from each other according to the Tukey Test (α=5%).

It is possible to observe that the *L** and *a** variables influence the color characterization of the leaves. They have relatively low standard deviation and coefficient of variation. The red-green variable showed differences only between the treatments with 30 and 60 mites. However, the lightness variable had a greater influence on color differentiation between treatments, making it possible to distinguish between the 30, 60, and 100 mite levels per leaflet. For the color difference means, there were differences only between the treatments with 30 and 100 mites per leaflet.

The regression analyses revealed a significant relationship for the values of lightness, red-green, yellow-blue, and color difference (p-value < 0.05 - Fig. 1). Considering these values, connections were sought between the data matrices (CIE-*L*a*b**) and the reference values (Y - Infestation Levels) to describe the relationship between the two variables.

Equations were generated for the variables of lightness (Eq. 1), red-green (Eq. 2), yellow-blue (Eq. 3), and color difference (Eq. 4). An initial dataset containing about 70% of the samples was used to calibrate the equations (Fig. 1).

Through variance and regression analyses, it was found that the red-green color variable presents the best relationship for estimating the number of mobile forms of mites on soybean leaves. Thus, only one equation for determining the number of mobile forms of mites was validated [Eq. (2)].

To validate the model's predictions, a second dataset, previously separated, containing approximately 30% of the samples, was used to evaluate the equation's performance in predicting unknown samples. Using the validation measurements in the calibrated model, the estimated number of mites was close to the actual measurements.

For better observation of the data, groups were created using clustering techniques (Fig. 2). The potential for grouping infestation levels and red-green values into ranges was evaluated. Infestation levels and red-green values were divided into three ranges. Thus, the ranges presented were obtained (Fig. 2). The predicted number of mobile forms of mites through [Eq. (2)] fell within the values of the groups created (Fig. 2).

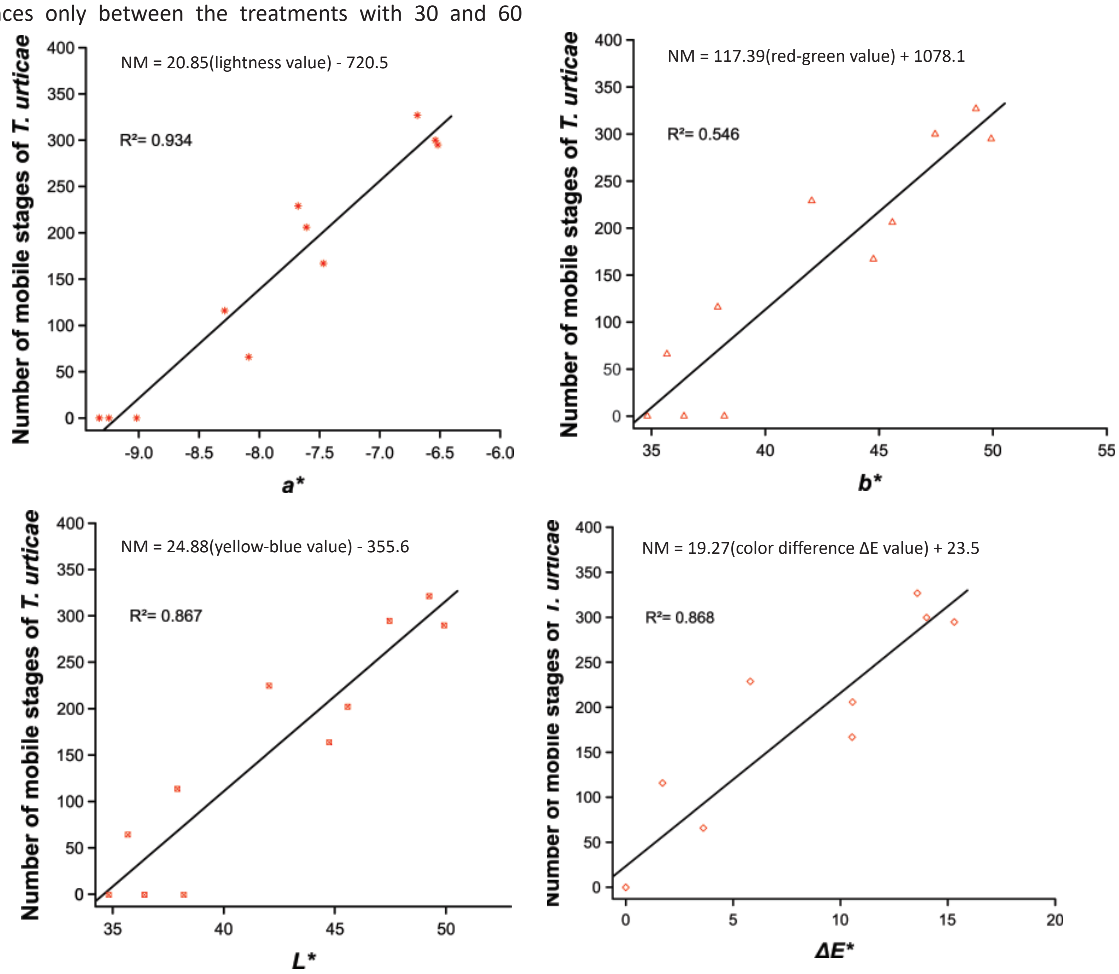

Figure 1. Linear dependencies between luminosity (L*), red-green (a*), yellow-blue (b*) and color difference (ΔE*) for number of mobile forms of Two-spotted Spider Mite. NM= number of mites.

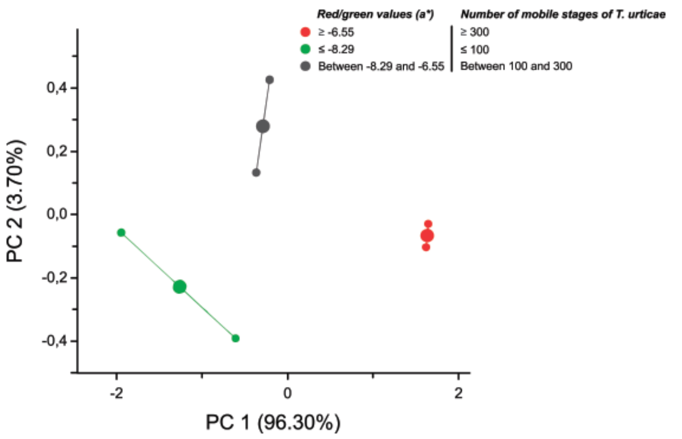

Figure 2. Classification of final infestations of Two-spotted Spider Mite and red-green values in clusters.

The values of the lightness variable in the treatments indicated an increase in leaf lightness with the increase in infestation levels. It was observed that as the number of mites from infestations on the leaves increased, the lightness also increased. Higher leaf lightness is related to various biochemical compounds, such as water, chlorophylls, and carotenoids. Lower concentrations of chlorophyll reduce the plant's use of sunlight (photosynthesis), thus increasing the reflection of light captured by the device (Ustin & Jacquemoud 2020).

Amarante et al. (2009), conducting measurements with a colorimeter on grape leaves, reported that there was an increase in leaf color with the increase in chlorophyll a, b, and total chlorophyll, indicating changes from yellow-green to deep green. The authors also observed that the lightness values decreased with the increase in chlorophyll a, b, and total chlorophyll content in the leaves, indicating a reduction in brightness. The opposite was reported in the present study, where the lightness values increased as infestation levels increased.

The red-green color parameters showed that they are strongly influenced by the density of mites, resulting in differences in the measurements taken. According to Meir et al. (1992), the differences in red-green color values are the result of changes in chlorophyll concentration as the leaves lose their green color. Mites feed on the cellular contents on the abaxial side of the leaves, resulting in a reduction in foliar chlorophyll. As the density of the two-spotted spider mite increases and the feeding period lengthens, the injuries lead to chlorosis in the leaves (Iatrou et al. 1995). The red-green color values became more positive in the later stages of chlorosis.

Various studies have identified color changes in leaves due to stress caused by *T. urticae*, as reported by Fraulo et al. (2009) in strawberries, Lan et al. (2013) in cotton, Luedeling et al. (2009) in peaches, and Herrmann et al. (2012) in peppers. The use of the CIE-*L*a*b* color space offers the possibility to estimate the number of mobile forms of mites by measuring the color changes in the leaves.

Clustering presented satisfactory results. Through clustering, it is possible to obtain homogeneous classifications within the same group. This method makes it easy to classify the number of mites that best represent the red-green color values.

Based on our results, we found that the colorimetric technique using the CIE-*L*a*b* color space is effective in differentiating infestation levels and color changes in leaves. The model used to estimate the number of mites on soybean leaves has the potential to be applied in the field, providing quick and accurate estimates.

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Authors' Contributions

EGS: Conceptualization, Methodology, Formal analysis, Writing - Original draft preparation, Writing - Review and editing; AJSN: Conceptualization, Methodology, Formal analysis, Writing - Original draft preparation, Writing - Review and editing; DCL: Conceptualization, Methodology, Formal analysis, Writing - Original draft preparation, Writing - Review and editing; PTNM: Writing - Original draft preparation, Writing - Review and editing; MAMF: Conceptualization, Writing - Original draft preparation, Writing - Review and editing.

Conflict of Interest Statement

The authors declare no conflict of interest.

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