Available online at http://www.ijims.com
ISSN - (Print): 2519 – 7908 ; ISSN - (Electronic): 2348 – 0343
IF:4.335; Index Copernicus (IC) Value: 60.59; Peer-reviewed Journal

# Studies on The Interaction of Predatory Insect and Transgenic Raised Prey

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**Abstract**

The objective of this study was to find out the impact of transgenic plant on predators, which forms one of the links in the tritrophic system. The life stages of *D. cingulatus* were raised on non- *Bt* and *Bt* cotton, and they were fed to the predator *R. fuscipes* and to find on different key biological factors like stadial periodicity, adult male and female longevity, fecundity, hatching percentage, chronological events of predation, mating and sex ratio. Based on the results of the present study the life history of the predator *R. fuscipes* was unaffected by prey that fed on, or by prey acquiring the toxin.

**Key words** : *R. fuscipes, D. cingulatus*, tritrophic interaction.

## Introduction

Among the different pests of cotton the cotton strainer *Dysdercus* sp. (Heteroptera : Pyrrhocoridae) is a serious pest, which feeds on developing cotton bolls, squares and ripe cotton seeds, and transmit fungi that develop on the immature lint and seeds[13] . These pests are difficult to manage merely by insecticide application in cotton fields, because they are highly mobile and have many alternative wild host plants[6] . Therefore, the use of natural enemies should be considered to manage these pests since natural enemies are applicable not only to cultivated cotton fields, but also to wild alternative vegetation hosting cotton strainers.

Arthropod natural enemies are very common in crops and play an important role in the regulation of many pest populations. They are taxonomically diverse and show considerable variation in life history[5] . Three major groups of predatory insects (heteropterans, coleopterans and neuropterans) are recognized as important natural enemies of key and secondary pests in cotton, and these predators are capable of consuming even non-pest arthropods to sustain their populations[7] .

Resistant plants, whether produced through conventional breeding or biotechnology, can potentially affect natural enemies in many different ways and their interaction may be additive, antagonistic or synergistic[9] . Effects of genetically modified plants on non-target entomophagous arthropods (predators and parasitoids) have been a major concern as these organisms often play an important role in natural pest regulation, and are considered to be of economic value. Moreover, the predatory organisms may be good indicators of potential ecological impact of transgenic plants as they belong to the third trophic level in the food chain[3] .

The non- target insect pest like *Dysdercus* sp survive on transgenic cotton even though there is systemic accumulation of the toxins produced by transgenics. These pests, supported by transgenic plants are consumed by predators not occupying those agroecosystems exposing them to the risk of *Bt* toxicity.

The non-target organisms can be potentially affected by the insect-resistant transgenic plants through various ways[10]. The impacts on non-target entomophagous arthropods can be due to direct toxic effects though exposure to the insecticidal protein, indirect effects through reduction in prey/host quantity and/or quality, or indirect effects due to unintended changes of plant properties (chemical or physical) caused by the insertion of a new gene.

Natural enemies feeding on *Bt*-intoxicated prey may have reduced longevity, and likely, fecundity that reduces their efficacy in controlling pest populations, and pest resurgence and secondary pest outbreaks may occur that may require insecticide use[4] .

*Bt* toxin may affect natural enemies either directly, or indirectly by feeding from sub-optimal food. The present study aims to find out the effect of *D. cingulatus* life stages raised on non- *Bt* and *Bt c*otton, when fed to the predator *R. fuscipes* and to find on different key biological factors like stadial periodicity, adult male and female longevity, fecundity, hatching percentage, chronological events of predation, mating and sex ratio.

**Materials and methods**

*R. fuscipes* adults were collected from Aralvaimozhi scrub jungles ($77^0 31^{''}$1” and $8^0$ 11” N). They were maintained in the laboratory in 200 ml plastic containers on *Corcyra cephalonica* Stainton caterpillars grown on wheat flour and on *Odontotermes obesus* Rambur under laboratory conditions (temperature 30-$35^0$ C; RH 75-85% and 11-13h photoperiod. The bugs were allowed to lay eggs. The eggs were incubated inside plastic containers protected from ants and other pests. The containers were placed in a cool area away from direct heat. The humidity was maintained by placing cotton stubs soaked in water inside the container. The hatched out nymphs were fed with *D. cingulatus* life stages raised on conventional and *Bt* cotton plants. The young *R. fuscipes* nymphs were fed with $1^{st}$ instar *D. cingulatus*. The nymphs were grown into adults. The adults were allowed to mate and start the second generation. In the second generation *R. fuscipes* females a record of the number of batches of eggs and the total number of eggs laid was maintained for each female. Each batch of eggs was allowed to hatch in individual containers giving optimum humidity and the hatched nymphs were fed ad libitum with first instar *D. cingulatus* raised on conventional and *Bt* cotton plants. Mortality as well as moulting of nymphs, sex ratio among laboratory emerged adults, and the adult longevity were recorded.

**Sequential events in predatory behavior:**

The chronology of different independent predatory events was chronicled. The starving predatory bugs were used in the study of predatory behavior since they were more active compared to the satiated ones. The hungry predator was allowed to settle in a feeding chamber and the prey was introduced. The moving prey which was reared on conventional and *Bt* cotton plant formed the switch point of the predatory behavior and it aroused the hungry predator. Arousal was followed by the orientation of the predator to the prey and subsequently the predator approached the prey. The next switch point was proximity of the prey. The second switch point lead to events like capturing and injection of saliva. The time taken for paralyzing the prey and the subsequent feeding time was recorded. Satiation is still another switch point at the end of the sequential events related to predation.

**Analysis of mating behavior**:

Mating behavior was analysed as timed independent events like arousal, approach, nuptial clasp, riding over, genetalia extension, intromission and emission of spermatophore capsule. The timing of these events was recorded. For analyzing the mating behaviour the mating chambers containing leaves, twigs and pebbles to suit the natural conditions. The mating pair was allowed- female first followed by the male. The mating events were recorded using an electronic stop clock. After the whole mating process is over, the time taken for postcoital and the time taken by the female foe ijecting the spermatophore capsule were recorded. The mating paradigm was separately followed for bugs fed with *D. cingulatus* reared on conventional and *Bt* cotton plants.

**Results**

The incubation period of *R. fuscipes* raised on *D. cingulatus* life stages fed with conventional cotton plant is 9.83±0.75 days and *Bt* cotton is 10.33±0.51 days. The preoviposition period of *R. fuscipes* raised on *D. cingulatus* life stages fed with conventional cotton is 8.0±0.63 and Bt cotton is10.3± 1.03. The oviposition period of *R. fuscipes* reared on conventional cotton fed *D. cingulatus* is24.42±1.16 and *Bt* cotton is 21.2±1.05. The post oviposition in conventional cotton is 9.02±1.03, where as in *Bt c*otton 8.66±1.01 days. The average, minimum and maximum number of eggs per batch in conventional cotton is 13.5±0.83 ; 5.83±0.40 and 18.83±0.98 and in *Bt* cotton 12.5±0.54; 5.33±0.51 and 18.16±0.98.

The male and female adult longevity and sex ratio of *R.fuscipes* were 39.05±2.9, 42.0±2.82 and 0.50±1 observed when the larvae fed with conventional cotton. When the larvae fed with *Bt* cotton the male and female adult longevity and sex ratio were 38.32±3.1, 41.1±2.6 and 0.48:1. The maximum adult longevity was decreased in the male *R. fuscipes* (1.86) when the larvae fed with *Bt* cotton (Table 1).

The maximum capturing (min), paralyzing (min), sucking (min), total duration (min) and piercing site (no) were (80.3±0.51, 3.9±1.03, 8.80±2.09, 100.2±8.45 and 6.62±0.81) observed when the *R.fuscipes* survive in the *Bt* cotton mature branch. The maximum decrease of capturing (16.1), paralysis (13.71), sucking (3.37), total duration and piercing sites (21.2) observed in these corresponding groups when compared to wild cotton mature branch (Table 2).

In chronological analysis of mating (min) in *R. fuscipes* chronological analysis such as arousal (2min 5sec) approach (2min 10 sec), nuptial clasp (21min 20sec) extension of genetalia (1 min 8 sec), duration of copulation (26 min 25 sec) and ejection of spermatophore capsule (54min 14 sec) were taken maximum time when the larvae fed with feed type B compared to feed type A (Table 3).

Statistically significant differences were observed only for total developmental period of *R. fuscipes* fed with *D. cingulates* raised on *Bt* cotton and conventional cotton. Incubation period, the different stadial period, male and female longevity and fecundity were not significant in comparison of *R. fuscipes* raised on the two feed types.

**Discussion**

The insecticidal proteins produced by the transgenic plants enter into herbivores that consume these genetically modified plants. The non-target herbivores that grow on transgenic plants show altered biology with specific changes in reproductive and feeding behavior[12] .The impact of transgenic plant raised pest on the reduviid predator, *R. fuscipes* has been studied. *Bt* plants have little direct effect on sucking insects, because Bt toxin uptake may be higher for chewing herbivores than sucking herbivores[11] .Laboratory studies suggest that the effect of *Bt* crops on natural enemies may depend upon whether the prey or host takes up the toxin and is affected by the toxin. Accordingly, no impact is expected for natural enemies attacking phloem-feeding herbivores. *Rhopalosiphum padi* L. a phloem feeder, did not take up the toxin and that the survival of *C. carnea* was not affected when provisioned with *R. padi* that had fed on *Bt* cotton [2].

*R. fuscipes* is a secondary consumer in the food chain, *Bt* cotton- *D. cingulatus* – reduviid predator. The secondary consumer is least affected by the endotoxins produced by *Bt* cotton. The laboratory experiments provided clear evidence supporting this hypothesis. Exposure of *S. subterraneus* to *Bt*-containing slugs had no effect on egg production rates, hatching success or time of hatching. Similarly, the fitness and fecundity of other generalist predators are not affected by the consumption of *Bt* containing prey[4,8] . However it is important to consider that although these studies indicate few changes in growth or survival parameters, generalists feed on a diverse spectrum of prey.

Based on the results of the present study the life history of the predator was unaffected by prey that fed on, or by prey acquiring the toxin. The results are comparable to the predator *Orius majusculus* (Reuter) feeding on the thrips *Anaphothrips obscures* (Muller) reared on *Bt*- maize[14] and *O. insidious* feeding on larvae of the lepidopteran *O. nubilalis* reared on diet mixed with *Bt* – toxins without any recognizable impact[1].

*R. fuscipes* is not greatly affected *by D. cingulatus* raised exclusively on transgenic cotton. The effects could be established only if many generations of *R. fuscipes* are continuously raised on variety of pests raised on *Bt* cotton.

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## TABLES

Table :1 : Incubation period, developmental period, oviposition pattern, hatchability, adult longevity and sex ratio of *R. fuscipes*

| **Parameters** | | **Conventional cotton** | ***Bt* cotton** |
|---|---|---|---|
| Incubation period | | 9.83±0.75 | 10.33±0.51 (5.08) |
| Total developmental period | | 43.0±10.72 | 46.28±2.15 (7.60) |
| Preovipositional period(days) | | 8.0±0.63 | 10.3±1.03 *(28.75) |
| Oviposition period(days) | | 24.42±1.16 | 21.2±1.05 *(-13.16) |
| Postoviposition period (days) | | 9.02±1.03 | 8.66±1.01 (-3.98) |
| Oviposition index | | 19.8±1.16 | 16.9±1.47 *(-14.64) |
| Total no. of batches of egg laid | | 7.0±0.63 | 6.83±0.75 (-2.43) |
| Total no. of eggs | | 87.16±7.05 | 86.90±5.72 (-0.29) |
| Average no. of eggs per batch | | 13.5±0.83 | 12.5±0.54 (-7.40) |
| Minimum no. of eggs per batch | | 5.83±0.40 | 5.33±0.51 (-8.57) |
| Maximum no. of eggs per batch | | 18.83±0.98 | 18.16±0.98 (-3.55) |
| Total no. of eggs hatched | | 85.12±0.72 | 83.43±0.69 (-1.97) |
| Hatching percentage | | 97.6 | 96.0-1.63 (-1.63) |
| Frequency of 0% hatching | | 0.9±0.11 | 1.1±0.32 *(22.22) |
| Frequency of 100% hatching | | 1.2±0.13 | 0.8±0.07 *(-33.33) |
| Adult longivity | Male | 39.05±2.9 | |
| | Female | 42.0±2.82 | 0.50:1 |
| Sex ratio | Male | 38.32±3.1(-1.86) | 0.48:1 |
| | Female | 41.42±2.6(-1.38) | |

Note : Values in parentheses are percent change over control values

* Significant at P≤0.05. All other deviations not significant (Student's t-test).

Table :2: Chronology of predation (in min) in *R. fuscipes*

| | Predatory acts | | | | |
|---|---|---|---|---|---|
| | Capturing(min) | Paralysing(min) | Sucking(min) | Total duration (min) | Piercing site(no) |
| Conventional cotton tender branch | 9.0±0.63 | 4.16±0.75 | 89.83±0.75 | 103.19±5.08 | 7.16±0.75 |
| Conventional cotton mature branch | 9.9±0.63 | 4.52±0.61 | 91.1±2.08 | 106.41±8.33 | 8.41±0.82 |
| Bt cotton tender branch | 7.66±0.51 *(-14.87) | 3.5±1.04 *(-15.8) | 81.83±1.94(-8.88) | 92.99±8.2(-9.79) | 6.16±0.40*(-13.96) |
| Bt cotton mature branch | 8.3±0.51*(-16.1) | 3.9±1.03*(-13.71) | 88.0±2.09(-3.37) | 100.2±8.45*(-5.83) | 6.62±0.81*(-21.2) |

Note : Values in parentheses are percent change over control values

* Significant at P≤0.05. All other deviations not significant (Student's t-test).

Table :3: Chronological analysis of mating (in min) in *R. fuscipes*

| Feed type | Arousal | Approach | Nuptial clasp including riding over | Extension of genetalia | Duration of copulation | Time taken for ejection of spermatophore capsule |
|---|---|---|---|---|---|---|
| A | 1.10±0.01 | 1.50±0.012 | 23.42±0.103 | 0.48±0.06 | 32.50±0.15 | 48.20±0.73 |
| B | 2.5±0.12*(127.26) | 2.10±0.10*(39.99) | 21.20±1.36(-9.45) | 1.8±0.01*(274.99) | 26.25±0.12*(-19.18) | 54.14±0.82*(12.29) |

Note :

Feed type A : *D. cingulatus* life stages raised on conventional cotton

Feed type B : *D. cingulatus* life stages raised on *Bt* cotton

Values in parentheses are percent change over control values

* Significant at P≤0.05. All other deviations not significant (Student's t-test).