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Understanding the Pink Bollworm Bioecology in Cotton Agroecologies of India to Laying a Sound Basis for Devising IPM Strategies

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ABSTRACT

Globally, the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechidae) is the most devastating insect pest of cultivated cotton (*Gossypium* sp.). Recently, the resurgence of pestilence in pink bollworm on transgenic cotton due to resistance development posed a serious threat to the sustainability of cotton production in India. During its recent epidemic in 2017-18, pink bollworm is reported to cause colossal cotton yield losses to the tune of 30% in Central cotton growing zone of India, thereby threatening the livelihood of thousands of resource poor farmers of rainfed agro-ecologies. Due to widespread infestations on Bt cotton fields of north zone since 2019, the pest has now became a major threat to Bt cotton cultivation in India. Considering the bio-ecology and spread of pink bollworm in Indian cotton ecosystem, practically it is difficult to manage this mysterious and notorious pest with any single tactic used in isolation with field-by-field approach adopted for a season or two. Thus, it requires an area-wide management approach that focuses on cropping systems of a region and essentially involves adoption of multipronged strategies based on crop growth window. The renaissance of the pink bollworm on Bt cotton bears significant ecological and economic consequences for cotton cultivation. In this context, understanding the ecobiological behaviour of pink bollworm is crucial to devise future climate resilient management strategies for this pest.

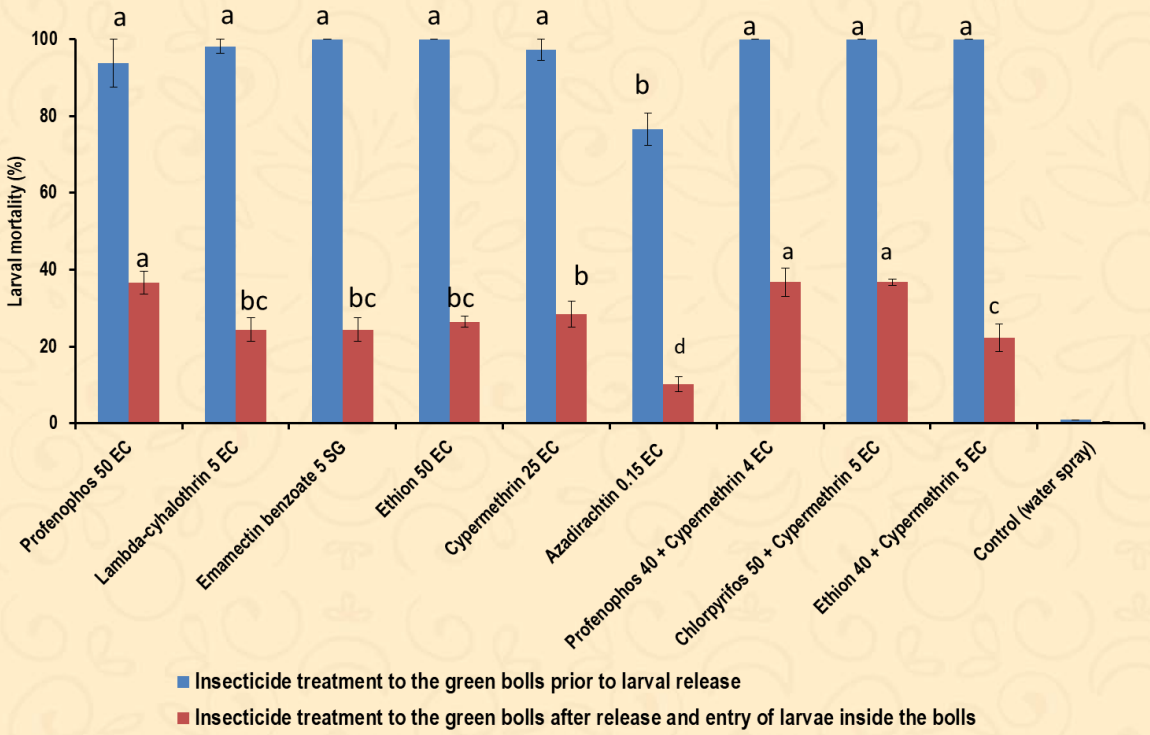
KEY WORDS: Bio-ecology, Cotton, climate resilient, IPM, pink bollworm, phenology.

KEY ASPECTS OF PINK BOLLWORM BIOECOLOGY FROM MANAGEMENT POINT OF VIEW

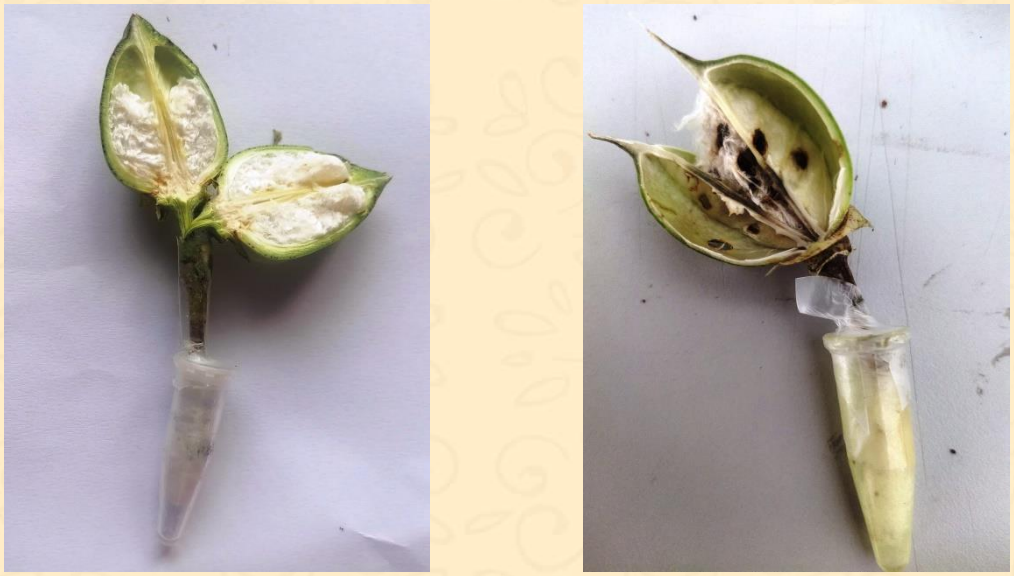
i. Cryptic feeding habitat hindering the effective chemical control

Consequent upon resistance development against Bt cotton, currently the management of pink bollworm in India has been effected mainly through use of synthetic insecticides; although a well-defined package of integrated management practices (IPM) has been advised by research institutions and agricultural universities of the respective regions (Kranthi, 2015; ICAR-CICR, 2023). However, chemical control strategies are many times inconsistent and unsatisfactory to control pink bollworm in field, mainly due to its cryptic habitat. The larvae enter the bolls within <4 hours of hatching from the eggs and feed internally by damaging the seeds and lint of cotton (Figure 1). The internal feeding habit of pink bollworm larvae increases the target site inaccessibility of applied chemical insecticides. This further leads to the adverse consequences like more frequent insecticide use, increased cost of control and poisoning of farmers due to repeated chemical exposures. In India, over thousands of cotton farmers, their families and farm labourers suffer from pesticide poisoning every year due to intensive and indiscriminate insecticide use against ravages of destructive cotton insect pests. Therefore, for ensuring maximum control efficacy, any action oriented towards management of pink bollworm should necessarily be initiated well before the larval entry into the green bolls. The experiment carried out at ICAR-CICR, Nagpur revealed a significant decrease in the pest control efficacy of the test insecticides from >80% in pre-larval release insecticide treatment of green bolls to <49% in the treatment of post-larval release and entry inside the green bolls (Figure 2) (Busnoor, 2021; Busnoor et al., 2023). This clearly demonstrates that insecticide use becomes relatively less useful against pink bollworm when the larvae find their way inside the green bolls (Figure 3). This reduced larvicidal activity due to larval burrowing inside the green bolls provides a sound basis for insisting on timely insecticide application before larval entry inside the bolls in order to avoid control failure. This information is crucial in framing an effective strategy using chemical insecticides for pink bollworm management in cotton.

Figure 1. Seeds and lint of cotton damaged by pink bollworm larvae feeding inside the green boll



Figur 2. Bioefficacy of insecticides against pink bollworm larvae as influenced by their internal boring habit. The larval mortality represents corrected values using Abbott's formula. The total sample size was. 75 larvae (25 larvae/ replication). (F test significant at 1% level of significance (ANOVA: SE(diff.) = 5.0 and 2.5; CD@ 5% = 10.4 and 5.3; CV (%) = 8.1 and 11.0, respectively for pre and post larval release insecticide treatment to the green bolls). Source: Busnoor, 2021; Busnoor et al., 2023.



a b

Figure 3. Larvicidal effect of emamectin benzoate 5% SG against neonate larvae of pink bollworm. Healthy boll with no internal damage when bolls treated prior to larval release (a), and boll with internal damage symptoms by pink bollworm larvae when insecticides were applied 24 h after larvae burrowed inside the bolls (b).

ii. Narrow window of opportunity for synchronizing the management actions with the pest activity

Following the emergence of moths, pink bollworm females generally takes 2-3 days to lay eggs, which typically hatch within 4-5 days of the incubation period (Figure 4) (Fand et al., 2020; 2021; Peddu et al., 2020; Busnoor et al., 2021; 2023). The neonate larvae of pink bollworm cannot withstand the prolonged periods of starvation and hence they find their way inside the green bolls within approximately 4-5 h of hatching by drilling a small hole (approximately 0.2 mm size) on the rind of bolls (Figure 5) (Fand et a., 2020; Busnoor et al., 2021; 2023). This highlights the critical importance of timings of pest control actions like insecticide treatments and or release of biological control agents aimed mainly at two stages of pink bollworm life cycle i.e., eggs and neonate larvae before burrowing inside the bolls in determining their effectiveness against pink bollworm control. Any mismatch between the pink bollworm phenological events (oviposition, egg hatching and entry of neonate larvae inside the bolls) and management actions (insecticide sprays or releases of biocontrol agents) initiated against its control will lead to field level control failures.

Usually, the insecticide sprays against pink bollworm are advocated upon crossing an economic threshold level (ETL) of eight moths per trap per day,

consecutively for three days recorded in sex pheromone traps baited with gossyplure as an active ingredient (Taneja and Jayaswal, 1981; Kranthi et al., 2015; ICAR-CICR, 2019; 2023). Mostly, the insecticides having strong ovicidal and or ovo-larvicidal actions like profenophos, chlorpyriphos, quinolphos, etc. (during crop stage < 120 days after sowing, i.e. DAS), and cypermethrin, lambda cyhalothrin, fenvelerate, etc. (during crop stage > 120 DAS) are recommended for the control of pink bollworm (Kranthi, 2015; ICAR-CICR, 2019; 2023). Normally, for monitoring the activity of pink bollworm moths, installation of five traps at 45 DAS are recommended for one hectare area of cotton field (ICAR-CICR, 2019; 2023). Considering the facts of pink bollworm bioecology described in above paragraph, it is clear that in order to achieve its desirable control, the insecticide sprays must be initiated within an available narrow window of approximate 7-8 days (2-3 days preoviposition and 4-5 days incubation periods) since the beginning of moth emergence as recorded in pheromone traps. Failure to compliance may lead to poor insecticidal control of pink bollworm resulting in increased crop damage.

Similar to the insecticide sprays, the internal feeding habit of pink bollworm larvae imposes restrictions on the pest suppressing activities of natural enemies. The studies conducted at ICAR-CICR, Nagpur have shown that the egg parasitoids like *Trichogramma bactrae* (Nagaraja) and *Trichogramma brasiliensis* (Ashmead) are promising bioagents of pink bollworm providing effective control of the pest comparable with insecticides (Asha et al., 2019; Naik et al., 2019). However, inundative field releases of these parasitoids must be guided by the timings of pheromone trap catches of moths so as to synchronise them with the egg laying activity of the pest. The field release of parasitoids after egg hatching and larval entry inside the bolls will lead to failure of pink bollworm control. Normally, three inundative releases of egg parasitoids are recommended during 45 – 75 DAS of crop stage to prevent early to mid-season colonization of the pink bollworm, thereby reducing the inoculum load for later part of the season.

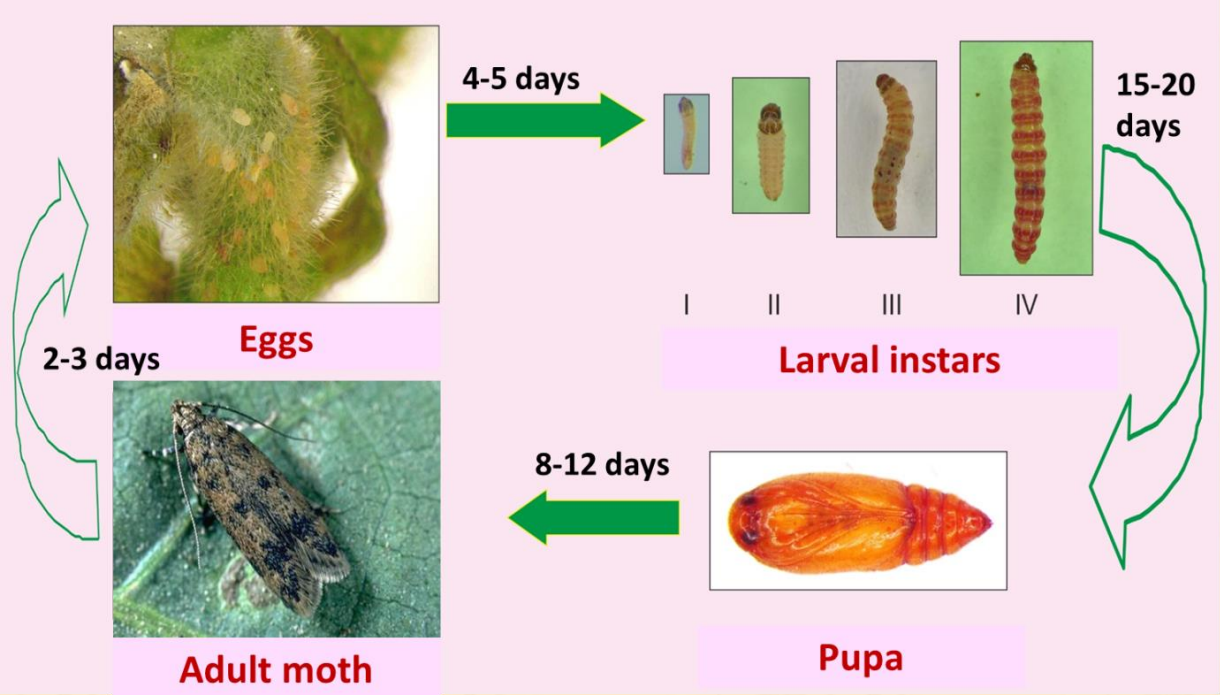


Figure 4. General life cycle of pink bollworm on cotton bolls (Source: Fand et al., 2020; Peddu et al., 2020; Busnoor, 2021).

a **b**

Figure 5. Larval entry inside the green bolls of cotton. Neonate larvae drilling a hole on the surface of boll rind (a), and a small (~ 0.2 mm) entry hole made by larvae on boll rind (b)

iii. Spatio-temporal variation in pest phenology in response to varied climate and agroecologies

In India, cotton is grown under both rain-fed and irrigated environments in three major growing regions viz, North, Central and South zones characterised by wide range of distinct agro-climatic conditions. This multifaceted diversity is reflected in terms of varying climatic conditions (arid, sub-humid, per-humid), different soil types (alluviums, vertisols, vertic intergrades, red, laterite), area under the cultivation of all four species (*G. hirsutum, G. arboreum, G. barbadense, and G. herbacium*) with a large pool of varieties and hybrids, year-round cropping, a wide range of seed rates and plant densities ranging from 10,000 to 100,000 plants per hectare, and a harvesting window that spans over eight to nine months (September to May) (Venugopalan and Prasad, 2022). This complexity significantly adds to the challenges of pest management in cotton. The spatio-temporal variability in temperature and microclimate impose restrictions on blanket use of any pest control measure across geographical locations that reflect wide bioclimatic variability (Fand et al., 2021).

The population dynamics and phenological events in pink bollworm life cycle such as emergence of moths from previous season's hibernating population, egg laying and hatching, and developmental durations of larvae and pupae are primarily dependent upon the microclimate in the crop ecosystem (temperature, relative humidity and soil moisture) and crop phenological events (initiation of squares, flower buds and green bolls) (Fand, 2021; Fand et al., 2021). Having originated from Indo-Pakistan region, the pink bollworm has acclimated to the diverse climatic conditions prevailing in the cotton growing areas across India. The pest can complete its life cycle between 20°C and 35°C temperatures, with the most favourable range for its development falling between 25°C and 30°C (Peddu et al., 2020). The environmental factors, mainly the temperatures prevailing during cotton growing season affects the length of pink bollworm life cycle, being shortest (35-37 days) in relatively warmer period during July to October, and longest (59-73 days) in cooler winter period during November to January (Fand et al., 2021). In the months following the main season (February to June), the pink bollworm assumes a dormant state as larvae. It can be found within the remaining bolls on cotton stalks, which might still be present in the field or stacked along field boundaries. Additionally, these hibernating larvae can also be located within the seeds of cotton lint that have been contaminated and transported to market yards and ginneries (Mallah et al., 2000; Kranthi, 2015).

A wide variability in diurnal and inter annual temperatures in the North, Central and South cotton growing zones of India was reflected by the latitudinal variation from south to north in degree day (DD) accumulations (4190 – 5407 DD) across different geographical locations falling in these zones (Figure

6). This has resulted in a large amplitude in number of days (27 -59) lapsed between two successive moth trap catch peaks (which were supposed to be equivalent to one in field generations of pink bollworm) (Fand et al., 2021).

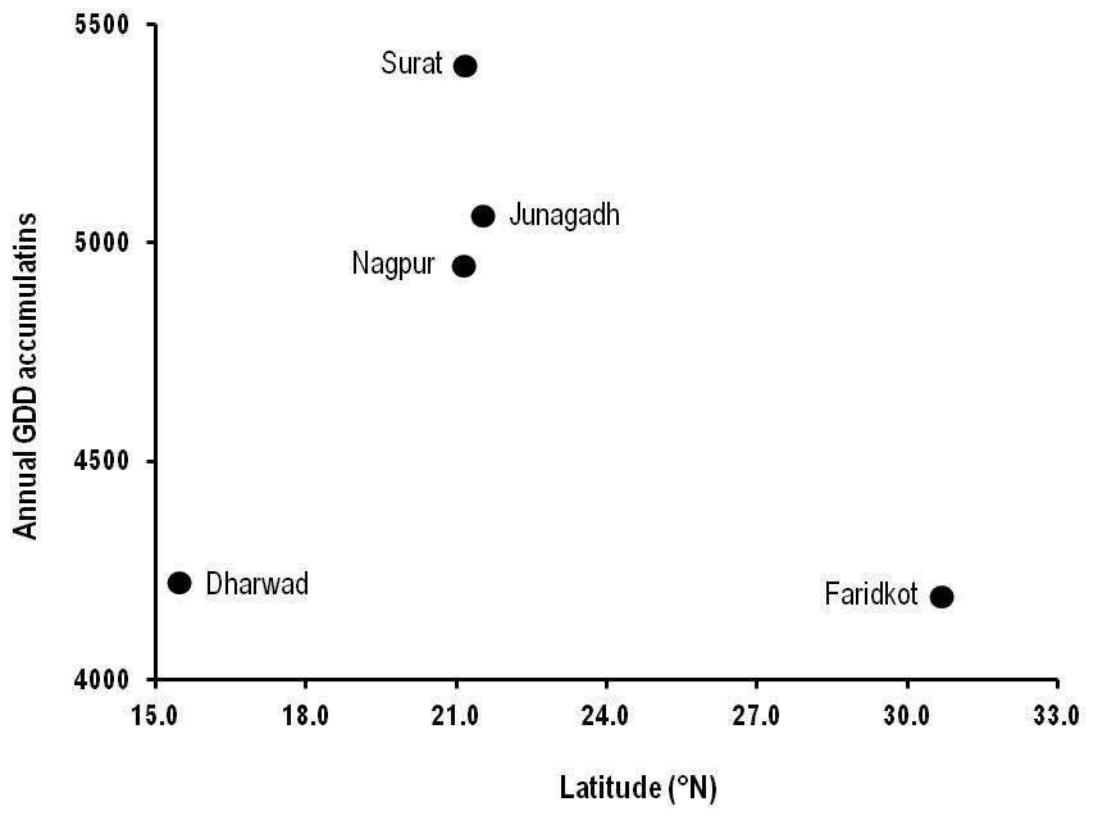


Figure 6. The latitudinal variation in mean growing degree days (GDD) accumulation for four different geographical locations falling in north, central and south cotton growing regions of India (Source: Fand et al., 2021).

The results of a time series analysis of data on peak moth catches of pink bollworm recorded in sex pheromone traps across different locations across cotton growing areas revealed a gradual transition with a successive monthly delay in the dates marking the onset of emergence and the peak captures of pink bollworm moths while moving from north to south. In the locations at north, central and south zones, the moth emergence started in the first week to the middle of July, August and September months, respectively. The population peaks at these locations were recorded during the mid of October (North zone), end of November to first week of December (Central Zone) and December end to first week of January (South Zone) (Figure 7). The seasonal peak in pink bollworm population at all the test locations was reached mostly in third generation (Fand et al., 2021). Considering the early-season low survival of pink bollworm populations on squares and flowers, and the time

required for ample availability of green bolls (Sevacherian and El-Zik, 1983; Sarwar, 2017), the pink bollworm populations are generally not expected to reach their seasonal peaks prior to third generation. This information holds significant implications from a management perspective. It enables the timely strategic targeting of the highest populations responsible for substantial damage, thus facilitating efficient control of the pest.

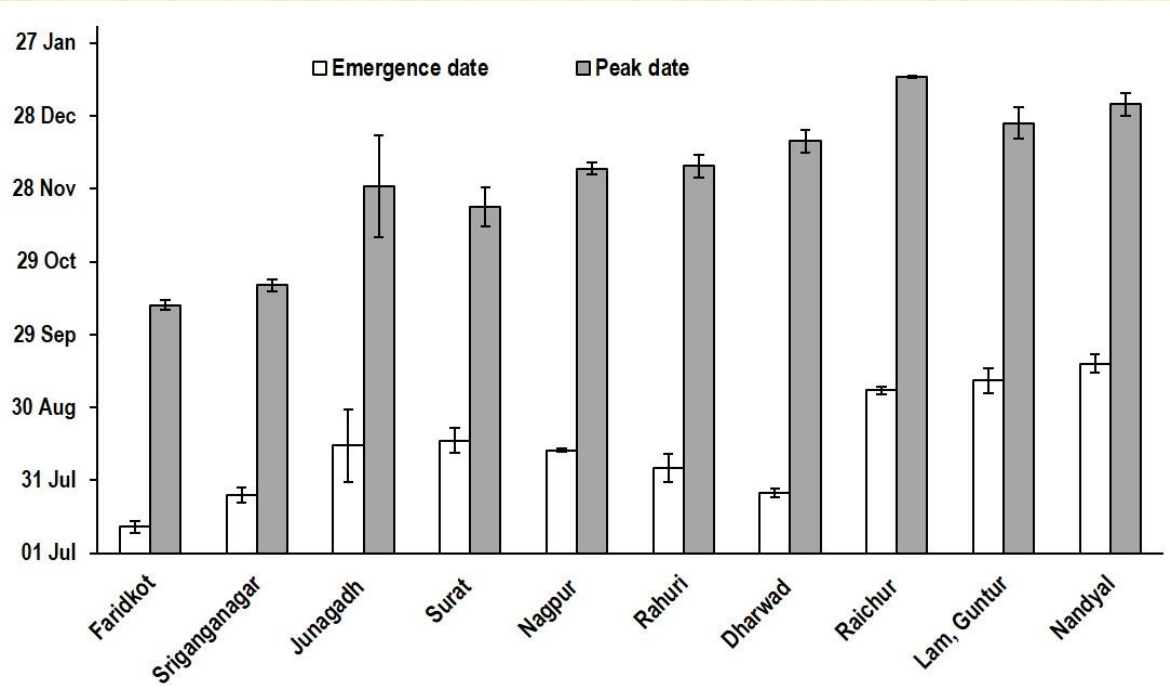


Figure 7. A gradual transition in the dates of beginning of emergence and peak moth catches observed from north to south cotton growing locations of India (Source: Fand et al., 2021).

iv. Unique feeding niche

The cotton crop is attacked by a bollworm complex consisting of three species: the American bollworm (*Helicoverpa armigera*), the spotted bollworm (*Earias* sp.), and the pink bollworm (*Pectinophora gossypiella*). Among these species, the pink bollworm distinguishes itself by inhabiting a unique ecological niche within the cotton ecosystem. This differentiation is evident both in terms of the physical space it occupies and the functional role it assumes. In contrast to the other two bollworm species, the pink bollworm exclusively occupies a specific ecological role. It refrains from attacking the vegetative parts of cotton plants such as tender leaves and growing shoots. Instead, it confines its feeding to the reproductive components, specifically squares, flower buds, and green bolls (Figure 8) (Lukefahr and Griffin, 1962; Singh et al., 1988; Fand et al., 2020).

This unique feeding behaviour of pink bollworm larvae could potentially be linked to the rapid development of resistance within Indian populations of pink bollworm towards Bt cotton that carries the Cry1Ac and Cry2Ab2 genes. Research conducted across various regions globally has revealed variations in toxin expression within different parts and organs of the cotton plant. It has been observed that the most significant expression of Bt toxin occurs in the leaves, succeeded by squares, bolls, and flowers (Kranthi et al., 2005; Chen et al., 2017, 2018; Liu et al., 2019). Feeding on tender foliage and shoots with elevated Bt toxin levels results in the mortality of neonate larvae of *H. armigera* and *Earias* sp., thereby preventing their survival on Bt cotton plants. In contrast, neonate pink bollworm larvae encounter relatively lower concentrations of Bt toxins in reproductive structures like squares, flower buds, and bolls. Consequently, extended exposure to sub-lethal Bt toxin levels since the introduction of Bt cotton in India, combined with the absence of non-Bt cotton refuges, likely initiated the early development of resistance in successive generations of pink bollworm.

a **b**

Figure 8. Feeding habitat of cotton pink bollworm. Rosette bloom formation due to flower infestation (a), and damage to developing seeds and lint of bolls due to internal feeding (b)

An additional crucial bio-ecological aspect which should essentially be added to the recent rise in pink bollworm infestations in India is that the larvae seldom attack the plant parts (such as squares, flowers, and bolls) that have already been damaged by other bollworms. Typically, pink bollworms prefer to feed on undamaged and healthy organs, avoiding those that have already been attacked by other pests. Prior to the

introduction of Bt cotton in India, *H. armigera* and *Earias* sp posed significant challenges to cotton cultivation (Armes, 1996; Kranthi et al., 2002). These pests would establish themselves within the cotton crop from its initial vegetative stage, targeting young leaves and shoots. As the crop matured they shift to attacking reproductive structures such as squares and bolls. Pink bollworm, on the other hand, is primarily late season pest attacking the crop towards the season end (Singh et al., 1988; Kranthi, 2015, Fand et al., 2019). However, in the pre-Bt cotton era, the cotton ecosystem was predominantly influenced by H. *armigera* and *Earias* sp, which would initiate infestations early. This early infestation by these pests would leave a smaller portion of healthy resources vulnerable to pink bollworm attack, given its occurrence at a later stage, leading to its competitive displacement. The advent of Bt cotton technology in India brought about a situation where the primary competitors (*H. armigera* and *Earias* sp) were eliminated from the cotton ecosystem. Alongside the factors outlined earlier, this circumstance resulted in the pink bollworm gaining prominence as a significant pest within the cotton environment upon its come back on cotton after a hiatus of about one and half decade.

v. *Early sowing renders suicidal emergence ineffective*

The recommended sowing window for cotton crop in north, central and south zones of India are April-May, June-July and July-August, respectively. A strict adherence to these regions specific sowing windows is of paramount importance in order to avoid early colonization of pink bollworm on new season's crop. In few scattered pockets of central cotton growing zone of the country (Maharashtra and Madhya Pradesh), farmers with access to protected irrigation facilities opt for pre-monsoon sowing of cotton between late April and mid-May. On reaching 40-45 DAS of crop age, the cotton plants starts bearing squares becomes favourable for establishment of pink bollworm infestation as moths prefer to lay eggs on young squares and floral buds (Fand, 2021; Fand et al., 2021). This developmental stage in case of early sown crop is attained by June end or first week of July. The data recorded on moth trap catches in pheromone traps at ICAR-CICR, Nagpur during 2017-2023 revealed that the pink bollworm moth emergence typically starts approximately 15-20 days after the first monsoon shower. The intial moth emergence will be in discrete form with an average of <1 moth observed per trap (Figure 9). However, the coincidence of squaring and flowering in cotton plants with thr beginning of moth emergence from the previous season's overwintering population facilitates the initial establishment and perpetuation of pink bollworm progeny in early-sown crops. Systematic surveys conducted by ICAR-CICR, Nagpur in cotton growing areas of Akola, Buldana, Jalgaon, Dhule and Jalna districts of Maharashtra state in central zone of India revealed 5-8% flower infestation of pink bollworm (rosette flowers) on early sown cotton

crop. This infestation subsequently spreads to rainfed cotton crops planted during the regular sowing period of June-July.

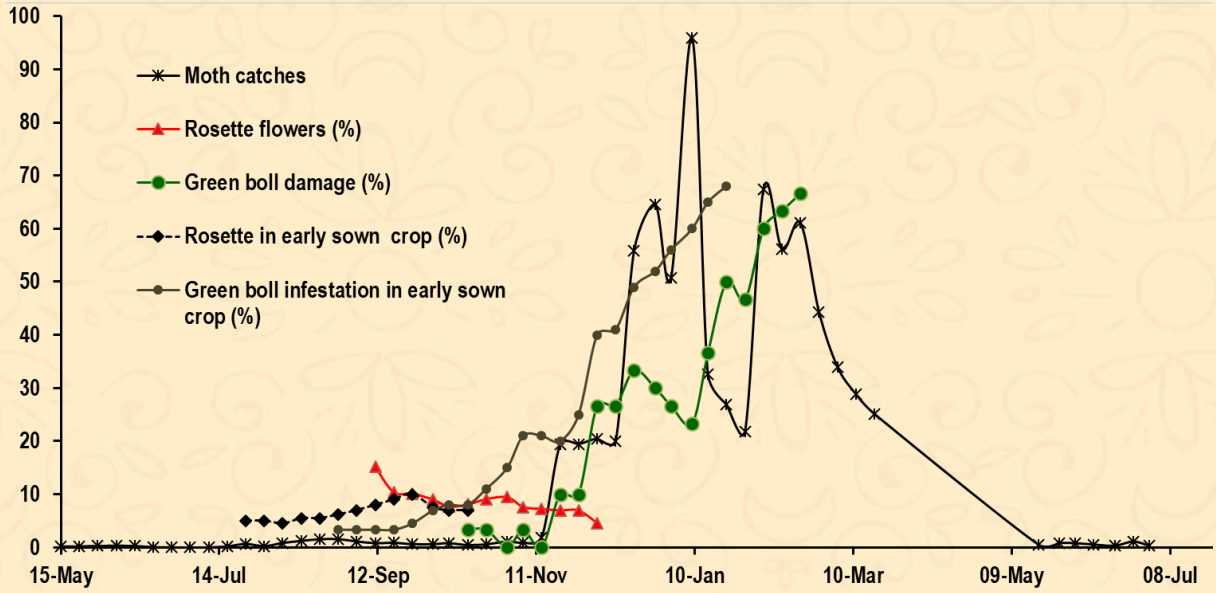


Figure 9. Simulation of pink bollworm infestation in early sown crop based on the moth emergence pattern and relationship observed between cotton and pink bollworm phenology

By avoiding early planting of cotton crops, the pink bollworm moths that emerge from hibernation have no suitable host stage to survive, resulting in 'suicidal emergence'. This phenomenon significantly reduces the pink bollworm's potential impact on the upcoming season's cotton crop. Therefore, considering the above bio-ecological aspects of pink bollworm life cycle, increasing the chances for suicidal emergence of pink bollworm moths by avoiding early sowing of cotton crop can be the best cultural practice to minimize the intensity of pink bollworm damage. A crop growth window based integrated pest management (IPM) strategy of ICAR-CICR strongly discourages early cotton sowing to minimize the pink bollworm infestation (Kranthi, 2015; Fand et al., 2019; ICAR-CICR, 2023).

vi. Crop – pest phenological synchrony

The successful establishment of pink bollworm infestation in the cotton field hinges on two essential conditions: the emergence of adult moths and the initiation of reproductive stage (onset of squares and flower buds) in cotton plants. Following this, the manifestation of damage symptoms of pink bollworm in the field, such as rosette flowers and/or infested green bolls, takes approximately two weeks (12-14 days) from the onset of moth emergence (Fand et al., 2021; Fand et al., 2021). The phenological relationship studies

between cotton crop and pink bollworm carried out at ICAR-CICR, Nagpur during 2018-2020 have shown that the first visible square appeared on cotton plants at 43 – 45 DAS which was developed into white bloom in about 12-14 days later. As the pink bollworm moths emerging from overwintering population prefer to lay eggs on the squares, coincidence of moth emergence and squaring in cotton plants results in the formation of rosette flowers instead of normal healthy flower, approximately in next 12-14 days (Figure 10) (Fand, 2021; Fand et al., 2021). This sequence of events is replicated over the progress of season, with remarkable shift in the preference of pink bollworm to feed on and damage the green bolls instead of squares and flowers (Ellsworth, 2006; Fand et al., 2021). This is evident from the gradual decline in the proportion of rosette flowers and corresponding increase in the infestation in green bolls. Therefore, this point onwards, the monitoring and surveillance programs should focus primarily on checking the pest's presence in green bolls of the cotton plant.

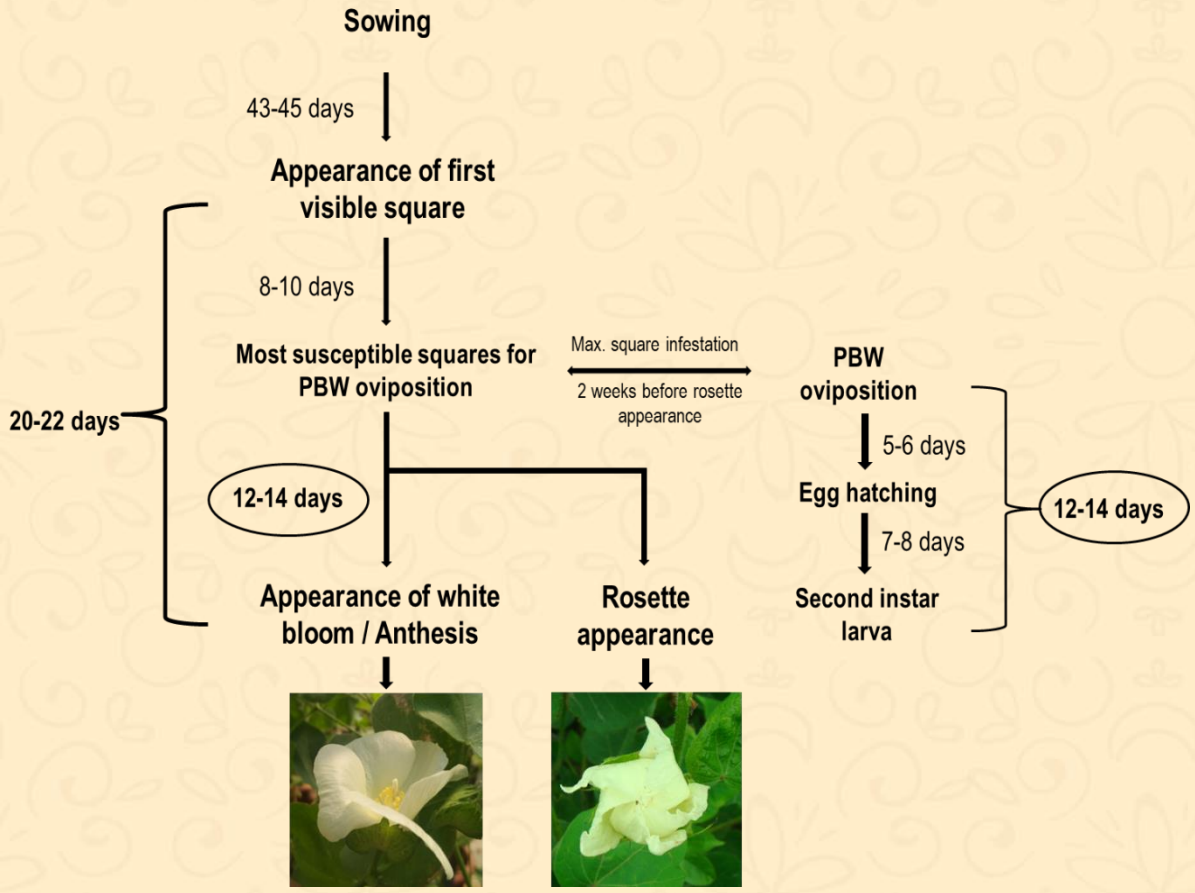


Figure 10. Relationship between the phenologies of cotton plant and pink bollworm (*Source*: Fand et al; 2021; Fand, 2021)

KEY INSIGHTS FOR ECOLOGICALLY BASED IPM STRATEGY FOR PINK BOLLWORM

Understanding the ecological intricacies of the pink bollworm's life cycle discussed above is fundamental to formulating the sustainable and effective strategies for its management. The crux of managing pink bollworm resides in identifying pivotal junctures in its life cycle and behaviour. This includes pinpointing the timing of moth emergence, understanding the preferred host stages, and recognizing the environmental factors that mould its propagation. The major insights for formulating ecologically sustainable management strategy for pink bollworm are given below:

1. The timings of management actions such as insecticide sprays or releases of bio-control agents aimed at egg and neonate larval stages before entering the bolls are highly crucial in determining the effectiveness of control measures
2. To ensure that the treatments achieve their desired efficacy, precise timing should be guided by consistent monitoring of pest activity. This allows for the strategic utilization of a limited window of opportunity available for implementing control measures.
3. Strategic targeting of the highest pest population responsible for significant damage, facilitating efficient control of the pest is crucial.
4. The seasonal peaks in the pink bollworm populations anticipated from third generation onwards varies spatio-temporally across north, central and south cotton growing areas of the country in response to the considerable influence wielded by the climatic elements. Therefore, formulating regions specific management strategies suiting to the local climatic conditions for targeting the most damaging pest populations is essential.
5. Asynchrony between the host and pest phenologies through discouraging early sowing and increasing the chances for suicidal emergence of the moths would be the essential strategy for checking the initial colonization and further perpetuation of pink bollworm infestation.
6. Moth emergence pattern and degree accumulations between successive moth peaks revealed that two in-field generations of pink bollworm could be prevented by following timely termination of cotton season as per the region specific recommend window.

CONCLUSION

Taking into account the bio-ecology and distribution patterns of the pink bollworm within the Indian cotton ecosystem, managing this enigmatic and destructive pest using standalone tactics on a field-by-field basis for a short duration proves challenging. An area-wide approach that takes into account regional cropping systems and is centred on the adoption of multifaceted strategies aligned with the crop's growth

window is necessary. An ideal strategic plan for pink bollworm IPM centred on ecological principles should necessarily be like:

A. Preseason management practices

i. Deep summer ploughing and other tillage operations

ii. Follow crop rotations to break the pest's life cycle

iii. Discouraging early (pre-monsoon) sowing

B. In-season management practices

i. Timely sowing

ii. Selection of early maturing, short duration cultivars

iii. Monitoring of pest activity by installation of pheromone traps @ 5 traps per hectare at 45 DAS

iv. Removal and destruction of infested (rosetted) flowers

v. Preventive sprays of botanical pesticides (e.g. neem oil)

vi. Use of bioagents like *Trichogrmma bactrie* in three splits @ 10-15 days interval during crop growth window of 45-75 DAS

vii. Monitoring of green boll infestation through random sampling

viii. Economic threshold level (ETL) based sprays of recommended insecticides

C. Post-season management practices

i. Discouraging the practice of ratooning

ii. Timely termination of the crop

iii. Destruction of infested crop residues

iv. Installation of pheromone traps in ginning mills for off-season catches of moths

v. Destruction of gin trash and infested seeds

vi. Quarantine measures for restriction of movement of seed cotton and cottonseed from infested areas to other regions

REFERENCES

Armes, N. J., Jadhav, D. R. and DeSouza K.R. 1996. A survey of insecticide resistance in *Helicoverpa armigera* in the Indian sub-continent. Bulletin of Entomological Research, 86, pp. 499 -514.

Asha, S., Naik, V.C.B., Neharkar, P.S., Ughade, J.D. and Sant, S.S., 2019, Parasitizing potential of four Trichogramma species on the eggs of pink bollworm, *Pectinophora gossypiella* (Saunders). *Journal of Pharmacology. Phytochemistry*, 8(5), pp. 857-859.

Busnoor, A.V. 2021. Evaluation of newer insecticides gaiant pink bollworm under laboratory condition. A Thesis submitted to Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India in partial fulfillments of the requirements for the degree of Master of Science (Agriculture) in Agricultural Entomology. Pp. 81.

Busnoor, A.V., Wadaskar, R.M., Fand, B.B., Tambe, V.J., Pillai, T., Mahule, D.J., Nagrare, V.S. and Prasad, Y.G. 2023. Timings of treatments determine the effectiveness of insecticides in controlling the cotton pink

bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). 31 March 2023, PREPRINT (Version 1) available at Research Square. https://doi.org/10.21203/rs.3.rs-2759076/v1.

Chen, W.B., Lu, G.Q., Cheng, H.M., et al. 2017. Transgenic cotton expressing Vip3A and Cry1Ac has a broad insecticidal spectrum against lepidopteran pests. Journal of Invertebrate Pathology, 149, pp. 59–65.

Chen, Y., Li, Y., Zhou, M.Y., et al. 2018. Nitrogen (n) application gradually enhances boll development and decreases boll shell insecticidal protein content in n-deficient cotton. Frontiers in Plant Sciences, 8(9), pp. 51. https://doi.org/10.3389/fpls.2018.00051.

Dhurua, S. and Gujar, G.T. 2011. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. *Pest Management Science*, 67, pp. 898–903.

Ellsworth, P., et al. 2006. Pink Bollworm Management. Newsletter of the Pink Bollworm Action Committee. A Project of the Cotton Foundation Produced by the University of Arizona – Cooperative Extension, 1(2), pp. 1–2.

Fand, B.B. 2021. Seasonal dynamics of pink bollworm in cotton ecosystems of India and key ecological aspects for its management. *ICAC Recorder*, 39(1), pp. 29-32.

Fand, B.B., Nagrare, V.S., Bal, S.K., Naik, V.C.B., Naikwadi, B.V., Mahule, D.J., Gokte-Narkhedkar, N. and Waghmare, V.N. 2021. Degree day-based model predicts pink bollworm phenology across subtropics and semi-arid tropics of India. *Scientific Reports*, 11(436), pp. 1-18. https://doi.org/10.1038/s41598-(020-80184-6.

Fand, B.B., Nagrare, V.S., Deshmukh, V., Naikwadi, B.V., Gokte-Narkhedkar, N. and Waghmare, V.N. 2020. A simple and low-cost laboratory rearing technique for cotton pink bollworm, *Pectinophora gossypiella* (Suanders) (Lepidoptera: Gelechiidae) using detached green bolls of cotton. *Phytoparasitica*, 48, pp. 25–33. https://doi.org/10.1007/s12600-019-00779-2

Fand, B.B., Nagrare, V.S., Gawande, S.P., Nagrale, D.T., Naikwadi, B.V., Deshmukh, V., Gokte-Narkhedkar, N. and Waghmare, V.N. 2019. Widespread infestation of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) on Bt cotton in Central India: a new threat and concerns for cotton production. *Phytoparasitica*, 47, pp. 313–325

ICAR-CICR, 2023. Cotton Pest and Disease Management Strategies 2023-24. ICAR-Central Institute for Cotton Research, Nagpur. Pp. 1-7. Avaliable online at: https://cicr.org.in/wp-content/uploads/Pest-and-disease-management-2023-24.pdf. Accessed on 15/08/2023.

ICAR-CICR. 2019. Technical Bulletin No 1/2019. Cotton: Integrated pest, disease and nematode management (English version). (Editors: Nagrare, V.S., Naik, V.C.B., Fand, B.B., Gawande, S.P., Nagrale, D.T., Gokte-Narkhedkar, N. and Waghmare, V.N.) ICAR-Central Institute for Cotton Research, Nagpur, Maharashtra, India. pp – 39.

Kranthi, K.R. 2015. Pink bollworm strikes *Bt* cotton. *Cotton Statistics News*, 35, pp. 1

Kranthi, K.R., Derek, R., Wanjari, R., Kherde, M., Munje, S., Lavhe, N. and Armes, N. 2002. In-Season Changes in Resistance to Insecticides in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in India. Journal of Economic Entomology, 95(1), pp. 34-142.

Kranthi, K.R., Naidu, S., Dhawad, C.S., et al. 2005. Temporal and intraplant variability of Cry1Ac expression in Bt-cotton and its influence on the survival of the cotton bollworm, *Helicoverpa armigera*. Current Science,89, pp. 291–298.

Kumar, R., Monga, D., Naik, V.C.B., Singh, P. and Waghmare, V.N. 2020. Incipient Infestations and Threat of Pink Bollworm *Pectinophora gossypiella* (Saunders) on Bollgard-II Cotton in the Northern Cotton-Growing Zone of India. *Current Science*, 118, pp. 1454-1456.

Liu Z., Abidallha E.H.M.A., Huimin, W.U, Zhou, M., Zhang X., Chen Y. and Chen D. 2019. Bt insecticidal efficacy variation and agronomic regulation in Bt cotton. Journal of Cotton Research, 2 (23), pp. 1.6. https://doi.org/10.1186/s42397-019-0042-1.

Lukefahr, M. J. and Griffin, J.A. 1962. Pink Bollworm Development in Relation to Age of Squares and Bolls with Notes on Biology, Journal of Economic Entomology, Volume 55, Issue 2, 1 April 1962, Pages 158–159, https://doi.org/10.1093/jee/55.2.158

Mallah, G.H., Soomro, A.R., Soomro, A.W., Kourejo, A.K. and Kalhoro, A.D. 2000. Studies on the leftover standing cotton as carry–over sources of pink bollworm in Sindh. *Pakistan Journal of Biological Sciences*, 3 (1), pp. 147–149.

Naik, V.C.B., Kumbhare, S., Kranthi, S., Satija, U., and Kranthi, K.R. 2018. Field-evolved resistance of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), to transgenic *Bacillus thuringiensis* (Bt) cotton expressing crystal 1Ac (Cry1Ac) and Cry2Ab in India. Pest Management Science, 7(75), pp. 2544-2553.

Naik, V.C.B., Nagrare, V.S., Wawdhane, P.A., Fand, B.B., Kranthi, S., Gotke-Narkhedkar, N. and Waghmare, V.N. 2019. Biocontrol agents for pink bollworm management. ICAR-Central Institute for Cotton Research Nagpur, pp. 06.

Peddu, H., Fand, B.B., Sawai, H.R., Lavhe, N.V. 2020. Estimation and validation of developmental thresholds and thermal requirements for cotton pink bollworm Pectinophora gossypiella. Crop Protection 127, 104984, pp. 1-15.

https://doi.org/10.1016/j.cropro.2019.104984

Sarwar, M. 2017. Pink bollworm *Pectinophora gossypiella* (Saunder) (Lepidoptera: Gelechiidae) and practices of its integrated management in cotton. International Journal of Plant Science and Ecology, 3, pp. 1-6.

Sevacherian, V., El-Zik, K.M. 1983. A slide rule for cotton crop and insect management. University of California, Division of Agricultural Sciences, Cooperative Extension Leaflet, 21361.

Singh, J.P., Lather, B.P.S. and Mor, B.R. 1988. Exit behavior of pink bollworm (*Pectinophora gossypiella*) Larvae. *Indian Journal of Agricultural Sciences*, 58(3), pp. 236-237.

Venugopalan, M.V. and Prasad, Y.G. 2022. Scalable Climate Smart Technologies for Sustainable Rainfed Cotton Production in India. *ICAC Recorder*, 11(2), pp. 2-10.

Molecular Basis of Resistance to Bt Toxins in Cotton Pink bollworm: Challenges and Way Forward

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ABSTRACT

Transgenic crops carrying insecticidal genes from a ubiquitous soil bacterium *Bacillus thuringiensis* (*Bt*) have certainly revolutionized the field of crop protection since 1996 when Bt-cotton and Bt-corn were first approved for commercial cultivation in the USA. The transgenic crops, more specifically the Bt crops have proven to be economically beneficial and ecologically safer solution to combat agricultural pest problems. This is because they exhibit an array of advantages, those including season-long protection against target insect pests, thereby curtailing the need for application of environmentally hazardous insecticides. This ultimately promotes the natural biological control through conservation of natural enemies of insect pests. Reduced cost of crop protection and increased yield and quality of the produce makes the Bt crops a preferred choice of farmers. Nevertheless, akin to insecticides, the target pests can also evolve a resistance to the Bt toxins expressed *in planta*. This risk of resistance evolution represents the most significant challenge faced by all Bt crops, questioning the viability and sustainability of the transgenic technology. Research spanning over two decades has extensively illustrated the adaptability of bollworms, more specifically, of pink bollworm to the Bt cotton. This pest has demonstrated the ability to evolve resistance to Bt toxins by undergoing mutational changes in the receptors to which these toxins bind. This adaptability accentuates the difficulty in managing resistance to Bt crops. A field-evolved resistance that results in undesirable levels of crop damage bear tangible implications for pest management. Till date, such occurrences have been meticulously documented in 26 cases involving 11 distinct pest species across seven

countries of the world. Consequently, genetically modified (GM) crops, particularly Bt crops, have come under intense scrutiny from entomologists, environmentalists and policymakers. Their focus encompasses a range of sustainability concerns tied to the technology, including the efficacy of the products, the emergence of resistance in target insects, the establishment of refuges, the advantages for farmers, and the broader environmental impact. Of note among the instances of field-evolved Bt resistance is the situation involving the pink bollworm, a highly destructive pest of cotton. This pest has developed resistance to both stacked toxins, Cry1Ac and Cry2Ab2, present in Bollgard II cotton in India. This particular case has caused substantial economic ramifications. This article traces the progression of our comprehension regarding the genetic and molecular underpinnings of Bt resistance in the pink bollworm, placing special emphasis on its manifestation within the Indian context.

KEYWORDS: Bt resistance, cotton, Cry1Ac, Cry2Ab2, genetics, pink bollworm, resistance management

UNDERSTANDING THE PROBLEM OF Bt RESISTANCE IN PINK BOLLWORM

The pink bollworm, *Pectinophora gossypiella* (Saunders) has always been an enigmatic cotton pest in terms of its high invasiveness, poor insecticidal control due to cryptic feeding habit, and propensity to evolve resistance to Bt crops due to high selection pressure accentuated by a narrow host range (Kranthi, 2015; Fand et al., 2019; CABI, 2023). Being an endemic and destructive pest of cotton, pink bollworm has been the subject of research investigations worldwide, which became more intense with the introduction of Bt cotton, especially in Bt cotton growing countries like USA, India and China. The Bt toxins have co-evolved with the host to use several host molecules as receptors to initiate the toxicity process. Populations of pink bollworm harbouring several mutational alleles of cadherin and transporter proteins have adapted to feed and multiply on Bollgard and Bollgard II cotton in India since 2009 and 2014, respectively (Dhurua and Gujar, 2011; Kranthi, 2015; Mohan et. Al., 2016; 2017; Naik et al., 2018). This has generated a large reservoir of homozygous resistant individuals feeding and multiplying on ~ 12 million ha of Bollgard II cotton. Consequently, the carryover of the Bt-resistant population from one kharif season to the next, grows larger and also the pest makes an early appearance on cotton with the initiation of bloom. The knowledge we have gained on the genetics and molecular basis of resistance to Cry1Ac and Cry2Ab2 in pink bollworm populations could be effectively used in monitoring and resistance management strategies of the future.

Bt TOXINS AND THEIR MODE OF ACTION

Bacillus thuriengiensis (Bt) toxins, commonly called "Cry toxins" due to their crystalline structure are natural proteins produced by the soil bacterium *Bacillus thuringiensis.* These toxins are essentially stomach poisons and specifically target insect digestive tracts. Cry toxins remain inactive until consumed by an insect. Once digested, the protein is activated in highly alkaline gut pH of insects and then binds to specific receptors present on gut epithelial wall. Once bound, the Cry toxins pierce holes (pore formation) in the insect's gut, ultimately causing the contents to leak and the insect to starve (cessation of feeding). The sequence of events that occur in insect body after Bt-intoxication are outlined below (Figure 1):

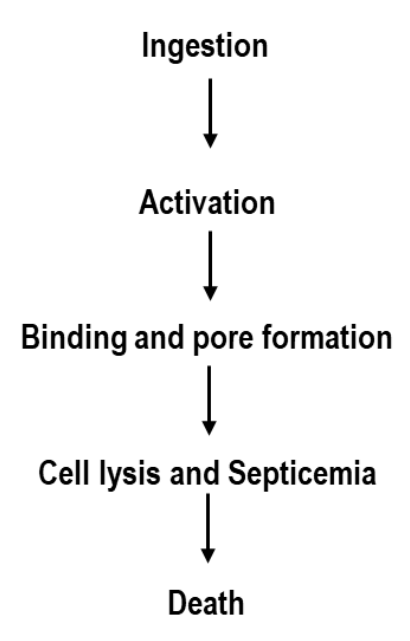

Figure 1: Sequence of events occuring during the action of Bt toxins in insect larva

The specificity of Bt toxins lies in their receptor binding affinity for insect midgut. Different Bt toxin variants target specific receptors present only in certain insect species. This specificity minimizes harm to beneficial insects and other non-target organisms. Additionally, the mode of action differs significantly from conventional chemical insecticides, which makes it less likely for insects to develop quick resistance against Bt toxins.

MECHANISMS OF RESISTANCE TO Bt TOXINS:

The transgenic cotton engineered to produce Bt toxins, has been successful in reducing bollworm damage in cotton crop for quite long period since their first release. Nonetheless, repeated exposure of insects to Bt toxins in the genetically engineered crop plants have resulted into the evolution of resistant pink bollworm strains (Tabashnik et al., 2000; Fabrick et al., 2009; 2020; 2023; Dhurua and Gujar,

2011; Wan et al., 2012; 2017; Naik et al., 2018). Alongside the introduction of Bt crops for commercial cultivation in major countries like USA (1996), China (1997), and India (2002), the studies were initiated in several key research laboratories exploring the genetic propensity of target insect pests to evolve resistance to Bt toxins. Pink bollworm was the subject of intense study at USA based institutions like the University of Arizona and USDA/ARS, and at ICAR-Central Institute for Cotton Research, Nagpur in India, primarily because of the high risk associated with the evolution of Bt resistance and increasing acreage of Bt cotton in pink bollworm endemic areas of western USA and throughout the Indian cotton growing belt across North, Central and South zones. Analysis of boll infestation data collected from Cry1Ac Bt cotton cultivated in Texas and Arizona in 1997, just a year following the commercial introduction of Bt cotton in the USA, revealed that pink bollworm larvae capable of surviving on Bt cotton were not rare. This observation was pivotal, as it highlighted the critical presumption supporting the effectiveness of non-Bt cotton refuge in delaying the progression of resistance development.

The fundamental approaches used by researchers in unravelling the Bt resistance mechanisms in insects are: (i) selective breeding of survivors on Bt toxin incorporated-diet over several generations in laboratory, (ii) repeated screenings of the field Bt-susceptible populations to select the rare Bt resistant phenotypes, and (iii) using survivors of F_2 screens because field-evolved resistance with reduced efficacy of Bt crops was a rarity then (Heckel et al., 2007; De Bortoli, 2019; Endo, 2022). Based on the various studies, the following mechanisms involved in conferring genetic and molecular basis of Bt resistance in pink bollworm have been identified.

i. Increased tolerance

In some studies, the researchers have attempted to induce the increased tolerance to Bt toxins in pink bollworm populations by the deliberate selection and breeding of survivors exposed to Bt toxin-incorporated diets over multiple generations (Tabashnik et al., 2000a; Ferre´ and VanRie, 2002). The resultant Bt-resistant pink bollworm strains provided unique opportunities to understand the genetics, including the inheritance and the molecular mechanisms governing Bt resistance. These insights, in turn, provided useful insights into the field-evolution of Bt resistance and its management through the implementation of refuge strategies. Research carried out involving pink bollworm strains sourced from the field (originating in Bt cotton fields of Arizona and Texas) but then selectively bred in a controlled laboratory setting demonstrated that resistance to Cry1Ac, a Bt protein lethal to bollworms and featured in the initial generation of Bt cotton, could be increased by around 300 folds over the span of six generations. This was in comparison to a susceptible strain (LC_{50} 0.53 ppm = ug/mL) (Tabashnik et al., 2000a). This discovery was among the initial indicators of the potential for pink bollworm to develop resistance to Cry1Ac. The frequency of the resistant allele, estimated to be associated with a single genetic locus, was in the range of 0.13 to

0.16, approximately a hundredfold higher than that of other bollworms. The inheritance pattern of resistance was autosomal, and the degree of dominance was dependent on the concentration of the Bt toxin, ranging from co-dominance at lower doses (0.1 ppm) to recessiveness at higher doses (10 ppm) (Tabashnik et al., 2000a). Empirical field data on the survival of Cry1Ac-resistant pink bollworm larvae on Bt cotton bolls clearly demonstrated that the inheritance mechanism was functionally recessive (Liu et al., 1999; Liu et al., 2001). Consequently, this emphasized the significance for producers of the Bt cotton seeds to ensure a "high dose" of Cry1Ac in Bt cotton target tissues, thereby maintaining the functional recessiveness of inheritance of Cry1Ac resistance.

ii. Target site mutations

Cadherin is a cell-surface protein with a role in cell adhesion. Certain strains of Bt have evolved to utilize cadherin as one of the receptors for binding with Bt proteins. This binding triggers the formation of pores in the midgut epithelial cells. Resistance can arise from mutations that alter the target site receptors of Bt toxins. For example, mutations in the cadherin gene and ATP-binding cassette transporter protein, which encodes a receptor for Bt toxins, have been identified in resistant pink bollworm populations (Fabrick et al., 2020; 2023; Naik et al., 2020). These mutations reduce the binding affinity of the toxins, rendering them ineffective.

In USA, the work of Tabashnik et al (2000a) and Ferre´ and VanRie (2002) on successive selection of pink bollworm strains on a diet containing Cry1Ac was further continued by Morin et al. (2003a) and developed a highly resistant strain of pink bollworm called AZP-R. This strain exhibited an impressive resistance ratio of > 3000-fold. The origin of this resistance was linked to genetic mutations found in the cadherin PgCad1 gene. Three distinct mutational alleles (r1, r2, and r3) of the cadherin gene were identified. In each of these three mutations, a minimum of 8 amino acids were deleted within the Cry1Ac toxin binding region of the cadherin molecule. These deletions were proven through experimentation to disrupt the toxic effects of Cry1Ac and provide resistance in individuals homozygous for the resistant alleles. A fourth mutational allele of the cadherin gene (r4) was discovered in a different strain of pink bollworm that had been selected on Cry1Ac-containing Bt cotton bolls for 42 generations (Fabrick et al., 2009). A total of four recessive mutations within the cadherin gene were identified as factors leading to resistance against Cry1Ac in five distinct strains of pink bollworm. This highlights that mutations in the cadherin gene constitute the primary mechanism driving resistance to Cry1Ac in pink bollworm strains that were specifically selected for this trait in laboratory settings in Arizona (Fabrick and Tabashnik, 2007). Moreover, the inheritance pattern of Cry1Ac resistance associated with all the cadherin mutational alleles was found to be recessive.

In China, many investigations into the molecular aspects of Cry1Ac resistance have been conducted on both naturally occurring and artificially selected strains

of pink bollworm. These studies revealed the presence of various mutant-PgCad1 alleles that were closely linked to pink bollworm's resistance against Cry1Ac. This resistance was confirmed through bioassays, where the larvae showed significant survival rates and successfully completed life cycle on Bt cotton plants containing the cry1Ac gene. Importantly, these newly identified cadherin mutation alleles differed from the cadherin mutant alleles (previously labeled as r1 to r13) documented in previous research efforts. These findings highlighted the potential of pink bollworm to adapt to practical resistance against Cry1Ac and contributed valuable insights for devising effective strategies to manage resistance in Bt cotton fields, particularly in China. In a pink bollworm strain obtained from the field, a mutational variant of the cadherin gene (PgCad1), referred to as the r14 allele, exhibited a 234-bp insertion within exon 12. This resulted in an altered PgCad1 protein characterized by the absence of a specific sequence of 36 amino acids within cadherin repeat 5 (CR5) (Wang et al., 2020). The individuals of a strain homozygous for this allele displayed a remarkable 237-fold resistance to Cry1Ac and were able to undergo complete development from neonate to adult stages on Bt cotton plants producing Cry1Ac. The inheritance pattern of Cry1Ac resistance associated with the r14 allele was found to be recessive and tightly linked with this particular mutation. While the abundance of PgCad1 transcripts in midgut tissues did not differ between resistant and susceptible larvae, there was a noticeable difference in the toxicity of Cry1Ac to insect cells that were transformed. Cells expressing the r14 allele had lower susceptibility to Cry1Ac compared to cells expressing the wild-type PgCad1. Importantly, the wild-type PgCad1 protein was properly transported to the cell membrane, whereas the PgCad1 protein produced by the r14 allele was not efficiently transported. In larval midgut tissue, the presence of PgCad1 protein on the brush border membrane was predominantly observed in susceptible larvae, suggesting that the r14 allele mediates resistance to Cry1Ac in pink bollworm larvae through mechanisms such as reduced translation, increased degradation, and/or mislocalization of the cadherin protein.

In the pink bollworm population collected from the Yangtze River Valley of China, a deletion of 207 base pairs within the transmembrane domain of the cadherin gene was observed, which had a significant impact on cellular trafficking. This genetic alteration led to a remarkable 220-fold resistance to Cry1Ac when present in a homozygous state (Wang et al., 2018a). Another case of cry1Ac resistance involving a transposon-mediated mutation was reported in a field-derived pink bollworm population in China (Wang et al., 2019a). In this instance, the mutation within the cadherin gene associated with the r15 allele consisted of a 3,370-bp insertion, comprising an inverted repeat transposable element that included two additional transposons. This genetic change resulted in the production of two mis-spliced transcript variants known as r15A and r15B. The pink bollworm strains carrying the homozygous r15 allele displayed a substantial 290-fold resistance

to Cry1Ac. Interestingly, this resistance did not extend significantly to Cry2Ab2 (no cross resistance), and these strains were capable of completing their life cycles on Bt cotton plants that produced Cry1Ac. The inheritance pattern of the Cry1Ac resistance associated with the r15 allele followed a recessive pattern and was tightly linked with the presence of the r15 mutation. Another study reported (Wang et al., 2019b) a novel mutational allele (*r16*) of the cadherin gene (PgCad1) in field-collected PBW associated with resistance to Bt toxin Cry1Ac, involving the insertion of a degenerate transposon in the exon 20 of PgCad1. This mutation generated a mis-spliced transcript containing a premature stop codon and interfered with the localization of cadherin on the surface of the brush border of the epithelial cells. A strain homozygous for r16 had 300-fold resistance to Cry1Ac and completed its life cycle on transgenic Bt cotton-producing Cry1Ac. Inheritance of Cry1Ac resistance was recessive and tightly linked with r16.

iii. Narrow spectrum of cross resistance

Subsequent investigations on the resistance mechanisms in pink bollworm revealed that the pink bollworm strains resistant to Cry1Ac also exhibited cross-resistance to Cry1Aa and Cry1Ab, while not displaying cross-resistance to Cry1Bb, Cry1Ca, Cry1Da, Cry1Ea, Cry1Ja, Cry2Aa, Cry9Ca, and hybrid variants resulting from the exchange of domains between Cry1Ab and Cry1C. This selective range of cross-resistance was attributed to the reduced binding of Cry toxins to specific target sites in the midgut (Tabashnik et al., 2000b), a prevailing mechanism of resistance observed in other Lepidopterans as well (Tabashnik et al., 2000c). Furthermore, conducting competitive binding studies utilizing brush border membrane vesicles (BBMV) from both Bt-susceptible and Cry1Ac-selected pink bollworm strains revealed a common binding site for Cry1Aa, Cry1Ab, Cry1Ac, and Cry1Ja. Interestingly, the binding affinity of Cry1Ca, Cry2Aa, or Cry9Ca remained unaffected with BBMV of Cry1Ac-resistant pink bollworm (Gonza´lez-Cabrera et al., 2003). The possibility of cross-resistance between Cry1Ac and Cry2Ab2, the toxins expressed in Bollgard II, appeared minimal due to their distinctly separate binding sites within the midgut. However, a form of asymmetrical cross-resistance was detected between these two toxins. The Cry1Ac-resistant pink bollworm strain did not exhibit cross-resistance to Cry2Ab2, whereas the Cry2Ab2-resistant strain displayed a certain level of tolerance to Cry1Ac, with a resistance ratio of 420-fold compared to the susceptible strain. In terms of inheritance, resistance to Cry2Ab2 followed a recessive pattern. Notably, the pink bollworm strain possessing resistance to both toxins could not survive on Bollgard II cotton bolls (Tabashnik et al., 2009).

CURRENT STATUS OF Bt RESISTANCE IN INDIAN POPULATIONS OF PINK BOLLWORM

Bt cotton expressing Cry1Ac was approved for cultivation in India in March 2002. Technically, among the three key bollworms, pink bollworm was in the high-risk category for evolving resistance to Bt cotton because conventional (non-Bt) cotton was the only productive host for

this pest in India. In the pre-Bt era, pink bollworm was a destructive late-season pest, and cotton farmers were finding it difficult to manage this pest with insecticides due to its secluded feeding habit within the developing bolls (Singh et al., 1988). The Bt cotton (single and dual Bt-gene) could effectively control damage by pink bollworm because of its high sensitivity to both, Cry1Ac and Cry2Ab2 proteins, which were expressed as 'high-dose' in the target tissues of transgenic cottons. However, in the absence of structured/natural refuge and integrated pest management (IPM) elements for this pest, high selection pressure hastened the evolution of field-resistance to first Cry1Ac in 2009 (Monsanto, 2009; Durua & Gujar, 2011; Mohan et al., 2016), and to Cry1Ac & Cry2Ab2 in 2014 (Naik et al., 2018), the sequence in which the pink bollworm was exposed to these toxins through commercialization of Bollgard in 2002 and Bollgard II in 2006 onwards (Choudhary and Gaur 2010; Mohan, 2017).

Pink bollworm field populations originating from Bollgard cotton exhibited notable survival rates when exposed to the diagnostic concentration of 10 ppm of Cry1Ac. The observed resistance was found to be autosomal in nature, with a partially recessive mode of inheritance at Cry1Ac concentrations present within Bollgard cotton tissues (Nair et al., 2016). It's important to note that this resistance did not extend to cross-resistance with Cry2Ab2. Furthermore, a significant decrease in the binding of biotinylated-Cry1Ac to brush border membrane vesicles of the resistant pink bollworm populations collected from the field strongly suggested an association with Cry1Ac resistance (Ojha et al., 2014). These findings underscored the higher vulnerability of single-gene Bt products relative to dual-gene products that express both Cry1Ac and Cry2Ab2. Additionally, the studies emphasize the increased risk of resistance evolution with non-compliance to refuge strategy. The molecular basis of this field-evolved resistance Cry1Ac in Bollgard cotton in India was elucidated through the analysis of pink bollworm samples originating from regions where Bollgard crops experienced damage (Fabrick et al., 2014). Hitherto, mutations in the cadherin gene linked to Cry1Ac resistance were primarily originated from laboratory-selected Cry1Ac strains of pink bollworm. DNA sequence of seven field-evolved Cry1Ac resistant pink bollworm populations revealed that none of the four-recessive cadherin mutant alleles of *PgCad1* were linked to Cry1Ac resistance in Arizona sourced and laboratory selected strain of pink bollworm, reported earlier (Morin et al., 2003; Fabrick et al, 2009). However, the analysis revealed the presence of eight novel allelic forms with significant disruptive mutations that were associated with Cry1Ac resistance. These mutations were determined to lead to severe alterations in the gene's function. Intriguingly, a multitude of transcript isoforms of the cadherin gene, indicative of alternative splicing, were observed. These isoforms were a consequence of substantial deletions (at least 99 bases in length) and the occurrence of new premature stop codons within the cadherin gene. This marks the first instance of alternative splicing being identified as the

molecular mechanism underpinning Cry1Ac resistance in pink bollworm populations (Fabrick et al., 2014).

Considering the widespread adoption of Bollgard II in large acreages globally, Fabrick et al. (2015) evaluated the propensity of pink bollworm for evolving resistance to the dual toxins expressed by Bollgard II cotton. Through selective pressure from a Cry2Ab2-enriched diet, Cry2Ab2 resistance in pink bollworm was found to escalate dramatically, reaching levels up to 150,000 times greater within merely two generations of selection. This resistance to Cry2Ab2 was determined to be recessive in nature and was confirmed to be independent of Cry1Ac resistance. Importantly, the pink bollworm strain that exhibited resistance to both toxins in laboratory managed to survive on Bollgard II bolls, underscoring the significant risk associated with the evolution of resistance to the toxins expressed by Bollgard II. This risk was particularly pronounced when Bt toxins were introduced sequentially and the practice of refuge planting was considerably compromised. In accordance with these predictions, instances of field resistance to Bollgard II emerged within pink bollworm populations in India (Monsanto, 2009). The molecular basis of resistance to Cry2Ab2 in pink bollworm populations collected from Bollgard II cotton fields in India was attributed to mutations in the ATP-binding cassette transporter gene, PgABCA2. Various mutational forms of this gene associated with Cry2Ab2 resistance were also detected in a laboratory-selected pink bollworm strain from Arizona, USA (Mathew et al., 2018). Notably, these mutations included a prevalent form involving the loss of exon-6 due to alternative splicing. To test the hypothesis linking ABCA2 gene mutations to Cry2Ab2 resistance, a series of disruptive mutations were deliberately introduced into the ABCA2 gene using CRISPR/Cas9 technology. As anticipated, these mutations were indeed found to be linked with resistance to Cry2Ab2, as confirmed through bioassays (Fabrick et al., 2021). Profiling of the central Indian PBW populations, sourced from Bollgard II cotton fields, for cry1Ac-resistant cadherin mutational alleles *r1, r2 & r3*, revealed that all three were present, both in the homozygous and hemizygous forms (Likhitha et al., 2023) in contrast to the findings of Fabrick et al. (2014), who ruled out the occurrence of none of the four-recessive cadherin mutant alleles of *PgCad1* linked to Cry1Ac resistance in Indian samples of pink bollworm.

WAY FORWARD TO TACKLE THE CHALLENGES

Since India is considered to be the region of origin of pink bollworm (Saunders, 1843; CABI, 2023), it would be interesting to probe further the multitude of resistance mechanisms to Cry1Ac and Cry2Ab2 toxins present in various populations. This exploration encompasses an investigation into their inheritance patterns as well as their varying prevalence across the eight cotton-producing states in the country. Studies on epistatic interactions among the mutational alleles associated with Bt resistance is particularly valuable, potentially elucidating increased dominance of resistance mechanisms. This accumulation of knowledge holds the

potential to facilitate the development of more effective insect resistance management (IRM) strategies.

Assessing the haplotype variability within the mitochondrial gene cytochrome oxidase-1 (COI) among pink bollworm populations sourced from nine cotton-growing states has indicated a low degree of genetic isolation based on geographic distance (Naik et al., 2020). Although the specific relationship between COI diversity and the distribution of mutational alleles associated with Bt resistance remains unclear, it can be postulated that the wide dispersal of these mutational alleles (Cry1Ac and Cry2Ab2) across the eight cotton-growing states is relatively uniform. This uniformity persists due to the absence of distinct genetically isolated groups within the pink bollworm populations in India, characterized by low mating compatibility. Such a scenario holds advantageous implications, especially if India were to adopt sterile male release strategies for suppression of Bt-resistant pink bollworm populations.

The studies have shown that Cry1Ab and Cry1Ac toxins undergo oligomerization post-binding to specific domains on the cadherin molecules located in the midgut brush border membrane. The process of oligomerization involves the removal of helix alpha-1. The resulting truncated and oligomerized Cry1Ab/Cry1Ac toxins initiate the pore formation in the epithelial membrane of the midgut. This leads to toxicity in pink bollworm larvae that are resistant to Cry1Ac due to cadherin deletions (Soberon et al., 2007; Tabashnik et al., 2013). The oligomerization process of Cry1Ac in susceptible pink bollworm was confirmed by Ocelotl et al. (2015) who observed that modified Cry1Ac, which was altered by removing 56 amino acids including alpha-1 helix, could oligomerize in a laboratory condition without cadherin involvement. These modified toxins induced toxicity in Cry1Ac-resistant pink bollworm larvae, which were highly resistant (over 1000-fold) to the original Cry1Ac toxin. The researchers suggested that these modified Bt toxins, capable of bypassing the cadherin binding stage, might have utility in scenarios where pink bollworm populations have developed resistance to field applications of Cry1Ac.

As learnt from the lessons in China, field-evolved resistance to Cry1Ac was detected in pink bollworm populations from the cotton fields of Yangtze Valley, but in the absence of crop damage, it was not termed as practical resistance. The study showed a steep rise in the proportions of pink bollworm population which survived a diagnostic dose of 9 ppm from 0% in 2005-2007 to 56% in 2008 -2010. This was an early detection report for timely action (Wan et al., 2012). However, the frequency of Cry1Ac resistance in pink bollworm populations across six provinces in China, continuously monitored for 11 years, dropped after the proportion of non-Bt cotton plant population increased to approximately ¼ the total plant population, implying that a substantial refuge size could reverse the resistance frequency in pink bollworm (Wan et al., 2017). However, considering the widespread nature of resistance in Indian population of pink bollworm to Cry1Ac and Cry2Ab2

produced by Bollgard II cotton, it does not seems practically feasible that the adoption of refuge strategy would lead to reversal of susceptibility in pink bollworm in a short span of time (Tabashnik, 2021). However, refuge strategy can still have its promise in delaying the process of resistance development in other two dreaded bollworm pests of cotton viz., *Helicoverpa armigera* (Hubner) and *Earias vitella* (Fabricious), which are still controlled effectively with the use of Bt cotton. In this context, implementation of refuge in bag (RIB) strategy (The Gazettes of India, 2016) seems to hold some promise for IRM strategies against cotton bollworms (Kranthi et al., 2017; Fand et al., 2019).

The future IRM strategy to be effective, should be invariably and rigorously practiced with new and potent Bt cotton events. Therefore, Indian research laboratories must invest more into the research and development on genetically modified cotton with stacked new Bt proteins which are toxic to the pink bollworm strains that are currently resistance to Bollgard II cotton producing Cry1Ac and Cry2Ab2 toxins.

CONCLUSION

The molecular underpinnings of Bt resistance in pink bollworm provide deeper insights into the complex mechanisms that pests employ to adapt and survive against the most effective transgenic technology. As we dig into the molecular intricacies of Bt resistance, we uncover a web of genetic changes and physiological adaptations that together allow pink bollworm populations to withstand the lethal effects of Bt toxins. Increase in the folds of resistance due continuous exposure to Bt toxins, mutations in the cadherin gene and ATP-binding cassette transporter protein, alterations in receptor binding sites and a narrow range of cross resistance have been identified as the key mechanisms of Bt resistance in pink bollworm populations studied across the different parts of the world including India. These studies uncovering the genetic basis of Bt resistance have laid the foundation for more effective resistance management strategies that included the resistance monitoring, refuge implementation, and the protein engineering of the Bt toxin allowing its binding with the mutated midgut receptors thus re-establishing the toxicity against resistant strains. This newfound knowledge illuminates our path forward. Armed with a deeper understanding of the mechanisms driving resistance, we are better equipped to develop strategic solutions that disrupt these mechanisms. Integrated pest management strategies, which blend diverse approaches, present a formidable arsenal against Bt resistance development in insects in general and pink bollworm in particular.

REFERENCES

CABI. 2023. Invasive species compendium: *Pectinophora gossypiella* (pink bollworm). Available online at https://www.cabi.org/isc/datasheet/39417#70AF7142-7A8B-4F36-A0BA-4F14FA270EED, Accessed on 08/08/2023.

De Bortoli, C.P. and Jurat-Fuentes, J.L. 2019. Mechanisms of resistance to

commercially relevant entomopathogenic bacteria. *Current Opinions in Insect Science*, 33, pp. 56–62.

Dhurua, S. and Gujar, G.T. 2011. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. *Pest Management Science*, 67, pp. 898–903.

Endo, H. 2022. Molecular and kinetic models for pore formation of *Bacillus thuringiensis* Cry toxin. *Toxins*, 14, pp. 433.

Fabrick, J., Unnithan, G., Yelich, A., DeGain, B., Masson, L., Zhang, J., Carrière, Y. and Tabashnik, B.E. 2015. Multi-Toxin Resistance Enables Pink Bollworm Survival on Pyramided Bt Cotton. *Scientific Reports*, 5, pp. 16554. https://doi.org/10.1038/srep16554

Fabrick, J.A. and Tabashnik, B.E. 2007. Binding of *Bacillus thuringiensis* toxin Cry1Ac to multiple sites of cadherin in pink bollworm. *Insect Biochemistry and Molecular Biology*, 37, pp. 97–106.

Fabrick, J.A., Forlow, J.L and Henneberry, T.J. 2009. Novel pink bollworm resistance to the Bt toxin Cry 1Ac: effects on mating, oviposition, larval development and survival. Journal of Insect Science, 9, pp. 24. https://doi.org/10.1673/031.009.2401. PMID: 19613847.

Fabrick, J.A., LeRoy, D.M., Mathew, L.G., Wu, Y., Unnithan, G.C., Yelich, A.J., Carrière, Y., Li, X. and Tabashnik, B.E. 2021. CRISPR-mediated mutations in the ABC transporter gene ABCA2 confer pink bollworm resistance to Bt toxin Cry2Ab2. *Scientific Reports*, 11, pp. 10377.

Fabrick, J.A., Ponnuraj, J., Singh, A., Tanwar, R.K., Unnithan, G.C., Yelich, A.J., Li, X., Carrière, Y. and Tabashnik, B.E. 2014. Alternative Splicing and Highly Variable Cadherin Transcripts Associated with Field-Evolved Resistance of Pink Bollworm to Bt Cotton in India. *PLoS ONE* 9(5), e97900. https://doi.org/10.1371/journal.pone.0097900

Fabrick, J.A., Li, X., Carrière, Y., Tabashnik, B.E. 2023. Molecular and Genetic Basis of Lab- and Field-Selected Bt Resistance in Pink Bollworm. *Insects*, 14, pp. 201. https://doi.org/10.3390/insects14020201.

Fand, B.B., Nagrare, V.S., Gawande, S.P., Nagrale, D.T., Naikwadi, B.V., Deshmukh, V., Gokte-Narkhedkar, N. and Waghmare, V.N. 2019. Widespread infestation of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) on Bt cotton in Central India: a new threat and concerns for cotton production. *Phytoparasitica*, 47, pp. 313–325

Ferre, ´ J., Van, Rie, J. 2002. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. *Annual Review of Entomology*, 47, pp. 501–533.

Gonzalez-Cabrera, J., Escriche, B., Tabashnik, B.E. and Ferre´, J. 2003. Binding of *Bacillus thuringiensis* toxins in resistant and susceptible strains of pink bollworm (*Pectinophora gossypiella*). *Insect Biochemistry and Molecular Biology*, 33, pp. 929–935.

Heckel, D.G., Gahan, L.J., Baxter, S.W., Zhao, J.Z., Shelton, A.M., Gould, F. and Tabashnik, B.E. 2007. The diversity of Bt resistance genes in species of Lepidoptera. *Journal of Invertebrate Pathology*, 95, pp. 192–197.

Likhitha, P, Naik, C.B., Moharil, M.P., Undirwade, D.B., Kulkarni, U.S. and Kolhe, A.V. 2023. Molecular profiling of resistance alleles in *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) collected from different locations. Egyptian Journal of Biological Pest Control, 33, pp. 55.

Kranthi, K.R. 2015. Pink bollworm strikes *Bt* cotton. *Cotton Statistics News*, 35, pp. 1

Kranthi, S., Satija, U., Pusadkar, P., Rishikumar, S. C. S., Ansari, S., Santosh, H. B., Monga, D., and Kranthi, K. R. (2017). Non-Bt seeds provided by seed companies in India – Are they suitable as refuge for Bt-cotton? *Current Science, 112*(10), 1992–1993

Liu, Y.B., Tabashnik, B.E., Dennehy, T.J., Patin, A.L. and Bartlett, A.C. 1999. Development time and resistance to Bt crops. *Nature*, 400, pp. 519.

Liu, Y.B., Tabashnik, B.E., Meyer, S.K., Carrie`re, Y. and Bartlett, A.C. 2001. Genetics of pink bollworm resistance to *Bacillus thuringiensis* toxin Cry1Ac. Journal of Economic Entomology, 94, pp. 248–252.

Mathew, L.G., Ponnuraj, J., Mallappa, B., Chowdary, L.R., Zhang, J., Tay, W.T., Walsh, T.K., Gordon, K.H.J., Heckel, D.G., Downes, S. et. al. 2018. ABC transporter mis-splicing associated with resistance to Bt toxin Cry2Ab2 in laboratory- and field-selected pink bollworm. Scientific Reports, 8, pp. 13531.

Mohan, K.S. 2017. An area-wide approach to pink bollworm management on Bt cotton in India – a dire necessity with community participation. *Current Science*, 112, pp. 1988-1989.

Mohan, K.S., Ravi, K.C., Suresh, P.J., Sumerford, D., Head, G.P. 2016. Field resistance to the *Bacillus thuringiensis* protein Cry1Ac expressed in Bollgard® hybrid cotton in pink bollworm, *Pectinophora gossypiella* (Saunders), populations in India. Pest Management Science, 72, pp. 738 -746.

Monsanto, 2010. Cotton in India. http://www.monsanto.com/monsanto_today/for_the_record/india_pink_bollworm.asp .

Morin, S., Biggs, R.W., Sisterson, M.S., Shriver, L., Ellers-Kirk, C., Higginson, D., Holley, D., Gahan, L.J., Heckel, D.G., Carrie`re, Y., Dennehy, T.J., Brown, J.K., Tabashnik, B.E. 2003. Three cadherin alleles associated with

resistance to *Bacillus thuringiensis* in pink bollworm. *Proceedings of the Nationa Academy of Scinces USA*, 100, pp. 5004–5009.

Naik, V.C.B., Kumbhare, S., Kranthi, S., Satuja, U., Kranthi, K.R. 2018. Field-evolved resistance of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) to transgenic Bt cotton expressing Cry1Ac and Cry2Ab2 in India. *Pest Management Sci*ence, 74, pp. 2544 - 2554, https://doi.org/10.1002/ps5038.

Naik, V.C.B., Pusadkar, P.P., Waghmare, S.T., Raghavendra, K.P., Kranthi, S., Kumbhare, S., Nagrare V.S., Kumar, R., Prabhulinga, T., Gokte-Narkhedkar, N and Waghmare, V.N. 2020. Evidence for population expansion of cotton pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) in India. *Scientific Reports*, 10, pp. 4740. https://doi.org/10.1038/s41598- 020-61389-1

Nair, R., Kamath, S.P., Mohan, K.S., Head, G. and Sumerford, D.V. 2016. Inheritance of field-relevant resistance to the *Bacillus thuringiensis* protein Cry1Ac in *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) collected from India. Pest Management Science, 72, pp. 558–565.

Ocelotl, J., Sánchez, J., Arroyo, R., García-Gómez, B.I., Gómez, I., Unnithan, G.C., Tabashnik, B.E. and Bravo, A. 2015. Binding and oligomerization of modified and native Bt toxins in resistant and susceptible pink bollworm. *PLoS ONE* 10(12), e0144086. https://doi.org/10.1371/journal.pone.0144086

Ojha, A., Sowjanya, Sree, K., Sachdev, B., Rashmi, M.A., Ravi, K.C., Suresh, P.J., Mohan, K.S. and Bhatnagar, R.K. 2014. Analysis of resistance to Cry1Ac in field-collected pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae), populations. *GM Crops Food*, 5, pp. 280–286.

Soberón, M., Pardo-López, L., López, I., Gómez, I., Tabashnik, B.E., Bravo, A. 2007. Engineering modified Bt toxins to counter insect resistance. *Science*, 318, pp. 1640–1642.

Tabashnik BE, Patin AL, Dennehy TJ, Liu YB, Carrie`re Y, Sims MA, Antilla, L (2000a). Frequency of resistance to *Bacillus thuringiensis* in field populations of pink bollworm. *Proceedings of National Academy of Sciences USA*, 97, pp. 12980–12984.

Tabashnik, B.E., Fabrick, J.A., Unnithan, G.C., Yelich, A.J., Masson, L., Zhang, J., Bravo, A., Soberón, M. 2013. Efficacy of genetically modified Bt toxins alone and in combinations against pink bollworm resistant to Cry1Ac and Cry2Ab2. PLoSONE, 8, pp. e80496.

Tabashnik, B.E., Johnson, K.W., Engleman, J.T. and Baum, J.A. 2000c. Cross-resistance to *Bacillus thuringiensis* toxin Cry1Ja in a strain of diamondback moth adapted to artificial diet. *Journal of*

Invertebrate Pathology, 76, pp. 81–83.

Tabashnik, B.E., Liu, Y.B., De, Maagd, R.A. and Dennehy, T.J. 2000b. Cross-resistance of pink bollworm (*Pectinophora gossypiella*) to *Bacillus thuringiensis* toxins. *Applied Environmental Microbiology*, 66, pp. 4582 - 4584.

Tabashnik, B.E., Unnithan, G.C., Masson, L., Crowder, D.W., Li, X. and Carriere, Y. 2009. Asymmetrical cross-resistance between Bacillus thuringiensis toxins Cry1Ac and Cry2Ab2 in pink bollworm. Proc. Natl. Acad. Sci. USA 16, 11889–11984.

The Gazette of India, 2016. Extraordinary. (2016) Part II - Section 3(ii), No. 1236. Regd. No. D. L.-33004/99, New Delhi, May 18, 2016, pp, 1-9.

Wan, P., Huang, Y., Wu, H., Huang, M., Cong, S. et. al. 2012. Increased frequency of Pink bollworm resistance to Bt toxin Cry1Ac in China. PLoSONE, 7(1), e29975. https://doi.org/10.1371/journal.pone.0029975

Wan, P., Xu, D., Cong, S., Jiang, Y., Huang, Y., Wang, J., Wu, H., Wang, L., Wu, K., Carriere, Y., Mathias, A., Li, X. and Tabashnik, B.E. 2017. Hybridizing transgenic Bt cotton with non-Bt cotton counters resistance in pink bollworm. *Proceedings of National Academy of Sciences USA*, 114 (21), pp. 5413-5418.

Wang, L., Ma, Y., Guo, X., Wan, P., Liu, K., Cong, S., Wan, J., Xu, D., Xiao, Y., Li, X., Tabashnik, B.E. and Wu, K. 2019b. Pink bollworm resistance to Bt toxin Cry1Ac associated with an insertion in cadherin exon 20. Toxins, 11, pp. 186.

Wang, L., Ma, Y., Wan, P., Liu, K., Xiao, Y., Wang, J., Cong, S., Xu, D., Wu, K., Fabrick, J.A., Li, X. 2018. Resistance to *Bacillus thuringiensis* linked with a cadherin trans-membrane mutation affecting cellular trafficking in pink bollworm from China. Insect Biochemistry and Molecular Biology, 94, pp. 28–35.

Wang, L., Ma, Y., Wei, W., Wan, P., Liu, K., Xu, M., Cong, S., Wang, J., Xu, D., Xia, D., Li, X., Tabashnik, B.E., and Wu, K. 2020.Cadherin repeat 5 mutation associated with Bt resistance in a field-derived strain of pink bollworm *Scientific Reports*, 10, pp. 16840. https://doi.org/10.1038/s41598-020-74102-z

Wang, L., Wang, J., Ma, Y., Wan, P., Liu, K., Cong, S., Xiao, Y., Xu, D., Wu, K., Fabrick, J.A. *et. al*. 2019. Transposon insertion causes cadherin mis-splicing and confers resistance to Bt cotton in pink bollworm from China. *Scientific Reports*, 9, pp. 7479.

The Battle against Pink Bollworm Resistance to Bt Cotton in India

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ABSTRACT

The introduction of genetically modified (GM) crops, specifically Bt cotton carrying genes that encode delta-endotoxin proteins derived from the entomopathogenic soil bacterium *Bacillus thuringiensis*, was a revolutionary solution to combat pest infestations and enhance crop yields. In India, where cotton is a major cash crop, Bt cotton swiftly gained popularity due to its inherent resistance to the dreaded bollworm complex comprising of American bollworm *Helicoverpa armigera* (Hubner), spotted bollworm *Earias* vitella (Fabricious) and pink bollworm *Pectinophora gossypiella* (Saunders). Bt cotton technology was highly effective in managing the bollworm complex. Twenty two years after its initial introduction in India, Bt cotton technology is still effective against the two major bollworm species, *H. armigera* and *E. vitella*. However, development of field-level resistance in pink bollworm was reported early within 7 years after its introduction. The pest exhibited a newfound ability to feed on and survive within cotton plants carrying single-gene (Bollgard I) and dual-gene (Bollgard II) Bt toxins. This unusual survival led to pink bollworm infestations being documented in multiple states of central and south cotton growing zones, including Gujarat, Maharashtra, Karnataka, Andhra Pradesh, and Telangana. The range of resistant pink bollworm populations has now recently extended to the north cotton growing zone of India. This article focuses on the current status of pink bollworm resistance to Bt cotton in India and explores potential strategies to manage this escalating issue.

KEY WORDS: Bt cotton, ICAR-CICR, India, pink bollworm, resistance development, community approach for pest management

ADOPTION OF Bt COTTON IN INDIA

Bt cotton is the only crop approved in India for commercial cultivation. After the approval of Genetic Engineering and Appraisal Committee (GEAC) of Government of India, single gene (Cry 1Ac) and dual gene (Cry 1Ac + Cry 2Ab) Bt cotton hybrids were introduced in India during 2002 and 2006, respectively, for commercial cultivation (Choudhary and Gaur 2010), targeting the dreaded bollworm complex of cotton i.e. American bollworm, spotted bollworm and pink bollworm. In India, the adoption of Bt cotton technology was spectacular with an increase of 4 million ha area coming under cotton cultivation within a span of 10 years (Figure 1). With 94% adoption rate achieved (Figure 2), cotton production increased to 39 million bales of 170 kg lint and productivity attained a peak of 560 kg lint/ha by 2013-14 (ICAC Cotton Data Book, 2022).

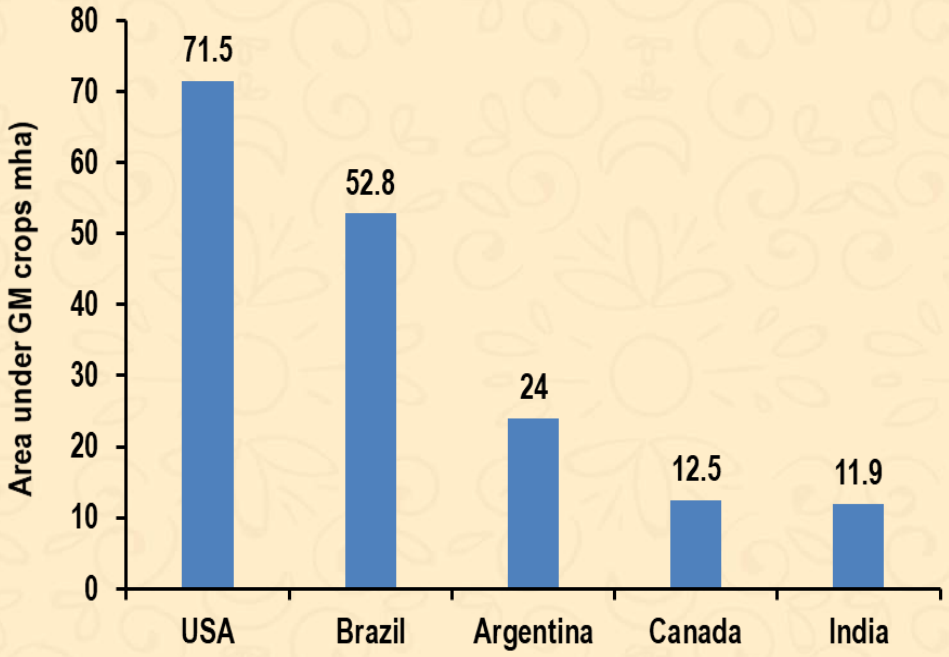


Figure 1: Top five countries of the world cultivating GM crops (Source: ICAC Data Book, 2022)

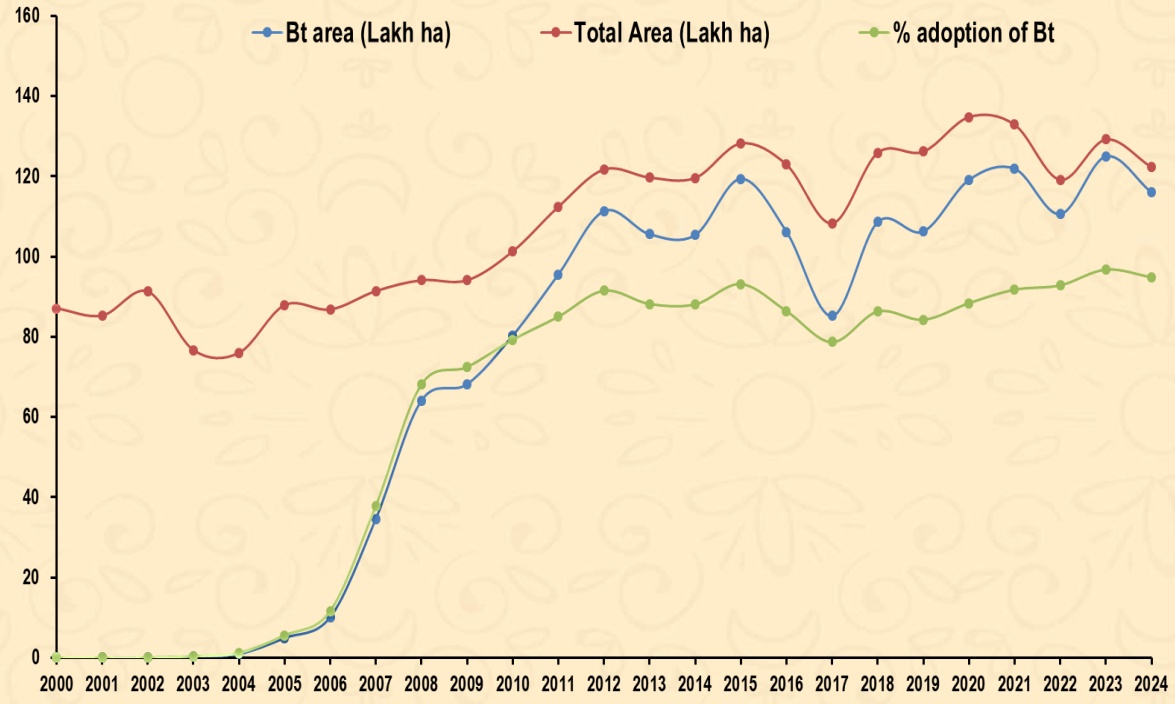


Figure 2. Adoption of Bt cotton technology in India from 2002 (Source: Directorate of Cotton Development, Nagpur. (Government of India), Consolidated Weekly Crop Weather Prospects Report in Respect of Nodal Crop : Cotton, Week Ending 25.08.2023)

CHANGING SCENARIO OF INSECT PESTS DURING POST-BT COTTON ERA

Since the introduction of Bt cotton for commercial cultivation, the Indian cotton ecosystem has witnessed phenomenal changes in its pest status. While the introduction of Bt cotton promised effective control against bollworm pests, notable changes have been observed among sap-sucking insects of the crop. Among the major sap sucking insects, the frequently damaging outbreaks of mealybugs (Nagrare et al., 2009) and whiteflies (Naveen et al., 2017) have been observed, and recent surge in damage attributed to thrips and jassids is increasing in the era of post Bt cotton phase and impending climate change. In the initial years following its debut, Bt cotton technology demonstrated its efficacy in controlling the bollworm complex until the year 2009. Impressively, even after more than 22 years of uninterrupted cultivation of Bt cotton in India, negligible resistance has been observed among the two primary bollworm species: *H. armigera and E. vitella*. This resilience to resistance in major bollworms is a credit to Bt technology and polyphagous feeding habit on multiple crop hosts of the American bollworm. However, after a substantial hiatus of around one and a half decades, the pink bollworm has staged an unforeseen comeback as a formidable pest challenge. This resurgence has primarily impacted the central and southern cotton-growing regions of India during 2015-2018

(Figure 3) (Kranthi, 2015; DurgaPrasad et al., 2016; Naik et al., 2018; Fand et al., 2019), followed by its surge in northern cotton growing belt from 2019 onwards (Kumar et al., 2020). Here, the pest's natural field infestations have been documented on both single-gene (Bollgard I) and dual-gene (Bollgard II) Bt cotton hybrids, marking a significant shift from the technology's initial success. The evolving pest scenario underscores the intricacies of managing pest populations in a dynamic agricultural landscape. As pests adapt and environmental conditions evolve, so must our strategies for pest control and crop protection. This calls for a comprehensive approach that encompasses research, innovation, and collaboration among stakeholders, aimed at maintaining the delicate balance between sustainable cotton cultivation and pest management.

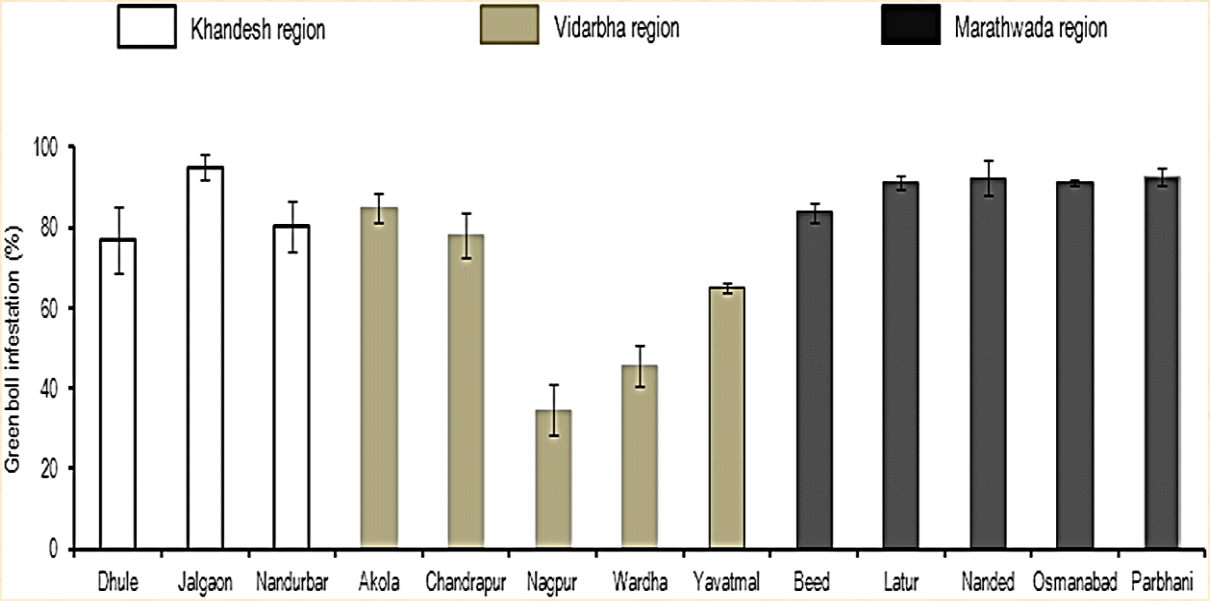
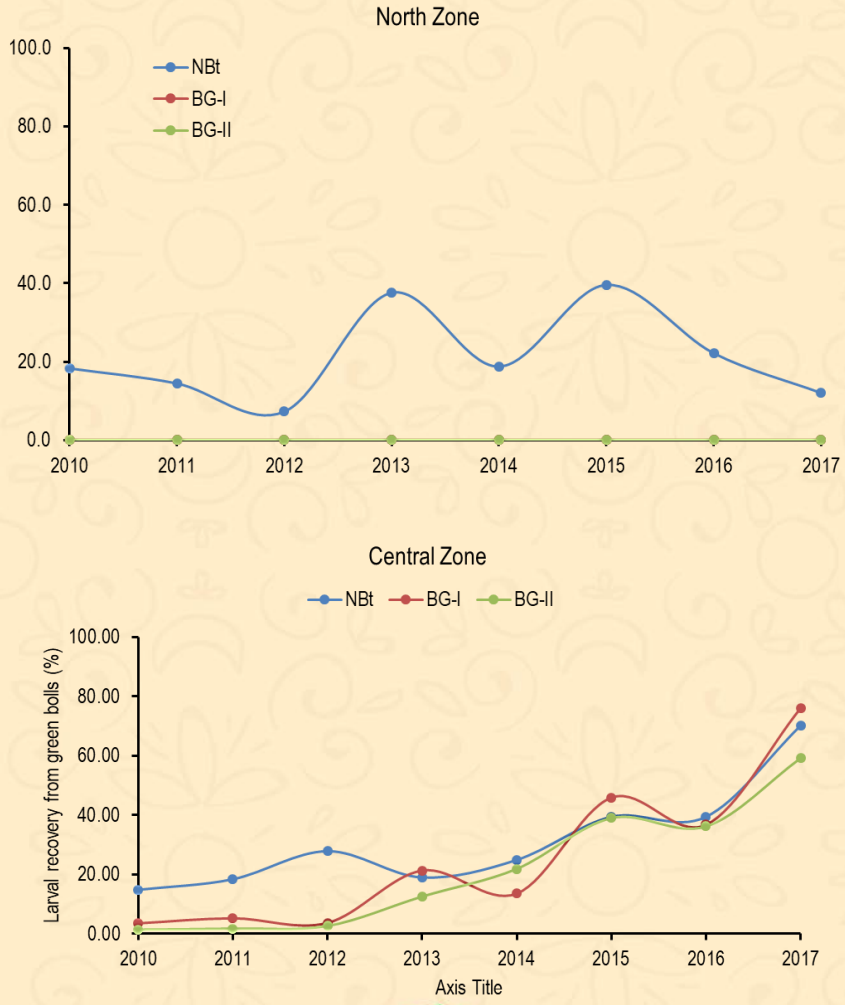


Figure 3. Pink bollworm infestation in green bolls (%) recorded during 2017-18 in major cotton growing districts of Maharashtra State. (Source: Fand et al., 2019**)**

CURRENT STATUS OF PINK BOLLWORM INFESTATION IN COTTON

The initial instance of pink bollworm developing field-evolved resistance to Bollgard (Cry1Ac) in India was recorded in 2010 (Dhurua and Gujar, 2011), followed by resistance to Bollgard II (Cry1Ac and Cry2Ab) from Gujarat state in 2014 (Kranthi, 2015) and other major states from central and south zones in 2017-18 (Naik et al., 2018; Fand et al., 2019). Over the past 5 to 6 years, there has been a notable increase in early occurrences of pink bollworm on BG-II hybrids, particularly in the largest cotton cultivation regions of Central and South India (Figure 4). Pink bollworm has now emerged as a major obstacle to cotton production in key states of central and south zones such as Maharashtra, Gujarat, Madhya Pradesh, Telangana,

Andhra Pradesh, and Karnataka. These states collectively contribute to 85% of the nation's cotton cultivation area. However, pink bollworm damage to Bt cotton in the Northern region was not witnessed on Bt cotton until 2018 (Figure 4). Reports of incidence in previously unaffected cotton areas of North Zone started in 2019 (Figure 5) (Kumar et al., 2020; Prasad and Kumar, 2022). A nationwide survey and monitoring data revealed that the onset of pink bollworm infestation displays a rising trend especially in the later part of the season coinciding with the months of late August to mid-October in north zone, and November to January in the central and south zones of the country (Annual Reports of AICCIP, 2010-2022; Naik et al., 2018, Fand et al., 2021). During 2010-2011, the occurrence of pink bollworm in green bolls was primarily confined to non-Bt cotton crops. The green boll samples collected from Bt cotton fields of central and south zones consistently revealed a higher prevalence of pink bollworm larvae from 2013 and 2014, respectively. The larval recovery rate from sampled green bolls sampled at > 100 DAS of crop stage increased sharply from < 50% during 2014-16 to > 70% from 2017 onwards (Figure 4). Similar trend was noticed in north zone from 2020 onwards (Figure 5) (Kumar et al., 2020; Prasad and Kumar, 2022).



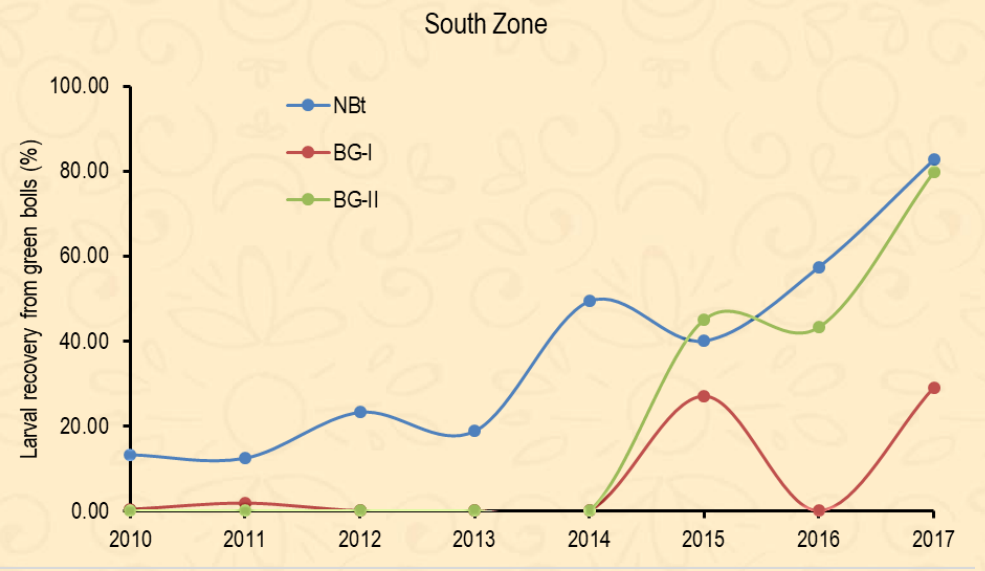


Figure 4. Progressive increase in pink bollworm infestation on Bt cotton in India from 2010-2017. Larval recovery recorded from green bolls sampled during 100-120 DAS. The values are means of the observations recorded at different locations in North (08), Central (18) and South (9) cotton growing zones of India. NBt = Non transgenic cotton, BG-I = single gene transgenic cotton with Cry1Ac gene, BG-II = dual gene transgenic cotton with Cry1Ac and Cry2Ab genes (Source: Naik et al., 2018).

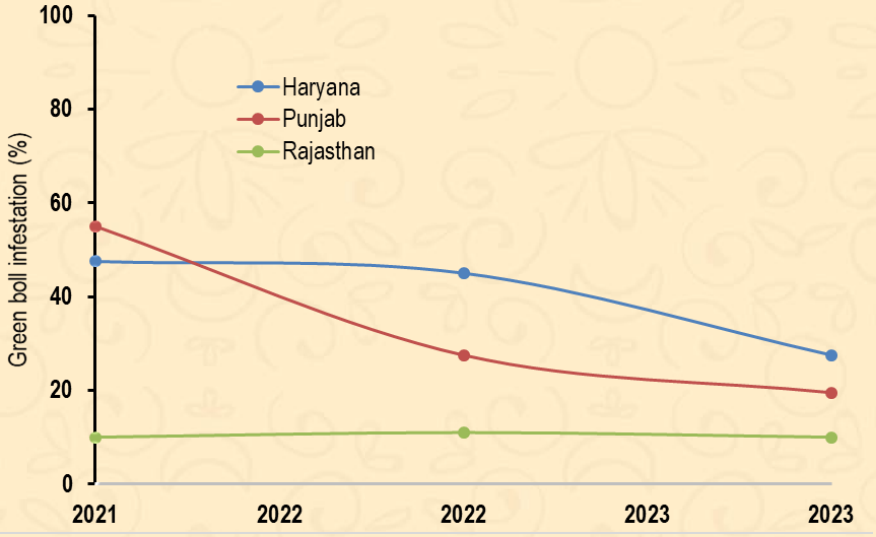


Figure 5. Upsurge of pink bollworm infestation in North Zone of India

Yield loss due to pink bollworm infestation in cotton crop of over 120 days with about two locules affected per boll has been estimated to the tune of 20-30% (Fand et al., 2019). Extending the cotton crop beyond normal recommended crop window leads to pink bollworm infestation in green bolls as high as 100% towards the fag end of the season (Prasad, 2021).

The resurgence of pink bollworm infestation against Bt cotton can be attributed to several key factors. These include the failure to adhere to the refuge strategy, the cultivation of extended-duration Bt cotton hybrids with varying flowering and fruiting periods, ensuring an ongoing food supply that sustains the pests. Additionally, the practice of extending the crop season beyond the typical planting window has exacerbated the pink bollworm infestations (Kranthi, 2015; Fand et al., 2019; Prasad, 2021).

PROACTIVE STEPS AT NATIONAL LEVEL TO TACKLE THE PROBLEM OF PINK BOLLWORM

To address the challenge posed to cotton production due to the resurgence of pink bollworm infestation on Bt cotton in India, several proactive steps have been taken at the national level. Some of the key initiatives in this direction implemented in India are given below:

i. Crop growth window based IPM strategy

The Central Insitute for Cotton Research (CICR) developed a crop-growth window based integrated pest management (IPM) strategy for pink bollworm considering the pest risk at different developmental stages of cotton crop (Kranthi, 2015; ICAR-CICR, 2023). The strategy emphasizes the adoption of management practices according to crop growth window of 0-60 days after sowing (DAS), 60-90 DAS, 90-120 DAS and >120 DAS. As the effective management of pink bollworm warrants season long practices to be followed at different critical stages of cotton crop, the window-based IPM strategy advocates various crop management options along with regular pest monitoring and surveillance coupled with spraying decisions guided by the economic threshold level (ETL) . The key elements of this IPM strategy are: timely sowing and termination with no extension of crop season beyond recommended period, no use of chemical insecticides before 60 DAS of crop stage and avoiding use of insecticides belonging to synthetic pyrethroid group prior to 120 DAS to avoid resurgence of sucking pests, monitoring of pest activity through pheromone traps and undertaking need based sprays guided based on pest infestations reaching economic threshold level (ETL).

The widespread dissemination and adoption of IPM strategy has been encouraged by the national agricultural research system, mainly through a larger network of State Agricultural Universities (SAU), Krishi Vigyan Kendras (KVKs), State Department of Agriculture and other line departments. The strategy has been endorsed by and

included in the national pest management programme of Directorate of Plant Protection, Quarantine and Storage (DPPQS), Ministry of Agriculture, Government of India.

ii. Nationwide regular monitoring of resistance development in pink bollworm

ICAR-CICR has well equipped state-of-the-art laboratory for resistance monitoring of major insect pests of cotton. The institute takes up extensive surveillance every year for collection of samples of green bolls from Bt cotton fields across the north, central and south cotton growing zones of the country to record the level of field infestations. The diet incorporation bioassays of pink bollworm larvae collected from different locations against cry toxins (both Cry1Ac and Cry2Ab2) are being conducted every year to assess the resistance development to cry toxins.

iii. Development of wax based slow release formulation of sex pheromone for monitoring and mass trapping of pink bollworm

ICAR-CICR, Nagpur has developed and commercialised an innovative slow-release pheromone formulation with extended field performance (Kranthi, 2015; Prasad, 2021). The ICAR-CICR pheromone formulation is effective on par with commercially available formations and is effective in catching the male moths of pink bollworm even at low population densities. The lure lasts for about 45 days after field deployment and is relatively cheaper. Annually over 5000 traps along with 15,000 pheromone lures have been distributed by the institute as critical inputs to the cotton farmers under various Government funded schemes/projects (Annual Reports of ICAR-CICR, 2018-2022).

iv. Large scale field demonstrations on mass trapping and mating disruption technology

ICAR-CICR has conducted large scale field demonstrations on > 250 acres in farmers' fields of Amravati, Chandrapur and Nagpur districts of Maharashtra state during cotton growing seasons of 2021-22 and 2022-23 (Figure 6). The trapping density of 30-35 traps per acre was found best for effective mass trapping of pink bollworm and reducing the green boll damage below economic threshold level (ICAR-CICR, Annual Report, 2021; 2022).

In order to best deliver the latest cutting-edge technologies for pink bollworm management to the cotton growers, ICAR-CICR has provided technical backup in Public-Private-Partnership (PPP) mode. Large scale field demonstrations of specialised pheromone application technology (SPLAT) with wax based formulation of gossyplure (Cremit PBW) were conducted for pink bollworm management in collaboration with ATGC Pvt Ltd, Hyderabad (ICAR-CICR Annual Report, 2022). The project was implemented on 500 acres of cotton area with a local support in 20 districts spread across the cotton areas of the country. In another endeavour in this direction, ICAR-CICR is providing technical backstopping for a mating

disruption technology product PB-Knot implemented on > 300 acres of cotton fields in Maharashtra since 2020 by Agrovision Foundation.

a **b**

Figure 6. ICAR-CICR's pheromone technology for pink bollworm management. A wax-based formulation of gossplure for monitoring and mass trapping (a), and view of traps installed in cotton fields for mass trapping (b)

v. Area wide dissemination of pink bollworm management strategies

A project worth 2.5 crores of annual budget financed by Department of Agriculture and Farmers' Welfare (Crops and PHMF Division), Government of India under a centrally sponsored scheme National Food Security Mission (NFSM): Commercial Crops is being implemented since 2018 in farmers' fields by ICAR-CICR as a Nodal institute in collaboration with State Agricultural Universities (SAUs) and farm science centres (KVKs) in 10 major cotton growing states of India. Every year, field demonstrations are being conducted on 1050 acres of cotton area and mass awareness is being created through outreach activities (Figure 7). With concerted and focused efforts during the period of last five years of project implementation, the average pink bollworm infestations could be reduced down by 39.0% in the demonstration plots over farmer practices. This has resulted in realization of mean benefit: cost ratios of 2.1:1 and increased yield of seed cotton (Annual Reports, IRM-PBW, 2018-2022).

Figure 7. Area wide dissemination of IPM technologies for pink bollworm management in farmers' fields.

vi. Initiatives on breeding programmes for short duration and early maturing cultivars

As the pink bollworm being a late season pest of cotton, adoption of early maturing and short duration varieties or hybrids is an effective strategy to escape the pink bollworm damage. In this direction, ICAR-CICR has already taken proactive steps by formulating well focused varietal breeding programmes for development of short duration and early maturing cultivars. The All India Coordinated Cotton Improvement Project (AICCIP) is sponsoring the entries for multiplication testing of early maturing cultivars paving the way for their potential commercial release (Prasad, 2021).

vii. Weather-based and crop growth stage specific weekly advisories

During cotton growing season of each year, ICAR-CICR issues weather-based and cotton crop growth window specific Weekly Cotton Advisory for providing real-time information enabling cotton farmers in making timely decisions on nutrient, pests and disease management in their crop. The basic inputs related to rainfall, situation, crop growth stage and pest situation required for the advisory are collected from various cotton scientists working in a national network of AICCIP spread over 21 different centres across the north, central and south cotton growing areas. The advisory is prepared in English language as base document which then is translated into nine different regional languages for the benefit of local farmers. Once finalised the document is uploaded on the institute's website and also shared with other stakeholders for its wider circulation. The document can be accessed from the link on ICR-CICR website: https://cicr.org.in/resource-weekly-advisory/.

viii. Centrally sponsored pilot project on cotton for targeting technologies to agro-ecologies

A key to the successful management of pink bollworm in cotton in India can be the adoption of early maturing, short duration and compact genotypes grown at higher density planting systems (HDPS). HDPS is one of the promising technology developed by ICAR-CICR for profitable cotton production on light shallow soils of rainfed areas. As the cultivars grown at high density (90 cm row spacing x 15 cm plant spacing) in light soils will mature early and thus helps in potential escape of pink bollworm damage which is otherwise likely to cause heavy toll in the later part of the season (Venugopalan and Prasad, 2022). Considering the potential of HDPS cotton in rainfed agro-ecologies of India, ICAR-CICR is currently implementing a mission mode project on "Large scale demonstrations of best practices and scalable technologies to enhance cotton productivity in India" during 2023-24. The project is funded by Ministry of Agriculture and Farmers' Welfare and supported by the Ministry of Textiles, Government of India. HDPS demonstrations are underway across 6 states in 34 districts covering >3500 ha area.

CONCLUSION

In India, nationwide consistent efforts are being made in implementing IPM strategies for managing the pink bollworm menace in cotton. Significant success in reducing pink bollworm infestation, pesticide usage and correspondingly improvement in yield, economic benefits and reduced environmental impact has been demonstrated through area wide IPM demonstrations. This success was achieved through collective efforts and active involvement of all concerned stakeholders of cotton production. Nevertheless, it is crucial to recognize that the battle is still ongoing and requires constant adaptation. Continued research and collaboration are essential to stay ahead of the pink bollworm's adaptability, ensuring that new resistance management strategies are in place and integrated effectively. Public awareness campaigns, farmer education, and government support remain integral to the sustained success of these endeavours.

REFERENCES

AICCIP Annual Reports, 2010-2022. ICAR-All India Cotton Improvement Project, Available online at: https://aiccip.cicr.org.in/aicrp/index.html. Accessed on 10/08/2023.

Choudhary, B. and Gaur, K. 2010. Bt cotton in India: A country profile. ISAAA series of biotech crop profiles. Ithaca, NY: ISAAA.

DCD. 2017. Status Paper of Indian Cotton, Directorate of Cotton Development Government of India, Ministry of Agriculture and Farmers Welfare, Department of Agriculture, Cooperation and Farmers Welfare (DAC & FW), Nagpur, Maharashtra. pp. 40.

Dhurua, S. and Gujar, G.T. 2011. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. *Pest Management Science*, 67, pp. 898–903.

DurgaPrasad, N.V.V.S., Sesha-Mahalakshmi, M., Sreelakshmi, B., Satish, Y., SivaReddy, K.V., Bharathi, S., RatnaKumari, S., ChengaReddy, V. and RatnaPrasad, P. 2016. Pink bollworm in Cotton, Acharya NG Ranga Agricultural University Regional Agricultural Research Station, Lam, Guntur – 522 034, Andhra Pradesh, India. Technical bulletin, OPMAS Project, pp. 28.

Fand, B.B., Nagrare, V.S., Gawande, S.P., Nagrale, D.T., Naikwadi, B.V., Deshmukh, V., Gokte-Narkhedkar, N. and Waghmare, V.N. 2019. Widespread infestation of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) on Bt cotton in Central India: a new threat and concerns for cotton production. *Phytoparasitica*, 47, pp. 313–325.

ICAC Cotton Data book, 2022. International Cotton Advisory Committee, Washington DC, USA. Pp. 949. https://icac.org/Content/PublicationsPdf%20Files/d5fa29c6_14534623_871b_1658c57dfebe/Intro-pages%20DATABOOK-2022.pdf.pdf

ICAR-CICR Annual Reports, 2018-22. ICAR-Central Institute for Cotton Research Nagpur, Maharashtra, India. Available online at: https://cicr.org.in/resources/resource-cicr-annual-reports/. Accessed on 10/8/2023.

ICAR-CICR. 2023. Cotton Pest and Disease Management Strategies 2023-24. ICAR-Central Institute for Cotton Research, Nagpur. Pp. 1-7. Avaliable online at: https://cicr.org.in/wp-content/uploads/Pest-and-disease-management-2023-24.pdf. Accessed on 15/08/2023.

Kranthi, K.R. 2015. Pink bollworm strikes *Bt* cotton. *Cotton Statistics News*, 35, pp. 1.

Kumar, R., Monga, D., Naik, V.C.B., Singh, P. and Waghmare, V.N. 2020. Incipient Infestations and Threat of Pink Bollworm *Pectinophora gossypiella* (Saunders) on Bollgard-II Cotton in the Northern Cotton-Growing Zone of India. *Current Science*, 118, pp. 1454-1456.

Monsanto. 2010. Cotton in India. http://www.monsanto.com/newsviews//Pages/india-pink-bollworm.aspx (subsequently revised to Pink Bollworm Resistance to GM Cotton in India. https://monsanto.com/ company/ media/ statements/pink-bollworm-resistance/, revised page accessed on November 15, 2017.

Nagrare, V.S., Kranthi, S., Biradar, V.K., Zade, N.N., Sangode, V., Kakde, G., Shukla, R.M., Shivare, D., Khadi, B.M. and Kranthi, K.R. 2009. Widespread infestation of the exotic mealybug species, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), on cotton in India. *Bulletin of Entomological Research*, 99, pp. 537–541.

Naik, V.C.B., Kumbhare, S., Kranthi, S., Satija, U. and Kranthi, K.R. 2018. Field-evolved resistance of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), to transgenic *Bacillus thuringiensis* (Bt) cotton expressing crystal 1Ac (Cry1Ac) and Cry2Ab in India. *Pest Management Science*, 7(75), pp. 2544-2553.

Naveen, N., Chaubey, R., Kumar, D., Rebijith, K., Rajagopal, R., Subrahmanyam, B. and Subramanian, S. 2017. Insecticide resistance status in the whitefly, *Bemisia tabaci* genetic groups Asia-I, Asia-II-1 and Asia-II-7 on the Indian subcontinent. Scientific Reports, pp. 7, 40634. https://doi.org/10.1038/srep40634.

Prasad, Y.G. 2021. Short Season Compact Genotypes Planted at Higher Density Will Hold Fort Against PBW in India. ICAC Recorder 39(1), pp. 15-16.

Prasad, Y.G. and Kumar, R. 2022. Pink Bollworm in North Cotton Growing Zone of India– It Is Time For Coordinated Corrective Measures. *Cotton Statistics and News*, Weekly Publication of Cotton Association of India, 3, pp. 1-8.

Venugopalan, M.V. and Prasad, Y.G. 2023. High Density Planting System in Cotton, Q&A. ICAR-Central Institute for Cotton Research. Technical Bulletin 2023/3.