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Built in refuge for the management of Pink Bollworm, *Pectinophora gossypiella* Saunders (Gelichidae: Lepidoptera) in Bt cotton

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Abstract: Field experiment was conducted at the Main Agricultural Research Station, University of Agricultural Sciences, Raichur during 2012-13 to identify and evaluate the suitable refuge strategy systems for pink bollworm resistance management in Bt cotton (*Gossypium hirsutum* L.) cultivation. The experiment was laid out in randomized complete block design with nine refuge systems : 0 % N-Bt (BIR), 5 % N-Bt (BIR), 10 % N-Bt (BIR), 15 % N-Bt (BIR), 20% N-Bt perimeter refuge, 100% Non Bt**,** 50% Non Bt, 10% structured refuge and 20% structured refuge as treatments with three replications in replacement series. Significantly lowest incidence (3.55 larvae/plant) and number of bolls with exit holes (11.22) due to pink bollworm was recorded in 0% BIR (41.25 q/ha) followed by 5 % N -Bt (BIR) with yield of 38.74 q/ha and this was on par with 10 % N-Bt (BIR) with yield of 37.79 is next best refuge systems with Bt cotton for higher production and greater economic benefits.

Keywords: BIR (Built in refuge), Cotton, Larval incidence, Pink bollworm, Structure refuge

INTRODUCTION

Extensive cultivation of Bt cotton (*Gossypium hirsutum* L.) can impose a continuous and intense selection pressure on bollworms of cotton leading to the latter's development of resistance to the toxin (Hardee *et al.,* 2001). One of the conditions for environmental release of Bt cotton is that each such field is to be surrounded by a belt of non Bt cotton of the same variety to serve as "refuge" for bollworm. Refuge is any host plant (non Bt cotton, alternate host) that does not produce Bt toxin and has not been treated with conventional Bt formulations (Qiao *et al.*, 2010). Refuge crops enable mating between resistant and susceptible adults, resulting in production of susceptible offspring (Kranthi *et al.*, 2002; Kranthi and Kranthi, 2004). The refuge cropping strategy is designed to ensure that Bt susceptible insects will be available to mate with Bt resistant insects, should they arise. Available genetic data indicates that susceptibility is dominant over resistance. The off springs of these matings would most likely be Bt susceptible, thus mitigating the spread of resistance in the population (Hardee *et al.*, 2001).

Early testing of transgenic Bt cotton included studies of seed blends offering a refuge for a resistance-management strategy. These blends contained a higher percentage of transgenic Bt cottonseed mixed with a smaller percentage of non-Bt cottonseed (Amy *et al.*, 2001). By placing a blend of the two cottonseed types in the same bag, Monsanto could ensure that a refuge would be planted by growers with Bt and non-Bt plants interspersed in the same rows within a field known as refuge in bag or built in refuge. The development of an effective resistance-management plan for these insect-resistant cottons will provide growers with another tool in an integrated pest-management scheme for cotton. Therefore, the experiment was conducted to examine the effects of plantings of pure and blended genotypes of Bollgard *Bt* cotton and non-Bt cotton on the incidence of pink bollworm.

MATERIALS AND METHODS

The investigation was carried out at the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka, India during 2004-05 and 2005-06. Bt cotton crop was grown as a rain fed crop with protective irrigation as on the stage of the crop. The climate of the study area is transitional in nature with bi-modal distribution of rainfall. The experiment was laid out in randomized complete block design with nine refuge systems : 0 % N-Bt (Built in Refugia), 5 % N-Bt (BIR), 10 % N-Bt (BIR), 15 % N-Bt (BIR), 20% N-Bt perimeter refuge,

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Table 1. Pink bollworm incidence and fruiting bodies damage in different Built in Refuge treatments at 100 to 140 days after sowing in non-Bt cotton.

Treatments	**100 DAS**		**120 DAS**		**140 DAS**	
	PBW Larvae/20 bolls	**No. of bolls with exit holes/50 bolls**	**PBW Larvae/20 bolls**	**No. of bolls with exit holes/50 bolls**	**PBW Larvae/20 bolls**	**No. of bolls with exit holes/50 bolls**
0% N-Bt (BIR)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
5% N-Bt (BIR)	2.38 (1.70)	6.11 (2.57)	2.56 (1.75)	8.61 (3.02)	4.38 (2.21)	13.57 (3.75)
10% N-Bt (BIR)	2.64 (1.77)	7.19 (2.77)	2.63 (1.77)	8.74 (3.04)	4.46 (2.23)	13.72 (3.77)
15% N-Bt (BIR)	2.69 (1.79)	7.36 (2.80)	2.65 (1.77)	8.72 (3.04)	4.61 (2.26)	13.76 (3.78)
20% N-Bt perimeter refuge	2.55 (1.75)	7.31 (2.79)	2.71 (1.79)	8.88 (3.06)	4.70 (2.28)	13.80 (3.78)
100% Non Bt	3.49 (2.00)	9.27 (3.13)	3.38 (1.97)	11.03 (3.40)	5.39 (2.43)	16.11 (4.08)
50% Non Bt	3.42 (1.98)	8.94 (3.07)	3.21 (1.93)	10.81 (3.36)	5.28 (2.40)	15.93 (4.05)
10% structured refuge	2.59 (1.76)	7.42 (2.81)	2.61 (1.76)	8.81 (3.05)	4.52 (2.24)	13.79 (3.78)
20% structured refuge	2.28 (1.67)	7.38 (2.81)	2.59 (1.76)	8.86 (3.06)	4.58 (2.25)	13.74 (3.77)
SEm ±	0.05	0.03	0.02	0.07	0.02	0.08
CD at 5 %	0.13	0.10	0.06	0.20	0.07	0.24

DAS: Days after sowing; Values in the parentheses are Vx + 0.5 transformed values.

Table 2. Pink bollworm incidence and fruiting bodies damage in different Built in Refuge treatments at 160 and 180 days after sowing and yield of Bt cotton.

Treatments	160 DAS		180 DAS		Yield (q/ha)
	PBW Larvae/20 bolls	No. of bolls with exit holes/50 bolls	PBW Larvae/20 bolls	No. of bolls with exit holes/50 bolls	
0% N-Bt (BIR)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	41.25
5% N-Bt (BIR)	4.62 (2.26)	13.83 (3.79)	3.55 (2.01)	11.22 (3.42)	38.74
10% N-Bt (BIR)	4.76 (2.29)	13.91 (3.80)	3.62 (2.03)	11.36 (3.44)	37.79
15% N-Bt (BIR)	4.81 (2.30)	13.85 (3.79)	3.67 (2.04)	11.35 (3.44)	37.26
20% N-Bt perimeter refuge	4.88 (2.32)	14.03 (3.81)	3.71 (2.05)	11.44 (3.46)	36.42
100% Non Bt	5.61 (2.47)	16.56 (4.13)	4.97 (2.34)	13.28 (3.71)	6.52
50% Non Bt	5.45 (2.44)	16.28 (4.10)	4.74 (2.29)	13.12 (3.69)	16.14
10% structured refuge	4.51 (2.24)	14.07 (3.82)	3.61 (2.03)	11.39 (3.45)	37.73
20% structured refuge	4.72 (2.28)	14.12 (3.82)	3.77 (2.07)	11.45 (3.46)	37.44
SEm ±	0.03	0.06	0.05	0.07	0.52
CD at 5 %	0.08	0.18	0.16	0.21	1.58

DAS: Days after Sowing; Values in the parentheses are Vx + 0.5 transformed values.

100% Non Bt , 50% Non Bt, 10% structured refuge and 20% structured refuge as treatments with three replications. The Bt cotton hybrid RCH BG-2 (Rasi seeds) was used in the study along with its respective non Bt cotton hybrid with a spacing of 90×60 cm.

The BIR treatments (5% BIR, 10% BIR and 15% BIR) were planted in such a way that each Non Bt plant is surrounded by four Bt plants and observations in these treatments were recorded on the ten tagged plants and their surrounding four plants for each Bt plant. In the treatments of 10% structured refuge and 20% structured refuge the plants were selected randomly on ten Non Bt plants and forty Bt plants. Whereas in the treatment 20% N-Bt perimeter refuge only plants surrounding the treatment plot was recorded and in 100% and 50% Non Bt fifty plants were randomly selected and observed. In all the refuge treatments the observation on pink bollworm incidence and number of bolls with exit holes were recorded by collecting green bolls at different time points starting from 100 days after sowing to 180 days after sowing. Finally yields were recorded in all the treatments and were statistically analyzed following standard methods using XLSTAT.

RESULTS AND DISCUSSION

In the present investigation, there was no incidence of pink bollworm in the sole Bt cotton without any seed mix of Non Bt at all the time points starting from 100 to 180 days after sowing. The incidence of larvae (2.38 larvae/plant) and number of bolls with exit holes (6.11) was lowest in the treatment 5% BIR and it continued up to 180 days after sowing where in lowest incidence was noticed compared to all other treatments and was on par with rest of the refuge treatments at all the time points. 10% BIR recorded the larval population of 2.64 larvae per plant and 7.19 bolls with exit holes and was on par with 15% BIR and also other refuge treatments. At all the time points the pink bollworm incidence and number of bolls with exit holes was highest in the treatment of 100% Non Bt which was followed by 50% non Bt and dint differ significantly. At 180 days after sowing the larval incidence in the pure stature of Non Bt plot recorded 4.97 larvae per plant and with 13.28 bolls with exit holes out of fifty bolls (Tables 1 and 2).

With respect to seed cotton yield in different refuge strategies differed significantly. Among the different refuge treatments sole Bt cotton without Non Bt (0% BIR) recorded highest yield (41.25 q/ha) and this treatment was followed by 5% BIR which recorded 38.74 q/ha which differed significantly. The treatment with 10 % BIR recorded yield of 37.79 q/ha and was on par with 5% BIR. The treatment with 50 % Non Bt recorded 16.14 q/ha and Lowest yield of 6.52 q/ha was noticed in the treatment with 100% Non Bt (Table 2).

Not much significant differences were noticed in the treatments of 5 % N-Bt (BIR), 10 % N-Bt (BIR), 15 % N-Bt (BIR), 20% N-Bt perimeter refuge, 10% structured refuge and 20% structured refuge with respect to larval incidence but with respect to yield all the refuge treatments differed statistically. Compare to existing refuge strategy 5% and 10% BIR recorded highest seed cotton yield and with respect to larval incidence these treatments were statistically on par with each other which implies that both the refuge treatments have same larval population and equal number of adults to mate with the resistance adults which emerge from Bt fields. Much of the concern surrounding the intrafield refuge, or mixed seed, strategy for resistance management is that seed mixtures may enhance resistance in mobile insects such as bollworm and tobacco budworm (Mallet and Porter, 1992). Seed blend efficacy trials by Durant (1995) also demonstrated that yields in seed treatments containing 100% Bt seed were significantly higher than some seed treatments containing mixtures of Bt and non-Bt seed. Durant (1995) concluded that seed mixes containing less than 90% Bt seed may not provide acceptable control of bollworm and tobacco budworm. The 0% Bt:100% non-Bt plots had significantly higher larval numbers and damaged fruit and significantly lower yields than all other seed treatments both years. Amy *et al.* (2001) revealed that the treatment containing 100% Bt seed had lower percent larval infestation and percent damaged fruit and higher yields than all other seed blends in both years. In general, mean percent larval infestation and mean percent damaged fruit increased in both years as the percentage of Bt seed in the blends decreased. Conversely, seed cotton yields decreased as the percentage of Bt seed in the blends decreased. Pink bollworm, Spotted bollworm and Spiny bollworm and, therefore, it becomes necessary to provide them with adequate cotton (non-Bt) crop itself as refuge to support the required susceptible populations. The later-instar larvae of *Pectinophora* and *Earias* hardly move between plants. So, the question of their moving from non-Bt to Bt plants is hardly a concern (Manjunath, 2012).

Conclusion

The present study concluded that of these different refuge treatment strategies tested, 5% or 10% built in refuge were considered as an effective refuge seed mix in delaying the resistance build-up in pink bollworm. Transgenic Bt cotton provided an effective and more environmentally sound means of controlling cotton lepidopteran insect pests. Further research is necessary to determine the most practical refuge options and to quantify amounts of refuge necessary to delay resistance to transgenic Bt cotton in bollworms.

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