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Research

Field and Forage Crops

Impact of *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae) on Soybean Yield and Quality Under Field Conditions

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Abstract

Bemisia tabaci MEAM1 (Hemiptera: Aleyrodidae) is a key insect pest in soybean fields in Brazil but data are lacking on the relationship between pest abundance and crop yield and quality. Controlled infestation studies were conducted on caged soybean plants in the field over a two year period at two sites in Brazil. Differences in temperature in the two years affected population growth of *B. tabaci*, reaching 413 nymphs per leaflet in the first year, and 179 the second year even when the average temperature was 3°C higher. Higher temperatures promoted a shorter lifecycle and nearly one more generation. Yield was affected with losses up to 500 kg/ha in 2017/2018 and 1,147 kg/ha in 2018/2019. A decrease in the weight of a thousand grains of 18 and 33 g was observed in the first and second year, respectively. No significant differences were observed in grain germination, but estimated losses in protein content were up to 440 kg/ha at the highest infestation level. Pest density and yield data were used to estimate economic injury levels (EILs). EILs ranged from 2.5 to 25.67 nymphs per leaflet and 0.17–1.79 adults per leaflet over a range of control costs, soybean production values, and control efficacies. These results should provide data useful toward development of pest management decision making tools.

Key words: *Glycine max*, IPM, yield, economic injury level, temperature

Bemisia tabaci Middle East-Asia Minor 1 (MEAM1) (Gennadius, 1889) (Hemiptera: Aleyrodidae) is a widespread pest, known for causing damage to diverse species of cultivated crops like cotton, potato, tomato, and recently gaining more attention in soybean (Oliveira et al. 2001, Baldin et al. 2017). The whitefly *B. tabaci* MEAM1 was first reported in Brazil in 1991 (Lourenção and Nagai 1994), and its importance in soybean noted in the 2000's (Moscardi et al. 2012, Vieira et al. 2016). Three other members of the species complex are present in Brazil, two from the New World (NW1 and NW2) group (Marubayashi et al. 2013) and the Mediterranean (MED) (Barbosa et al. 2015). However, MEAM1 is the predominant species in the country and in open fields, and is present in all soybean production regions. MEAM1 also is the only member of the complex detected in west central Brazil. Although MED species are found in the South and Southeast mainly infesting ornamentals plants and greenhouses (de Moraes et al. 2018, Pozebon et al. 2020), it was found colonizing soybean plants in open fields in São Paulo and Paraná States in Brazil (Bello et al. 2021).

Soybean (*Glycine max* (L.) Merr.) is a major vegetable protein source (USDA 2019) and the fifth most produced crop in the world (FAO 2017). Brazil and United States are the main soybean producers, representing 70% of world production, with an expected production of 139 and 120 million tons respectively in 2022 (USDA 2022). The damage caused by *B. tabaci* MEAM1 include losses of up to 30% in soybean yield (Vieira et al. 2013), with total control costs of US$75/ha and economic losses of more than US$3 billion in Brazil since the establishment of the pest (Pozebon et al. 2020).

Climatic factors play an important role in the performance and development of both crops and pests. Agricultural zoning was established in Brazil to assist farmers in making decisions regarding the optimal sowing time and cultivars best adapted to their region in relation to climatic conditions, such as temperature (MAPA 2020a).

West central Brazil is the main soybean production area in the country and the average annual temperature in the region is one of the factors that makes it an excellent location for this crop. Temperature also influences the insect's lifecycle length. In the case of *B. tabaci*, the lifecycle can vary from 70 d at 15°C to 21 d at 30°C on soybean (Albergaria and Cividanes 2002). These variations in lifecycle length may affect whitefly density, which would in turn have consequences on damage and yield.

Despite the importance of soybean and the relevance of *B. tabaci*, there are no consistent economic injury levels (EILs) established for this pest in the crop. Studies from central Brazil suggested an action threshold of 40 nymphs per leaflet was found (Vieira et al. 2013), while in southern Brazil an EIL of 1.465 combined nymphs and adults per trifoliate was proposed (Padilha et al. 2021). The goal of this study was to evaluate the damage caused by different densities of *B. tabaci* in soybean under field conditions to quantify the population level capable of causing damage to soybean yield and to verify changes caused by pest feeding on plant vigor and grain protein content.

Material and Methods

Insects

The initial *B. tabaci* MEAM1 population was obtained from a colony of the Agronomic Institute of Campinas, Campinas, São Paulo, Brazil in 2017, which was previously identified as *B. tabaci* MEAM1. Molecular characterization of the insects was made periodically during the study, for confirmation of the insect biotype by amplifying individual samples of DNA by polymerase chain reaction using the primer pair Bem23: Bem23F (5′-CGGAGCTTGCGCCTTAGTC-3′) and Bem23R (5′-CGGCTTTATCATAGCTCTCGT-3′) (De Barro et al. 2003). Insects were maintained under greenhouse conditions (approximately 13 hr photoperiod at 29 ± 5°C and 40 ± 10% RH) on cabbage (*Brassica oleracea* L.) at the Dept. of Entomology and Acarology of Luiz de Queiroz College of Agriculture (ESALQ). For infestations, adults were collected by selecting mating pairs to obtain an approximate 1:1 sex ratio (Byrne and Bellows 1991).

Experimental Design

Experiments were conducted using soybean variety BRS 232 (EMBRAPA, Londrina, Brazil) during two seasons, 2017/2018 and 2018/2019, from mid-December to early March. The first study was conducted at an experimental field in the Federal University of São Carlos (UFSCar), Buri, São Paulo, Brazil (W 48°32′59.54″ and S 23°36′28.07″) and the second at an experimental field at ESALQ, Piracicaba, São Paulo, Brazil (W 47°37′24.7″ and S 22°42′55.4″). The fields were tilled and fertilized with nitrogen, phosphorus, and potassium, following standard practices (Malavolta 1992). The soil is classified as dystrophic red-yellow latosol. The experiment was a randomized complete block design with four treatments, consisting of three levels of infestation and a control treatment (no infestation) replicated four times (Table 1). Each plot consisted of a whitefly-proof cage (2.0 × 1.7 m × 1.6 m high) supported by four bamboo stakes (2 m), and enclosing three 50 cm rows with an average of 25 plants spaced at 7 cm intervals.

The number of adults released inside the cages was determined based on previous work to reach an expected population density (Table 1) (Vieira et al. 2013, 2016). All cages were erected when soybean was in the V1 phenological stage (Fehr et al. 1971) and the infestations were made at stage V2. Potted cabbage seedlings in acetate cages enclosed on the top with voile fabric were used to transport insects to the field cages. *B. tabaci* adults were collected from the rearing plants in a greenhouse with an aspirator made with 0.79 cm diameter clear plastic hose, voile fabric, and a 10 ml pipette tip. The specimens were counted and transferred to the acetate cages 24 hr before infestations. In the field, the cage was removed from the pots inside the field cages, leaving the cabbage seedlings exposed and the whiteflies free to disperse.

The infestation level in each plot was evaluated weekly throughout the development of the soybean crop, counting the number of adults present on 10 leaflets from the middle third of the plants. Ten leaflets were collected and evaluated in the laboratory, where the number of eggs and nymphs of *B. tabaci* on each leaflet were counted and the leaflet area was measured using a Leaf Area Meter (LI-3000C, LI-COR Inc., Lincoln, NE). When the expected population density was reached (Table 1) the soybean plants from treatments were sprayed with cyantraniliprole (Benevia) at a dose of 125 g a.i./ha using a backpack sprayer. The cage was large enough to allow a person inside to spray.

Determination of Soybean Yield

At the full maturity stage (R8), the ideal time of harvest, all pods from the plot were collected, stored in paper bags, and dried (up to 13% moisture) using air circulation at 40ºC to evaluate yield (kg/ha) and weight of 1,000 grains (g).

Grain evaluation. These analyses were conducted using the grains harvested from the second study (2018/2019) that were stored in a cooling chamber (20°C and 40% RH) until analysis. The samples were weighed to an average of 22 g of soybean seeds per plot and placed in aluminum containers. Each container and its lid were previously weighed and identified. The samples were then evenly distributed in the containers and placed in the oven on their respective lids and kept at 105°C for 24 hr. After the drying period, the samples were removed from the oven, quickly covered, and placed in a desiccator with silica gel until it cooled and then weighed again, following the recommendations contained in the Rules for Seed Analysis (MAPA 2020b). For each sample, two sub-replicates were performed. Equation 1 was used to calculate the moisture percentage based on the wet weight of the grains.

$$\%\ \text{Moisture}\ (\text{M}) = 100\,(\text{P} - \text{p})\,/\text{P} - \text{p} \qquad (1)$$

Where:

P = initial weight, weight of the container and respective lid plus the weight of the wet sample;

p = final weight, weight of the container and respective lid plus the weight of the dry sample;

Germination Test

For the germination test, two sub-replicates of 50 seeds each were assessed for each plot. Seeds were homogeneously distributed through a 50-cell seed tray on two sheets of paper for seed germination (28 × 38 cm) moistened with an amount of water equivalent to 2.5 times the weight of the substrate. After sowing, the seeds were covered with another sheet of paper under the same conditions.

Table 1. Infestation levels of treatments, corresponding to the number of adult *Bemisia tabaci* released on soybean and expected population density of nymphs per leaflet in field experiments

Treatment	Adults released	Expected population density (nymphs/leaflet)
Control (no infestation)	0	0
Low infestation	300	60
Medium infestation	600	120
High infestation	1,200	240

Subsequently, individual rolls were made for each sub-replicate and then grouped into four rolls joined by elastic at the upper and lower ends. The rolls were then placed in a germination chamber at 25°C, and evaluations were carried out four and seven days after sowing, according to Rules for Seed Analysis (MAPA 2020b). The evaluations consisted of counting the normal germinated seeds, the abnormal germinated seeds, the seedlings, and the ungerminated seeds. Germinated seeds were considered normal when all essential structures such as the hypocotyl and primary root had developed, cotyledons had less than 50% damage, the primary leaves were green, and the apical bud was in expansion. Seeds that had all these structures developed, complete and proportionate were considered healthy. Seeds that did not have such characteristics were considered abnormal. The seeds that did not germinate 4 d after sowing were kept in the rolls for evaluation at 7 d after sowing. The results were expressed as a percentage of normal seedlings in each plot.

Grain Vigor Evaluation

An accelerated aging test was conducted by distributing 50 seeds in plastic germination boxes (11 × 11 × 3.5 cm) on a single layer of wire mesh above 40 ml of water. The boxes were transferred to a Biological Oxygen Incubator at 41°C and maintained for 48 hr, after that the germination test was performed at 25°C, as described above, during 4 d after sowing. After this period the percentage of normal seedlings for each plot was counted. Two sub-replicates were completed for each plot.

Electrical Conductivity

The conductivity test is a measurement of electrolytes leaking from seeds, higher vigor seeds are able to reorganize their membranes more rapidly, reflecting their membrane integrity (Fatonah et al. 2017). Samples were placed in disposable plastic cups with a capacity of 200 ml, containing 75 ml of distilled water. The cups were placed in a Biological Oxygen Incubator at 25°C for 24 hr before conductivity meter readings were performed and a mean electrical conductivity (µs/cm/g) was estimated. Two sub-replicates were performed per plot, each containing 50 weighed seeds.

Grain Protein Content Evaluation

Two sub-replicates of approximately 15 g from each plot were separated and ground in an electric knife mill (IKA mill model: A11BS32) until they acquired the consistency of flour. Samples were then stored individually in a universal collector with a lid. The moisture content was determined by weighing the fresh mass (around 5 g) in a watch glass that was previously dried for 60 min in an oven at 40°C. After weighing, the ground soybeans were placed in a ventilated oven at a temperature of 60°C overnight. After being cooled in a desiccator, they were weighed again until a constant weight was obtained. These measurements were performed with two sub-replicates for each sample. The percentage of humidity (U%) was obtained by Equation 2.

$$\mathrm{U}\ \% \ = (\mathrm{M_f} - \mathrm{M_d}) * 100/\mathrm{M_f} \tag{2}$$

Where:

M_f = initial fresh mass,

M_d = dry mass after drying in an oven.

Crude Protein Content Determination

The crude protein content was determined by the Kjeldhal method (Bataglia et al. 1983). The ground soybeans were weighed and transferred to a test tube where approximately 0.5 g of potassium sulfate catalyst and 3 ml of concentrated sulfuric acid were added. The digestion process started when the temperature of the digesting plate reached a temperature of 350ºC (ca. 12 hr). After cooling the tubes, 2 ml of hydrogen peroxide was added, and the acid solution was reheated at a temperature of 70ºC until the residual hydrogen peroxide evaporated. Nitrogen was distilled in a basic medium with 15 ml of sodium hydroxide at 30%, collecting the distilled ammonia in boric acid solution in the presence of the indicators. The nitrogen content was determined by titrating the samples in boric acid with 0.1 N hydrochloric acid solution until the color change from greenish blue to pink. Equation 3 was used to calculate the protein content.

$$\%\ \text{protein} = \frac{(\mathrm{V_{HCl}} - \mathrm{V_{blank}}) * \mathrm{N_{HCl}} * 14 * 100 * 6.25}{\mathrm{DM_g}} * 1000 \tag{3}$$

Where:

V_{HCl} = volume of HCl solution spent on sample titration;

V_{blank} = volume of HCl solution spent titrating solution with no sample added;

N_{HCl} = normality of HCl;

DM = dry mass.

Statistical Analysis

Data were subjected to exploratory analyzes to verify normality, homogeneity, and suitability to the Shapiro-Wilk (Shapiro and Wilk 1965) and Bartlett (Bartlett 1952) models. Treatments were compared with a mixed model analysis of variance (ANOVA); treatment was a fixed effect and block was random. The Tukey test was used for post-hoc mean separation (SAS Institute 2001). To examine the relationship between yield and insect abundance, mean yield per treatment was regressed on seasonal average nymph or adult density per leaflet per treatment in each year. A combined regression of mean yield per treatment on mean insect density per treatment was performed pooling both years.

Economic Injury Levels

Stern et al. (1959) conceptualized the EIL and Norton (1976) and Pedigo (1986) formalized equations for its estimation. Based on relationships between nymphs and adults per leaflet and soybean yield, we used the method of Norton (1976) to estimate the EIL as:

$$EIL = \frac{C}{(V \times D \times K)} \tag{4}$$

Where:

EIL = Economic injury level (nymphs or adults of *B. tabaci* per leaflet);

C = control cost ($/ha);

V = soybean crop market value ($/kg);

D = soybean yield loss per unit of pest (kg/ha/insect);

K = efficacy of control (proportion);

D was estimated as the slope coefficient from regression models described above. A range of control costs ($10–40/ha) were estimated from IMEA (2022) and EMBRAPA (2021). Probably production values for soybeans (300–600 $/mton) were estimated from EMBRAPA (2021) and CEPEA (2022) and control efficacy values of 0.7 and 0.9 were used to calculate EIL for nymphs, adults, and both life stages combined.

Results

Pest Population Dynamics

In the first season in Buri, all stages of *B. tabaci* occurred at roughly twice the density compared with the second season in Piracicaba (Fig. 1) despite equal levels of initial infestation. The initial egg density was similar in both years, but dropped in the first weeks and rapidly increased at the beginning of the soybean reproductive stage (R1–R2) (Fig. 1). Nymph density increased during soybean reproductive stages with the highest densities occurring during R5 in both years (Fig. 1). In both years, densities declined in association with the end of the crop cycle, when fewer leaves were available. The adults of *B. tabaci* that initially infested the cages laid the first eggs in the soybean and remained in the crop for the first weeks, with some present until the emergence of a new generation of adults after

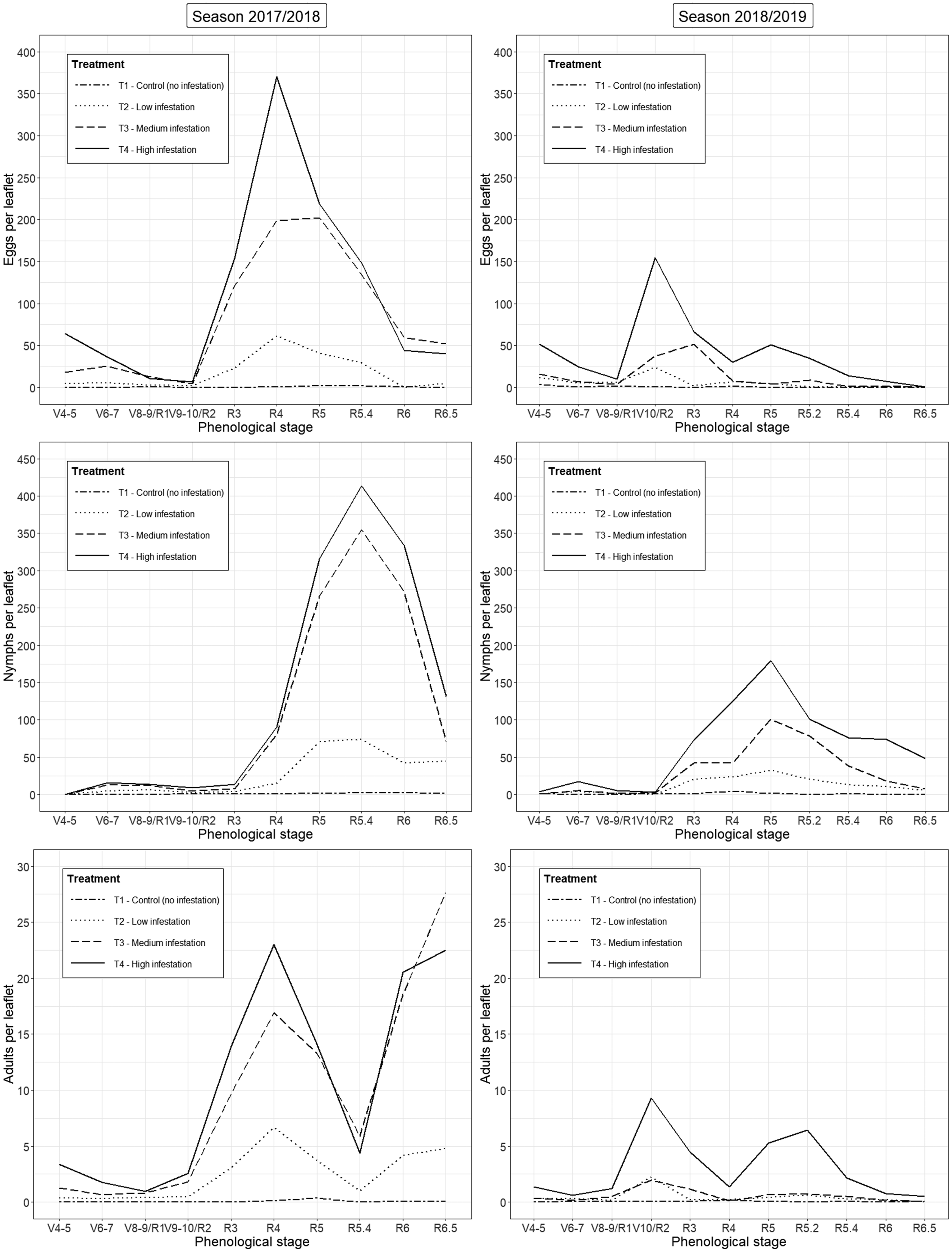

Fig. 1. *Bemisia tabaci* eggs, nymphs, and adults per leaflet over the phenological stages of soybean in two crop seasons.

3–4 wk (Fig. 1). We observed similar patterns of *B. tabaci* population dynamics among infestation treatments but densities increased as expected with increasing levels of initial infestation (Fig. 1).

Despite the higher insect densities observed in the first crop season (Fig. 1), higher temperatures were observed in the second crop season (Fig. 2). Based on degree-days for development (472.6°C) and base temperature (8.3°C; egg-adult) from Albergaria and Cividanes (2002), accumulated degree-days in 2018/2019 (1315.7°C) were 350 higher than in 2017/2018 (966.6°C), resulting in nearly one more *B. tabaci* generation (2.8 and 2.0 generations, respectively).

Determination of Damage and Yield

Yields different greatly between the two years of study. In 2017/2018, the mean yield in the control treatment was 1,947 kg/ha, but in 2018/2019 the control yield was nearly twice as high at 3,787 kg/ha(Fig. 3). A similar differential also was observed in mean weight of 1000 grains for the control treatment between years (Fig. 3). Both yield [2017/2018 ($F_{(3,\ 12)}$ = 3.89, p = 0.05) and 2018/2019 ($F_{(3,\ 12)}$ = 4.79, p = 0.03)] and the weight of 1,000 grains [2017/2018 ($F_{(3,\ 12)}$ = 3.52, p = 0.05) and 2018/2019 ($F_{(3,\ 12)}$ = 16.19, p = <0.001)] were significantly depressed in the highest infestation treatment compared with the control in both years.

There were negative relationships between yield and insect density in both years (Fig. 4). Slightly more of the variation in yield could be explained by insect density in 2017/2018 [nymphs ($F_{(1,\ 3)}$ = 37.30, p = 0.02), adults ($F_{(1,\ 3)}$ = 22.66, p = 0.04) and combined life stages ($F_{(1,\ 3)}$ = 35.81, p = 0.03)] compared with 2018/2019 [nymphs ($F_{(1,\ 3)}$ = 12.18, p = 0.07), adults ($F_{(1,\ 3)}$ = 4.05, p = 0.18) and combined stages ($F_{(1,\ 3)}$ = 11.63, p = 0.08)]. A general relationship between yield and *B. tabaci* density was estimated by combining the data over both years [nymphs ($F_{(1,\ 3)}$ = 25.01, p = 0.04), adults ($F_{(1,\ 3)}$ = 26.43, p = 0.04) and combined stages ($F_{(1,\ 3)}$ = 25.14, p = 0.04)].

Grain Quality and Vigor Evaluation

There were no significant differences in the quality and vigor among infestation treatments for moisture content ($F_{(3,\ 12)}$ = 0.1505, p = 0.9268), germination ($F_{(3,\ 12)}$ = 0.9831, p = 0.4431), accelerated aging ($F_{(3,\ 12)}$ = 0.5804, p = 0.6425) or electrical conductivity ($F_{(3,\ 12)}$ = 2.8611, p = 0.0966; Table 2).

Grain Protein Content

No differences in the protein content in grains was observed among treatments ($F_{(3,\ 12)}$ = 1.759, p = 0.2247; Table 3). However, there were significant difference among estimated total amounts of protein per hectare ($F_{(3,\ 12)}$ = 6.380, p = 0.013; Table 3). The highest infestation level resulted in a mean loss of 440 kg of protein per hectare compared with the uninfested control.

EIL Estimates

Regression analyses of data combined over both years showed that yield was reduced by 7.42 and 106.13 kg/ha for each nymph and adult per leaflet, respectively. Without regard to life stage, the yield reduction was 6.94 kg/ha per insect. Based on a range of realistic estimates of control cost, production value and control efficacy, EILs varied considerably for both life stage individually and combined (Table 4). As expected, EILs were lower when control costs were low and/or when control efficacy and production value were high. Values ranged from 2.5–25.67 for nymphs, 0.17–1.79 for adults and 2.67–27.45 when insect counts are made without respect to life stage.

Discussion

Soybean is a suitable reproductive host for *B. tabaci* (Musa and Ren 2005). The increase in the area planted with soybean is credited with being one of the main factors driving the development of large populations of *B. tabaci* in Brazil (Costa 1975, Morales 2006), ultimately leading to increasing impacts on crop production observed decades later (Vieira et al. 2011, Bortolotto et al. 2015). Our results show that the insect can have negative impacts on yield and seed size that increase proportionally with density. However, measures of quality, such as moisture content, germination rate, electrical conductivity, and protein content of seeds are generally unaffected. Soybean plants were able to compensate for the loss of production capacity by reducing the weight of the grains but maintaining the qualitative characteristics evaluated. Isaacs et al. (1998) showed that *B. tabaci* feeding tends to concentrate the plants' nutrients, thereby maintaining a similar total nutritional content by reducing water, resulting in smaller grains. However, at the field scale, overall protein content can be negatively affected by high pest infestations through reduced yields.

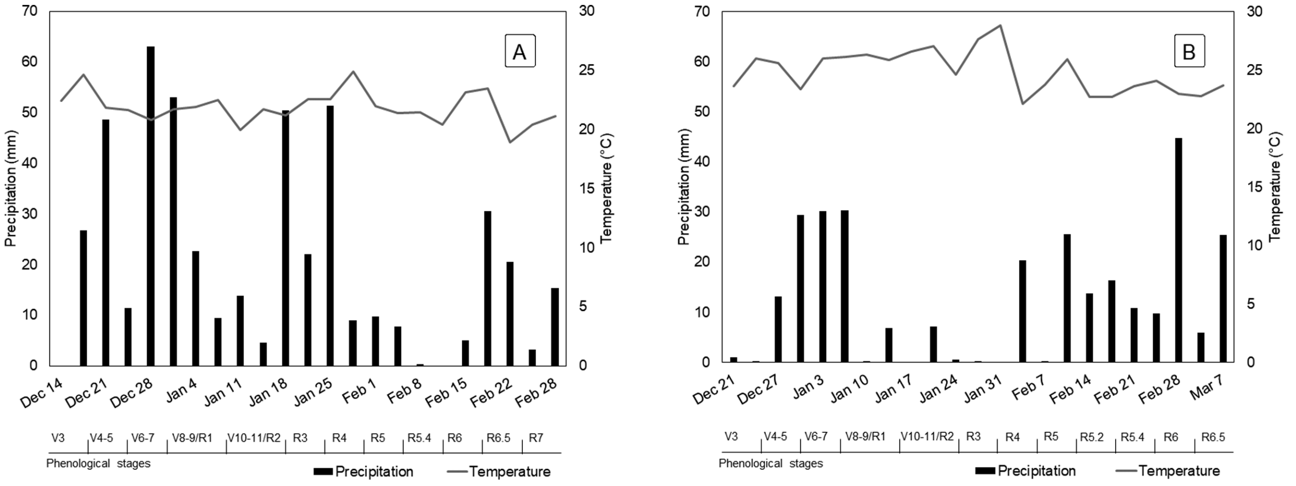

Fig. 2. Precipitation (mm) and average temperature (°C) in the municipality of Buri, São Paulo, Brazil, during the crop season 2017/2018 (A) and Piracicaba, São Paulo, Brazil, during the crop season 2018/2019 (B).

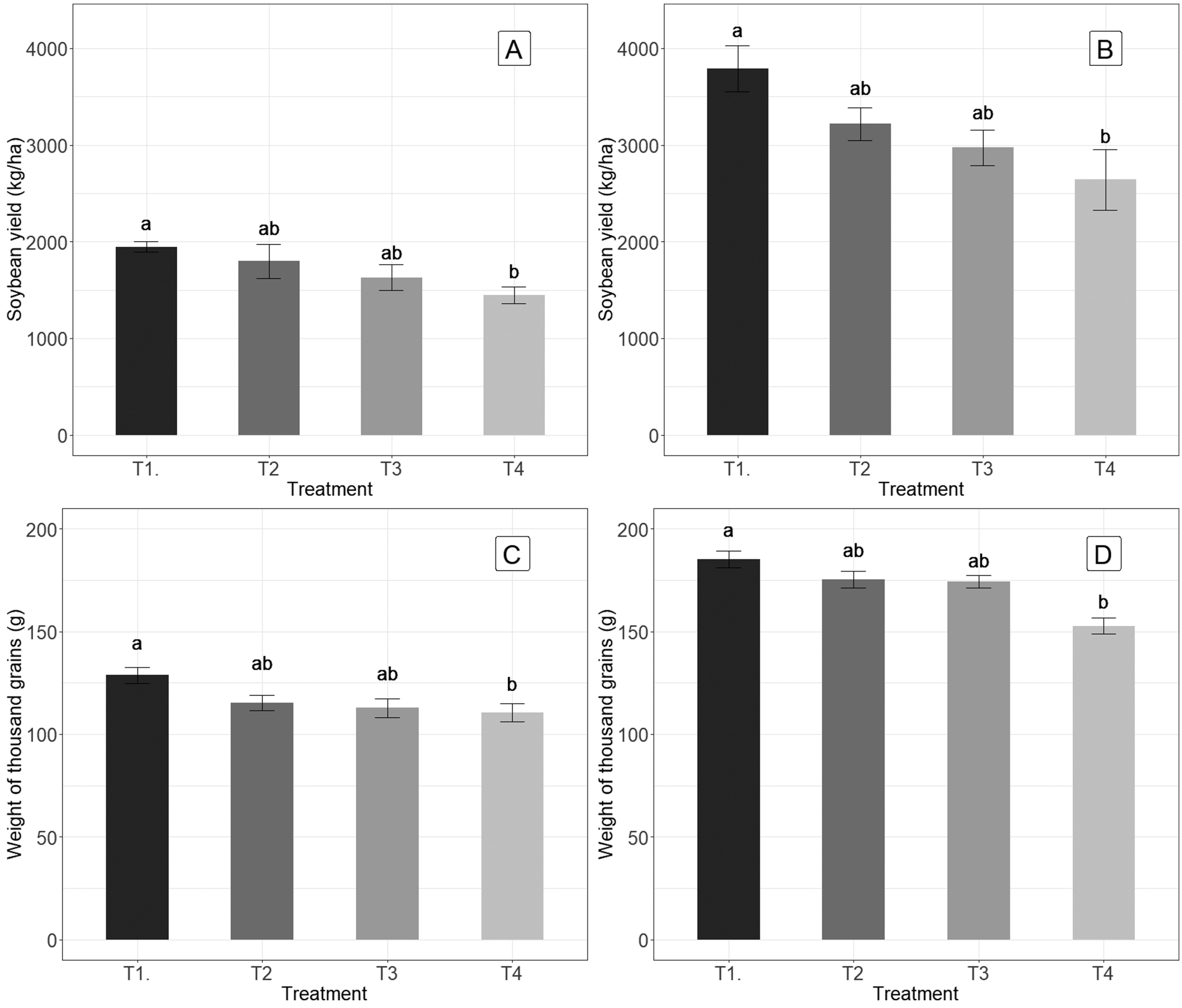

Fig. 3. Yield of soybean (kg/ha) and mean weight of a thousand grains of soybean (g) exposed to different *B. tabaci* infestation levels (T1 control, no infestation, T2 low, T3 medium, and T4 high infestation) in two crop seasons; A and C, 2017/2018; B and D 2018/2019. Error bars are standard error ($p < 0.05$, Tukey test).

The initial number of eggs in the first weeks were similar in both years, but the abrupt decline in adult density, likely caused by the early rain in the 2019 soybean cycle, led to lower numbers of eggs; the rain fell later in the crop cycle of 2018. Heavy rain at the end of January in the Buri region in 2018 (INMET 2022) and in Piracicaba in 2019 (LEB 2020) likely caused a decline in the number of ovipositing adults present on the leaves. The immobile egg and nymph stages did not appear to be affected to the same degree. Previous studies have shown a decrease in *B. tabaci* populations after rainfall and/or wind in the field for soybean (Lima et al. 2002), cotton (Naranjo and Ellsworth 2005, Kataria et al., 2019), tomato (Chaudhuri et al. 2001), and chili (Chaubey and Mishra 2018). Prolonged periods of rainfall also can be an adverse factor in the survival of the eggs and nymphs (Villas Bôas et al. 1997).

Temperatures around 30°C are optimal for *B. tabaci* development and the insect can complete its life cycle within about 21 d with a survival rate of 90% at this temperature in the laboratory (Albergaria and Cividanes 2002). In the state of São Paulo, where the trials were conducted, the annual maximum temperature ranges from 27 to 31°C (INMET 2022). We observed differences in the two crop seasons in terms of production and the density of *B. tabaci*. In the first year, lower average temperatures (~22°C) were observed during the period of the trial, while in the second year, the average temperature was about 3°C higher (~25°C). This differential in temperature was large enough to result in almost an entire additional generation of the pest. Nonetheless, the densities we measured were lower in the second year suggesting that perhaps other environmental factors led to higher mortality rates in the insect population. In any case, yields were higher in the second year, reflecting a smaller amount of damage from the smaller overall pest population.

Temperature-dependent laboratory studies on soybean (Albergaria and Cividanes 2002) supports the timing of the density of adults observed in this study. In the first crop season, the highest number of adults occurred at the sixth week of evaluation, 42 d after infestation. While in the second year, the population peaked 28 d after infestation, the peak of *B. tabaci* population occurred during the reproductive stage of soybean in both years, R5.4 (2017/2018) and R5 (2018/2019). Previous studies also have documented peak densities occurring during these plant stages (Vieira et al. 2013, Czepak et al. 2018). The reproductive stage of the crop is the most susceptible to *B. tabaci* attack, and critical to grain filling and yield determination. Here, the *B. tabaci* peaks were mostly observed between R1 and R5 in the second season where yields were higher, but where we also observed the largest reductions in yield compared

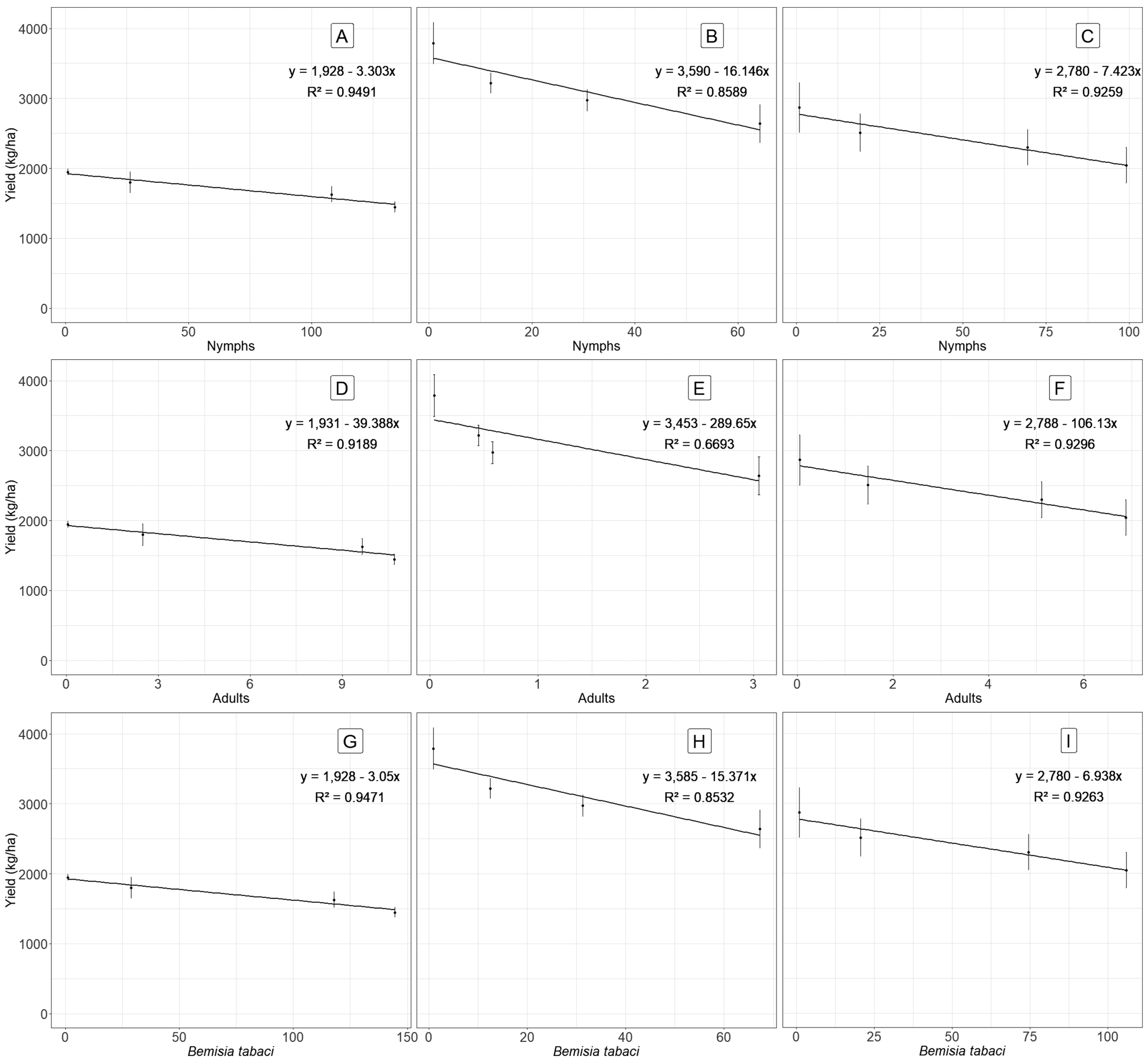

Fig. 4. Regression of mean yield on mean number of *Bemisia tabaci* per leaflet per treatment in each year and for years combined; (A) nymphs in 2017/2018, (B) nymphs in 2018/2019, (C) nymphs in combined years; (D) adults in 2017/2018, (E) adults in 2018/2019, (F) adults in combined years; (G) both life stages 2017/2018, (H) both life stages 2018/2019, (I) both life stages, years combined.

Table 2. Quality and vigor of soybean grains from plants exposed to *Bemisia tabaci* infestation (Mean ± S.E.)

Treatment	Moisture content (%)	Germination (%)	Germ. Accelerated Aging (%)	Electrical conductivity (µs/g/cm)
Control (no infestation)	7.3[a] ± 0.34	98.25[a] ± 0.90	96.75[a] ± 0.16	82.8[a] ± 2.8
Low infestation	7.3[a] ± 0.34	99.00[a] ± 0.90	97.25[a] ± 0.16	86.8[a] ± 2.8
Medium infestation	7.1[a] ± 0.34	97.00[a] ± 0.90	98.00[a] ± 0.16	78.9[a] ± 2.8
High infestation	7.1[a] ± 0.34	98.00[a] ± 0.90	95.00[a] ± 0.16	75.8[a] ± 2.8

[a] Means followed by the same letter within a column are not significantly different (p > 0.05, Tukey test).

with the uninfested control. A similar pattern was reported by Padilha et al. (2021).

Here, we found a yield difference of 500 kg/ha (average of 133.75 nymphs per leaflet) in the first crop season and 1,147 kg/ha (64.28 nymphs per leaflet) in the second, and a reduction of 18 and 32.5 g, respectively, in the weight of 1,000 grains between the control and the high infestation treatments. In a study conducted in Goiás, a difference of 1,505 kg/ha (58.28 nymphs per leaflet) between control (4,992 kg/ha) and weekly sprayed (3,486 kg/ha) treatments was obtained in soybean yield when exposed to *B. tabaci* (Vieira et al. 2013). That reduction was 30% higher than found here and, although the weight of the grains was not affected in that trial, the average number of whiteflies was around 10% lower than observed here. This suggests that the higher temperatures in Goiás intensified the damage caused by *B. tabaci*, perhaps due to a greater potential for population growth.

The higher the soybean yield, the more sensitive soybean plants appear to be to *B. tabaci*. In both years, a high density of nymphs was observed during the seed fill stage (R5–R6), considered the most critical for soybean seed evaluation due to the impact of the movement of nutrients from leaves to seeds (Bellaloui 2013). To maintain the nutritional intake, *B. tabaci* actively controls the feeding rate, and thus, their developmental rate (Isaacs et al. 1998). In the second year, when a larger yield was observed even with a lower density of nymphs, the pest's impact on yield was greater. One hypothesis is that the competition between *B. tabaci* feeding and the movement of nutrients to fill the seeds reduced the concentration of nutrients in the leaves. Thus, increased feeding rates by nymphs, resulting in fewer nutrients available for the plant to produce grain.

Table 3. Protein content of soybean seeds and total protein in soybean yield from plants exposed to *Bemisia tabaci* infestation (Mean ± S.E.)

Treatment	Grain protein content (%)	Total protein (kg) in soybean yield
Control (no infestation)	$36.11^{a} \pm 0.44$	$1365.96^{a} \pm 73.01$
Low infestation	$34.85^{a} \pm 0.44$	$1120.22^{ab} \pm 73.01$
Medium infestation	$35.58^{a} \pm 0.44$	$1057.43^{ab} \pm 73.01$
high infestation	$34.58^{a} \pm 0.44$	$925.92^{b} \pm 73.01$

Means followed by different letters within a column are significantly different ($p < 0.05$, Tukey test).

Accurate relationships between pest densities and their consequent impact on yield loss are essential for the calculation of the economic injury level (EIL). Under our experimental conditions and with reasonable estimates of control costs of US$20/ha, a crop value of US$500/ton, and 90% control efficiency we estimated an EIL of 5.99 *B. tabaci* nymphs per soybean leaflet (Table 4). For adults and both stages combined, the EIL was 0.42 and 6.41 insects per leaflet under these same conditions (Table 4). Padilha et al. (2021) estimated an EIL of 1.33 *B. tabaci* (adults plus nymphs) per trifoliate with a control cost of US$20/ha, a crop value of US$500/ton, and 90% control efficacy. Converting to density per leaflet, their EIL for combined life stages would be 0.44 insects, nearly 15 times lower than our estimates. In contrast, Vieira et al. (2013) recommended not spraying the soybean until reaching 40 nymphs per leaflet. However, this value is an action threshold not derived from a basic understanding of the effects of control costs, production value and control efficacy, and so direct comparisons are not possible. To determine reliable economic thresholds based on our EIL estimates would require additional research into the dynamics of the pest in open field conditions in our region without the caging restrictions we imposed in our studies.

The EILs discussed here were calculated for different regions and biomes of Brazil, and with different cultivars and climatic conditions. Padilha et al. (2021) conducted their trials in Santa Maria, Rio Grande do Sul, in southern Brazil where the average minimum and maximum temperatures were respectively 18.4 and 29°C in 2019, and 16.5 and 29.9°C in 2020, and rainfall 424,4 mm and

Table 4. Economic injury levels for *B. tabaci* per soybean leaflet in relation to control costs, crop value, and control efficacy based on combined studies from two years

Control Cost ($/ha)	Efficacy	Crop Value ($/mton)	Nymphs	Adults	Nymphs+Adults
10	0.7	300	6.42	0.45	6.86
10	0.9	300	4.99	0.35	5.34
10	0.7	400	4.81	0.34	5.15
10	0.9	400	3.74	0.26	4.00
10	0.7	500	3.85	0.27	4.12
10	0.9	500	2.99	0.21	3.20
10	0.7	600	3.21	0.22	3.43
10	0.9	600	2.50	0.17	2.67
20	0.7	300	12.84	0.90	13.73
20	0.9	300	9.98	0.70	10.68
20	0.7	400	9.63	0.67	10.30
20	0.9	400	7.49	0.52	8.01
20	0.7	500	7.70	0.54	8.24
20	0.9	500	5.99	0.42	6.41
20	0.7	600	6.42	0.45	6.86
20	0.9	600	4.99	0.35	5.34
30	0.7	300	19.25	1.35	20.59
30	0.9	300	14.97	1.05	16.01
30	0.7	400	14.44	1.01	15.44
30	0.9	400	11.23	0.79	12.01
30	0.7	500	11.55	0.81	12.35
30	0.9	500	8.98	0.63	9.61
30	0.7	600	9.63	0.67	10.30
30	0.9	600	7.49	0.52	8.01
40	0.7	300	25.67	1.79	27.45
40	0.9	300	19.97	1.40	21.35
40	0.7	400	19.25	1.35	20.59
40	0.9	400	14.97	1.05	16.01
40	0.7	500	15.40	1.08	16.47
40	0.9	500	11.98	0.84	12.81
40	0.7	600	12.84	0.90	13.73
40	0.9	600	9.98	0.70	10.68

126 mm respectively, over the seasons (INMET 2022). The Vieira et al. (2013) study was conducted in Paraúna, Goiás with minimum and maximum temperatures of 20.3 and 30.8°C, respectively, and precipitation of 821.8 mm, less than of that expected in Santa Maria. In our region, precipitation was 473.8 mm in 2017/18 and 325.8 mm in 2018/19, with a minimum and maximum temperatures ranging from 21.3–24.2°C and 22.5–25.6°C, respectively, over the two years. Higher temperatures and lower humidity favor the development of *B. tabaci* (Sharma et al. 2013), which could influence the variable estimates of EIL and control thresholds so far developed. Overall, the available data suggests that EILs and eventual associated thresholds might need to be region specific. EILs also will, of course, vary depending on control costs, production values, and control efficacy and these factors would need to be considered in developing IPM strategies.

The lack of an EIL has led soybean growers to spray insecticides indiscriminately in their fields (Bortolotto et al. 2015), likely leading to higher production costs and the potential acceleration of new cases of insecticide resistance in *B. tabaci* populations. For some crops, such as tomato and common bean, where *B. tabaci* plays an important role as vectors of disease, EILs and associated thresholds are generally not likely to be useful due to the very low density of pests able to transmit such diseases (Pedigo et al. 1986). Currently, outside of occasional instances of stem necrosis in soybean, disease transmission is not a major issue in pest control decision-making. Much higher densities of the pest can be tolerated before control actions are needed, thus, reducing production costs and preserving the effectiveness of insecticides available in the market.

B. tabaci has recently become a primary pest to soybean fields in Brazil, and there are challenges for establishing effective and efficient IPM programs. Our results provide foundational information that will aid the eventual development of decision-making strategies for management of *B. tabaci* in Brazilian soybean systems. The results here also indicate that the densities of *B. tabaci* capable of inflicting economic damage to soybean yield may depend on the climatic factors, such as rainfall and temperature, that directly affect pest population development. While high densities of *B. tabaci* can cause yield losses in soybean fields, evidence suggests that various quality parameters may be unaffected outside of some effects on the protein content of overall yields.

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