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Cover plants with potential to reduce two-spotted spider mite population in soybean

Abstract – The objective of this work was to evaluate the potential of cover plants in reducing the population of the two-spotted spider mite, *Tetranychus urticae*, in soybean. Seven host plants – cotton (*Gossypium hirsutum*), *Urochloa ruziziensis*, crotalaria (*Crotalaria juncea*), corn (*Zea mays*), radish (*Raphanus sativus*), soybean (*Glycine max*), and sorghum (*Sorghum bicolor*) – were evaluated for their effect on *T. urticae* biological parameters (oviposition, development stages, and survival) and preference for volatile odors (using a Y-tube olfactometer). *Tetranychus urticae* preferred cotton and showed a higher population growth rate on this host plant. The survival and oviposition of *T. urticae* was prolonged in radish and crotalaria plants, whereas juveniles did not complete their immature development on *U. ruziziensis* leaves. No preference was observed between the volatile odors of *U. ruziziensis*, radish, and crotalaria. *Urochloa ruziziensis* can reduce *T. urticae* populations in soybean plants.

Index terms: *Glycine max*, *Tetranychus urticae*, *Urochloa ruziziensis*, intensive agricultural production systems.

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Plantas de cobertura com potencial de reduzir a população do ácaro-rajado na soja

Resumo – O objetivo deste trabalho foi avaliar o potencial de plantas de cobertura na redução da população do ácaro-rajado, *Tetranychus urticae*, em soja. Foram avaliadas sete plantas hospedeiras – algodão (*Gossypium hirsutum*), braquiária (*Urochloa ruziziensis*), crotalária (*Crotalaria juncea*), milho (*Zea mays*), rabanete (*Raphanus sativus*), soja (*Glycine max*) e sorgo (*Sorghum bicolor*) – quanto ao seu efeito nos parâmetros biológicos (oviposição, estágios de desenvolvimento e sobrevivência) e na preferência por odores voláteis (com uso de olfatômetro em forma de Y) de *T. urticae*. *Tetranychus urticae* preferiu o algodão e apresentou taxa de crescimento populacional mais alta nesta planta hospedeira. A sobrevivência e a oviposição de *T. urticae* foram prolongadas nas plantas de rabanete e crotalária, enquanto os juvenis não completaram seu desenvolvimento imaturo nas folhas de braquiária. Nenhuma preferência foi observada entre os odores voláteis de braquiária, rabanete e crotalária. A braquiária pode reduzir as populações de *T. urticae* em soja.

Termos para indexação: *Glycine max*, *Tetranychus urticae*, *Urochloa ruziziensis*, sistemas intensivos de produção agrícola.

Introduction

Among the various arthropods that attack soybean [*Glycine max* (L.) Merr.], the two-spotted spider mite, *Tetranychus urticae* Koch

(Acari: Tetranychidae), stands out as a pest in several producing regions (Guedes et al., 2007; Padilha et al., 2020). The increase in crop infestations by *T. urticae* may be the result of different factors, such as: the intense use of pesticides, leading to a reduction in populations of natural enemies (Castro et al., 2016); hormesis (Cordeiro et al., 2013; Guedes et al., 2014); the hot and dry climate during harvests, allowing of the multiplication of mites; and the ability of these herbivores to adapt to different host plant species (Grbic et al., 2011). Another factor that may contribute to the greater occurrence of *T. urticae* is the intensification of the use of extensive agricultural areas, resulting in a continuous supply of food and shelter for the pest (Danne et al., 2010).

In production systems, the cultivation of two to three consecutive crops may favor the population growth and dispersal of arthropod pests, especially of polyphagous-generalist ones, and the surrounding vegetation may be a source of insects and mites (Vassaeur et al., 2013). According to Bianchi et al. (2006), although this vegetation can harbor natural enemies, contributing to the natural control of pests, it usually provides food and shelter for several herbivorous arthropods, favoring their permanence in the agricultural landscape.

Therefore, to break the cycle of pests in intensive production systems, it is important to adopt control strategies such as the use of fallow areas (e.g., sanitary breaks), crop rotation, and cover crops, among others (Emery et al., 2021). The use of cover crops, for example, can significantly reduce the population of arthropod pests, and the mulch formed by these plants promotes soil protection and reduces the proliferation of weeds (Quintanilla-Tornel et al., 2016). However, for an effective pest management, studies are needed on the interactions between pest arthropods and the cultivated and spontaneous host plant species that make up the agricultural landscape (Östman et al., 2001; Bommarco et al., 2013).

Plants inserted in the landscape can impact herbivore-plant interactions in agricultural crops, attracting or repelling pest-arthropods. Gravois et al. (2014) concluded that corn (*Zea mays* L.) cultivars susceptible to *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae) should be cultivated far from sugarcane (*Saccharum officinarum* L.) fields in order to avoid the dispersal of moths. Other cover crops, such as crotalaria (*Crotalaria juncea* L.) that produces nematode-repellent allelochemicals in the soil, are desirable since they are less palatable to or suitable for shelter or reproduction of pest populations (Danne et al., 2010).

Although cover crops are commonly used in agricultural systems, there are still few researches on their use for the management of polyphagous pests, such as phytophagous mites from the Tetranychidae family. Even though these plants are generally grown in the inter-harvest period to protect the soil, provide nutrients to the successor crop (Pacheco et al., 2017; Ordónez-Fernandes et al., 2018), and minimize the incidence of weeds (Witter et al., 2019), they must be evaluated as possible hosts for pests. This assessment is even more necessary for species of polyphagous-generalist pests as the *T. urticae* spider mite.

The objective of this work was to evaluate the potential of cover plants in reducing the population of the two-spotted spider mite, *T. urticae*, in soybean.

Materials and Methods

Two experiments were carried out at the Laboratory of Agricultural and Forestry Entomology of Universidade Federal de São João del-Rei, located in the municipality of Sete Lagoas, in the state of Minas Gerais, Brazil. The first bioassay aimed to evaluate the biological parameters of *T. urticae* when fed with different host plants over a generation, from the egg to adult death. The second evaluated the behavioral response of *T. urticae* to the volatile odors of these host plants.

The mites for *T. urticae* rearing were collected in 2018 from infested sorghum [*Sorghum bicolor* (L.) Moench] leaves and kept in a greenhouse, also in the municipality of Sete Lagoas, in the state of Minas Gerais (19°27'57"S, 44°14'49"W). Subsequently, using a soft bristle brush, the mites were transferred to the abaxial surface of leaves of jack bean [*Canavalia ensiformis* (L.) DC.] plants, which were kept in the laboratory, in cages sealed with organza mesh to avoid contamination and infestation by other herbivores. The jack bean plants were irrigated as necessary.

To carry out the rearing of *T. urticae* mites in the laboratory, the jack bean leaves (with the abaxial side facing upwards) were individually placed on a moistened sponge (approximately 3.0 cm thick) in

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20x12 cm plastic trays, which were kept in a BOD incubator at 25±2°C, with a relative humidity of 70% and a 12-hour photoperiod. A total of 30 adult females of *T. urticae* were transferred to each leaf. To ensure turgor and prevent the mites from escaping, the edges of the leaves were contoured with moistened cotton. The leaves were changed whenever necessary, by placing the old leaves over the new ones, allowing of the mites to migrate.

The evaluated cover plants were: *Urochloa ruziziensis* (R.Germ. & C.M.Evrard) Crins, crotalaria (*C. juncea*), 'Cati AL 1000' radish (*Raphanus sativus* L.), 'BRS 380' sorghum (*S. bicolor*), Fibermax FM cotton (*Gossypium hirsutum* L.), L3 MS corn, and '8579RSF IPRO' soybean. Seeds of each species were sown in 1.0 L plastic pots containing Terral Solo substrate (Terral, Inhaúma, MG, Brazil). The pots were kept in the greenhouse and watered as needed. After two weeks, thinning was performed, keeping only the most vigorous plant in each pot. The plants were used when at least three non-cotyledonary leaves were formed and they were approximately 25 cm tall. The leaves were removed as needed and used as a food substrate for *T. urticae*.

To determine the biological parameters of *T. urticae*, bioassays were carried out in a completely randomized design with seven treatments (host plants) and 30 replicates, totaling 210 experimental units. First, the leaves of the evaluated plants were collected, washed in running water, and then wrapped in paper towels to remove excess moisture.

To assemble the arenas, a metal perforator (25 mm Ø) was used to section 30 leaf discs of the cover crop plants. The leaf discs, with the abaxial side facing upwards, were individually placed in plastic Petri dishes (35 mm Ø) lined with a thin layer of moistened cotton. Subsequently, with the aid of a soft bristle brush, two one-day-old eggs of *T. urticae* were transferred to the center of each leaf disc. To ensure that the eggs were all of the same age at the beginning of the bioassay, 40 females at oviposition age were separated 24 hours before the experiment was set up and kept on the jack bean leaves to obtain batches of the same age. During the evaluation period, the cotton on the plates was moistened in order to ensure the turgor of the leaf discs and prevent the mites from escaping. The old leaf discs were replaced by new ones whenever necessary, using a bristle brush to gently transfer the mites to the new leaf disc of the corresponding host plant species.

After hatching, only one larva was maintained on each leaf disc. During the immature stage, two daily evaluations were carried out, one at 8 a.m. and the other at 3 p.m. After reaching adulthood, the evaluation was performed once a day, at 8 a.m. until the individual's death. Initially, the incubation period of the eggs was evaluated. Subsequently, from larva hatching to the end of the adult stage, the following biological variables were determined: duration of the development stages, survival, and number of eggs, which were removed after counting. Sex was identified after adult emergence, discarding males and maintaining the females that were not mated.

Intrinsic growth rate (r_m) was obtained using the data of the female cycle (Janssen et al., 2022). The following equation was used to estimate parameters and compare treatments, based on the Jackknife method:

$$\sum_{x=0}^{T} 1x\ mx\ e^{-rm.(x+1)} = 1$$

where x is the time interval, T is the oldest age group, 1x is the survival rate, and mx is the number of female offspring produced.

For the olfactometry tests, a completely randomized design was used, with five treatments and three true replicates (= series of plants). In each replicate, 20 releases of the mites that responded to the analyzed odor sources were performed. The treatments, comparing the cover crops with soybean, were: radish vs. soybean, crotalaria vs. soybean, *U. ruziziensis* vs. soybean, and sorghum vs. soybean, with air vs. air as the control.

To evaluate the preference of *T. urticae* for the odor of the cover and cultivated plants, a Y-tube olfactometer was used, with dimensions of 21 cm in length for each arm and 3.5 cm in diameter. Hoses were fitted on each of the two upper arms of the Y-tube, connecting to glass boxes where the plants used as the odor sources were stored. A third hose was fitted to the base arm of the Y-tube, connecting to the vacuum pump responsible for generating the continuous flow of air inside the olfactometer. A flowmeter was used to regulate the direction of the air flow from the containers at a velocity of 0.5 L s^{-1}. Plants with at least three non-cotyledonary leaves formed and a height of approximately 25 cm were evaluated one at a time in the olfactometer.

Adult females of *T. urticae* were released individually on a copper wire positioned along the inside of the Y-tube, to facilitate the displacement of the individual in the olfactometer. The females used in the experiment were deprived of food 24 hours before being tested and kept in Petri dishes. The behavior of adult females of *T. urticae* inside the olfactometer was observed for up to 5 min, classified as “no response”, when the mites showed no preference after 5 min, and “response”, when they exceeded 1/3 of one of the arms of the tube. For every ten responses, the positions of the hoses through which the odors were released were inverted and the Y-tube was cleaned with water to avoid choice trends.

The data obtained for duration of immature stages and number of eggs were subjected to the analysis of variance by the F-test, and averages were compared by the Scott-Knott test, at 5% probability. Female longevity data were analyzed using survival models to assess the effect of the evaluated plants (explanatory variable) on survival (response variable) according to Crawley (2013). The data of olfactometry tests were subjected to chi-squared tests (α=5%) for categorical data. The R software (R Core Team, 2014) was used for the statistical analyzes.

Results and Discussion

The egg stage of *T. urticae* was significantly longer on the leaf discs of crotalaria, corn, radish, and sorghum ($F = 19.4$; $df = 203$; $p<0.001$), when compared with those of cotton, *U. ruziziensis*, and soybean (Figure 1). The larval stage of *T. urticae* lasted longer on *U. ruziziensis*, was intermediate on sorghum, radish, corn, and cotton, and shorter on crotalaria and soybean. The duration of the protochrysalis stage was significantly longer on *U. ruziziensis* and radish, whereas the protonymph stage lasted longer on *U. ruziziensis* ($F = 9.2$; $df = 154$; $p<0.001$). Moreover, the duration of the deutochrysalis stage only differed

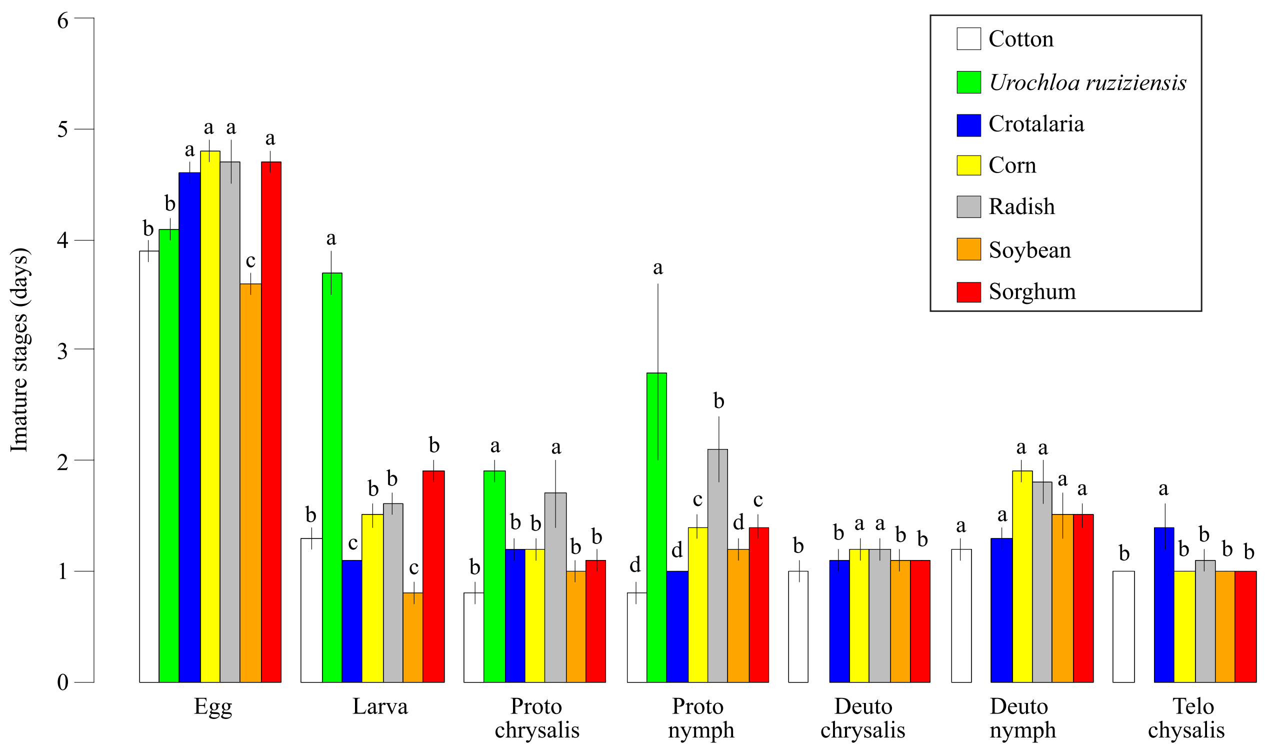

Figure 1. Mean duration in days (± standard error) of the development of the immature stages of the *Tetranychus urticae* spider mite fed on the abaxial surface of leaf discs of cotton (*Gossypium hirsutum*), *Urochloa ruziziensis*, crotalaria (*Crotalaria juncea*), corn (*Zea mays*), radish (*Raphanus sativus*), soybean (*Glycine max*), and sorghum (*Sorghum bicolor*) plants under laboratory conditions. Bars followed by equal letters do not differ statistically according to the Scott-Knott test, at 5% probability.

significantly (F = 1.32; df = 149; p = 0.258) on corn and radish, while the deutonymph stage did not differ (F = 4.7; df = 142; p<0.001) between the cover plants. Finally, the telochrysalis stage showed significant differences only on crotalaria.

Mite longevity was greater on leaf discs of the crotalaria and radish cover plants, but shorter on soybean (Figure 2). Furthermore, the mites kept on the sorghum, corn, and cotton cultivated species showed a greater longevity than those on soybean. Puspitarini et al. (2021) concluded that longevity is one of the life-table parameters of spider mites that is strongly influenced by host plant species, particularly by traits such phytochemical components and morphological and histological leaf structure (Ali et al., 2015). In the present study, the longer longevity of *T. urticae* on the tested plants species, when compared with soybean, says more about the establishment of the pest population than about its growth. Considering that its peak is reached around the seventh day after adulthood, the oviposition of long-lived *T. urticae* females contributes little to population growth (Janssen et al., 2022). However, the use of the host plants on which *T. urticae* lives longer may favor the persistence of this pest between soybean crop seasons.

The host plant species affected the average number of eggs produced per female (F = 14.7; df = 33; p<0.001) (Figure 3). The number of *T. urticae* eggs fed on radish was significantly higher than that on the other plants. Regarding total oviposition, no significant differences were observed between cotton and crotalaria, nor between corn, soybean, and sorghum. Ali & Agrawal (2012) found that, due to plant physical and chemical differences, many herbivores were selected to a specialized diet on one or a few host species. Although this is not the case for *T. urticae*, a generalist pest that feeds on more than 1.100 plants species belonging to more than 140 different botanical families (Grbic et al., 2011), after many generations on one plant, the mites may show a reduced performance when forced to feed on a new host species (Marinosci et al., 2015). In the present study, the population of *T. urticae* was collected from sorghum and then reared on jack bean plants for a few generations, meaning that the used

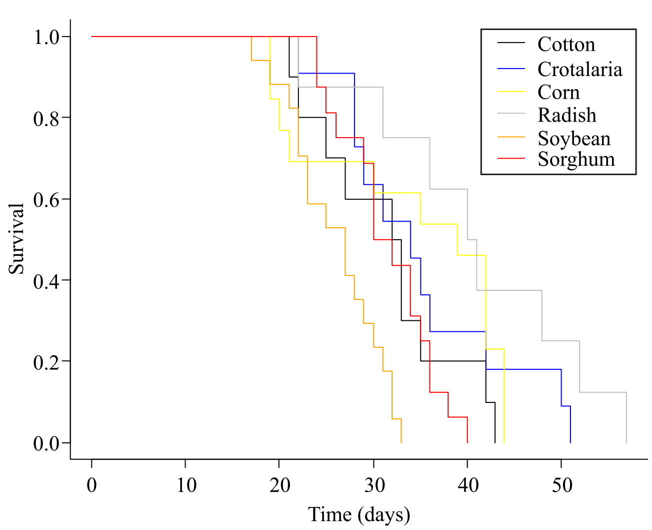

Figure 2. Survival curves of adult *Tetranychus urticae* females until death when fed on the abaxial surface of leaf discs of cotton (*Gossypium hirsutum*), crotalaria (*Crotalaria juncea*), corn (*Zea mays*), radish (*Raphanus sativus*), soybean (*Glycine max*), and sorghum (*Sorghum bicolor*) plants under laboratory conditions.

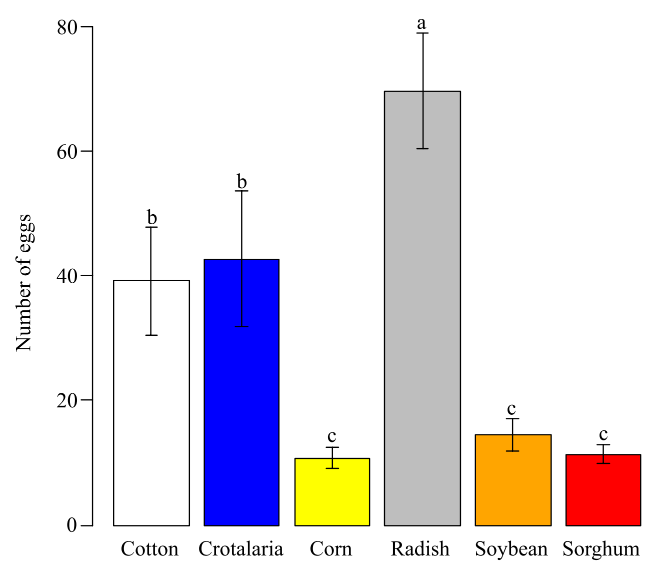

Figure 3. Average number of total eggs (± standard error of the mean) produced by female *Tetranychus urticae* fed on the abaxial surface of leaf discs of cotton (*Gossypium hirsutum*), crotalaria (*Crotalaria juncea*), corn (*Zea mays*), radish (*Raphanus sativus*), soybean (*Glycine max*), and sorghum (*Sorghum bicolor*) plants under laboratory conditions. Means (± standard error of the mean) followed by equal letters do not differ statistically according to the Scott-Knott test, at 5% probability.

experimental design produced a strong challenge for host plant adaptation to *T. urticae*. However, the females reached a high fecundity on radish, cotton, and crotalaria, which was even higher than that on their original host plant, i.e., sorghum. In addition to reinforcing how *T. urticae* has the ability to adapt to a new host plant, the obtained results aid in the establishment of criterium for the selection of cover plants and rotational crops. An important finding is that radish, cotton, and crotalaria plants should be avoided in areas where soybean is cultivated as a strategy to minimize the risk of attack by *T. urticae*.

The r_m of the *T. urticae* population on cotton, crotalaria, corn, radish, soybean, and sorghum was 0.247, 0.177, 0.123, 0.183, 0.176, and 0.150 females per day, respectively. However, it was not possible to calculate the r_m of *T. urticae* on *U. ruziziensis*, since the individuals kept on the leaf discs of this plant species did not reach the adult stage.

The *T. urticae* spider mite showed the highest r_m when fed on radish, crotalaria, and cotton leaves. The analysis of the r_m of *T. urticae* on the leaf discs of the radish and crotalaria cover crops showed that the females survived and reproduced successfully. Although crotalaria is widely used as a cover crop to improve soil quality due to its role as a nitrogen fixer and has been shown to control nematodes (Nascimento et al., 2020), in the present study, it did not reduce the population of *T. urticae*. Therefore, this plant species favors the maintenance of *T. urticae* in crop areas, as does cotton if grown in rotation with soybean.

Contrastingly, the *T. urticae* spider mite performed poorly when fed on *U. ruziziensis* leaves, as shown specifically by its inability to complete its immature development on this plant species. This suggests that *U. ruziziensis* has mechanisms of resistance to herbivory by *T. urticae*, such cuticle, thick epidermis, thorns, and trichomes that could be associated with the result of *T. urticae* biology on that plant.

Regarding plant odor, *T. urticae* showed no preference between the arms of the olfactometer in the air vs. air treatment, without external interference on the choices of the mites (Figure 4 A). There was also no significant preference between the sources of odor in the radish vs. soybean, crotalaria vs. soybean, and *U. ruziziensis* vs. soybean treatments (Figure 4 B, C, and D). As reported for several herbivores, *T. urticae* also uses volatile organic compounds from plants to assess their quality by distance before settling on them (Pallini et al., 1997), i.e., odor cues allow of this pest to evaluate the different nutritional and/or toxic compounds in plants. The quantitative and qualitative difference in the blend of volatile organic compounds of different plant species (Van Den Boom et al., 2004) could explain why *T. urticae* showed no preference between the plants of the radish vs. soybean, crotalaria vs. soybean, and *U. ruziziensis* vs. soybean treatments. Another possible explanation for this observed lack of preference could be the absence of previous experience of the tested mites with the evaluated plants.

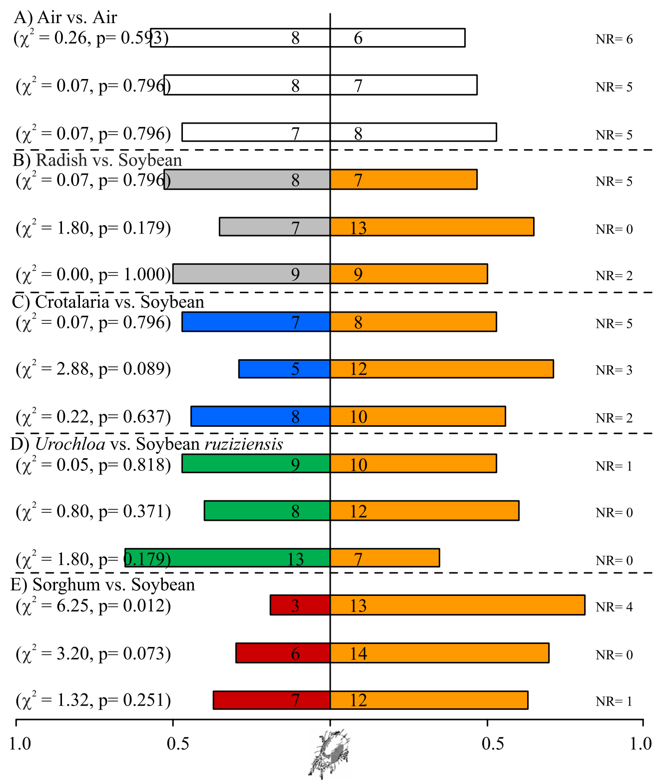

Figure 4. Olfactory response of the *Tetranychus urticae* spider mite in a Y-tube olfactometer according to the following treatments: A, air vs. air (control); B, radish (*Raphanus sativus*) vs. soybean (*Glycine max*); C, crotalaria (*Crotalaria juncea*) vs. soybean; D, *Urochloa ruziziensis* vs. soybean; and E, sorghum (*Sorghum bicolor*) vs. soybean. Bars represent a replicate of the experiment equivalent to 20 mite responses. The number of mites that did not respond (NR) after 5 min is accounted for outside the bars by the chi-squared test (χ^2).

Conclusions

1. *Urochloa ruziziensis* reduces the development of *Tetranychus urticae*.

2. Radish (*Raphanus sativus*) and crotalaria (*Crotalaria juncea*) favor the maintenance of *T. urticae* populations.

3. Cotton (*Gossypium hirsutum*) favors the development of *T. urticae*.

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