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Control of thrips (*Enneothrips flavens* Moulton.) with synthetic and biological insecticides in different peanut genotypes

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Abstract

In peanut, pests are controlled by application of insecticides throughout the crop cycle, boosting production costs. This study compared the control of thrips (*Enneotrhips flavens* Moulton) in 10 peanut genotypes by biological and synthetic insecticides in the field in tropical area in Southeastern Brazil (21°13'29.9''S 48°54'33.0''W). The experiment was arranged in a randomized block, 10 x 3 factorial design with three replications. The first factor corresponded to 10 peanut genotypes and the second to different insecticides (synthetic, biological and control). The plants were evaluated 36, 44, 51, 59, 65, 72, and 79 days after sowing to determine the number of thrips individuals and the damage on leaflets caused by *E. flavens*. The plant height (cm), branch length (cm) and pod yield (kg ha-1) were also determined. The data were subjected to analysis of variance and averages compared by Tukey's test. In prior evaluation carried out before the start of the applications of insecticides (25 DAS), natural infestation of thrips was observed in all genotypes. The genetic variability of lines L. 386 and L. 314 and cultivar IAC 113 was not significant, whereas yield difference was not observed among treatments of insecticides and control, showing tolerance to *E. flavens.* The synthetic insecticide Engeo PlenoTM showed of 39% higher efficiency (as average) for controlling thrips in peanut. The insecticides *Beauveria bassiana* (Bals.) (AUIN®) and *Metarhizium anisopliae* (Metch.) (GR-INN®) have potential to control *E. flavens* in peanut, but further studies are needed to better define the application date and products doses.

Keywords: *Arachis hypogaea* L.; biological control; chemical control; plant resistance; thrips.
Abbreviations: a.i._active ingredient; L_liters; kg_kilograms; m_metros; m²_square meters; g_grams; ha_hectares; DAS_days after sowing; L._line; IAC_Agronomic Institute of Campinas.

Introduction

The peanut (*Arachis hypogaea* L.) is considered the fourth largest oilseed crop after soybean, rapeseed and sunflower (USDA, 2018). Grown in tropical and subtropical regions, there is great demand for peanuts and peanut products based on the international market, especially for the confectionery industry (Varshney et al., 2017). Currently, world production is around 44 million tons, with China and India the main growers. In Argentina and Brazil it accounts for 1.07 and 0.53 million tons, respectively (USDA, 2018). Of this total, 19.5 to 62.7% can be lost annually due to damage caused by *Enneothrips flavens* Moulton (Thysanoptera: Thripidae), the main pest of crop (Moraes et al., 2005). This pest causes widespread damage due to its broad occurrence and high population levels (Lourenção et al., 2007). Symptoms of Thrips attack are visible after opening the leaflets, when the leaves show deformation, curling and silvering. This damage impairs light absorption by the plant, reducing photosynthesis and, consequently, the development and production of peanut (Almeida and Arruda, 1962). In addition, the physical damage of thrips to leaves makes plant susceptible to attack by other phytopathogens, causing diseases commonly called late leaf spot [caused by *Cercosporidium personatum* (Berk. & Curt.) Deighton], early leaf spot (caused by *Cercospora arachidicola* Hori), which according to are considered the main diseases of peanut and can cause yield losses of 10% to 70% (Clevenger et al., 2018: Ramesh and Zacharia, 2017). Also, some species of thrips are vectors of viruses such as *Tomato spotted wilt orthotospovirus* (TSWV) and *Groundnut ringspot orthotospovirus* (GRSV), recently reported by Godoy et al. (2017) and Camelo-García et al. (2014) causing damage in areas of peanut production in Brazil. To date, there are no reports of *E. flavens* as virus transmitter. However, there is a hypothesis that it can potentially be a possible carriers. Thus, the peanut must be protected from pests to ensure maximum yields.

Currently, control strategies for this insect are predominantly based on systematic applications of synthetic insecticides, to maintain the pest population at tolerable levels. In addition, the restricted number of available insecticides with different modes of action makes the pest management inefficient and the use of non-selective and often unregistered insecticides aggravates problems related to environmental waste and contamination (Whalon et al., 2008). In addition, the constant use of insecticides can lead to the emergence of insects resistant to insecticides. In peanuts, there are no reports of insects resistant to insecticides. However, we need a sustainable way to minimize the use of insecticides in agriculture. For this, an integrated pest management (IPM) to control the pest has been proposed, integrating other control tools such as plant resistance, cultural practices and the use of biological insecticides.

According to Godoy et al. (1999), the use of cultivars with *E. flavens* resistance could induce additional yield gains or significantly decrease production costs, for leading to the disuse or reduction of chemical control. In the field, researchers observed moderate resistance to this thrips in some cultivars (Boiça Junior et al., 2012; Moraes et al., 2005), although the impact of this advantage with regard to a possible reduction in insecticide applications has not been experimentally evaluated.

Another alternative to circumvent the chemical control of insect pests is the use of entomopathogenic fungi. Although several studies demonstrate the effectiveness of fungi for thrips control in different crops (Cavalcanti et al., 2008; Ganga Visalakshy and Krishnamoorthy, 2012; Wraight et al., 2016), so far no studies have addressed the efficiency of entomopathogenic fungi for *E. flavens* control on peanut.

Therefore, the use of entomopathogenic in the peanut crop can be a viable alternative to control thrips species. Thus, this study evaluated (1) the interaction between insecticides and peanut genotypes resistance to *E. flavens*, comparing the association of two biological insecticides with a synthetic insecticide, applied to different peanut genotypes, (2) to evaluate the efficiency of insecticides and to select resistant genotypes.

Results and Discussion

Number of thrips and visual damage symptoms of E. flavens on leaflets of 10 peanut genotypes

In an initial evaluation which carried out before the start of the applications of insecticides (25 DAS), natural infestation of thrips was observed in all genotypes (Table 1). However, we observed significant differences between genotypes, highlighting Runner IAC 886 cultivar as more infested (susceptible) and L.318 and L.322 lines as less (tolerant) (Table 1).

Considering the mean of 10 genotypes, all evaluations differed significantly between the insecticides, both for number of thrips and visual damage symptoms (Fig 1).

Insect infestations were most intense mainly in the evaluations 59 and 72 DAS. For the damage symptoms, the grades were highest in the evaluations 36, 65, 72, and 79 DAS. In an evaluation of six peanut cultivars, Lourenção et al. (2007) observed a severe *E. flavens* infestation at 56 DAS, with a decreasing trend until the end of the crop cycle.

These fluctuations in the *E. flavens* population may be associated with the planting seasons, region and climate. Climatic factors such as dry spells can benefit the insect, while rainy periods can reduce its population by mechanical action (soaking and drowning of individuals) and by ensuring favorable moisture levels for the activity of micro-organisms that kill these insects (Michereff Filho et al., 2012). According to Gallo et al. (2002), the critical period of thrips infestation for peanut is between 25 to 60 days after planting.

In the mean of the genotypes, the use of synthetic insecticides reduced the number of thrips and grades of visual symptoms, confirming results of Calore et al. (2012), who found that applying thiamethoxam + lambda-cyhalothrin insecticide was highly efficient to control *E. flavens*, resulting in an increase in peanut production. The biological insecticides, AUIN® and GR-INN®, provided an intermediate level of control.

When analyzed separately, high thrips infestation was observed in all genotypes in control treatment in at least one evaluation, with peaks of more than 10 thrips per 10 leaflets (Fig 2). In the synthetic insecticide treatments (Engeo Pleno™), infestation was always below or close to 2 thrips per 10 leaflet.

Application of biological insecticides treatment showed no significant difference in population/number of thrips compared to the use of synthetic insecticide treatment which was detected in at least one of the evaluations in all genotypes (for L. 318 at 36, 51, 59, 65 and 72 DAS; L. 322 at 59 and 72 DAS; for L. 389 at 59 and 65 DAS; L. 386 at 51 and 59 DAS; L. 383 at 59 DAS; L. 314 at 59 DAS; IAC Caiapó at 59, 65 and 79 DAS; Runner IAC 886 at 44, 59, 65, and 79 DAS; IAC 113 at 51, 59, 65, and 79 DAS; and IAC 503 at 59 DAS).

Interestingly, in all genotypes, the biological insecticides was as efficient as synthetic insecticide at 59 DAS evaluation. In onion, Maniania et al. (2003) observed higher efficiency of control of *Thrips tabaci* Lind. using entomogenous fungus *Metarhizium anisopliae*, compared to synthetic insecticides (dimethoate). Moreover, these authors reported a higher number of non-target insects in the plots, where *M. anisopliae* was applied, demonstrating the advantages of using biological control methods.

The L.386 line presented the largest number of thrips in the control treatment. The smallest number of thrips was observed on IAC 113, IAC 886 Runner and IAC Caiapó cultivars and L.318 and L.314 lines using the biological insecticides (Table 2).

In contrast to the synthetic insecticide, the biological insecticides (AUIN ® and GR-INN ®) showed a reduction of 26.4% in population of thrips compared to the control. The synthetic insecticide provided 87.1% reduction relative to the control (Table 2). However, for the biological insecticides, this percentage varied from 0 to 59.0%, evidencing that variability of genotypes which were efficiency influenced.

For the visual symptom scores of thrips damage, the pattern observed was the same as for number of thrips, but at a lower intensity. The biological insecticides, AUIN® and GR-INN®, did not differ from the synthetic insecticide treatment in some evaluations for the genotypes L.318 at 36, 44 and 59 DAS; L. 322 at 44 DAS; L.389 at 79 DAS; L.383 at 36, 59 and 65 DAS; L.314 at 36 and 44 DAS; IAC Caiapó at 36 and 44 DAS; Runner IAC 886 at 36 DAS; and IAC 113 at 59 DAS) (Fig 3).

The infestation of *E. flavens* nymphs and adults has an

Table 1. Number of thrips of *E. flavens* on leaflets, in 10 peanuts genotype before the start of the applications of insecticides (25 DAS). Pindorama, state of São Paulo, Brazil, growing season 2014/15.

	Numbers of thrips
Genotypes (G)	6.38**
Runner IAC 886	8.0 a
L.314	7.5 ab
IAC 503	6.8 ab
IAC 113	6.3 abc
L.386	6.2 abc
L.389	5.5 abc
IAC Caiapó	5.0 bc
L.322	4.3 c
L.318	4.1 c
Insecticides (I)	0.68^{ns}
Synthetic	5.8 a
Biological	5.5 a
Control	6.1 a
G x I	1.37^{ns}
CV(%)	10.89

Mean values followed by the same letter in the column do not differ statistically by the Tukey test (p< 0.05). ** , * significant at the 0.01 and 0.05 level, respectively.

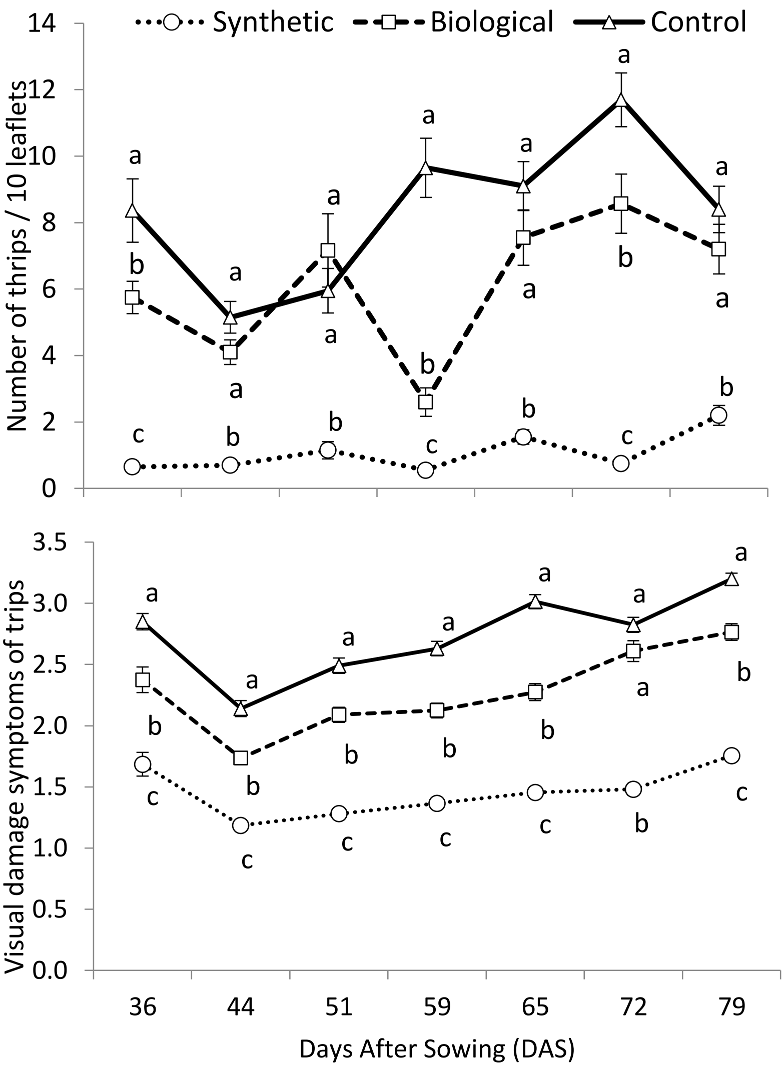

Fig 1. Number of thrips and visual damage symptoms of *E. flavens* on leaflets (mean of 10 genotypes), in seven evaluations of plants after application of synthetic and biological insecticides and control (without application). Pindorama, state of São Paulo, Brazil, growing season 2014/15. *Same letter at each evaluation date do not differ by the Tukey test (p > 0.05). Vertical bars correspond to standard error.*

Table 2. Total thrips (nymphs + adults) in 10 peanut genotypes, after application of synthetic and biological insecticides and control (without application). Pindorama, state of São Paulo, Brazil, growing season 2014/15.

Genotypes	Number of thrips										
	Control			Biological			(%R)	Synthetic			(%R)
L. 318	58.5	b	A	24.0	d	B	(59.0)	6.5	a	C	(88.9)
L. 322	57.5	b	A	59.2	ab	A	(0)	6.5	a	B	(88.7)
L. 389	50.5	b	A	45.8	bc	A	(9.2)	9.5	a	B	(83.2)
L. 386	81.0	a	A	49.0	bc	B	(39.5)	9.0	a	C	(88.9)
L. 383	56.0	b	A	66.8	a	A	(0)	13.0	a	B	(76.8)
L. 314	58.7	b	A	39.5	cd	B	(32.7)	2.5	a	C	(95.7)
Runner IAC 886	63.0	b	A	36.0	cd	B	(42.9)	7.5	a	C	(88,1)
IAC 503	53.5	b	A	45.5	bc	A	(15.0)	4.5	a	B	(91.6)
IAC Caiapó	48.5	b	A	39.0	cd	A	(19.6)	9.5	a	B	(80,4)
IAC 113	56.0	b	A	24.5	d	B	(56.3)	8.0	a	C	(85.7)
Means	58.3 A			42.9 B			(26.4)	7.6 C			(87.1)
CV(%)	14.59										

Means followed by the same lowercase letter in the column and upper case in the row do not differ by the Tukey test ($p > 0.05$). (%R): Percent reduction in thrips population relative to control.

important and peculiar aspect once they are lodged on the young leaflets of still closed buds, where they feed and only migrate to another leaflet when a new leaflet is produced by the plant. This may be a reason for the lower efficiency of entomogenous fungi than those with synthetic nature, which has systemic action of contact and ingestion (Antunes-Kenyon and Kennedy, 2001). Entomogenous fungi; however, do not have this systemic action, acting only through either contact or ingestion (Senthil-Nathan, 2015), and take a relatively long time to induce mortality of the target pests (Zhang et al., 2015).

These variations observed in control efficacy of entomogenous fungus can be explained by the influence of ambient and temperature in the foliage in these genotypes (Skinner et al., 2012). With regard to the latter factor, the leaf hair density and structure and waxiness of the leaf surface can be important (Head et al., 2004). In addition, the plant hosts may also mediate pathogen-insect interactions, increasing or decreasing the effectiveness (Barbercheck et al., 1995). In this sense, Barros and Vendramim (1999) warn that the genotypes can positively or negatively affect the performance of biological control agents by creating favorable or unfavorable environments.

Growth and yield of peanut plants

Reductions in the plant height and the mean length of lateral branches are considered indirect indicators to quantify the damage caused by peanut pests, since these changes reflect the *E. flavens* attack. The damage caused by these pests affects the absorption of light energy by plants, leading to a decreased photosynthetic activity with consequent reduction in plant development and productivity.

Analyzing the main stem height, no changes were observed in line L.386 and cultivar IAC Caiapó after spraying synthetic and biological insecticides, compared to control (Fig 4). However, in the genotypes L.318 and L.322 and cultivar Runner IAC 886, synthetic and biological insecticides induced a taller main stem height. For the other genotypes, synthetic insecticide provided the tallest main stem height.

The lines L.318, L.322 and L.386 did not differ between the insecticides treatments, indicating that the insecticides induced no significant increase in branch length (Fig 4). For cultivar Runner IAC 886, the biological insecticides, AUIN® and GR-INN®, increased branch length, with no statistical differences from the synthetic insecticide. For the other genotypes, synthetic insecticide promoted a greater length of lateral branches.

Numerous uncontrolled factors often interfere with the final yield and make it difficult to study the influence of one or a few factors. In this experiment, the type of insecticides, except for line L. 386 and cultivar IAC 113 (Table 3), influenced all genotypes. For the lines L.383 and L.314 and cultivar IAC Caiapó, there was no significant yield difference between the treatments with synthetic and biological insecticides, and line L.383 and IAC Caiapó stood out with yields exceeding 5,000 kg ha^{-1}, where they showed good performance with applications of the biological insecticide. Resistance to *E. flavens* in cultivar IAC Caiapó had already been detected in field experiments at two locations in the State of São Paulo, where the cultivar was exposed to a longer infestation period due to its long cycle, but the yield loss was lowest when no thrips control was applied (Moraes et al., 2005). However, with applications of the synthetic insecticide, the peanut yield was higher than 6,000 kg ha^{-1} for lines L.383 and L. 389, differing only from L.314. Compared to control (the treatment without insecticide applications), the control efficiency were 39% and 11% (as mean value), when synthetic insecticide and biological insecticides were applied, respectively.

Final considerations

Another possibility, which was not addressed in this study, is the use of entomogenous fungi associated with synthetic insecticide (Kivett et al., 2015). According to Cuthbertson et al. (2005), the use of biological agents used in sub-lethal doses associated with the neonicotinoid insecticide imidacloprid, increased mortality of *Thrips palmi* Karny, indicating possible synergistic effects. In a study of Al-Mazra'awi (2006), the effectiveness of *B. bassiana* in *T. tabaci* was increased when sub-lethal doses of imidacloprid insecticide were used.

This study demonstrated the potential use of entomopathogenic fungi *M. anisopliae* and *B. bassiana* (AUIN® and GR-INN® products) to control *E. flavens* in peanut, regardless of the genotype. Further research on the use of these fungi associated with synthetic insecticides as possible strategy for integrated pest management in peanut is suggested.

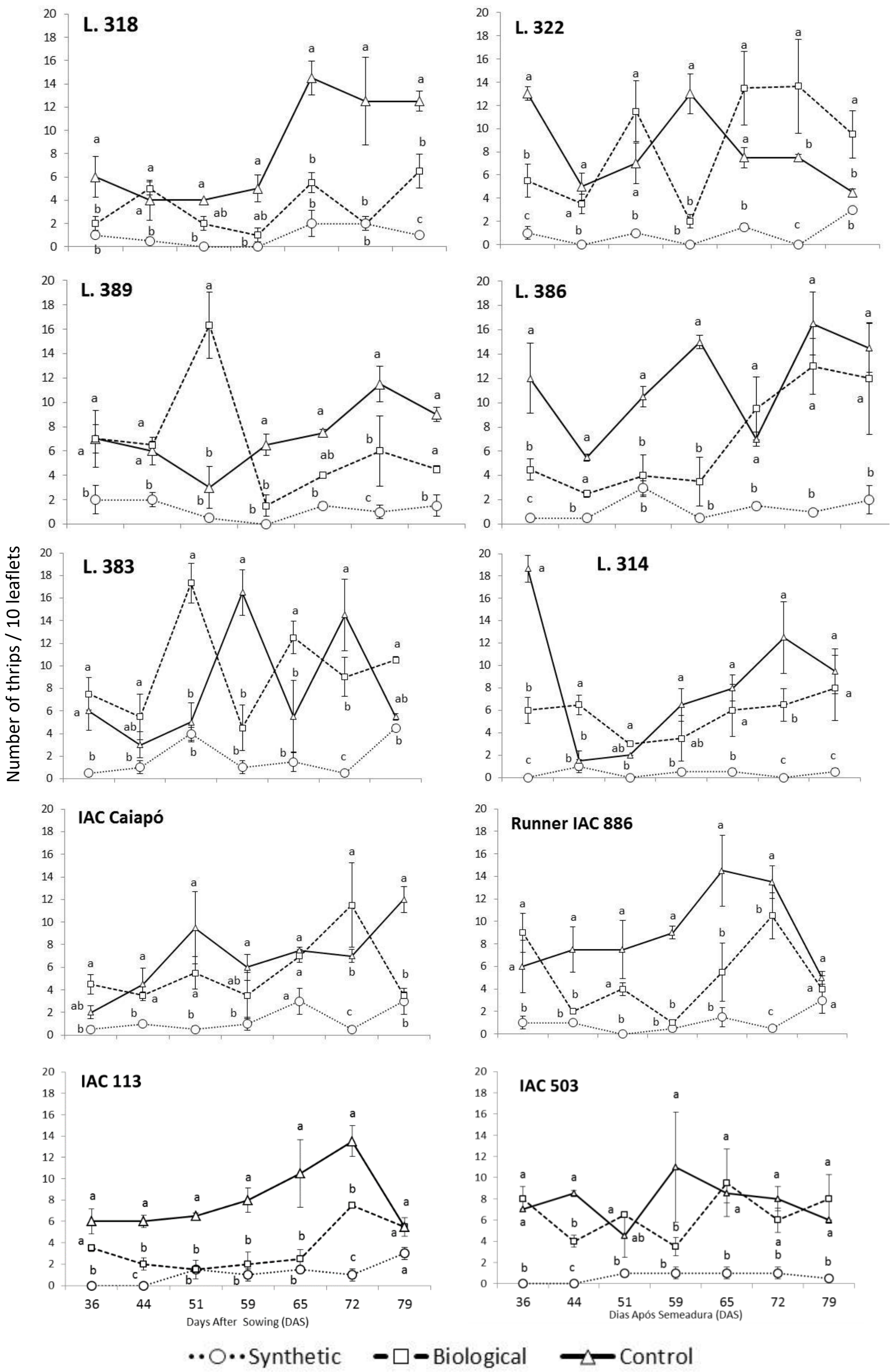

Fig 2. Number of thrips (*E. flavens*) on leaflets of 10 peanut genotypes, in seven evaluations of plants after application of synthetic and biological insecticides and control (without application). Pindorama, state of São Paulo, Brazil, growing season 2014/15. *Same letter at each evaluation date do not differ by the Tukey test (p > 0.05). Vertical bars correspond to standard error.*

Table 3. Yield (kg ha^{-1}) in 10 peanut genotypes, after application of synthetic and biological insecticides and control (without application). Pindorama, state of São Paulo, Brazil, growing season 2014/15.

Genotypes	Yield (kg ha^{-1})										
	Control			Biological			(%I)	Synthetic			(%I)
L. 318	4,178.5	ab	AB	3,622.7	ab	B	(0)	5,208.3	a	A	(24.6)
L. 322	3,737.2	ab	B	4,184.6	ab	B	(12.0)	5,553.2	a	A	(48.6)
L. 389	4,726.7	a	B	4,405.1	ab	B	(0)	6,268.5	a	A	(32.6)
L. 386	4,040.8	ab	A	4,122.7	ab	A	(2.0)	4,601.8	ab	A	(13.9)
L. 383	4,216.7	ab	B	5,333.3	a	AB	(26.5)	6,063.0	a	A	(43.8)
L. 314	2,006.5	c	A	2,700.4	b	A	(34.6)	3,238.4	b	A	(61.4)
Runner IAC 886	3,756.7	ab	B	4,381.9	ab	A	(16.6)	5,988.4	a	A	(59,4)
IAC 503	3,550.9	abc	B	3,791.7	ab	B	(6.8)	5,754.6	a	A	(62.1)
IAC Caiapó	2,929.4	bc	B	5,319.4	a	A	(81,6)	4,898.3	ab	A	(67,2)
IAC 113	4,604.2	ab	A	4,148.1	ab	A	(9.9)	4,942.1	ab	A	(7.3)
Means	3,774.8 C			4,201.0 B			(11.3)	5,251.7 A			(39.1)
CV(%)	14.59										

Means followed by the same lowercase letter in the column and upper case in the row do not differ by the Tukey test ($p > 0.05$). (%I): Percent increase in yield relative to control.

Materials and Methods

The experiment was set up in an experimental area of the Agência Paulista de Tecnologia dos Agronegócios (APTA), Polo Centro Norte, in Pindorama, state of São Paulo, Brazil (21°13'29.9''S 48°54'33.0''W) in the 2014/15 growing season.

Plant materials

The tested peanut genotypes consisted of six lines derived from crosses between cultivar Runner IAC 886 and accession 69007, introduced from India by the IAC breeding program (Godoy et al., 2009), and Runner IAC 886, IAC 503, IAC Caiapó and cultivar IAC 113. Cultivar Runner IAC 886 is a runner type with typically spreading branching, determinate growth habit, and long cycle (125 -130 days), recommended for the state of São Paulo. The yield potential is considered high, although production costs tend to be higher due to its disease and pest susceptibility, requiring heavy chemical control (Godoy et al., 1999). The Indian accession 69007 carries disease resistance genes of the species *Arachis cardenasii* Krapov wild. and W.C. Greg., and is therefore of interest to introgress these resistance factors into Runner IAC 886 to breed for resistant cultivars (Godoy et al., 2009; Michelotto et al., 2013).
To break dormancy, the seeds were previously treated with Ethephon (Ethrel® - commercial product), at 0.2 L kg-1 seeds, and as protection against soil-borne fungi, the mixture carboxin + thiram (Vitavax®-Thiram 200 SC - commercial product) was applied at a rate of 0.25 L kg^{-1} seeds.

Population of thrips

Evaluations were carried out before the start of insecticide applications and also from 25 DAS (Days After Sowing) and afterwards. The number of thrips (nymphs + adults) on 10 young still-closed-leaflets were measured. The plants sampled randomly in each plot. This was performed to check the presence and distribution of the insect in the area.

Experiment design and treatments

The experiment was arranged in randomized complete blocks, in a 10 x 3 factorial design, with three replications. The first factor consisted of six peanut lines and four cultivars and the second factor was the use of insecticides (T1: synthetic insecticide; T2: biological insecticides and T3: control). The plots consisted of four 4-m rows spaced 0.9 m apart, covering a total area of 4,200 m². For the treatment T1, synthetic insecticide, thiamethoxam + lambda-cyhalothrin (Engeo™ Pleno, Syngenta), at a rate of 21.15 + 15.90 g a.i./ha (0.15 L ha^{-1}) was used. In the treatment T2, biological insecticide, two products were used together, *Beauveria bassiana* (Bals.) (AUIN®, AgriValle) and *Metarhizium anisopliae* (Metch.) (GR-INN®, AgriValle), both at a rate of 50 g/kg a.i. ha^{-1}. T3 was considered control with no insecticide product was applied in the plots. The synthetic and biological insecticides were applied 28, 37, 45, 52, 58, and 66 days after sowing, simulating management used by Brazilian growers, who perform weekly applications without monitoring and follow only a calendar of applications. The synthetic and biological insecticides were applied with a 20 L electric backpack sprayer, calibrated to apply 300 L ha^{-1}.

Parameters evaluated

Evaluations were carried out 36, 44, 51, 59, 65, 72, and 79 DAS to determine the number of thrips (adults + nymphs) on 10 young, still closed leaflets, sampled randomly in each plot. The trial period was determined according to the period of most intense insect attack on the crop, as described by Lourenção et al. (2007). The damage on the leaflets was assessed by assigning scores of visual symptoms of thrips attack on peanut plants, on a 1-5 grade scale proposed by Moraes et al. (2005): 1 - leaflet without symptoms; 2 - leaflet with a few silvery-white streaks, without malformation; 3 - leaflet with some silvery-white streaks, with crinkling of the edges of young leaflets; 4 - silvery-white streaks all over the leaflet and beginning of shrinkage; 5 - silvery-white streaks all over and complete shrivelling of the leaflet. For this purpose, 10 mature leaflets in each plot were sampled and applied to the scale.

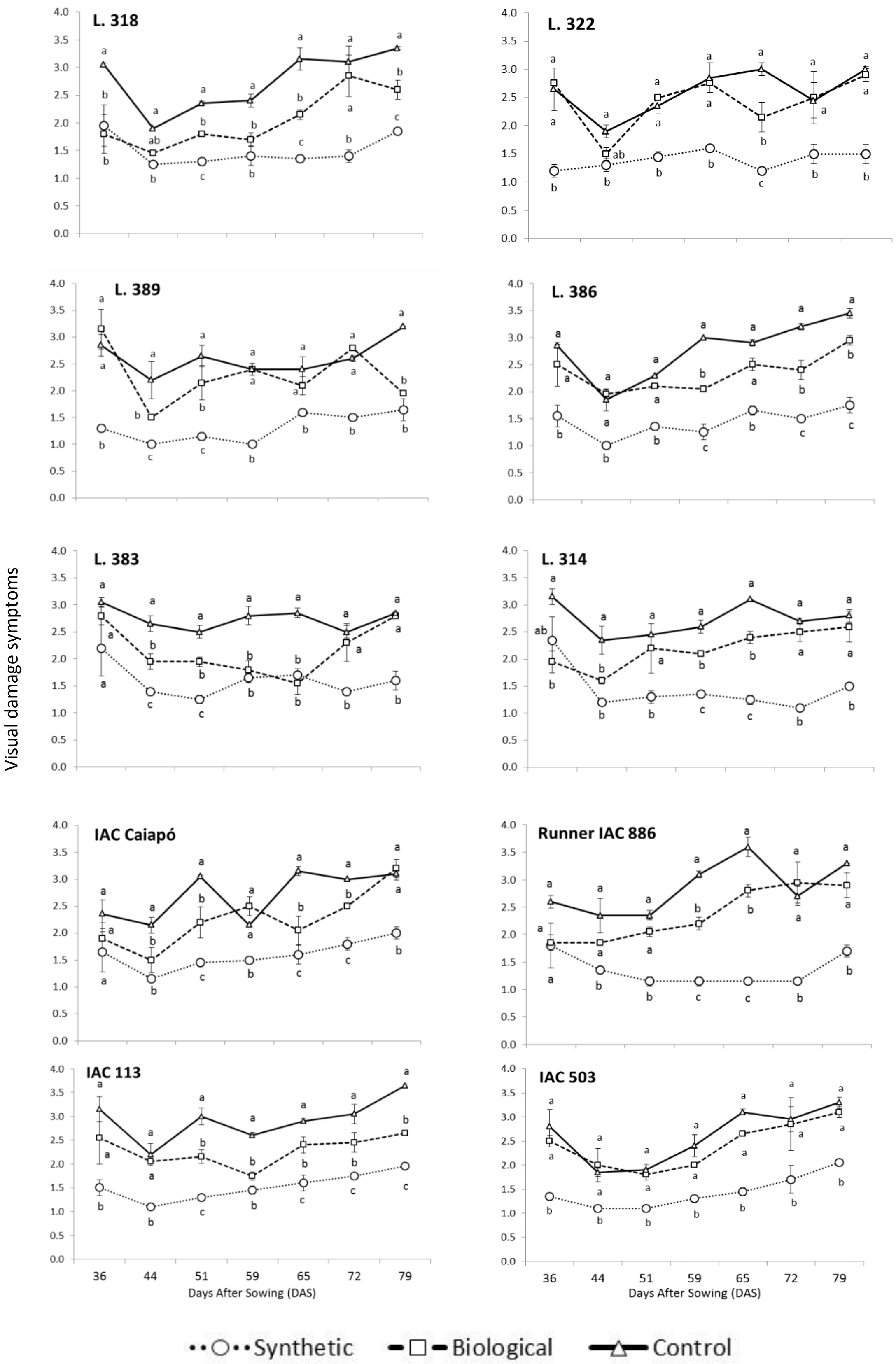

Fig 3. Degree of visual symptoms of *E. flavens* damage on leaflets of 10 peanut genotypes, in seven evaluations of plants after application of synthetic and biological insecticides and control (without application). Pindorama, state of São Paulo, Brazil, growing season 2014/15. *Same letter at each evaluation date do not differ by the Tukey test (p > 0.05). Vertical bars correspond to standard error.*

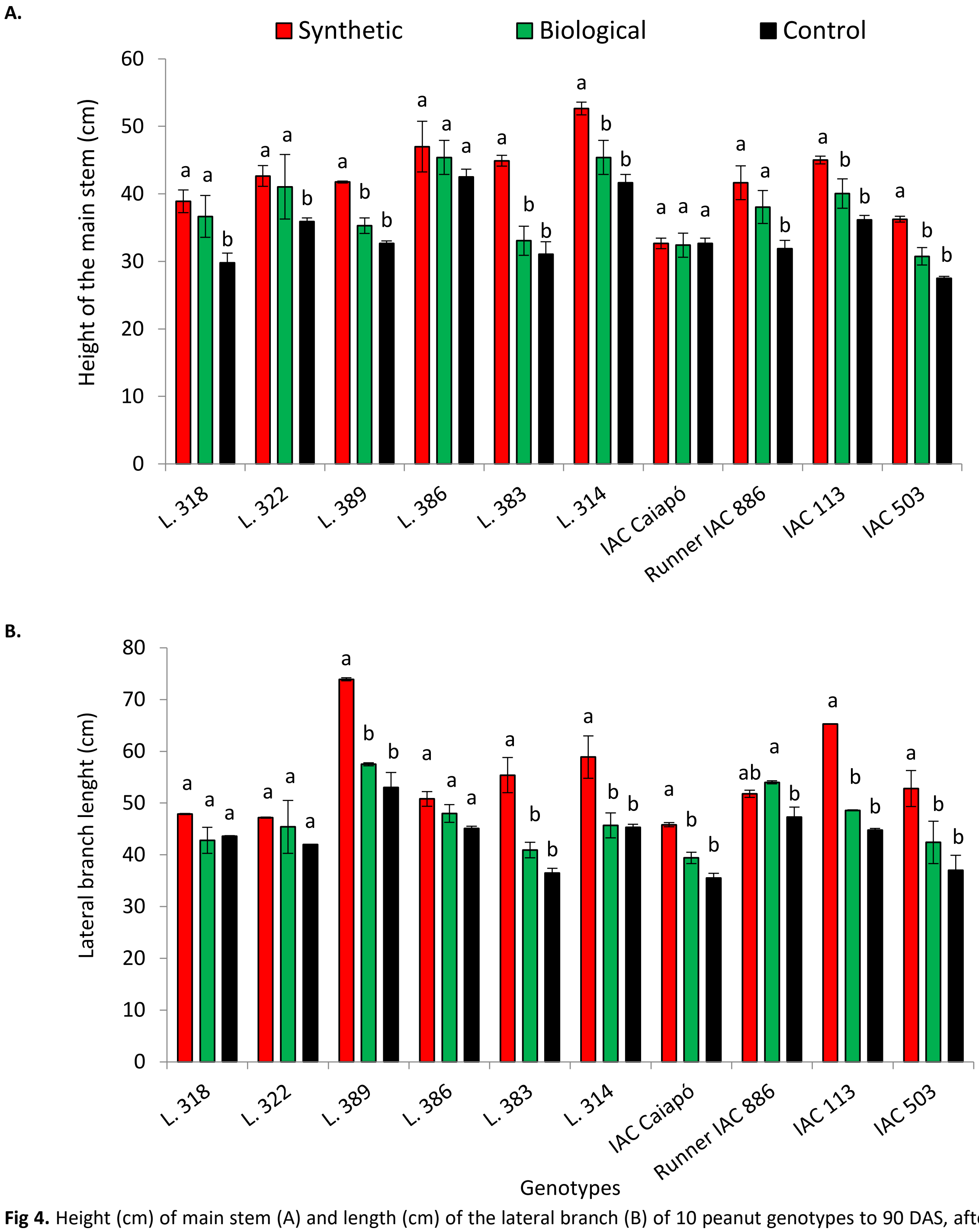

Fig 4. Height (cm) of main stem (A) and length (cm) of the lateral branch (B) of 10 peanut genotypes to 90 DAS, after application of synthetic and biological insecticides and control (without application). Pindorama, state of São Paulo, Brazil, growing season 2014/15. *Same letter at each evaluation date do not differ by the Tukey test (p > 0.05). Vertical bars correspond to standard error.*

The height (cm) of the main stem and length (cm) of the intermediate lateral branch were evaluated 90 DAS in 10 plants per plot. At the end of the crop cycle, the plots were harvested to determine the yield (kg ha^{-1}) by harvesting the two center rows and weighing the dry pods at a moisture content of approximately 13%.

Statistical analysis

The data were subjected to analysis of variance and the F-test, and the means compared by the Tukey test at 5% probability. The values of number of insects were transformed into $(x + 0.5)^{1/2}$.

Conclusion

The control efficiency of the synthetic insecticide thiamethoxam + lambda-cyhalothrin of *Enneothrips flavens* in peanut yield is greater, with means 39%, while the combination of the biological insecticides *Beauveria bassiana* and *Metarhizium anisopliae*, showing control efficiency of 11%. The *Beauveria bassiana* (Bals.) (AUIN®) and

Metarhizium anisopliae (Metch.) (GR-INN®) have potential to control *E. flavens* in peanut, but further studies are needed to better define the application date and products doses. The application of two biological insecticides induced yield increases in line L.383 and cultivar IAC Caiapó. The genetic variability of lines L. 386 and L. 314 and cultivar IAC 113 did not show significant yield difference between the treatments of insecticides compared to control, showing tolerance to *E. flavens.*

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