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DIRECT DEFENSE ELICITED BY *Tetranychus urticae* KOCH (Acari: Tetranychidae) IN Bt MAIZE PLANTS

DEFESA DIRETA ELICITADA POR Tetranychus urticae *KOCH (Acari: Tetranychidae) EM MILHO Bt*

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ABSTRACT: Direct and indirect induced defense mechanisms against herbivores can be manifested in maize (*Zea mays* L.) plants. Furthermore, there are constitutive defenses in which plants continuously express resistance traces. In recent decades has increased the production of transgenic maize plants that constitutively express proteins with insecticide action (Bt maize). The increase of the use of transgenic maize cultivars with the Bt (*Bacillus thuringiensis*) gene demand studies that evaluate the impacts caused by this technology on plant defense mechanisms and their impact on non-targeted organisms, as the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). We tested the hypothesis that Bt maize plants (expressing Cry1F protein) would be capable of inducing direct defenses to *T. urticae* after being attacked by these mites. Thus, we used plants of a commercial maize hybrid (30F35 Hx - expressing Cry1F protein) and plants of its respective non-Bt isogenic line (control). We compared the survival and reproductive performances of *T. urticae* on plants of both lines that were previously infested with conspecifics and on plants that did not suffer pre-infestations. The previous infestation of maize plants by *T. urticae* did not impacted the survival and reproductive abilities of adult and immature forms of the conspecific in both genotypes. These results suggest that, Bt maize expressing the Cry1F insecticidal protein, does not interfere in the induction of direct defense by the *T. urticae* when compared with conventional maize plants.

KEYWORDS: Plant resistance. *Zea mays*. Genetically modified organisms. Mite

INTRODUCTION

Among cultivated plants, the genetically modified maize with the Bt gene from the bacterium *Bacillus thuringiensis* is between the most commonly grown plants (KRUGER et al., 2012; VAN DEN BERG, 2013). The *B. thuringiensis* bacterium is the most widely used organism as a gene source for plant transformation seeking resistance, mainly to Lepidoptera and Coleoptera (PERLAK, 2001). The proteins of *B. thuringiensis* are expressed in the green tissues of genetically modified plants and may also be expressed in pollen, seeds and roots (KOZIEL et al., 1993). This may have adverse effects, in different ways, on non-target herbivores and natural enemies. For example, sap-sucking herbivores may acquire the protein expressed in the sap of the transgenic plants (SIMS; BERBERICH, 1996). Some herbivores may have long-term exposure to genetically modified crops but are not direct targets of Cry proteins (ANDOW; HILBECK, 2004).

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a polyphagous herbivore, having a wide range of hosts with over 1,100 species of plants (GRBIĆ et al., 2011), including maize plants (BYNUM et al., 2017). However, the technical literature which recommends pest management strategies in Brazilian maize (*Zea mays* L.; Poales: Poaceae) fields has not yet recorded mite populations at economic damage levels. The presence of phytophagous mites in maize has mostly been studied as indicators of Bt maize impacts for non-target organisms (FADINI et al., 2010b). In previous studies, the induction of direct defense by *T. urticae* in plants was reported as resulting in reduced performance of these organisms in plants that were previously infested (KARBAN; CAREY, 1984; KANT et al., 2004; DE OLIVEIRA et al., 2015).

The introduction of foreign genes that confer plants resistance against insects shall not interfere with induction of direct and indirect defense in genetically modified plants. Such approaches are promising for pest management and for future generations of transgenic plants (BATES et al., 2005; FERRY et al., 2006). Understanding the interaction between plant defense mechanisms

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facilitates the development of cultivars resistant to pests, as well as the determination of the biochemical and evolutionary interaction between plants-herbivores-natural enemies (WALLING, 2001; BALE et al., 2008; CHEN, 2008).

In this study, we investigated whether the transgenic Bt maize plants could interfere with the induction capacity for direct induced defense against the *T. urticae* mite. Then, our objective was to evaluate the effect of infestation of Bt maize plants by *T. urticae* on development and reproduction of conspecifics in re-infestation.

CONTENTS

We studied the biology of *T. urticae* on maize plants pre-infested by conspecifics and on plants not previously infested (= control, clean plants). Thereafter, for assessing the possibility of direct defense induction in Bt maize plants we compared the biological traits of *T. urticae* on Bt maize plants and conventional maize pre-infested by conspecifics, according with Karban; Baldwin, (1997).

The rearing of mites was started with leaves of maize plants infested by *T. urticae* collected from a greenhouse at Embrapa Milho e Sorgo, Sete Lagoas, Minas Gerais, Brazil. Adult mites, observed through a magnifying glass (40X) were collected individually by brush and transferred to bean plants (*Phaseolus vulgaris* L.) for rearing maintenance. The bean seeds were sown in 500-gram plastic pots using Terral Solo® substrate and separated into infested plants and maintained in screened cages. Uninfested bean plants were added to the mite rearing as necessary, for replacement of mite-infested plants.

The maize hybrids evaluated were 30F35 Hx (Pioneer® commercial hybrid), which expresses the Cry1F protein, and its respective non-Bt iso-hybrid, 30F35, as control. Seeds were sown in 500-gram plastic pots, using Terral Solo® substrate and maintained in a greenhouse. They were used in two steps: the first one, conventional clean maize plants (30F35) and pre-infested conventional maize plants (30F35); the second one, conventional maize plants (30F35) and Bt (30F35 Hx); both were pre-infested. Each treatment consisted of 30 maize plants (= experimental units), separated onto two worktops, resulting in a total of 60 plants. Two seeds per pot were used.

Conventional maize plants (30F35) were submitted to the first infestation with 10 *T. urticae* females, for a period of five days in greenhouse (25±5 °C, 65 ± 10% RH, L16:D8). Infestation of 30 maize plants was conducted, which compose the group of pre-infested plants. One leaf of each of the 30 plants was infested with 10 *T. urticae* females that were transferred and maintained in an entomological glue (Biocontrole®, Indaiatuba, São Paulo, Brazil) barrier to prevent escape of the mites. The leaves chosen for the pre-infestation were marked for identification. Maize plants that did not undergo pre-infestation by *T. urticae* composed the group of clean plants, representing the experimental control. Five days after pre-infestation, the 30 pre-infested maize plants were re-infested, as well as the clean plants. For that, ten *T. urticae* females were transferred to the abaxial surface of leaves. An entomological glue barrier was used to prevent mite escape. The infested leaves were marked for identification.

After five days of the last infestation, the reading of clean and pre-infested ones was randomly done. The pots, numbered 1 to 30, were chosen in such a way that each pot with a maize plant became an independent sampling unit. Counting was done by removing the previously identified leaves. These leaves were cut at their tips with scissors, and they were taken to the laboratory for viewing under a magnifying glass. The evaluations were conducted for 15 days, reading a total of four leaves per day, two belonging to the group of clean plants and two belonging to the pre-infested plant group. The number of alive and dead female mites, the number of immatures and number of eggs were counted.

The conventional (30F35) and Bt (30F35Hx) maize plants were submitted to the first infestation with 10 *T. urticae* females for a period of five days. Infestation was conducted in the 30 conventional maize plants and the Bt. One leaf of each of the 60 plants was infested with 10 *T. urticae* females, coming from the rearing stock, that were transferred and maintained within an entomological glue barrier to prevent their escape. The leaves chosen for the pre-infestation were marked for identification. After five days the conventional (30F35) and Bt 30F35Hx maize plants, previously infested, were re-infested with 10 *T. urticae* females maintained within an entomological glue barrier to prevent mite escape. The leaves chosen for re-infestation were marked for future identification.

After five days of re-infestation, random reading of the pre-infested conventional (30F35) and Bt (30F35Hx) maize plants was carried out. The pots, numbered 1 to 30, were chosen in such a way that each pot with a maize plant became an independent sampling unit. The counting was conducted removing the leaves from the previously identified chosen plants. These leaves were cut at the tips with scissors and taken to the laboratory for viewing under a magnifying glass. The evaluations were performed daily for 15 days, reading two leaves belonging to the conventional maize plant group (30F35), and two belonging to the Bt plant group (30F35Hx). The number of alive and dead female mites, the number of immatures and number of eggs were counted.

The statistical design was completely randomized with two treatments and 30 replications per treatment. Generalized linear models were used (α = 0.05%). These models are suitable for count data that do not meet the assumptions of parametric tests. The explanatory variables (x's) were time after the second infestation and plant quality [*i.e.* conventional (30F35) or Bt (30F35Hx), pre-infested or clean]. As response variables (y's) the number of alive and dead female mites, number of immatures and number of eggs were evaluated. The data were submitted to normality tests and residual analysis to assess the assumptions of the analysis and adequacy of adjusted models. We used the R program (R DEVELOPMENT CORE TEAM, 2014) for exploratory data analysis and statistical analysis (CRAWLEY, 2013).

The pre-infestation of *T. urticae* in non-Bt maize plants (30F35) reduced survival of adult females on re-infestation, when compared to clean maize plants (Figure 1A, χ^2 =26.8, df=58, *P*<0.001). Similar results were found for the number of immatures (Figure 1B, χ^2=30.5, df=58, *P*=0.001), and eggs (Figure 1C, χ^2=48.6, df=58, *P*=0.193), in which the pre-infestation of *T. urticae* reduced the number of conspecific immatures and eggs produced on re-infestation. The number of dead adult females was higher in pre-infested plants than in the clean plants (Figure 1D, χ^2=10.9, df=58, *P*<0.001).

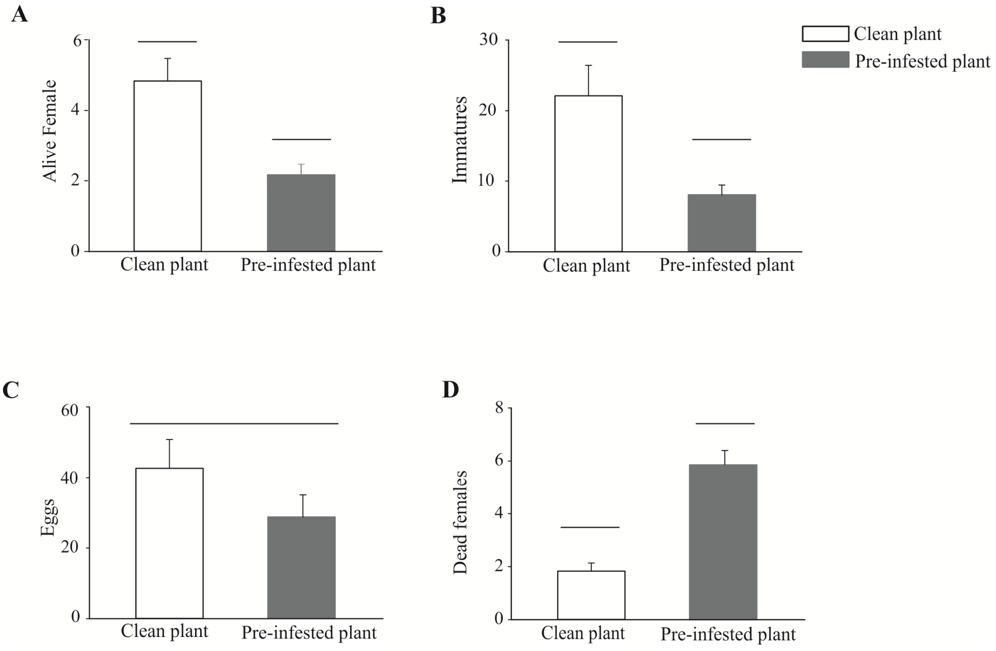

Figure 1. Number (mean ± SE) of alive females **(A)**, immatures **(B)**, eggs **(C)** and dead females (**D**) of *T. urticae* on clean (white bar) and conspecific pre-infested (gray bars) conventional maize (=30F35) plants.

As demonstrated in the first part of the present investigation and in previous investigations (BALDWIN; SCHMELZ, 1996; FADINI et al., 2010a; PAULO et al., 2017), the pre-infestation of non-Bt maize plants by *T. urticae* reduced the survival and reproduction of the conspecific adult females, and it also reduces the survival of immatures. This suggests that maize plants which have undergone pre-infested by *T. urticae* altered the survival pattern of conspecific adult forms, reducing the performance of the population as a result of direct defense induction. Similarly, Szczepaniec et al. (2013) found increased expression of defense genes induced by phytophagous mites and the expression of genes related to induced defense and reduction in the population of *T. urticae* in tomato, maize and cotton plants. These results corroborates to the hypothesis that plants that have suffered previous *T. urticae* infestations are able to respond to injuries faster, compared to those that were not attacked.

In this first stage of the work, it was thus possible to detect the direct defense induction in conventional maize by the pre-infestation of *T. urticae*. After observation, it was decided to assess whether the direct defense induction would also occur in Bt maize plants. Towards this end, we proceeded to the second and final stage of the work and the results are reported below.

The pre-infestation of *T. urticae* in Bt maize plants showed no significant difference in the number of conspecific adult females on re-infestation when compared with its non-Bt isoline (Figure 2A, χ^2=45.2, df=58, *P*=0.110). In addition, the pre-infestation of *T. urticae* in Bt maize showed no significant difference in the number of immatures (Figure 2B, χ^2=64.7, df=58, *P*=0.745), and the number of eggs (Figure 2C, χ^2=50.1, df=58, *P*=0.240). The pre-infestation in Bt maize plants showed no significant difference in the number of dead adult conspecific females compared to its non-Bt isoline plants. The number of dead adult females increased in Bt maize plants and in conventional maize plants that were previously infested (Figure 2D, χ^2=76.9, df=58, *P*=0.951).

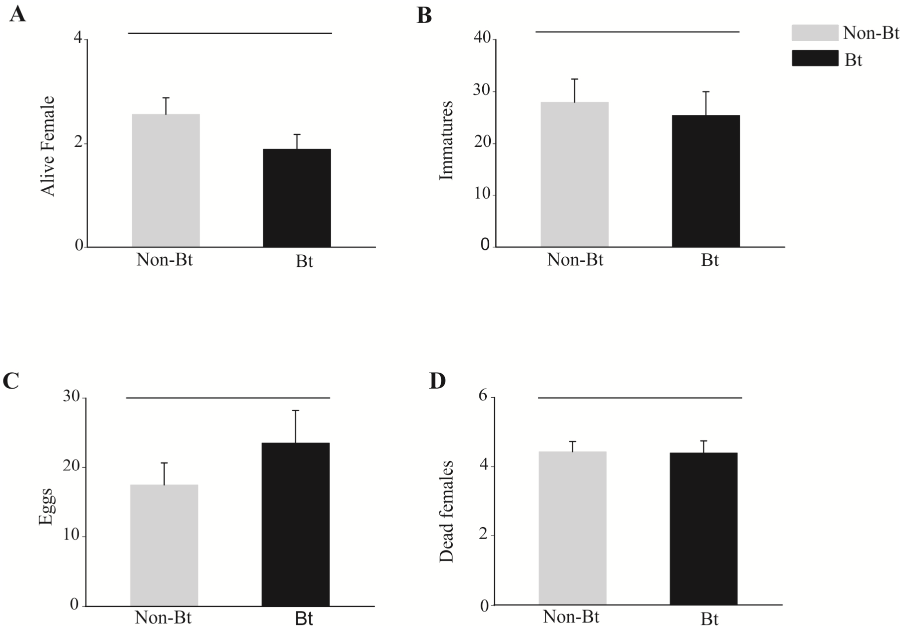

Figure 2. Number (mean ± SEM) of alive females (**A**), immatures (**B**), eggs (**C**) and dead females (**D**) of *T. urticae* on conventional maize (=30F35, gray bars) and Bt (=30F35 Hx, Cry 1F, black bars) conspecific pre-infested plants.

In this second stage of the work, we could then see that the biological parameters evaluated for *T. urticae* upon re-infestation did not differ significantly between the conventional and Bt maize plants pre-infested with the conspecific. The expression of Cry1F protein did not impact the abilities of maize plants to induce direct defenses against the *T. urticae*. Therefore, the plant defense conferred by inserting the Bt gene did not negatively interfere in the pre-existing direct defense induction capacity of maize plants. Ferreira et al. (2017) reported that *T. urticae* showed no preference between the Bt or conventional maize leaves, and did not affect the abundance of species in phytophagous mite. Similarly to the results non-Bt maize plants results, the pre-infestation of Bt maize plants by *T. urticae* did not resulted in significant reductions of the mite survival and reproductive abilities. Although not directly addressed here, other investigations (HAGENBUCHER et al., 2014) have shown that Bt and non-Bt cotton plants did not differ in terpenoid levels when they were previously subjected to jasmonate treatments.

Contradictorily, other investigations have pointed out to the potential synergy between the Bt gene and direct induced defense triggered by jasmonates (FENG et al., 2007; WU et al., 2000; ZHANG et al., 2000). However, Wu et al. (2000) reported that the content of tannins and phenolic acid content were significantly lower in R93-1 and R93-4 Bt cotton compared with their non-Bt parental varieties. In Bt maize, the authors also found that the expression of genes related to direct induced response was significantly higher than in conventional plants. The quality of the host plant is a determining factor for the performance of an herbivore; changes in plant physiology and biochemistry directly affect their fertility Awmack; Leather (2002). However, in our work the pre-infestation of Bt maize plants did not change the number of immatures and eggs of *T. urticae* when compared to the conventional.

Our work suggests that the defense of the plant conferred by expression of the Cry1F protein, should not affect the induced of direct defense to *T. urticae* when the plants are submitted to previous infestations.

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RESUMO: Mecanismos diretos e indiretos de defesa induzida contra herbívoros podem manifestar-se em plantas de milho (*Zea mays.*). Além das defesas induzidas, existem as defesas constitutivas, nas quais as plantas expressam a resistência de forma contínua. Nas últimas décadas vem se difundindo a produção de plantas de milho geneticamente modificadas que expressam proteínas com ação inseticida de forma constitutiva (milho Bt). Com o crescente uso de cultivares de milho transgênico com o gene Bt (*Bacillus thuringiensis*), há uma demanda por estudos que avaliem os impactos causados por essa tecnologia sobre os mecanismos de defesa das plantas e seu impacto sobre organismos não alvo, como o ácaro-rajado *Tetranychus urticae* Koch (Acari: Tetranychidae). Testou-se a hipótese de que plantas de milho *Bt* (expressando a proteína Cry 1F) seriam capazes de induzir defesas diretas a *T. urticae* após o ataque por esses ácaros. Assim, foram utilizadas plantas de milhos híbridos comerciais (30F35 Hx expressando a proteína Cry 1F) e seu respectivo isogênico não-Bt (controle). Nós comparamos a sobrevivência e o desempenho reprodutivo de *T. urticae* em plantas de ambas as linhagens que foram previamente infestadas com coespecíficos e em plantas que não foram pré-infestadas. A infestação prévia de plantas de milho *Bt* por *T. urticae* não apresentou diferença nos padrões de sobrevivência de formas adultas e formas jovens do coespecífico em comparação com o milho convencional. Os resultados sugerem que, o fato de o milho *Bt* expressar a toxina inseticida Cry 1F, não interfere na indução de defesa direta pelo ácaro-rajado *T. urticae* quando comparado com plantas de milho convencional.

PALAVRAS-CHAVE: Resistência de plantas. *Zea mays*. Organismos geneticamente modificados. Acaros.

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