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Temporal and Spatial Variations in Susceptibility of *Helicoverpa armigera* (Hub.) From Different Agronomic Hosts to Bt Cotton

Shanmugam, P.S., R. Balagurunathan and N. Sathiah
Department of Entomology, Tamil Nadu Agricultural University, Coimbatore-641 003,
Tamil Nadu, India

Abstract: Refuge is widely recommended as a resistant management strategy in Bt cotton. As an alternative to refuge, the other host crops may be used. The susceptibility of *H. armigera* larvae collected from different agronomic hosts (bhendi, red gram, chickpea, tomato and cotton) to Bt cotton hybrids were studied. *H. armigera* larvae collected from different agronomic hosts were highly susceptible to Bt cotton hybrids at 60 DAS and the susceptibility decreases at 90 and 120 DAS. The toxicity of different plant parts of Bt cotton hybrids to the larvae from different agronomic hosts was in the order of top leaves > middle leaves > squares > bolls. *H. armigera* neonates from chickpea recorded maximum mortality to MECH 184 Bt at 60 DAS. Susceptibility of second and third instar *H. armigera* collected from different hosts to Bt cotton hybrids was in the order of chickpea > red gram > bhendi > tomato > cotton. The order of effectiveness of different Bt cotton hybrids to *H. armigera* collected from different hosts were MECH 184 Bt > MECH 162 Bt > RCH 2 Bt. The possibilities of these alternate host as a refuge to Bt cotton was discussed.

Key words: Bt cotton hybirds, *H. armigera*, different agronomic hosts

INTRODUCTION

Cotton plant is infested by 162 species of insects at various stages of growth, of which 15 are considered to be key pests in India. The average loss in yield of seed cotton due to insect pests ranges 50-60% (Dhaliwal *et al*., 2004). The minimum losses were caused by sucking pests (4.6%) whereas bollworms (51.3%) cause maximum loss (Satpute *et al*., 1985). Among these *H. armigera* causes US $ 290-350 million worth of damage every year in India (Gujar *et al*., 2000).

Nearly Rs. 12 billion worth of pesticides are used in India to control the bollworm complex of cotton (Barwale *et al*. 2004). Though cultural practices, resistance traits (Sadras, 1995) and beneficial fauna activity (Wilson *et al*., 1994) make it possible to reduce the insect pest damage, the economic sustainability of the cotton crop is not realized. Biocontrol agents including several biopesticides have not been included in the schedule of the bollworm complex management (Khadi *et al*., 2001).

Bacillus thuringiensis Berliner (Bt) based biopesticide is now being increasingly used in India in cotton and is thought to restrict application of chemical insecticides. Bt produces crystalline inclusions sporulation composed of one or several proteins known as insecticidal crystal proteins (ICPs) or δ-endotoxins. These toxins kill the insects by binding to and creating pores in midgut membranes (Gill *et al*., 1992).

Global adoption of Bt cotton has risen dramatically from 80,000 hectares in 1996 to 5.7 m ha (alone and stacked with herbicide tolerant cotton) in 2002 (James, 2003).

In India, Bt cotton comprises 0.78% of the hybrid cotton area in 2002-03. Barwale *et al.* (2004) projected that during 2003-04 and 2004-05 Bt coverage will be of 6.40 and 11.65%, respectively in India.

As the area under Bt cotton increases, it will exert high selection pressure on target pest species and accelerate the build up of resistance (Gould, 1998). This may seriously diminish the value of *B. thuringiensis*.

Provision of spatial refuge increases survival of susceptible pests and slows the evolution of resistance. It is effective only when resistance is inherited as recessive trait (Metz *et al*., 1995). In refuge strategy, the isogenic lines (non Bt cotton hybrids) are widely used. But the larvae from this refuge may be less susceptible because of the same allelochemical composition in Bt and non-Bt cotton hybrids. *H. armigera* is a polyphagous pest with a wide host range of 376 plant species including

Corresponding Author: Shanmugam, P.S., Research Associate, Department of Agril. Entomology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India

cultivated crops *viz.* cotton, red gram, tomato, chickpea, maize and several vegetable crops (Singh *et al.*, 2002). The susceptibility of *H. armigera* from these agronomic hosts to Bt cotton may vary (Gore *et al.*, 2003).

Fitness costs to Bt resistance in insects seem common (Ferre and van Rie, 2002). The growth potential of resistant population can be negatively affected by reduced fecundity, survivorship and toxic development. These fitness costs can be substantial, affecting most life history traits, as well as mating success and sperm precedence (Carriere *et al.*, 2004). Fitness costs may help to delay or prevent the spread of alleles conferring resistance to Bt crops when refuges of non-Bt host plants are present (Lenormand and Raymond, 1998).

MATERIALS AND METHODS

Insects: *H. armigera* larvae were collected from cotton, chickpea, red gram, bhendi and tomato fields during August, 2004 from Coimbatore and Dharmapuri districts of Tamil Nadu. Only late instar larvae were collected during the collection as suggested by Gore *et al.* (2003). They were reared on chickpea based artificial diet (Shorey and Hale, 1965). The colony from each hosts were maintained separately until F_1 generation. The bioassays were carried out at F_1 generation.

Host plants: The transgenic cotton cultivars MECH-162, MECH-184, RCH-2 were raised in mud pots (20 liters capacity). The mud pots were filled with soil, farm yard manure (FYM) and sand mixture. The experiment was conducted in completely randomized design (CRD). The fully expanded terminal leaves ($\cong$ 3 cm diameter and 5 cm from tip to petiole), middle leaves, squares and bolls were selected randomly after thorough examination and detached using sterilized sharp blades. The leaf samples collected from 30 days after sowing (DAS) at monthly intervals up to 120 days (Hui *et al.*, 2002). The reproductive parts were collected from 60 DAS onwards. The boll samples were collected by tagging the flower and collected seven days after tagging for attaining uniform size and age of the bolls. The samples were collected in the individually labeled polyethylene zip covers under ice cold conditions. The samples were disinfected using 0.1% sodium hypochlorite for 30 seconds and then washed with water and excess water was reduced using layers of tissue papers. The leaves were dried in open air for 20 min.

Bioassays: Leaf disc of diameter 3.0 cm were cut from respective treatments and placed in polypots, containing moistened filter paper. The squares and bolls bioassays were conducted as described by Gore *et al.* (2003). Five neonate larvae were released in to each polypot and 30 neonates were released per replication (Hui *et al.*, 2002). In case of second and third instars one larva per polypot was released. Then the polypots were closed with lids. The surviving larvae were transferred to semi synthetic diet third day after treatment. The bioassays were conducted at 27±2°C, 75±5% RH and natural photoperiod conditions. The observation on mortality was recorded at 24 h intervals and continued upto seven days. The data obtained in percentages were transformed to corresponding angles (Arc sine percentage). The analysis of variance in different experiments were carried out in IRRISTAT ver. 3.1. Biometrics unit, International Rice Research Institute, Philippines in HCL Busbee 2000 com pits.

RESULTS AND DISCUSSION

Among the Bt cotton hybrids MECH 184 Bt was highly effective against the *H. armigera* neonates from different hosts followed by MECH 162 Bt and RCH 2 Bt. The *H. armigera* neonates from chickpea and red gram were highly susceptible to Bt cotton top leaves followed by bhendi, tomato and cotton (Fig. 1). The neonates were highly susceptible to Bt cotton hybrids at 60 DAS and at later stage of crop the susceptibility decreased. In case of middle leaves the maximum neonate mortality (98.33%) was recorded to chickpea against MECH 184 Bt at 60 DAS (Fig. 2). The susceptibility of neonates increased upto 90 DAS and then decreased. The mean larval mortality to Bt cotton middle leaves at 60 DAS were 90.00-96.67, 86.66-96.67, 90.00-93.33, 80.00-83.33 and 70.00-73.33% for the neonates from bhendi, red gram, chickpea, tomato and cotton, respectively (Fig. 2). The

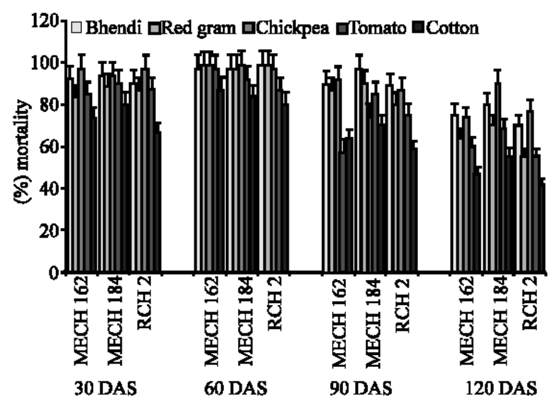

Fig. 1: Response of *H. armigera* neonates from different agronomic hosts to Bt cotton top leaves

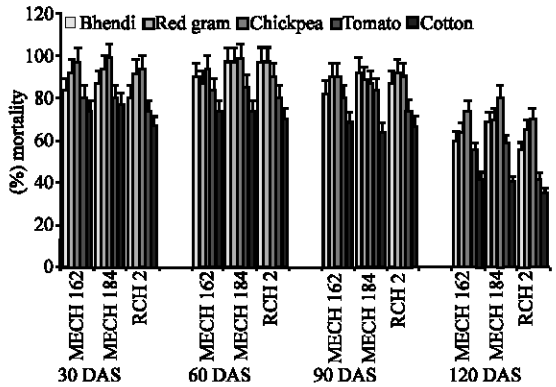

Fig. 2: Response of *H. armigera* neonates from different agronomic hosts to Bt cotton middle leaves

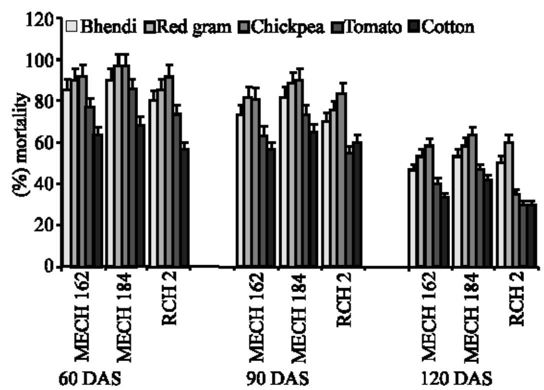

Fig. 3: Response of *H. armigera* neonates from different agronomic hosts to Bt cotton squares

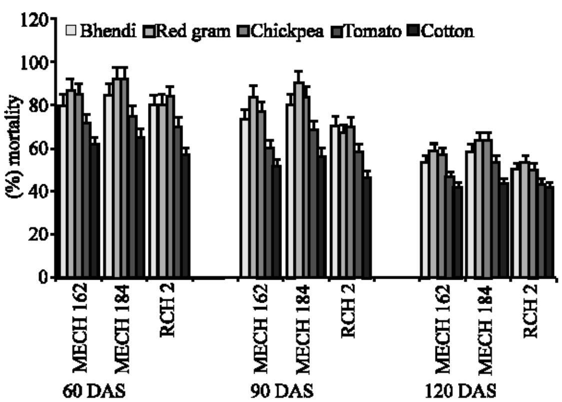

Fig. 4: Response of *H. armigera* neonates from different agronomic hosts to Bt cotton bolls

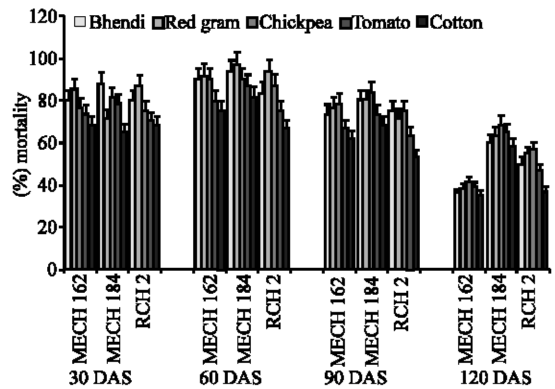

Fig. 5: Response of second instar *H. armigera* from different agronomic hosts to Bt cotton top leaves

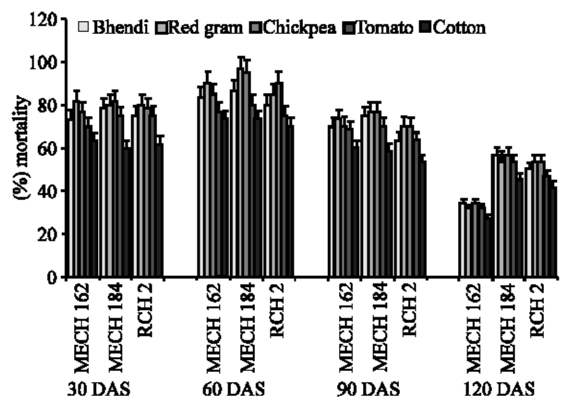

Fig. 6: Response of second instar *H. armigera* from different agronomic hosts to Bt cotton middle leaves

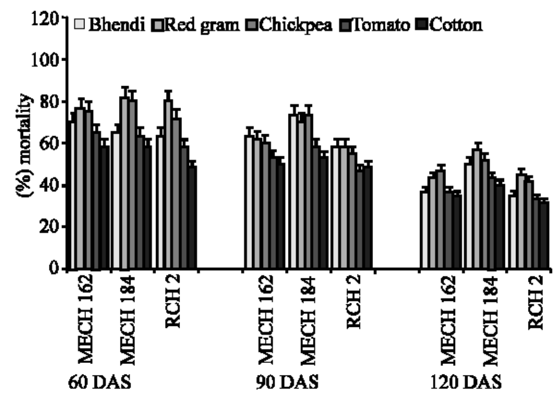

Fig. 7: Response of second instar *H. armigera* from different agronomic hosts to Bt cotton squares

larvae from the different hosts were highly susceptible to Bt cotton squares and bolls at 6 0 DAS. The MECH 184 Bt was equally effective against the neonates from chickpea (96.66%) and red gram (96.66%). The susceptibility of *H. armigera* neonates grown on different hosts decreased to Bt cotton squares and bolls at 120 DAS. The *H. armigera* neonates from cotton were

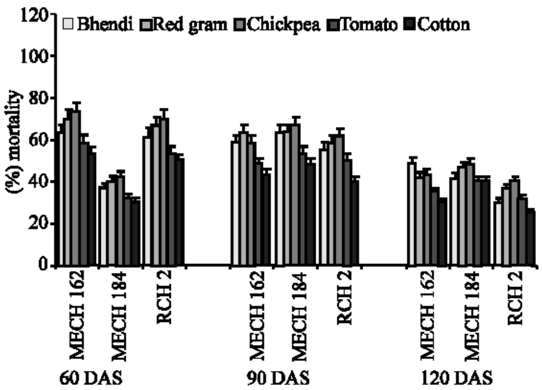

Fig. 8: Response of second instar *H. armigera* from different agronomic hosts to Bt cotton bolls

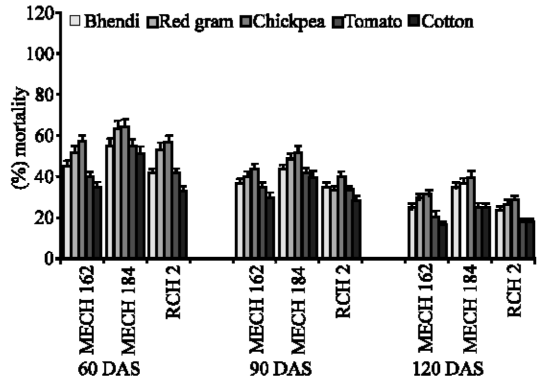

Fig. 11: Response of third instar *H. armigera* from different agronomic hosts to Bt cotton squares

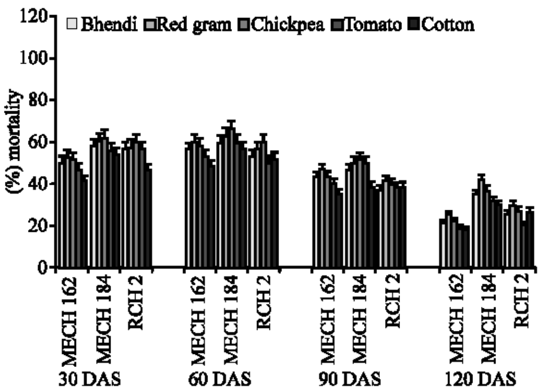

Fig. 9: Response of third instar *H. armigera* from different agronomic hosts to Bt cotton top leaves

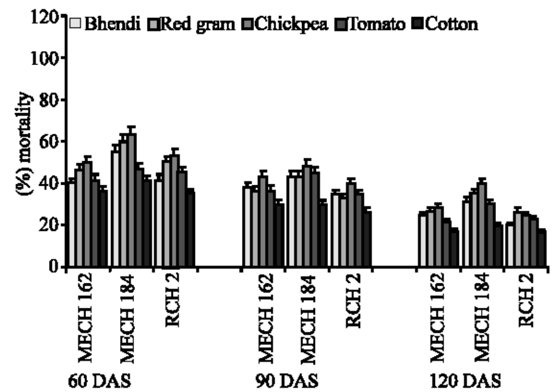

Fig. 12: Response of third instar *H. armigera* from different agronomic hosts to Bt cotton bolls

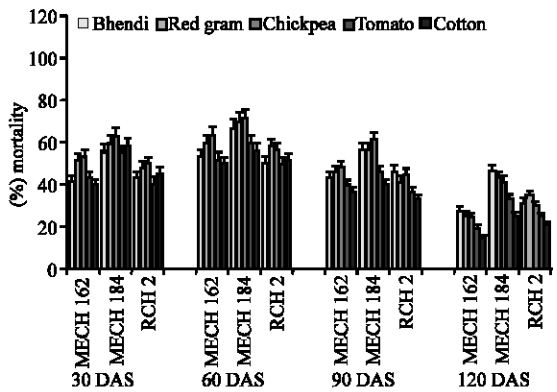

Fig. 10: Response of third instar *H. armigera* from different agronomic hosts to Bt cotton middle leaves

less susceptible to Bt cotton hybrids at various stages of the crop (Fig. 3 and 4).

The susceptibility of second instar *H. armigera* from different agronomic hosts was in the order of red gram > chickpea > bhendi > tomato > cotton at 60 DAS. The mean mortality of second instar *H. armigera* collected from bhendi, red gram, chickpea, tomato and cotton to Bt cotton top leaves at 60 DAS were 83.33-93.33, 91.66-96.66, 86.67-90.00, 75.00-86.66 and 66.67-81.67%, respectively (Fig. 5). In case of middle leaves the MECH 162 Bt and MECH 184 Bt were equally effective against the second instar *H. armigera* from chickpea and red gram (Fig. 6). The larvae from different hosts were highly susceptible to MECH 184 Bt followed by MECH 162 Bt and RCH 2 Bt. The susceptibility of second instar *H. armigera* from different hosts to Bt cotton squares was in the order of red gram > chickpea > bhendi > tomato > cotton (Fig. 7). In case of Bt cotton bolls also the second instar *H. armigera* from different hosts showed the similar trend. Among the hybrids MECH 184 Bt was highly effective against larvae collected from bhendi, red gram, chickpea and tomato, whereas it was less effective against the second instar *H. armigera* from cotton (Fig. 8). The susceptibility of second instar *H. armigera* decreased to Bt cotton squares and bolls at 120 DAS.

Third instar *H. armigera* larvae from cotton were less susceptible to different Bt cotton top leaves, whereas larvae from chickpea (22.00-66.66%) and red gram (25.00-66.66%) were highly susceptible (Fig. 9). Among the Bt cotton hybrids the order of efficacy was MECH 184 Bt > MECH 162 Bt > RCH 2 Bt for the third instar *H. armigera* to Bt cotton top leaves (Fig. 9). The MECH 162 Bt (63.33%) and MECH 184 Bt (71.67%) middle leaves were highly effective against the third instar larvae from chickpea (Fig. 11).

The mean mortality of third instar *H. armigera* from bhendi, red gram, chickpea, tomato and cotton were ranged between 41.67-45.00, 51.66-56.67, 56.67, 40.00-46.66 and 33.33-35.00, respectively to Bt cotton squares (Fig. 11). The susceptibility of third instar *H. armigera* from different hosts to Bt cotton bolls was in the order of chickpea > red gram > tomato > bhendi > cotton (Fig. 12). The maximum mean mortality 56.67% was recorded for the larvae from red gram and chickpea to MECH 184 Bt bolls. The larvae from the cotton were least susceptible to Bt cotton bolls.

The refuge strategy is widely recommended to delay the resistance development in target pests to Bt cotton. The *H. armigera* from the non Bt (isogenic) may less be susceptible to the Bt cotton, because of the same parental background except the Cry1Ac expression. In the lateral stages the tannins and phenolics reducing the Cry1Ac expression may also aggravate the problem (Olsen and Daly, 2000). For any pest management to be effective knowledge on the population dynamics of the target pests in relation to their various host plant is necessary (Dent, 1991; Fitt, 1998). As an alternative strategy, other than non Bt (isogenic), the other host plants like bhendi, red gram, chickpea and tomato may be used as an alternative source for refuge. Apart from serving as refuge these crops also serve as trap crops (Hokkanen, 1991). Singh *et al.* (1993) reported that *H. armigera* and jassids preferred the bhendi and sunflower than cotton for their oviposition in cotton + bhendi and cotton + sunflower inter cropping systems. Trap cropping of bhendi in cotton field at 10:1 reduced the incidence of cotton pests (Thontadarya and Rao, 1980).

Gujar *et al.* (2000) reported variation in susceptibility of *H. armigera* collected from different hosts and regions to Btk. Gore *et al.* (2003) recorded variable levels of mortality among F_2 *Helicoverpa zea* from cotton (76.8%), soybean (64.5%), grain sorghum (63.0%), field corn (89.7%) and meridic diet (75.6%) on Bt cotton (Deltapine 50B).

Plant hosts and meridic diets can influence the activity of various mortality factors including insecticides (Tan and Guo, 1996), bacteria (Moldenke *et al.*, 1994), nuclear polyhedrosis viruses (Peng *et al.*, 1997), fungi (Kulkarni and Lingappa, 2001) and nematodes (Barbercheck *et al.*, 1995). Several factors were associated with the susceptibility of insects to toxic substances. The induction of detoxifying enzymes in insect by host plants is an important factor in reducing efficacy of synthetic insecticides (Tan and Guo, 1996). In the present study, the difference in mortality may not be due to detoxification enzymes, because the induction is temporary and non hereditary to Bt cotton (Brattsten *et al.*, 1998).

Nutrition is an another factor, which influences the host susceptibility to Bt cotton. Jayaraj (1982) observed that *H. armigera* adults from larvae fed on soybean and cotton lived longer than those fed on tomato and corn. Contrary to that Mullick and Singh (2001) reported that adults lived for long time in cotton than the larvae reared on flower buds of pigeonpea and black gram. In the present study, as Gore *et al.* (2003) mentioned host plants influenced *H. armigera* survival on Bt cotton depending on their relative nutritional value.

In the integrated pest management and resistance management strategies the alternative host plants plays an important role. These host crops would serve as refuge, to decrease the initial frequency of resistance alleles to Bt cotton in field population and to maintain recessive character of Bt resistance in *H. armigera*. However, before these crops can be considered for refuges in a resistance management strategy, studies are necessary to determine specific numbers of target adults contributed by each of these hosts under field conditions.

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