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## Phytophagous mites on genetically modified maize with *Bacillus thuringiensis* genes

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**ABSTRACT:** The study of non-target organisms such as mites, can provide information about the possible effects of transgenic maize cultivars on the arthropod community. This study aimed to evaluate the abundance of phytophagous mites in the area of Bt maize and evaluate the instantaneous population growth rate, and food preference of phytophagous mites on Bt maize. We registered the species occurrence and the number of adult mite individuals on four maize cultivars, one non-Bt 30F35, and three containing the proteins Cry1Ab (30F35 Yg), Cry1F (30F35 Hx) and Vip3a (Impacto Viptera). Cry proteins disrupt the midgut epithelium of insect pests. The food preference and instantaneous population growth rate (ri) were evaluated using the two-spotted spider mite *Tetranychus urticae* as model. The species *Catarhinus tricholaenae* and *Aceria zeala* were recorded. The Bt maize cultivars did not significantly affect the average number of *C. tricholaenae* and *A. zeala* mites compared to conventional cultivar. The population growth rates of *T. urticae* were similar for the different maize cultivars. *T. urticae* showed no preference between the leaf of Bt or conventional cultivars. Bt maize did not affect the abundance of species in phytophagous mite.

**Key words:** plant defense, Non-target organisms, Genetically modified organisms Bt protein, Diptilomiopidae, Eriophyidae, Tetranychidae.

## Ácaros fitófagos em milho geneticamente modificado com genes de *Bacillus thuringiensis*

**RESUMO:** O estudo de organismos não-alvo, tais como ácaros, pode fornecer informações sobre os possíveis efeitos de cultivares de milho transgênico na comunidade de artrópodos. Este estudo teve como objetivo avaliar a abundância de ácaros fitófagos na área de milho Bt e avaliar a taxa de crescimento populacional instantânea e preferência alimentar de ácaros fitófagos em milho Bt. Registramos a ocorrência de espécies e número de indivíduos adultos de ácaros em quatro cultivares de milho, um não-Bt 30F35, e três contendo a proteína Cry1Ab (30F35 Yg), Cry1F (30F35 Hx) e VIP3A (Impacto Viptera). A preferência alimentar e taxa instantânea de crescimento populacional foram avaliadas utilizando fêmeas adultas do ácaro-rapado *Tetranychus urticae*. As espécies *Catarhinus tricholaenae* e *Aceria zeala* foram registradas. As cultivares de milho Bt não afetaram significativamente o número médio de ácaros *C. tricholaenae* e *A. zeala* em relação ao cultivar convencional. As taxas de crescimento populacional de *T. urticae* foram similares para os diferentes cultivares. *T. urticae* não mostrou preferência entre a folha de milho Bt ou cultivares convencionais. Sugere-se, então, que o milho Bt não afeta a abundância de espécies, preferência alimentar e taxa de crescimento de ácaros fitófagos. **Palavras-chave:** defesa de plantas, organismos não-alvo, organismos geneticamente modificados, proteína Bt, Diptilomiopidae, Eriophyidae, Tetranychidae.

## INTRODUCTION

Genetically modified (GM) plants that express genes with insecticidal activity represent an efficient control for insect pests, in addition to being an important component of integrated pest management (IPM) (PROKOPY, 1994). The first generations of GM plants resistant to chewing insects were developed with genes encoding insecticidal proteins from the bacterium *Bacillus thuringiensis* (FISCHHOFF et al., 1987), targeting resistance, mainly to Lepidoptera and Coleoptera (PERLAK

et al., 2001). Proteins to be expressed become also exposed to other non-target phytophages and their natural enemies (i.e. predators and parasitoids) (DUTTON et al., 2002, SCHRIJVER et al., 2016). Nevertheless, GM plants containing the Bt gene interact with non-target organisms from different trophic levels, harboring not only the insect pests (SCHULER et al., 1999, SCHRIJVER et al., 2016).

Scientific literature that recommends strategies for pest management in maize (*Zea mays* L.) does not refer to mite populations as key pest (MORAES & FLECHTMANN, 2008, FADINI et

al., 2010). The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is an important polyphagous pest of vegetables and other crops (BOLLAND et al., 1998, CAPINERA 2001, OPIT et al., 2004, LIBURD et al., 2007), and can be also found on maize. This mite can cause eventual infestations in leaves of GM and conventional plant, but it is not the target of modified plants with the *Bt* protein.

We evaluated the occurrence of phytophagous mites in conventional and *Bt* maize in a field. The null hypothesis that plant defense conferred by *Bt* maize proteins would not affect the abundance of phytophagous mites was also tested. We also evaluated, under laboratory conditions, if *Bt* proteins would affect the instantaneous population growth rate and the feeding preference of the phytophagous mite *T. urticae*.

## MATERIALS AND METHODS

We recorded the occurrence of phytophagous mite species on four maize cultivars: a non-*Bt* 30F35, and three containing the proteins Cry1Ab, (30F35 Yield Gard, Yg, Pioneer®) and Cry1F (30F35 Herculex, Hx, Pioneer®) and Vip3a (Impacto Viptera, Syngenta®). These cultivars have as target pest some caterpillars, as fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). The experimental area used was in Embrapa Milho e Sorgo, Sete Lagoas, Minas Gerais State, Brazil (19° 28' south latitude, and 44°15'08" west longitude GrW).

From June 2013 to September 2013, fortnightly, *Bt* and non-*Bt* maize leaf samples were collected in the experimental plot. The experiment was a completely randomized design with maize cultivars as treatments. The field conditions were homogeneous and do not justified the adoption of blocks. Each cultivar was located in a 100x100m plot area. Four maize leaves were considered as a sampling unit (=replication). For data analysis we used a split-plot analysis of variance, having the factor cultivar with the main plot and the sample unit collection times at 5% significance in the sub-plot (CRAWLEY, 2007). The statistical program R (R Development Core Team 2015) was used for the data analysis.

Mites specimens reported were mounted on slides with Hoyer medium (MORAES & FLECHTMANN, 2008) and the best mountings were sent for taxonomic identification confirmation. Slides with *Catarhinus tricholaenae* specimens were deposited in the mite collection of the Laboratory of Entomology of the Federal University of São João Del-Rei, Campus Sete Lagoas, in the state of Minas Gerais, Brazil.

The food preference and instantaneous growth rate of phytophagous mite *T. urticae* adult females were evaluated in four maize cultivars, three of them expressing Cry and Vip proteins, as described above. Tests were conducted in insect growth chamber incubator at 25±1°C, 70±10% RH and 12h photophase. Mites were collected on sorghum (*Sorghum bicolor* L.) plants grown in 5L plastic pots, containing sandy soil and maintained in a greenhouse. Mites were also reared in laboratory, on non-transgenic maize leaves in plastic cups with water. Although, the *T. urticae* mite is not a key pest in maize, this species was used as a biological model.

Three cm diameter leaf discs, obtained from the central part of median leaves of *Bt* and non-*Bt* maize were used. Plants were in the phenological stage V4. Discs were cut and individually placed in three cm diameter Petri dishes with the underside surface upwards. A piece of water-soaked cotton was inserted inside the Petri plate to keep the leaf moist and prevent the escape of the mites. Five fertilized *T. urticae* females with about three days were obtained from the rearing and maintained in insect growth chamber incubator were released in the middle portion of the leaf with the help of brush. After 96h the number of females and the number of eggs deposited were evaluated. Four treatments of *Bt* variety vs non-*Bt* were used in 30 replications (150 females per treatment).

The effect of the cultivars on the biology of *T. urticae* was evaluated by the instantaneous population growth rate (ri) (STARK et al., 1997). Three *Bt* vs non-*Bt* (Impacto Viptera (transgenic) x 30F35 Conventional, 30F35 HX (transgenic) x 30F35 Conventional and 30F35 YG (transgenic) x 30F35 Conventional) treatments were used in 30 females liberations. Leaf discs of three cm in diameter obtained from the central part of median leaves a non-*Bt* 30F35 maize, and three containing the proteins Cry 1F (30F35 Hx), Cry 1AB (30F35 Yg) and Vip3a (Impacto Viptera) were used. The discs (2cm Ø) were cut out and attached with plastic adhesive tape (Durex®, 3M). *Bt* and non-*Bt* (leaf discs) were glued in adjacent faces; so that, *Bt* face as well as the non-*Bt* face were present on both sides of the disc. Discs were individually placed in a 3cm diameter petri plate, on a piece of cotton soaked in water to keep the leaf wet. One fertilized female of *T. urticae* was released on the plastic adhesive tape with the aid of brush. Light was homogeneous into the rearing chamber and it does not influenced the mite behavior. After 24h and 48h food preference per leaf surface and number eggs deposited on each face of the disc were evaluated. Females not found and who did not have choice were removed from the analysis.

Results were evaluated by Kruskal-Wallis test (non-parametric) and the chi-square test at 5% significance (CRAWLEY 2007), using the R statistical package (R Development Core Team, 2011).

## RESULTS

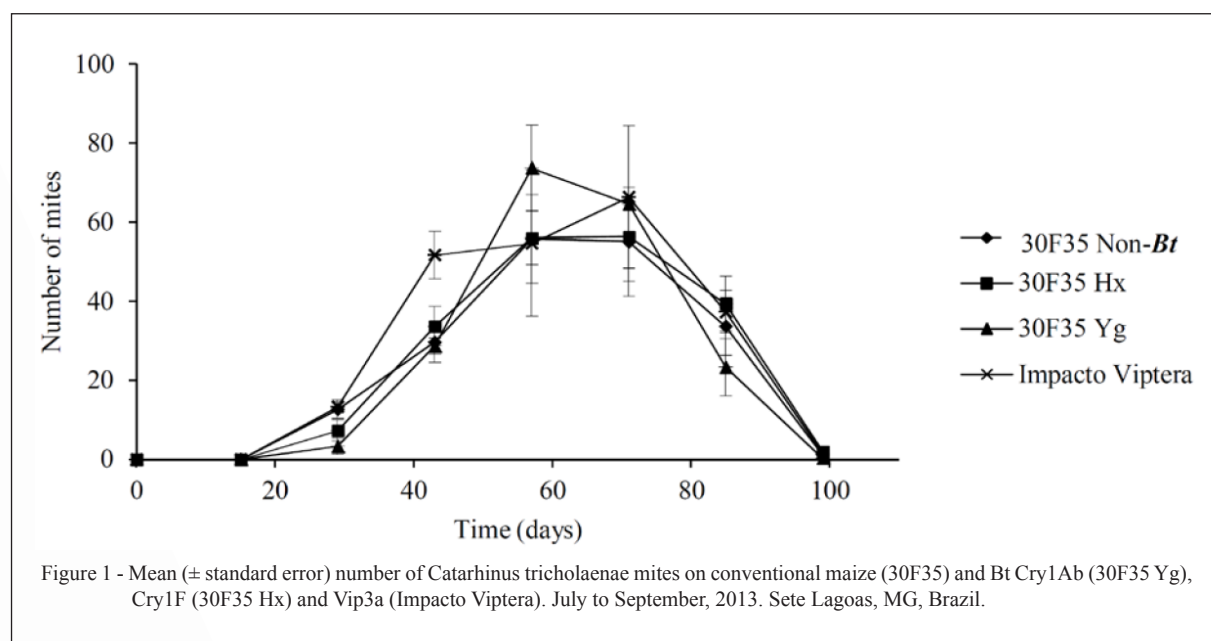
Phytophagous mite species found on maize leaves were *Catarhinus tricholaenae* Keifer (Acari: *Diptilomiopidae*) and *Aceria zeala* (Keifer) (Acari: *Eriophyidae*). No predatory mite species were reported. The Bt maize varieties (Cry1Ab (30F35 Yg), Cry1F (30F35 Hx) and Vip3a (Impacto Viptera) did not affect the average number of mites *C. tricholaenae* compared to the conventional cultivar (30F35) ( $F_{(3,98)}=0.36$ ,  $P=0.78$ ). The highest population abundances of mites occurred in the months of August and September, 60 days of experiment. This result suggested that the Cry protein present in Bt maize plants did not affect the abundance of *C. tricholaenae*. As expected, the time factor affected the *C. tricholaenae* abundance ( $F_{(3,98)}=50.3$ ,  $P<0.01$ ) (Figure 1).

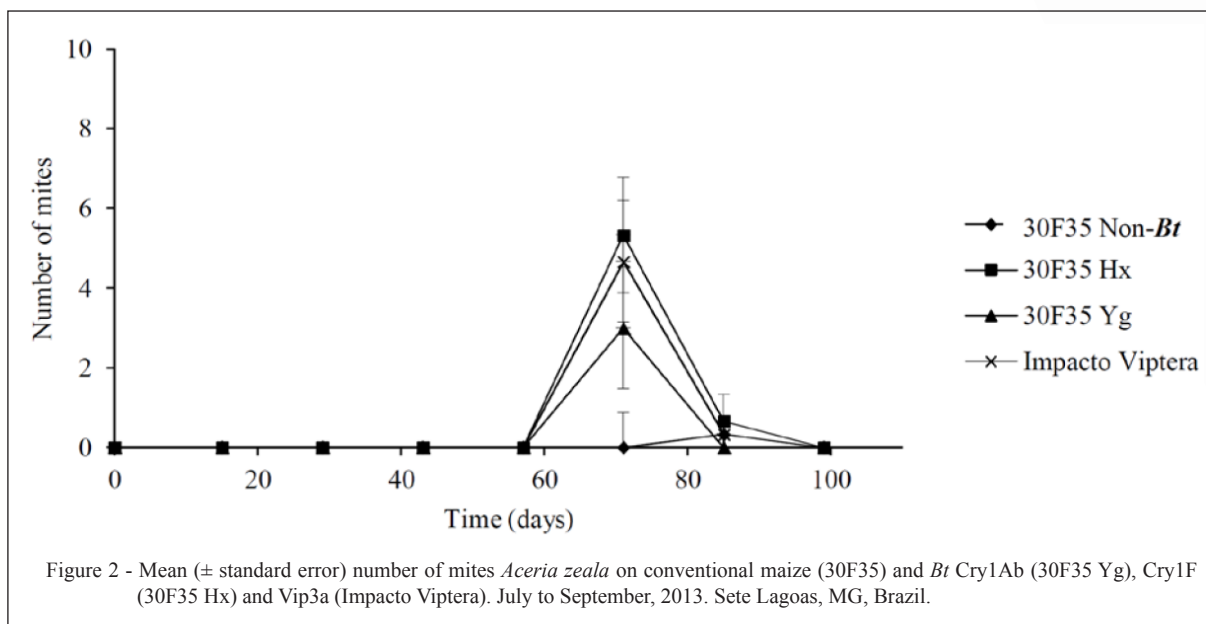
The varieties of Bt maize expressing Cry1Ab (30F35 Yg), Cry1F (30F35 Hx) and Vip3a (Impacto Viptera) also did not affect the average number of *A. zeala* mites in relation to the conventional cultivar (30F35) ( $F_{(3,98)}=1.12$ ,  $P=0.05$ ). Time factor affected the abundance of *A. zeala* ( $F_{(3,98)}=28.0$ ,  $P<0.01$ ). This result demonstrated that the Cry protein present in the Bt maize varieties did not affect the abundance of *A.*

*zeala* (Figure 2). From 93 adults of *A. zeala* collected,  $0.46\pm 1.21$  individuals were reported on non-Bt maize leaves. On Bt maize; with Cry1Ab (30F35 Yg) having  $0.37\pm 1.28$  individuals, Cry1F (30F35 Hx)  $0.75\pm 1.96$  and Vip3a (Impacto Viptera)  $0.62\pm 1.81$  individuals.

Instantaneous population growth rate (ri) for *T. urticae* did not differ when females were maintained on leaf discs of varieties of conventional maize (30F35) and Bt (Hx 30F35, 30F35 Yg and Impacto Viptera) ( $\chi^2=67.37$ ,  $df=74$ ,  $P=0.69$ ) (Figure 3) suggesting that the Cry and Vip proteins, present in Bt maize cultivars, did not affect the instantaneous population growth rate of the phytophagous mite *T. urticae*.

These results demonstrated also that the Cry protein present in Bt varieties did not affect the *T. urticae* foraging per the maize leaf surface. The preference of the phytophagous mite *T. urticae* on leaf surface for feeding and oviposition was not affected by Bt maize varieties (30F35 Hx, 30F35 Yg and Impacto Viptera) when compared to the 30F35 Conventional: 30F35 Hx vs 30F35 ( $\chi^2=0.53$ ,  $df=1$ ,  $P=0.47$ ); 30F35 Yg vs 30F35 ( $\chi^2=0.25$ ,  $df=1$ ,  $P=0.65$ ) and Impacto Viptera vs 30F35 ( $\chi^2=0.00$ ,  $df=1$ ,  $P=1$ ) 24 hours after the release of the females (Figure 4). After 48 hours of the release of the *T. urticae* females on the leaf surfaces, the preferences of the mites was also not affected by Bt maize varieties ( $\chi^2=0.05$ ,  $df=1$ ,  $P=0.83$ ); ( $\chi^2=0.05$ ,  $df=1$ ,  $P=0.82$ ) and 30F35 ( $\chi^2=0.22$ ,  $df=1$ ,  $P=0.62$ ), to the same treatment above (Figure 5).

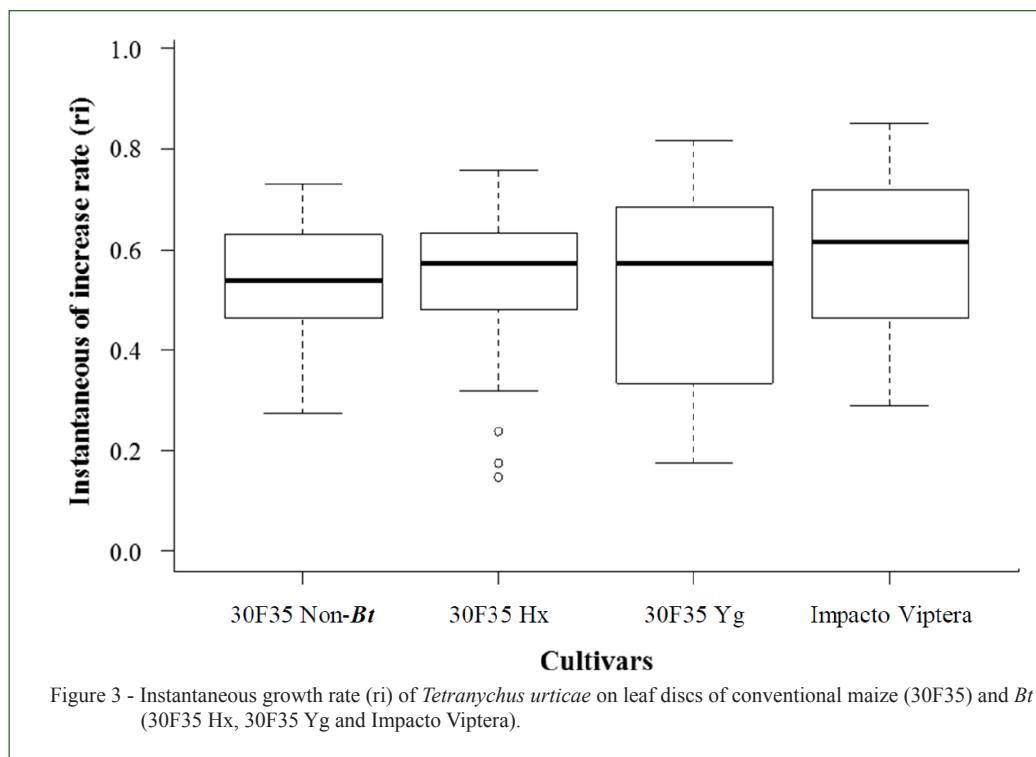




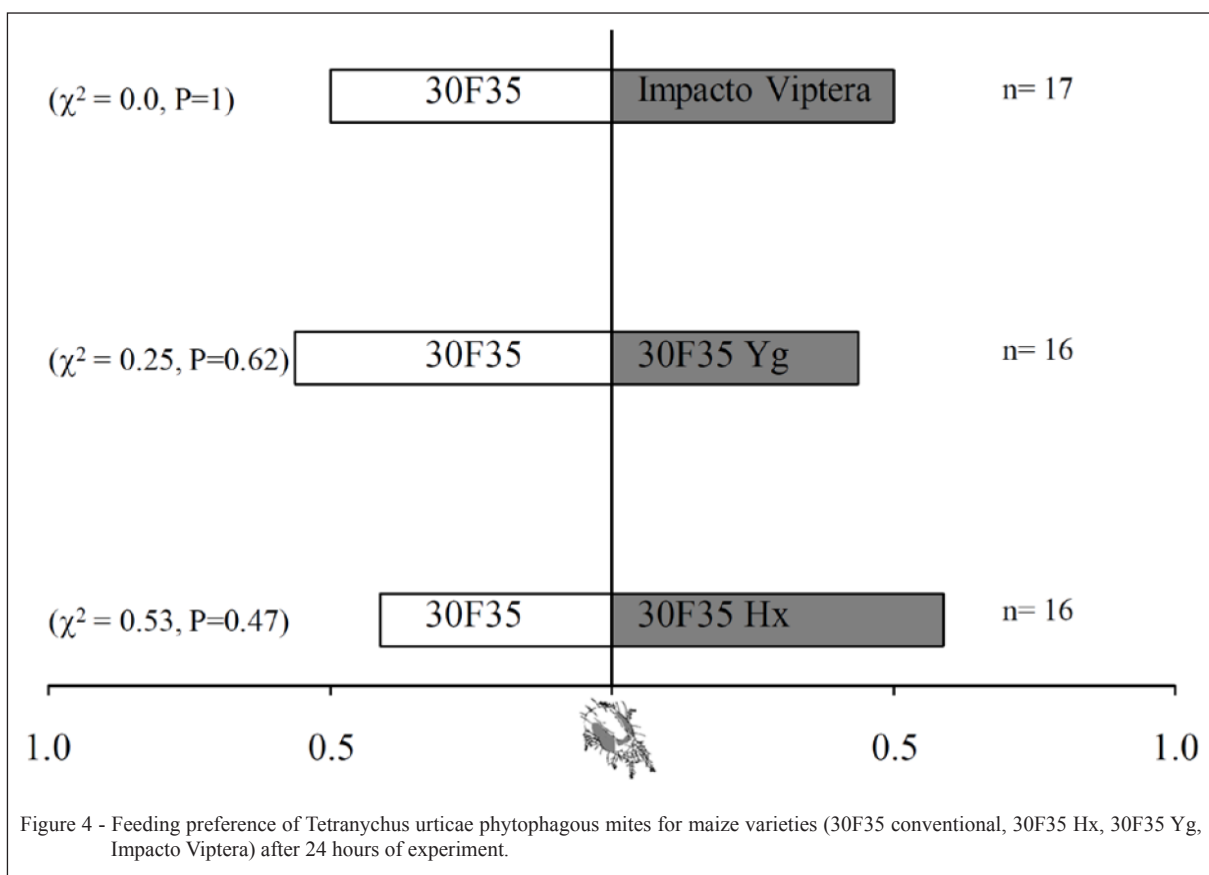
## DISCUSSION

The fact that the numbers of *C. tricholaenae* individuals did not differ among the *Bt* maize varieties Cry1Ab (30F35 Yg), Cry1F (30F35 Hx), Vip3a

(Impacto Viptera) and the non-*Bt* variety, 30F35 Conventional suggested that the Cry protein did not affect the population abundance of that species. FADINI et al. (2012) reported; however, that the population averages of *C. tricholaenae* on the cultivar







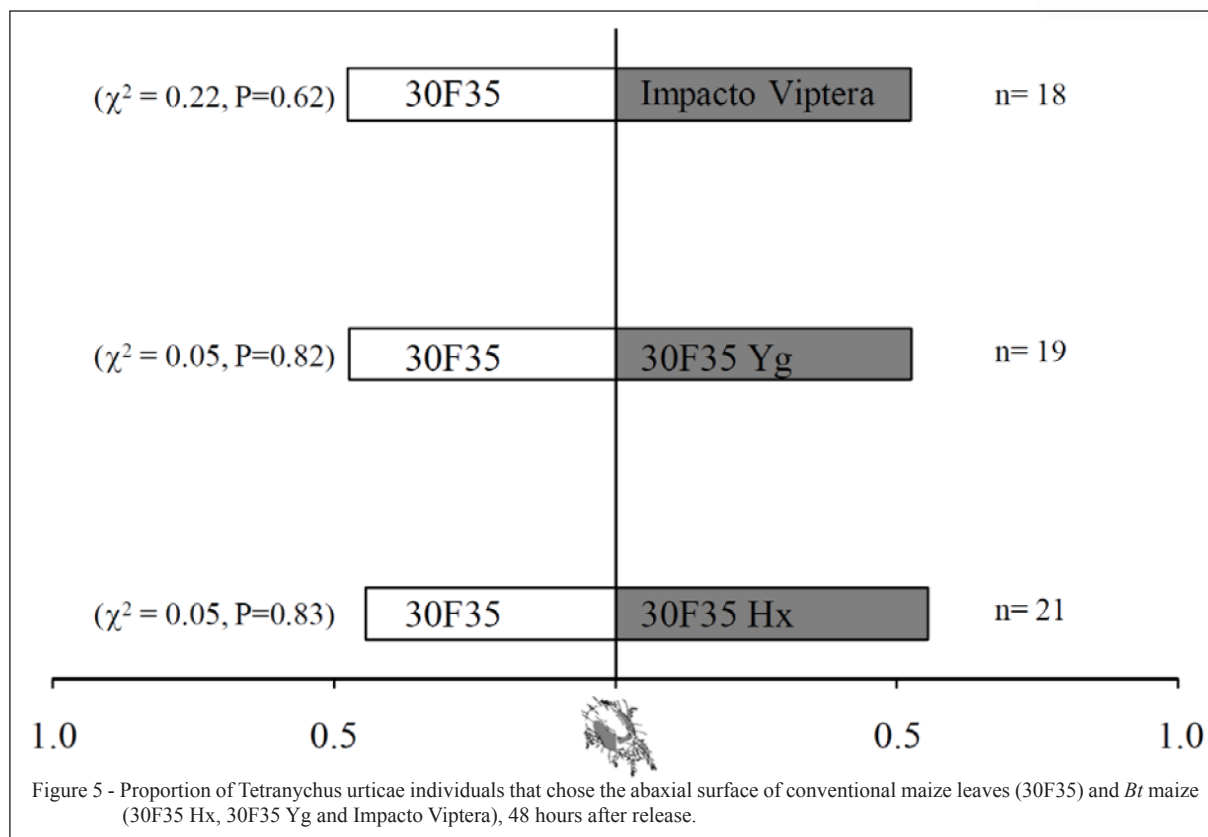
expressing the Cry1Ab protein were lower when compared to the other cultivars evaluated. A similar effect of the varieties was expected on the abundance of phytophagous mites. The *Bt* maize cultivars Cry1Ab (30F35 Yg), Cry1F (30F35 Hx) and Vip3a (Impacto Viptera) also did not affect the abundance of *A. zeala*, similar to that reported for *C. tricholaenae*.

The estimated average number of mites reported greater population densities of *C. tricholaenae* in August and September. These occurrences may be associated with low rainfall during this period since phytophagous mites correlate to hot and dry periods, in general. In low relative humidity conditions, mites have intensified feeding activity, because they need to ingest more fluid to compensate for their water loss, favoring population growth (VAN DER GEEST, 1985).

The lack of variation in the instantaneous growth rate between treatments may be related to physiological characteristics of the host plant, where the nutritional value of the plant is a determining factor in the choice by phytophagous mites, conferred by secondary compounds. The Cry and Vip proteins in

maize plants could promote changes in plant secondary compounds. Thus, the difference in the secondary compound composition could alter the attractiveness of the plant. However, these effects were not observed. There was no significant difference in the instantaneous population growth rate (*ri*) for *T. urticae* female mites maintained on leaf discs of *Bt* and non-*Bt* varieties when subjected to an environment without food choice. This result demonstrated that the Cry protein present in *Bt* maize cultivars (30F35 Hx, 30F35 Yg and Impacto Viptera) does not affect the instantaneous growth rate and the feeding preference of *T. urticae* females while feeding on *Bt* maize plants.

In food preference tests, there was no difference in free choice combinations of *T. urticae* mites between varieties 30F35 Hx vs 30F35, 30F35 Yg vs 30F35 and Impacto Viptera vs 30F35. The *T. urticae* mite demonstrated no preference to the leaf surfaces of both *Bt* and non-*Bt* at 24 hours and 48 hours after release. The non-preference for *Bt* and non-*Bt* maize variety leaf surfaces suggested that the presence of the Cry and Vip proteins in plants did not affect the choice of the feeding site by *T. urticae*.



## CONCLUSION

Plant defense conferred by the *Bt* maize, Cry and Vip proteins, did not affect the abundance of phytophagous mites in the crop. Proteins present in the *Bt* maize also does not affect the instantaneous growth rate and the feeding preference of *T. urticae*. *Bt* maize cultivar does not affect the abundance of phytophagous into mite communities.

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