Advance Journal of Food Science and Technology 4(6): 393-397, 2012
ISSN: 2042-4868; E-ISSN: 2042-4876

Submitted: September 11, 2012 Accepted: October 19, 2012 Published: December 20, 2012

Effect of Transgenic Bt Cotton on Abundance of Cotton Spider Mites and Total Phenolic Content of Leaves and their Relationship

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Abstract: The differences of the total phenolic content in leaves and percentage of cotton plants infested with cotton spider mites between in transgenic Bt (Ezamian No. 24F1) and in non-transgenic Bt cotton (Ek 9 parental line of Ezamian No. 24F1) plots with and without spraying acaricides were systematically investigated in Tai Lake farm, Hubei Province, China, over the period 26 May and 11 September 2011. In acaricide treated plots, transgenic Bt cotton does not result in a change of the abundance of cotton spider mites compared to that in non-transgenic Bt cotton, however, without acaricide treated plots, transgenic Bt cotton significantly increases the abundance of cotton spider mites compared to those of non-transgenic Bt cotton. The number of eggs, larva-nymph-adults, egg-larva-nymph-adults and the plant damage index are independent of the total phenolic content in leaves. The results are also discussed in relation to integrated pest management. It was very necessary for nontarget cotton spider mites of transgenic Bt cotton fields to control in wetland agricultural area.

Keywords: *Gossypium hirsutum*, *Tetranychus cinnabarinus*, total phenolic content, transgenic Bt cotton

INTRODUCTION

Cotton spider mites are the most destructive herbivorous arthropods of cotton, Gossypium hirsutum L. in the world and are the nontarget herbivores of Transgenic Bt cotton (Esteves *et al*., 2010). The group is composed of 5 dominant species in China, namely *Tetranychus cinnabarinus* Boisduval, *T. truncates* Ehara, *T. urticae* Koch, *T. turkestani* Ugarov et Nikoskil, *T. dunhuangensis* Wang (Acari: Tetranychidae) (Krantz and Walter, 2009; Xu *et al*., 2011). *T. cinnabarinus*, the dominant species in the Middle Reaches of the Yangtze River of China, has a 15-18 days life-cycle. Cotton spider mites prefer the underside of leaves. The mites pierce the leaves to suck the sap causing a range of foliar symptoms, such as red leaves, commonly referred to as "the dragon's fire". Cotton spider mites can cause important yield losses and even total yield loss during outbreaks.

Recent studies reported impact of transgenic Bt cotton on population dynamics of spider mites in cotton plants (Cui *et al*., 2004; Wang *et al*., 1999). However, the effect of transgenic Bt cotton and total phenolic content of cotton leaves on the abundance of cotton spider mites, in wetland agricultural area with and without acaricide sprays, remains largely unknown.

As the area planted with transgenic Bt cotton in the Middle Reaches of the Yangtze River increases, whether or not so does the damage caused by cotton spider mites? This study looked at the population dynamics of cotton spider mites and the relationship between the total phenolic content of leaves and the number of cotton mites, plant damage indices in transgenic Bt cotton and non-transgenic Bt cotton fields with and without acaricide sprays. Tests were carried out in 2011 at Tai Lake farm (Longitude 111.97º-112.15º, North latitude 30.34º-30.42º), a typical wetland agricultural area adjacent to Yangtze River and Han River, Hubei Province, China. The groundwater level is usually 100-150 cm in winter-spring and 50-100 cm in summer. Current cotton fields, is transformed from the lakes in the 1950s of the 20th century. In 2011, in Jingzhou city there were some 14100 km², of land used to grow rice, cotton, fish, rape and vegatables, etc., with 108000 hm^2 in cotton. At the Tai Lake farm, there were 1330 hm² of agricultural planting with some 540 hm² of cotton.

It is easy and common practice for land owners to use their land interchangeably as intensive culture ponds, cotton fields, lotus fields or rice paddy fields. The use of the land is often determined by changes in the market price of fish, lotus, cotton or rice paddy. It is a legal practice in Jingzhou city. Some 20 hm² cotton of land have been transformed from rice paddy fields to cotton production since 2008 at the Tai Lake farm test site.

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The objective of this study were to evaluate effect of transgenic Bt cotton on the abundance of cotton spider mites in wetland agricultural area and to provide a theoretical basis for integrated pest management of cotton spider mite in the Middle and Lower Reaches of the Yangtze River of China.

MATERIALS AND METHODS

Treatment: The study was conducted between 26 May and 11 September 2011. There were two treatments consisting of transgenic Bt cotton (Ezamian No. 24F1) and non-transgenic Bt cotton (Ek 9 parental line of Ezamian No. 24F1).

Monitoring: The experiment field was divided into acaricides spray and no-spray area. The same experimental design at each area consisted in a randomized complete block with two factors (transgenic Bt and non-transgenic Bt cotton) replicated 4 times (acaricides spray area) or 5 times (acaricides no-spray area). Acaricides spray and no-spray area measured approximately 133.3 and 166.7 m^2, respectively. Acaricides were sprayed 8 times in the spray area.

Monitoring was carried out 17 times throughout the season. Before 16 July 2011, mites were counted on each cotton plant leaves of a total of 25 cotton plants per sampling date. After 16 July 2011, mites were counted using the same manner as above before 16 July 2011, however, the number of cotton spider mites was counted on 3 randomly chosen leaves (upper, middle and lower) per plant and the percentage of plants with mites calculated. Acaricides were applied throughout the season in spray areas but only until 15 July 2011 in no spray areas. Monitoring of the no spray areas was carried out 8 times between 17 July and 4 September 2011 using the same method as in the spray area.

In the acaricide sprayed and no-sprayed fields, the total phenolic content of each of 10 cotton leaves per treatment, randomly collected (third complete leaf from the top of the plant) on 17 August 2011, was determined. In the fields without acaricide sprays after July 15, on 22 July 2011, thirty cotton leaves were randomly collected in the same manner as above on 17 August 2011. The number of eggs, larva, nymphs and adults of cotton spider mites and the damage index of each leaf were recorded before they were dried, then dried, ground and stored at -60ºC prior to the determination of total phenolic content. Total phenolic content of each cotton leaf was determined for each leaf (three replicates for each sample) using Folin-Ciocalteu's assay with gallic acid as standard (Folin and Denis, 1915).

The damage level of erythematous area in cotton leaves was estimated using a 1 to 5 scale (Zhang *et al*., 1993).

Statistical analysis: All data was analyzed using DPS (Tang and Feng, 2002). Average percentage of cotton plants infested with cotton spider mites and total phenolic content of leaves were transformed by arcsine of the square root before using Student's test for comparison. A regression analysis was carried out to determine if the number of eggs larva-nymph-adults and egg-larva-nymph-adults was correlated with the total phenolic content in leaves. The correlation between plant damage level and total phenolic content in leaves was measured using multiple sequence correlation.

RESULTS AND DISCUSSION

Population dynamics of cotton spider mites in acaricide treated plots: Over the period 26 May-11 September 2011 (Fig. 1), the average percentage of cotton plants infested with cotton spider mites was not change between in transgenic Bt (23.187±5.21%) and in non-transgenic Bt cotton plots (23.47±5.28%) (t = 0.153, df = 16, p = 0.880). Also, there were no significant differences in the number of mites on cotton between in transgenic Bt (15.50±7.96%) and in non-transgenic Bt cotton plots (14.5±5.66%), in the period 27 July-28 August 2011(t = 0.350, df =5, p = 0.740). The average percentage of infested cotton plants was the same very high in two types of cotton plots on 1 July and 17 August 2011. However, it was so very low on 20 June 2011 and in mid-season, 17-28 August 2011.

Population dynamics of cotton spider mites in without acaricide treated plots: On 27 July-28 August 2011 (Fig. 2), there were significant differences in the average percentage of cotton plants infested with cotton spider mites between in transgenic Bt (40.67±8.67%) and in non-transgenic Bt cotton plots (20.00±4.73%) (t = 3.47, df = 5, p = 0.018). On 24 August 2011, the average percentage of infested cotton plants was the highest in transgenic Bt cotton plots in the period 27 May-4 September 2011. However, on 28 August 2011 was the lowest during the period in non-transgenic Bt cotton plots.

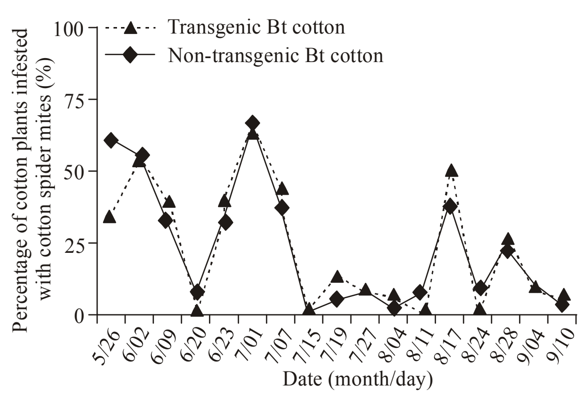

Fig. 1: Population dynamics of cotton spider mites in transgenic Bt cotton and non-transgenic Bt cotton fields sprayed throughout the season with acaricides (26 May-11 September 2011)

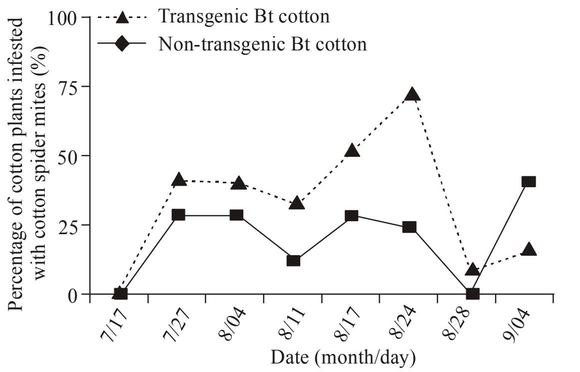

Fig. 2: Population dynamics of cotton spider mites in transgenic Bt cotton and non-transgenic Bt cotton fields with no acaricide sprays after July 15, 2011

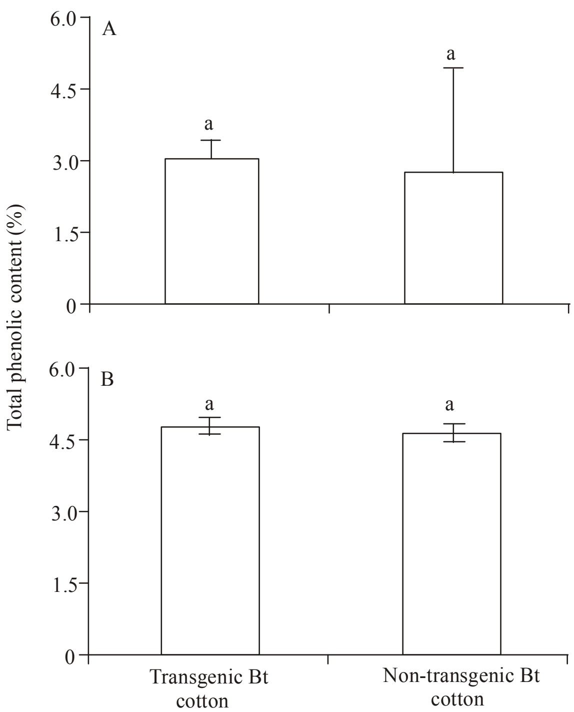

Fig. 3: Total phenolic content of cotton leaves (replicated 10 times) from transgenic Bt and non-transgenic Bt cotton fields with (A) and without (B) spraying acaricides on 17 August 2011

Total phenolic content of cotton leaves from transgenic Bt and non-transgenic Bt cotton fields with and without spraying acaricides: The total phenolic content in cotton leaves didn't vary significantly between transgenic Bt (3.04±0.34%) and non-transgenic Bt cotton fields (2.80±2.10%) with spraying acaricides (t = 0.549, df = 29, p = 0.585, Fig. 3(A)). There was also no significant difference in the total phenolic content of cotton leaves between in transgenic Bt (4.78±0.18%) and in non-transgenic Bt cotton plots (4.62±0.18%) without spraying acaricides (t = 0.655, df = 29, p = 0.521, Fig. 3(B)).

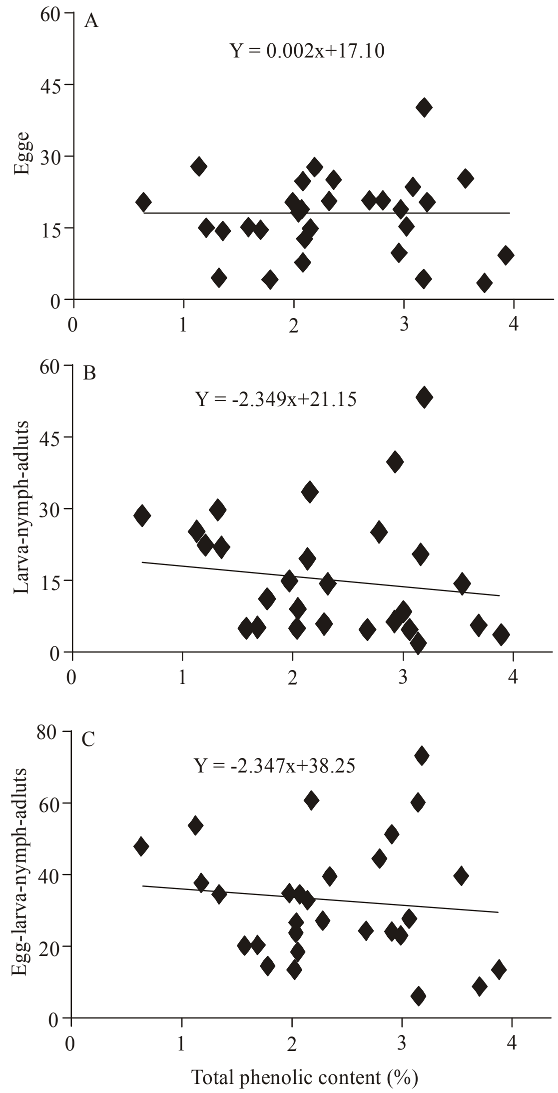

Fig. 4: The relationship between the numbers of eggs (A), larva-nymph-adults (B), egg-larva-nymph-adults (C) respectively and total phenolic content in of leaves

Relationship between different indicators of population abundance and total phenolic content of leaves: There was no significant linear correlation between the number of eggs (y = 0.002x+17.10, r = 0.006, $r_{28,0.05}$ = 0.349, p = 0.098, Fig. 4(A)), larva-nymph-adults (y = -2.349x+21.15, r = -0.181, $r_{28,0.05}$ = 0.349, p = 0.347, Fig. 4(B)), or egg-larva-nymph-adults (y = -2.348x+38.25, r = -0.135, $r_{28,0.05}$ = 0.349, p = 0.486, Fig.4(C)), respectively and total phenolic content of leaves. There was also no significant multiple sequence correlation between plant damage indices and total phenolic content (r = -0.136, df = 1, 28, p = 0.244).

CONCLUSION

Our results showed clearly that, in acaricide treated plots, transgenic Bt cotton does not result in a change of the abundance of cotton spider mites compared to that in non-transgenic Bt cotton, however, without acaricide treated plots, transgenic Bt cotton significantly increases the abundance of cotton spider mites compared to those of non-transgenic Bt cotton. Some authors suggested that Bt cotton has no effect on spider mites populations (Sims, 1995; Ma *et al*., 2006; Xu *et al*., 2011). Averages of the three generations showed that the Bt-cotton does not affect the development, survival of immature stages, and reproductive output of *T. urticae* (Esteves *et al*., 2010). However, the results of Cui *et al.* (2004) showed Bt cotton results in the increase on quantity of *T. cinnabarinus* in cotton field.

There were not differences between transgenic Bt cotton and non-transgenic Bt cotton field in total phenolic content of cotton leaves whether or not acaricides were used. The number of eggs, larva-nymph-adults, egg-larva-nymph-adults and the plant damage index were independent of the total phenolic content in leaves. Zhang *et al.* (1993) reported a similar absence of correlation between red spider mite, *T. urticae*, plant damage level and total phenolic content in leaves. However, some authors have found that an increase in total phenolic content in cotton canresult in an increased repellency to red spider mites and reduced number of eggs (Wu *et al*., 1996). Also, an aqueous solution of hydroxyphenylalanine and quinic acid was found to increase the content in phenolic compounds of strawberry leaves and high concentrations of catecholamines significantly delayed the development time of two spotted mites, *T. urticae* (Luczynski *et al*., 1990).

One can only speculate on the factor (s) responsible for the differences of the abundance of cotton spider mites in cotton leaves between in transgenic Bt and in non-transgenic Bt cotton field. The most likely factors would be the effect of Bt cotton gene and pesticides (acaricide) on natural enemies of cotton spider mites. In acaricide treated plots, pesticides (acaricide) reduce numbers of mites and their natural enemies (Wang *et al*., 1999). Without acaricide treated plots, transgenic Bt cotton result in a decrease of natural enemies of cotton spider mites and a change of cotton nutrition and secondary compounds to be unfavorable to outbreaks of cotton spider mites (Cui *et al*., 2004). Such as some authors reported Bt cotton resulted in the decrease on the total phenolic content in cotton leaves (Wu *et al*., 2000; Wang *et al*., 1999; Olsen *et al*., 1998).

Results have shown that it was very necessary for nontarget cotton spider mites of transgenic Bt cotton fields to control in wetland agricultural area.

ACKNOWLEDGMENT

This study was supported by grants from Public-benefit Industry Special Foundation No. 201103020 for Ministry of Agriculture of the People's Republic of China.

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