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Notes and Comments

Cry protein in the predatory mite *Neoseiulus californicus* and spider mite *Tetranychus urticae* prey fed with transgenic maize

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(With 1 figure)

The use of genetically modified insect-resistant crops (GM) with the gene from *Bacillus thuringiensis* (*Bt*) that express toxic proteins is an efficient means to control pests. Among these, *Bt* maize is the most widely grown crop (Ramirez-Romero et al., 2008).

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), an organism non-targeted by the *Bt* maize protein, is one of the most important polyphagous pests in agriculture (Bolland et al., 1998; Capinera, 2001; Liburd et al., 2007; Moraes and Flechtmann, 2008; Opit et al., 2004). Several studies have studied the effects of Bt plants on insects (Dutton et al., 2002; Lozzia and Rigamonti, 1998; Lozzia, 1999; Pilcher et al., 1997). However, there are only a limited number of evaluated on phytophagous mites and predators (Castro et al., 2013; Fadini et al., 2012).

It is probable then that the Cry protein can be transferred from the mites to the third trophic level, the predators. The predatory mite *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) feeds on mites of the family Tetranychidae (McMurtry and Croft, 1997) and can acquire the Cry protein by ingesting *T. urticae* mites that feed on Bt plants (Dutton et al., 2002; Obrist et al., 2006). Thus, aimed to evaluate the presence of Cry1F protein expressed in maize plants genetically modified, in phytophagous mite *T. urticae* and its predator *N. californicus*.

The experiment was conducted in a greenhouse and Agricultural Entomology Laboratory of the Federal University of São João del Rei (UFSJ), Sete Lagoas, Minas Gerais. It conducted the test with Bt-Cry1F Kit ImmunoStrip® Test for Cry 1F protein detection 30F35HX Bt maize leaves in phytophagous mite *T. urticae* fed Bt maize 30F35HX leaves, and predator *N. californicus* fed *T. urticae* 30F35HX maintained in Bt maize leaves. The control was made by conventional isoline 30F35, wherein the test was carried out in conventional maize leaves 30F35 in *T. urticae* mite maintained in conventional corn plants, and predators *N. californicus* fed *T. urticae* kept in conventional maize leaves

The results were negative for the control group samples [i.e. conventional corn leaf discs (30F35), *T. urticae* and *N. californicus*], there was the formation of a control line on the Cry 1F ImmunoStrip® strips, as expected, confirming the absence of the Cry protein in the conventional maize plants (30F35) (Figure 1a). The results were positive with Bt maize leaf discs samples (30F35 Hx), in which a second test line was developed in the region between the control line and the lower end of the strip. This result confirms the presence of the Cry 1F protein in leaves of the commercial hybrid maize 30F35 Hx (Figure 1b). The samples with phytophagous *T. urticae* mites that were fed with Bt maize, after contact with the Cry 1F Immunustrip® strips, presented the formation of the control line and the test line, indicating the presence of the Cry 1F protein in these mites (Figure 1c). Similarly, samples with the predator *N. californicus* which fed on *T. urticae* maintained on Bt maize leaves (30F35 Hx), presented a positive result, indicating the presence of the Cry protein in the *N. californicus* predatory mites (Figure 1d).

Results are from a qualitative test which reveals the presence or absence of the Cry 1F protein. Thus, they indicate that the Cry 1F protein present in Bt is transferred and accumulated in two-spotted spider mite *T. urticae* and the *N. californicus* predatory mite. The results also show that the Cry1F protein is transferred from the second to the third trophic level, i.e. to the predatory mite *N. californicus*. When feeding on *T. urticae* that fed on 30F35 Hx maize plants, *N. californicus* predatory mites accumulated protein from Bt Cry 1F maize plants.

Although there was Cry protein accumulation in phytophagous mite *T. urticae* and predator *N. californicus*, as found, the protein does not affect biological and behavioral parameters of *T. urticae* (Ferreira, 2014). The Cry protein, present in varieties of Bt maize (Hx 30F35, 30F35 YG and Viptera Impact) did not alter the abundance of phytophagous

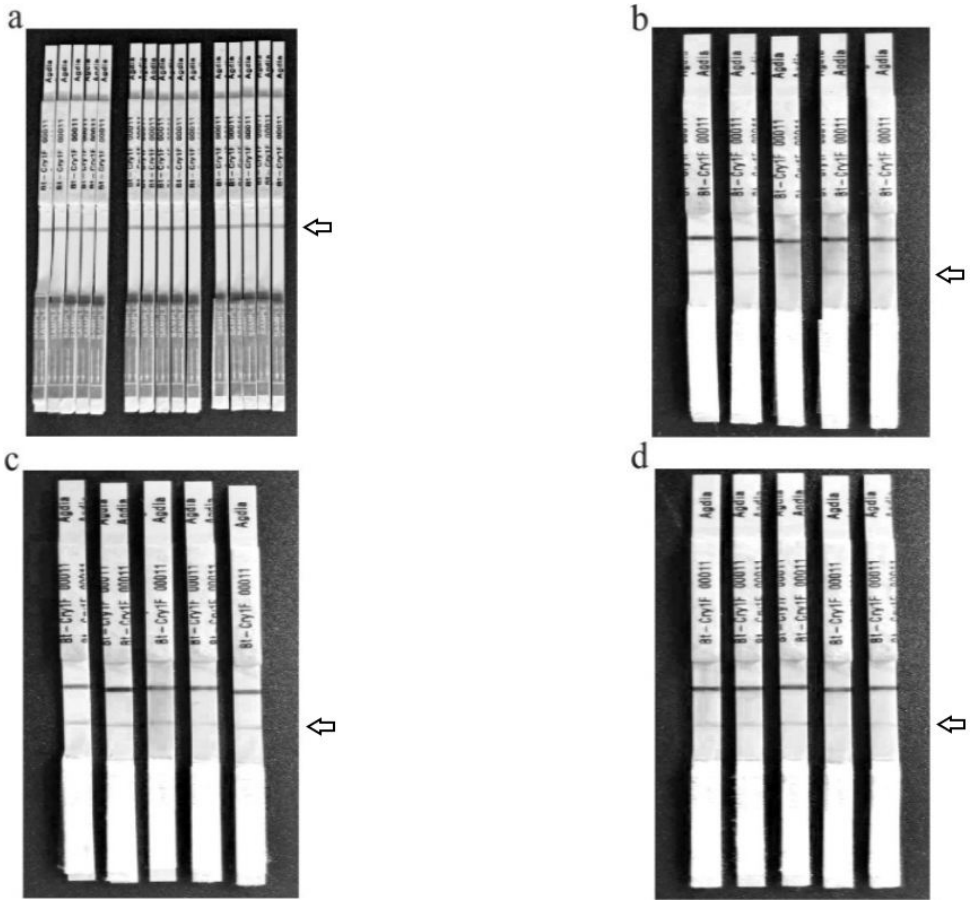

Figure 1. The arrow indicates positive band to cry protein presence. (a) ImmunoStrip® strips for Cry 1F from the control sample (conventional maize leaves, *T. urticae* and *N. californicus*); (b) Strips ImmunoStrip® Cry1F from the sample with maize leaf discs Bt (30F35 Hx); (c) Strips ImmunoStrip® Cry1F from the sample with *T. urticae* fed Bt maize plants (30F35 Hx); (d) Strips ImmunoStrip® Cry1F, from the sample with predator *N. californicus* fed *T. urticae* kept in Bt corn plants (30F35 Hx).

mites in the field, as well as the instantaneous rate of growth and food preference of *T. urticae* not differing in Bt and conventional maize (Ferreira, 2014). This work allowed us to assess the Cry protein expressed in Bt maize plants is transferred to the mite *T. urticae* and its predator *N. californicus*.

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