to IPM. However, IPM can be a practical option only if taken seriously. There is a need now to scientifically reflect on the past performance of cotton IPM so that lessons can be learnt to reinvent and reconsolidate it with the help of new scientific findings. For sure, the worms would not have gained an upper hand, if IPM strategies were implemented right from the inception, incorporating *Bt*-cotton as one of the IPM tools. Unfortunately, *Bt*-cotton in many countries was seen as 'the silver-bullet-panacea' to the bollworm problem, consequently IPM was ignored almost completely. It is never too late to wake up. Amalgamation of all available pest management strategies that work in consonance with ecosystem engineering could provide a robust roadmap towards sustainability. The article 'Beware of the boll weevil, bollworms, whitefly and leaf curl virus' attempts to throw some insights on potential remedies that are available in scientific literature, and a few that could be available in the future.

Beware of the Boll Weevil, Bollworms, Whitefly and Leaf Curl Virus

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In the last ten years, five countries, India, China, USA, Pakistan and Brazil together constituted 72 to 76% of the global cotton area annually and contributed to more than 75% of the global cotton production each year. In the recent past, there have been clear signals from research publications that pest problems are brewing up in these top five cotton producing countries. These insect pests are notorious. Concerted efforts are required to address the problems at the earliest before they become more serious. The boll weevil, cotton bollworm, pink bollworm, whitefly and leaf curl virus are known to cause heavy economic crop losses and are difficult to manage. Needless to state, changes in production of the five countries will impact global production proportionately.

The past ten years were characterized by global yield stagnation and trends towards increasing chemical usage. Increase in pesticide use during this period indicates the increased levels of attention drawn by insect pests and an insect-vector transmitted disease. Yields reached a plateau in India, USA, Pakistan and Brazil over the past 10-12 years, but, have been on the ascent consistently during the past several years in China. Though, everything looks deceptively normal as of now, the different undercurrents of biotic threats in the five major countries can potentially destabilize cotton yields in the near future, if unattended to. One of the major concerns is that, while insect pest infestation levels in major cotton growing countries continue to increase, new technologies to control them do not seem to be in place. This is reflected in the data of yield stagnation and increases in pesticide use that happened despite the introduction of new technologies such as improved varieties, new biotech products and new potent pesticides during the past ten years. Will the major countries be able to effectively combat the imminent biotic threats? Can the yields increase hereafter as we move towards 2020? Are there technologies at hand now, or short-listed for the near future to prevent decline in yields or to enhance yields, reduce chemical usage and bring down the cost of production? The answers to these questions will largely depend on the technological changes related to pest management and yield enhancement that may take place, especially in the five major cotton producing countries.

The Five Major 'Biotic-Stress' Challenges

The five major cotton growing countries are now plagued with either new insect pests or with *Bt*-resistant bollworms or herbicide resistant weeds and increasing pesticide usage. Incidentally, in all the five countries, the major yield-limiting factors are biotic threats. These relate to insects and an insect transmitted disease.

The most serious threats are:

- The boll weevil, *Anthonomus grandis* (Boheman) and the cotton bollworm, *Helicoverpa armigera* (Hűbner) in Brazil;
- The whitefly, *Bemisia tabaci* (Gennadius), the leaf curl virus and the *Bt*-resistant pink bollworm, *Pectinophora gossypiella* (Saunders) in India and Pakistan;
- *Helicoverpa zea* (Boddie) in USA and impending threat of *Bt*-resistance in *H. armigera* in India, Pakistan and China.

The problems are very serious and so is the potential threat to cotton production systems

The threats are being addressed efficiently by some countries, while others react only when the problem gets worse. While some countries are aware of the incoming risks, others may have only perceived some initial indications of the possible implications. Outstanding research and administrative efforts in USA paved the way for successful planning and implementation of effective programs on area-wide management of whiteflies, bollworms and boll weevils. These insects would have otherwise caused heavy economic losses to cotton production in the country. The problem of boll weevil in Brazil and the impending threats of bollworms in India, Brazil, Pakistan, USA and China deserve more attention in these countries. Incidentally, India, China, USA, Brazil and

Table 1. Biotic stress factors in the 5 major cotton growing countries

Insect pest / disease	USA	Brazil	China	India	Pakistan
1. Boll weevils		*****			
2. *Bt*-resistant bollworms					
a. Pink bollworm		*	**	*****	***
b. *Helicoverpa* & *Heliothis* spp.	**	*	**	***	**
3. Whiteflies	*	**	**	****	****
4. Leaf curl virus disease				*****	*****

*Number of asterisks indicate intensity of the threat, as surmised from research papers

Pakistan are the largest users of insect-resistant biotech cotton technologies. In addition to the insect-resistant biotech cotton, USA and Brazil have also been the major users of herbicide tolerant cotton. Interestingly, all the five countries are almost completely dependent now on biotech traits in their cotton varieties. But, technologies have a life of their own. They are seldom expected to continue to remain effective forever. Over the recent past, researchers across the world have been constantly issuing alerts on the declining efficacy of the biotech traits in many countries due to either bollworm resistance to *Bt* (*Bacillus thuringiensis*) proteins or the development of herbicide resistant weeds. Another concern is the emergence of minor pests as major threats to production systems. Mostly these are insect pests that are not affected by *Bt*-proteins. Consequently *Bt*-resistant bollworms and the newly emerging pests lead to increases in pesticide applications. Publications indicate that researchers are working out the best possible solutions to combat the biotic stress problems in their respective countries. These technologies are in various stages of development. It remains to be seen whether they will succeed in time to prevent a negative impact on global yields. This article attempts to evaluate the risks and examine the recent scientific advances that reflect hope for the future, and potential remedies that are at various stages of development in each of the five countries.

The Boll Weevil in Brazil

The boll weevil has been one of the menacing factors in the whole of South America starting from the mid 1980s. Grubs of the weevil feed inside the developing bolls. This cryptic feeding habit of boll weevil is the main hurdle in pest management. Boll weevils can cause severe economic losses up to 100% in the absence of management. Adults start colonizing cotton at the time of squaring. They feed on squares, flowers and oviposit inside them causing complete damage due to abscission. Boll weevil grubs reside and feed inside fruiting parts and thus are not vulnerable to naturally occurring predators, parasitoids and pesticide applications. Thus far, the main methods of boll weevil management are based on cultural control, pheromone traps and insecticide applications to break reproductive cycles and diapause. Very recently, a new Biotech-cotton technology expressing the *Bt*-gene *cry10Aa* was developed in Brazil and was reported to be highly effective in controlling the boll weevil (Ribeiro *et al*., 2017). A few other biotech-cotton based technologies were also reported recently (Silva *et al*., 2015, Macedo *et al*., 2017, Araujo *et al*. 2005 and Medel *et al*. 2015). These technologies add new dimensions to the existing boll weevil management arsenal. To ensure long term sustainability and profitability of cotton production systems in South America, cotton IPM programs must incorporate essential ingredients of boll weevil eradication program, coupled with the new biotech-cotton technologies that target boll weevils and bollworms.

The incidence of pest and disease attacks is high in Brazil, since most of the land used for cotton is in a tropical climate that is conducive for pests. High humidity attracts fungal diseases. Some of the important biotic stress elements are nematodes, fungal diseases, boll weevil, *Anthonomus grandis* (Boheman), pink bollworm, *Pectinophora gossypiella* (Saunders), tobacco budworm, *Heliothis virescens* (Fabricius), cotton bollworm *Helicoverpa armigera* (Hübner), cotton leaf worm, *Alabama argillacea* (Hübner), fall armyworm, *Spodoptera frugiperda* (J.E. Smith) and whitefy, *Bemisia tabaci* (Gennadius). Boll weevils pose the most formidable threat with potential to cause complete damage if left unchecked. The cotton blue disease caused by aphid transmitted luteoviruses is yet another threat that looms large if left unattended.

Farmers are inclined to use insecticides as the main remedial measure for insect pest control, thereby rendering the systems highly chemical intensive. *Helicoverpa armigera* can easily emerge as a major pest in Brazil and elsewhere in South America due to indiscriminate use of synthetic pyrethroids, as was the case in other countries across the world. The arrival of the boll weevil in the mid-1980s and the bollworm *Helicoverpa armigera* in 2012-13, tilted the balance enormously towards chemical dependence. At least one-third of insecticide usage is directed towards boll weevil control. High use of fertilizers, especially in an unbalanced manner, can aggravate pest and disease problems. Repeated applications of insecticides early in the season, mainly for aphid and boll weevil control, disrupt the ecosystem severely to prompt resurgence and emergence of a series of insect pests, including mites, aphids, whiteflies and bollworms, thereby necessitating further repeated applications of insecticides. A substantial portion of about

80% of the cost of cultivation at US$ 2,500 per hectare in Brazil is towards agrochemicals (Barbosa and Ramos, 2014). Recent reports suggest over-dependence of cotton cultivation on pesticides. Historically, indiscriminate use of pesticides has invariably caused a decline of cotton production systems across the world. Currently, Brazil is facing a huge challenge and needs to develop integrated Pest Management (IPM) systems through judicious pesticide applications for effective pest control while ensuring sustainable ecology and a healthier environment. At this point in time, only IPM can orient cotton towards sustainability.

Barbosa and Ramos (2014) clearly emphasize the need for initiatives towards the development of sustainable cotton programs. "*Today, cotton growing in Mato Grosso, West Bahia and elsewhere in the Cerrado is a extremely high-input activity with the costs to produce one hectare surpassing 2,500 US$.....Because the uncontrolled high use of modern inputs in cotton growing, considering their side effects on the economy, on human health and on the environment, may not be sustainable over the long run, caution must be taken against their overuse. What should count in the future is not the number of cotton bales in the barn but the number of dollars in the farmer's pocket after all the costs are paid for, with due concern to human health and the environment. Uncertainties on future cotton marketing trends and a growing concern on consumers in relation to the use of out-of-the-farm inputs, cotton farmers associations, universities, and research & extension systems must joint efforts and develop more benign and more sustainable cotton production methods to guarantee the sustainability of this very noble activity over the years to come*."

Recent Exciting Research Advances

At least three promising 'boll weevil-resistant' biotech *Bt*-cotton varieties were developed in Brazil in the past two years. A recent paper '*Transgenic cotton expressing Cry10Aa protein confers high resistance to the cotton boll weevil*' in Plant Biotechnology Journal, by Ribeiro *et. al.* (2017) generated considerable excitement for boll weevil management. The Brazilian scientists reported successful transformation of a Brazilian cotton cultivar (BRS 372) with *cry10Aa* gene derived from *Bacillus thuringiensis* subsp. *israelensis* (*Bt*i) strain using particle bombardment method. The Cry10Aa protein was found to be highly toxic to *Anthonomas grandis* (Aguiar *et al*., 2012). The biotech plants were found to express the Cry10Aa protein at 3.0 to 14.0 ppm, in leaves and flower buds adequate to cause up to 100% mortality of the grubs and adults of the boll weevil. The biotech cotton variety presents a great opportunity for boll weevil management. Previously Silva *et al*. (2015) had reported the development of biotech cotton variety BRS 293 using Cry1Ia that could cause 56.7 to 83.7% mortality of boll weevils and 88.1% mortality of the fall army worm *Spodoptera frugiperda*. Oliveira *et al*. (2016) developed a *Bt*-cotton variety BRS Cedro using Cry1Ia12 through pollen tube pathway method and reported up to 60% mortality in boll weevils and 40% mortality in the fall army worm with severe growth regulating effects in the surviving larvae. These scientific advances signal new hope for boll weevil management.

New genes were identified for use in the development of biotech cotton varieties. Navas *et al*. (2014), identified two Cry proteins Cry8Pa3 and Cry8QA2 from a *Bt* strain INTA Fr-7-4, that were toxic to *Anthonomus grandis*. Oliveira *et al*. (2011) developed a mutant protein Cry8Ka5 with high levels of median leathal concentration LC_{50} toxicity at 2.83 ppm. Macedo *et al*. (2017) reported that gene silencing of *chitin synthase2* through ribonucleic acid interference (RNAi) caused 100% mortality of the boll weevil. They also reported that gene silencing caused inhibition of oviposition by 93%. The results create a new promise for the development of biotech cotton expressing dsRNA (double stranded RNA) of *AgraCHS2* (*Anthonomus grandis chitin synthase 2*) for boll weevil management. Previously, based on transcriptome studies of mid-gut genes, Firmino *et al*. (2013) had reported effective growth regulating effect with gene silencing of *chitin synthase* through RNA interference. A few papers (Araujo *et al*., 2005 and Medel *et al*., 2015) also indicate that protease inhibitors had detrimental effects on weevils. It may be interesting to evaluate the Cowpea trypsin protease-inhibitor (CPTi) expressing biotech-cotton varieties developed by the Chinese Academy of Agricultural Sciences (CAAS), China, for their toxicity to boll weevils.

Identification of novel *Bt* genes such as *cry1Ia* and *cry8* for boll weevil control, coupled with the promise of protease inhibitors and RNAi (Ribonucleic acid interference) based gene silencing of *chitin synthase* genes, provide additional options for the development of new highly potent biotech cotton varieties by combining these with the newly developed Cry10Aa protein based biotech cotton varieties. Also, pyramiding the Cry10Aa biotech cotton event with the currently available bollworm-resistant biotech cotton events expressing other Cry proteins such as Cry1Ac, Cry2Ab, Vip3A, Cry1F, protease inhibitors (CPTi CAAS event) and RNAi based *chitin-synthase* silencing events, would result in robust biotech varieties that can combat the boll weevil and bollworms more effectively. The multi-gene biotech cotton varieties can be valuable components of sustainable integrated pest management (IPM) to keep the boll weevil and bollworms under control for the longest possible time.

Bt-Resistant Pink Bollworms in India and Pakistan

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), is one of the major concerns now in India, Pakistan and China. The pink bollworm developed resistance to *Bt*-cotton in India recently and has been causing significant damage to the crop over the past two to three years (Kranthi, 2015). Though pink bollworm is a major pest also in the USA, implementation of effective monitoring and

management programs using pheromones and release of male sterile moths (Simmons *et al*., 2011; Morrison *et al*., 2012; Tabashnik *et al*., 2012), refuges and appropriate gene pyramids (Head and Dennehy, 2010) ensured that the pest is still under control and resistance development is delayed. Cry1Ac is highly toxic to the pink bollworm (Mohan *et al*., 2015). *Bt*-cotton expressing Cry1Ac was able to control pink bollworm very effectively until the worm developed resistance to Cry1Ac in India (Dhurua and Gujar, 2011; Ojha *et al*., 2014; Mohan *et al*., 2015), China (Wan *et al*., 2012; Tabashnik *et al*., 2012) and Pakistan (Akhtar *et al*., 2016). Pink bollworm resistance to Cry1Ac in *Bt*-cotton grown in the central Indian state of Gujarat was first recorded in 2009 (Dhurua and Gujar, 2011). Surveys conducted by ICAR-CICR (Central institute for cotton research, India) in 2014, 2015 and 2016 clearly established that pink bollworm larvae were able to survive on the two-gene *cry1Ac+cry2Ab* based Bollgard-II (BG-II) *Bt*-cotton hybrids. The surveys showed that about 40-80% of the bolls were found to contain surviving larvae. Resistance monitoring conducted with larvae collected from Gujarat state showed that pink bollworms developed resistance to Cry1Ac, Cry2Ab and Cry1Ac+Cry2Ab proteins (Chinnababu *et al*., unpublished). Subsequently, pink bollworm damage was reported from central and south India, mostly from irrigated regions, where *Bt*-cotton was grown as a long-duration crop. Recent reports from India (Chinnababu Naik, CICR,personal communication & Kranthi, 2015) unequivocally confirm the development of pink bollworm resistance to the dual gene *Bt*-cotton expressing the two Cry proteins, Cry1Ac+Cry2Ab.

Pink bollworm with its Indo-Pak origins is considered as a traditional pest in the Indian sub-continent. It was first described in 1842 as a cotton pest in India (Ingram 1994). Prior to its scientific nomenclature as *Pectinophora gossypiella* (Saunders), the insect was known as, *Depressaria gossypiella* (Saunders), *Ephestia gossypiella* (Saunders), *Gelechia gossypiella* (Saunders), *Gelechiella gossypiella* (Saunders), and *Platyedra gossypiella* (Saunders). Generally the pink bollworm is a pest of long-season cotton. Historically cotton varieties in India, Pakistan and China were of long duration. The pink bollworm feeds on cotton, bhendi (okra), *Hibiscus*, and jute. Infestation starts in early winter and continues for 4-5 months depending on the availability of host crops. Moths are dirty brown in colour and about 5 mm in length. Eggs are laid mainly on floral parts or young bolls and hatch in 3-4 days. Within two days of hatching, the young larvae bore inside flowers or young bolls. Larvae turn pink in colour soon after feeding on tender seeds. Larvae feed on developing seeds thereby damaging bolls and leading to premature boll opening or rotting. Infested flowers get 'rosetted' and twisted. Fibre quality in the infested bolls is badly affected due to secondary fungal infection and staining. Transportation of seed cotton containing pupae or moths spreads infestation. Ginned and baled cotton is unikely to harbour live larvae or pupae that would be able to survive and infest new areas. Pink bollworm enters into a diapause for 6-8 months. Long duration crop allows multiple cycles of the pest which gets carried over into the next season. Experience across the globe shows that longer the cotton crop duration, higher is the probabilty of potential damage by pink bollworms in the subsequent crop season. Potential losses due to pink bollworms were estimated to be 61% in the USA (Schwartz, 1983), 20.2% in India (Agarwal and Katiyar, 1979), 17-26% in China (Luo *et al*., 1986) and 10.7% in Sudan (Darling, 1951).

Fig.1 Pink bollworm *Pectinophora gossypiella* damaging a boll

Generally, pink bollworms cause maximum economic damage late in the season, which coincides with post-130 days old crop in many countries. Pink bollworm moths prefer oviposition on 10-20 days old bolls mostly and seldom cause fresh infestation on older or mature bolls. Therefore a short season crop of 150-160 days duration can be cultivated in a specified time-window of the cropping season to escape pink bollworm infestation by creating a mismatch between the boll maturation and seasonal peaks of the pink bollworm. Early-sown short duration crop is likely to attract pink bollworms slightly during the flowering period, but, this peak is small and can be managed. However, the first picked cotton of early sown crop would in all likelihood escape pink bollworms, more so if the variety is of short or medium duration. Late-sown crops of medium and late duration varieties suffer the most. Staggered flowering phase in a continuous manner over an extended period also creates a long vulnerable window for pink bollworm attack. Some varieties may have morphological features of boll rind and allelochemical factors which may be more congenial for pink boll worm infestation thereby resulting in more damage compared to others. Excessive application of nitrogen and irrigation during the vegetative phase results in extended phase of the crop and stretches the crop duration further, wherein the staggered boll formation phase coincides with pink bollworm infestation.

The pink bollworm larvae feed on the developing seeds inside devloping green bolls. Therefore, they are not amenable for insecticide control. However, good pest control is achieved with the use of contact poisons such as the synthetic

pyrethroids (Sabry *et al*., 2014), mainly because of the contact toxicity to moths. Subsequent to the introduction of synthetic pyrethroid insecticides in the early 1980s, pink bollworm was effectively controlled and was replaced by the cotton bollworm *Helicoverpa armigera* in many countries. The change happened due to high susceptibility of pink bollworms and rapid development of *H. armigera* resistance to pyrethroids. Other factors that influenced the pest-shift were, slightly hastened crop maturity due to pyrethroid sprays, and disruption of the ecology of naturally occurring biological control that had otherwise kept the cotton bollworm populations much below the economic threshold levels. Indiscriminate use of pyrethroids also caused severe resurgence of whiteflies. Thus, the cotton bollworm and whitefly problems are considered as a consequence of excessive usage of pyrethroids for pink bollworm control (Kranthi *et al*., 2001; Kranthi *et al*., 2001a; Kranthi, 2012). Both pests are often referred to as insecticide-induced problems in India.

Causal factors for pink bollworm occurrence and resistance to *Bt*-cotton are listed below:

1. Pink bollworm resistance in China and Pakistan

Pink bollworm resistance in China and Pakistan is mainly due to the intensive exposure of the insect only to a single *Bt* protein Cry1Ac and not to a combination of proteins as in other countries. However, resistance development has been slow compared to India primarily because both countries relied on short to medium duration cotton varieties compared to long-duration *Bt*-cotton hybrids in India, and also while India cultivated hemizygous *Bt*-cotton hybrids, China and Pakistan opted for homozygous *Bt*-varieties (Kranthi, 2012). Hemizygous *Bt*-cotton hybrids are those which contain only one copy of the transgene, unlike the homozygous *Bt*-cotton varieties which contain two copies of the transgene. Pink bollworm was reported to have caused considerable damage to the single gene *cry1Ac* based *Bt*-cotton in Pakistan during the 2015-16 cropping season. Pink bollworm resistance to Cry1Ac was reported to have been one of the main causes for the high level of infestation (Abbas *et al*., 2016). Additionally, cotton duration increased after the introduction of *Bt*-cotton in Pakistan (Naveed *et al*., 2017) and India (Kranthi, 2015) due to which pink bollworm was exposed to multiple cycles of selection, thereby accelerating resistance. The pink bollworm caused an estimated loss of US$ 1.2 billion in Pakistan during the 2015-16 cropping season (Naveed *et al*., 2017). In China, though a small but statistically significant increase in resistance of the pink bollworm to Cry1Ac was reported in 2012 in the Yangtze River Valley from 2005–2007 to 2008–2010 (Wan *et al*., 2012) subsequent studies showed that resistance did not increase over the years until 2015 (Wan *et al*., 2017).

2. Resistance in India was rapid due to a variety of factors as listed below (Kranthi 2015)

- **Long season cotton:** Extended duration of long season cotton for 160 to 240 days coincided with favourable conditions for pink bollworm infestation and allowed multiple cycles of multiplication. It was not very uncommon in India, mostly in the irrigated regions, to find the crop being extended up to 8-10 months. Excessive use of nitrogenous fertilizers, coupled with application of a few organophosphate insecticide sprays such as monocrotophos and acephate led to extended vegetative phase and staggered flowering. This provided a continuous source of flowers and bolls as food supplies, almost all through the year and facilitated pest-multiplication in overlapping generations that were subjected intensive selection pressure of Cry proteins, thus accelerating resistance development (Kranthi 2012).
- **Large number of *Bt*-hybrids:** Several hundreds of *Bt*-cotton hybrid varieties were released every year. These hybrids have variable windows of flowering and fruiting. These are grown adjacent to each other in the same region in small-scale farming systems, thereby providing a constant supply of flowers and young bolls as continuous attractants and food sources almost all through the season.
- **Segregating seeds in bolls of *Bt*-cotton hybrid plants:** In India only F-1 hybrid *Bt*-cotton varieties were permitted for cultivation. The F-1 *Bt*-cotton hybrids are hemizygous for the transgenes. Because of which, bolls formed on the F-1 plants of single gene based *Bt*-cotton contain seeds which segregate in a ratio of 3:1 (Cry1Ac in 3 and none in 1) for the Cry1Ac protein, and in the two gene based Bollgard-II the bolls contain seeds that segregate in the ratio of 9:3:3:1 (Cry1Ac+Cry2Ab in 9; Cry2Ab alone in 3; Cry1Ac in 3 and none in 1) for the two *Bt*-proteins. Thus, a single boll contains a mixture of non *Bt* seeds, seeds with Cry1Ac alone, seeds with Cry2Ab alone and seeds with Cry1Ac+Cry2Ab. This situation is ideal for resistance development, due to selection of resistance to independent proteins. The segregating seeds in bolls of F-1 hybrid plants and low expression of *Bt*-proteins in buds, flowers and developing seeds in young bolls allow survival of larvae that are heterozygous for the resistant genes, thereby accelerating resistance development.
- **Decline in pyrethroid usage**: The use of synthetic pyrethroids declined in India after late 1990s due to *H. armigera* resistance to pyrethroids and also due to the introduction of *Bt*-cotton. Decline in pyrethroid usage on long duration hybrid-varieties coupled with the presence of non-*Bt* seeds in bolls of F-1 *Bt*-cotton hybrids, are believed to be the main factors that may have allowed pink bollworms to survive and gradually emerge as a major pest on *Bt*-cotton.
- **Poor refuge compliance:** The compliance of refuge planting of non-*Bt* cotton was poor (Kranthi *et al*., 2017).

Management Strategies

The following IPM strategies are proposed based on the available literature on the subject:

Pest monitoring

Pink bollworm infestation must be regularly monitored using 'pheromone traps' and 'green boll dissection' to initiate control interventions at economic threshold levels of 8 moths per trap per night and/or 10% damage in green bolls (Sevacherian and el-Zik, 1983; Dhawan and Simwat, 1993).

Resistance monitoring

Monitoring the development of bollworm resistance to *Bt* cotton is a key component of management, especially when it alerts the impending restance risk, so that appropriate management strategies can be initiated (Mohan *et al*., 2015).

Biological control

Pink bollworms are controlled in cotton fields by naturally occurring *Rogas* and *Apanteles* spp. (Singh *et al*., 1988), *Chelonus blackburni* (Jackson *et al*., 1979), *Trichogramma brasiliense* (Tuhan *et al*., 1987) *Trichogramma bactrae* (Malik, 2001) and *Trichogramma evancens* (Saad *et al*., 2012). Cheema *et al*. (1980) found 22 parasites of *P. gossypiella* in a survey in Pakistan: of these, only *Apanteles angaleti* was a major parasitoid. Based on practical field experiences in India, deployment of biological control using the parasitoid *Trichogramma bactrae* (Nagaraja) was found to be most economical, feasible and effective, especially in the initial stages of pest infestation.

Refuge

Compliance of refuge planting with 5 to 20% area of non-*Bt* cotton plants in the vicinity of *Bt*-cotton fields is one of the most important strategies to manage pink bollworm resistance to *Bt*-cotton (Kranthi *et al*., 2017).

Avoid extending the crop

Termination of the crop immediately after final harvest without resorting to ratooning or extending the crop through extra irrigation is an important practice to prevent availability of the host plants for multiple cycles of the pink bollworm. Crop termination also helps to reduce the chances for pink bollworm diapause where the end of diapause is triggered by day length (Frisbie *et al*., 1989).

Destruction of crop residues

Cotton stalks habor diapausing and non-diapausing pupae. It is important to destroy stalks to prevent carry-over of the pupal stages into the subsequent season. Stalks should be chopped and incorporated back into the soil, or used as fuel or industrial purposes.

Short season crop

Short duration (140-160 days) varieties, when sown early or on time mature before the peak onset of pink bollworm infestation thereby escaping damage. Further, pink bollworm populations get substantially reduced by breaking the pest cycles when combined with crop rotation (Beasley and Adams, 1995).

Mass trapping

Pheromone traps using gossyplure resulted in 60-80% reduction of the pink bollworm population in China (Gao *et al*., 1992). Pink bollworm eradication programmes were attempted in India (Simwat *et al*., 1988) but were found to be expensive. However, early-season use of pheromone at low pink bollworm population densities, coupled with insecticides was found to be most profitable (Frisbie *et al*., 1989). Currently 20 pheromone traps per acre are being recommended to be set up early in the season for mass trapping.

Mating disruption

Gossyplure was used successfully for mating disruption along with biological control in Pakistan (Ahmad *et al*., 2001). Pheromone SPLAT technology or PB Ropes can be used effectively for mating disruption (Miller *et al*., 2015).

Varieties resistant to sap-sucking insects

With varieties that are endowed with resistance to sap-sucking pests, it is possible to avoid the use of systemic insecticides such as monocrotophos, acephate, thiomethoxam, acetamiprid, imidacloprid or clothianidin early in the season. Avoidance of these insecticides helps to conserve the naturally occurring biological control for season long pest management and to obtain synchronous early maturity of bolls which helps in the escape of pink bollworm infestation (Kranthi and Kranthi 2010).

Insecticides

Synthetic pyrethroids may be used for the control of pink bollworm (Sabry *et al*., 2014) late in the season at economic threshold levels of damage. Spraying pyrethroids early in the season, as insecticide mixtures and in excess at any time in the season must be avoided to prevent outbreaks of *H. armigera* and whiteflies.

F-1 hybrids with homozygous *cry* genes

In countries where *Bt*-cotton hybrids are used, seed companies must be encouraged to ensure that both parent varieties of the hybrid are homozygous for the *cry* genes so that Cry proteins are present in the hybrids in a homogeneous form, instead of the segregating heterogeneous form as in the current F-1 hybrids (Kranthi, 2012). A recent paper (Wan *et al*., 2017) showed that 'hybridizing transgenic *Bt* cotton with non-*Bt* cotton countered resistance in pink bollworm'. The authors used F-2 *Bt*-seed to demonstrate that the 25% non-*Bt* plants served as an effective refuge to delay resistance development. It is interesting that the proposal is analogous to the existing crop situation in India wherein the bolls on F-1 hybrid plants actually contain seeds that segregate in F-2 ratios, but resistance developed in an accelerated manner.

Male sterile release techniques

A large-scale, four-year field deployment of a male-sterile strategy, where refuges are scarce or absent, as in Arizona was successful in controlling the pink bollworm. Male-sterile

insects were released to mate with resistant pink bollworm in *Bt* cotton fields to reduce pink bollworm abundance by >99%, while eliminating insecticide sprays against the invasive pest (Tabashnik *et al*., 2012a). Such techniques need to be examined for India, Pakistan and China.

Recent Exciting Research Advances

Pink bollworm continues to be susceptible to *Bt*-cotton despite 20 years of extensive cultivation. The main factors that delayed resistance in Arizona appear to be, timely crop termination; deployment of abundant non-*Bt* cotton refuges; recessive inheritance of resistance; fitness costs associated with resistance and incomplete resistance (Tabashnik *et al*., 2012a). From 2006–2011, refuge abundance was greatly reduced in Arizona, while mass releases of sterile pink bollworm moths were made, to delay resistance as part of a multi-tactic eradication program. Sustained susceptibility of pink bollworm to *Bt* cotton in Arizona provided a cornerstone for the pink bollworm eradication program and for integrated pest management in cotton. Reduced insecticide use against pink bollworm and other cotton pests has yielded economic benefits for growers, as well as broad environmental and health benefits (Tabashnik *et al*., 2012a)

The Cotton Bollworm *Helicoverpa armigera*

The cotton bollworm *Helicoverpa armigera* (Hübner) was a major problem in China, India, Pakistan and Australia until biotech *Bt*-cotton was introduced into these countries. Recent data show that the worm is adapting to *Bt*-cotton. Adaptation is believed to have slowed due to the deployment of two independently acting Cry-proteins, Cry1Ac and Cry2Ab, each having a different receptor site in the midgut of the bollworm. Due to intensive planting of *Bt*-cotton, frequency of resistant individuals to Cry1Ac increased from 0.93% in 2010 to 5.5% in 2013 in field populations of *H. armigera* from northern China (Zhang *et al*., 2011; Jin *et al*., 2015). *H. armigera* populations in India (Kranthi, 2012; Kranthi, 2015) and Pakistan are showing initial symptoms of resistance to *Bt*-cotton.

H. armigera is known to cause serious economic losses to a wide range of crops including cotton and warrants tremendous efforts to prevent it from causing catastrophic losses to agriculture. The problem can recur again and exacerbate in China, India and Pakistan, once the cotton bollworm develops resistance to *Bt*-cotton. Very recently, a few years ago, the cotton bollworm *Helicoverpa armigera* was detected for the first time in South America from Brazil, Argentina, Uruguay and Paraguay. This news rings alarm bells for agriculture not only in South America but also signals a possible invasion into USA. Two related species, *Heliothis virescens* and *Helicoverpa zea* are present in USA, but compared to *H. armigera*, are less devastating in their host range, propensity for insecticide resistance and potential to damage crops. So far *H. armigera* was ubiquitous in almost all other parts of the world, except America. But, with the recent reports of its presence in South America, pest management challenges can surmount for the entire American region. *H. armigera* easily reaches major pest status in many cropping systems because of a unique combination of four biological characteristics: polyphagy, high mobility, high fecundity, and facultative diapause (Fitt 1989) and due to indiscriminate use of synthetic pyrethroids (Kranthi and Kranthi, 2010).

The Heliothine pest complex of cotton bollworms comprises of at least four major species, namely, the cotton bollworm, *Helicoverpa armigera* (Hübner), the tobacco budworm, *Heliothis virescens* (Fabricius), the corn earworm *Helicoverpa zea* (Boddie) and the Australian bollworm, *Helicoverpa punctigera* (Wallengren). Undoubtedly, the first three species represent a constant threat to cotton production across the globe. Amongst the three, the cotton bollworm *Helicoverpa armigera* is considered to be a monster because it is one of the most difficult insect pests to manage.

Amongst all agriculturally important insect pests, *Helicoverpa armigera* has one of the widest distributions, occurring throughout Africa, the Middle East, southern Europe, India, central and south-eastern Asia, eastern and northern Australia, New Zealand, and many Pacific Islands (Fitt, 1989). *Helicoverpa armigera* moths are known to fly long distances with an ability to disperse widely under favourable conditions (Pedgley, 1985; Pedgley *et al*., 1987, Fitt, 1989, Colvin, 1990; Riley *et al*., 1992), Previously endemic to Asia, Africa, Europe and Australasia, it has now been reported in Brazil (Czepak *et al*., 2013), Puerto Rico, Argentina (Murúa *et al*., 2014) and Paraguay and Uruguay (Arnnemann *et al*., 2016) with a likely arrival date of between 2006 and 2008 (Tay *et al*., 2013; Sosa-Gomez *et al*., 2015). It is possible that the ability to disperse long distances has helped the species to establish itself in almost all the cotton growing countries of the world such as China, India, Pakistan, Australia, Brazil, Uzbekistan, Kazakhsthan, Kyrgyzstan, Brazil, Argentina,

Fig.2 Cotton bollworm *Helicoverpa armigera* damaging a bud

Egypt, Burkina Faso, Mali, Mozambique, Myanmar, Sudan, Tanzania, Morocco, Thailand, Togo, Turkey, Uganda, Vietnam, Zambia, Zimbabwe, Cameroon, Côte d'Ivoire, Greece, Iran, Israel, Kenya, France, Spain and Portugal, with the probable exception of USA.

Before its current scientific name as *Helicoverpa armigera*, the species was initially named as *Noctua obsoleta* (Fab.) in 1793, after which it underwent a series of nomenclature changes as *Chloridea obsoleta* (Fab.), *Chloridea armigera* (Fab.), *Heliothis obsoleta* (Hubner), *Helicoverpa obsolete*, (Auct.), *Heliothis fusca* (Cockerell), *Heliothis rama* (Bhattacherjee & Gupta) and *Noctua armigera* (Hübner). Although frequently called American bollworm in north Asia, it has no connection with the Americas other than the fact that it became an increasingly important pest of a range of Asian crops after the introduction and widespread cultivation of American cotton (*Gossypium hirsutum*) early in the 20th century (Russell and Kranthi, 2006).

H. armigera lays eggs initially during peak vegetative phase of the crop, but the damage is minimal due to heavy natural mortality of the larvae. Peak oviposition occurs when the crop starts producing squares and flowers. About 70-80% eggs are laid generally on the upper canopy and stems, while rest of the 20-30% eggs are laid on fruiting parts with preference on square and flower bracts. After hatching, the young larvae scrape on the leaf surface before they bore into squares, flowers and bolls. Larvae prefer fruiting parts to leaves. Each larva can feed on 8-10 squares/flowers and 2-3 bolls in a single life cycle. Typically the larva feeds inside bolls keeping half of its body outside. The larva feeds for two weeks on the fruiting parts before it turns into pupa. Moths emerge from pupae after 7-10 days and start oviposition within 3-4 days.

Helicoverpa armigera is known to feed on an extensive range of more than 180 plant hosts from about 45 families, (Manjunath *et al*., 1989; Tay *et al*., 2017) including agriculturally important crops such as cotton, chickpea, pigeon pea, peas, cowpea, sunflower, sorghum, groundnut, field beans, tomato, tobacco, maize (Gowda 2005) in addition to wheat, okra, castor and a wide range of vegetables**.** The species is known for its high propensity to rapidly develop resistance to insecticides (Kranthi *et al*., 2002; Yang *et al*. 2013). *Helicoverpa zea* is similarly polyphagous but demonstrates lesser capabilities than *H. armigera* for damage and resistance development. It is thought to be derived from *H. armigera* founders approximately 1.5 – 2.0 million years ago and the difference in economic damage and resistance development may be the consequences of a genetic bottleneck (Behere *et al*., 2007).

The pest causes significant damage to cotton and other crops in Asia, Europe, Africa and Australasia, causing losses to crops estimated at greater than US$ 2 billion annually, excluding socio-economic and environmental costs associated with its control (Tay *et al*., 2013). In India, crop losses due to *H. armigera* are commonly more than half the yield, and annual losses to cotton and pulses alone have been estimated at US$ 300-500 million (King, 1994). Several estimates of crop losses point out to the enormous magnitude of damage that the pest is capable of causing. Sharma (2001) estimated total global crop losses to this pest at around US$ 5 billion globally, with losses of over US$ 900 million in chickpea and pigeon pea in addition to US$ 1 billion spent on insecticides for its control. *H. armigera* caused huge crop losses to cotton with at least four devastating outbreaks that occurred in a cyclic manner at 3-4 year intervals starting from 1986 to 2001 in India, Pakistan and China. During this period, an estimated US$ 8-10 billion worth insecticides were used for its control in India alone (Russell and Kranthi, 2006). About 50% of the pigeonpea crop was estimated to be lost annually to the pest in east Africa (Hillocks *et al*., 2000; Youm *et al.*, 2005). In Queensland, Australia, cotton crop losses were estimated to be 7.7% despite the expenditure of US$ 4.2 million for its control (Fitt, 1994).

Helicoverpa armigera is known for its strong capability to develop resistance to insecticides recommended for its control. Indian and Chinese *H. armigera* recorded the highest levels of resistance to insecticides, as compared to any region of the world. Extensive studies were conducted during the period 1980 to 2000 on insecticide resistance status of *H. armigera*, mainly in India (Armes *et al*., 1996; McCaffery, 1999; Kranthi *et al*., 2002), China (Shen *et al*., 1994; Tan 1999), Pakistan (Ahmad *et al*., 1999) and Australia (Forrester *et al*., 1993; Gunning *et al*., 1998).

Table 2. Examples of *H. armigera* resistance factors (x-fold) to insecticides in China, India, Pakistan and Australia

	Pyrethroids	Organophosphates	Carbamates	Endosulfan	Reference
India	10 to 26,151	>59	>30	4 to 37	Armes *et al*., 1996; Kranthi *et al*., 2001, 2001a; McCaffery *et al*., 1989
China	120 to 56,911	32 to >200	>300		Mu and Wang, 1995; Shen and Wu, 1995; Wu *et al*. 1997; Shen *et al*., 1993; Cheng & Liu, 1996, Ren *et al*., 2002
Pakistan	25 to 205	>720	19 to 105	5 to 36	Ahmad *at al*., 1995; 1997; Hussain *et al*., 2014; Faheem *et al*., 2013
Australia	>1000	>92	>40	Up to 163	Forrester *at al*., 1993; Gunning and Easton, 1994: Gunning *et al*., 1996, 1998

In many countries, *Helicoverpa armigera* attained the status of a major pest, replacing the traditional cotton pests, pink bollworm and the tobacco caterpillar *Spodoptera litura*, mainly after introduction of the synthetic pyrethroids. Synthetic pyrethroids are supposed to have suppressed populations of *Spodoptera litura,* pink bollworm and spotted bollworm, but caused upsurges of *H. armigera* and the whitefly. The changes in pest dynamics were mostly due to the high levels of pyrethroid toxicity to the pink bollworm and also on natural enemies of *H. armigera* and the whitefly *Bemisia tabaci*.

Several reviews listed out the major parasitoids and predators of Africa that affected *H. armigera* in India (Romeis and Shannower, 1996), Africa (Youm *et al*., 2005) and Australia (Johnson *et al*., 2000; Gregg and Sorocco, 2000). Van den Berg (1993) showed that Pheidole ants caused high mortality of *H. armigera* in Kenya. In India, Pakistan and many other countries the egg larval parasitoid *Microchilonus curvimaculatus* and larval parasitoid *Campoletis* were regular mortality-causing factors in larvae collected from cotton fields. Some of the important naturally occurring parasitoids on *H. armigera* are *Trichogramma chilonis* (Ishii), *Chelonus curvimaculatus* (Cameron), *Campoletis chloridae* (Uchida), *Palexorista laxa* (Curran), *Eucarcelia illota* (C.) and *Goniopthalmus halli* (Mesnil). Some major predators include *Geocoris ochropterus* (Fabricius), *Coranus spiniscutis* (Reuter), *Chrysoperla carnea* (Stephens), *Orius* spp., *Polistes* spp., *Chilomenes sexmaculatus* (Fabricius) and spiders (*Oxyopes* spp., *Clubiona* spp and *Thomisus* spp.).

Causes of Outbreaks

The following causes are generally attributed for outbreaks of *H. armigera*:

- **Disruption of ecosystems:** Synthetic pyrethroids and insecticide mixtures have broad spectrum activity and are extremely toxic to some key parasitoids and predators, notably the *Orius* spp., which were known to predate on at least 60-90% of bollworm eggs. *Helicoverpa armigera* was subjected to intensive and extensive chemical applications across the globe, which ironically resulted in outbreaks due to strong disruption of naturally occurring biological control. It was also observed (Kranthi and Kranthi, 2010) that avoidance of insecticides for the first three months helps in build-up of entomophage populations such as *Chrysoperla, Campoletis chloridae, Microchilonis curvimaculatus* and Tachinids, which contribute to the management of *H. armigera.*
- **Insect resistance to insecticides:** Compared to its predators and parasitoids, *Helicoverpa armigera* developed resistance much faster to gain the upper hand in the cotton ecosystem thereby leading to frequent outbreaks. Implementation of insecticide resistance management strategies, is essential to keep this pest under check, especially during years of outbreak.
- **Weather factors:** Prolonged drought followed by rainfall during the late vegetative phase of the cotton crop can cause synchrony of moths and a subsequent outbreak (Kranthi and Kranthi, 2010).

Prolonged Absence of Outbreaks

Over the past few years, cotton pest management stabilized due to the introduction of biotech *Bt*-cotton and novel eco-friendly insecticides. As a result there have been hardly any reports of outbreaks from any part of the world, including countries that did not adopt biotech *Bt*-cotton. Apart from the introduction *Bt*-cotton, chemicals such as spinosad, indoxacarb, emamectin benzoate, chlofenapyr, chlorantraniliprole, flubendiamide, novaluron and lufenuron ensured effective control of *H. armigera.* Some of the new insecticides were relatively less toxic to predators and parasitoids in the cotton ecosystem.

Over the past 15-16 years, *H. armigera* populations were found to be on the decline in most parts of the world. Clearly this is a result of some of the following important interventions.

- Introduction of *Bt*-cotton contributed to effective bollworm control
- Decline in the use of synthetic pyrethroids and insecticide mixtures
- Introduction of some very useful eco-friendly molecules belonging to different chemical groups such as spinosyns, avermectins, pyrroles, chitin synthesis inhibitors, oxadiazines and diamides which are effective in controlling *H. armigera*
- Weather conditions and a combination of the above strategies

Management Strategies

Recently, sucking pests such as leaf hoppers, thrips and whiteflies have developed resistance against recommended insecticides. The cotton bollworm *H. armigera* is adapting to *Bt*-cotton and the pink bollworm has already developed high levels of resistance to *Bt*-cotton in India (Kranthi, 2015). If unattended to, cotton pest management could revert back into the uncertain phase, reminiscent of the earlier years. The following set of pest management strategies are based on general principles of IPM and can be adapted to many cotton growing countries.

Short season cotton

Short duration varieties enter into flowering phase earlier and escape bollworm attacks during squaring-flowering stage. A narrow flowering and fruiting window in short season cotton, especially in early-sown crops helps the crop to escape bollworm infestation by creating a mismatch between the insect-seasonal-cycles and the vulnerable reproductive phase of the crop. The varieties get adequate soil moisture during the critical flowering and fruiting phase and give rise to higher yields. A shorter season of 150-160 days makes it easier for nutrient and pest management. Adequate nutrients such as N, P and K applied in a balanced manner, especially

during flowering and early boll formation stage keep the crop healthy. It is important to strictly avoid excessive nitrogenous fertilizers and foliar application of systemic insecticides such as organophosphates and neonicotinoids during peak vegetative phase and just prior to squaring-phase which could lead to delayed flowering, staggered flowering, prolonged vegetative phase and delayed harvest, especially in combination with irrigation.

Conserve naturally occurring biological control

Naturally occurring biological control can be most effective in pest management if the predators and parasitoids are left undisturbed to the greatest possible extent. There are several generalist predators and parasitoids that thrive in the cotton eco-systems to control a wide range of insects such as the sucking pests and bollworms. Most of these natural enemies of insect pests are highly susceptible to insecticides and get easily killed with several insecticides at low dose applications. It takes a long time for the natural enemy populations to revive again, to be effective enough for biological control of major insect pests.

There are three major strategies to protect the naturally occurring biological control in the cotton ecosystem.

- **Cultivate varieties that are resistant to sap-sucking insects** so that early season insecticide use can be avoided. Insecticide seed treatment will reduce the infestation levels of sucking pests, especially in resistant varieties.
- **Avoid application of chemical insecticides early in the season.** In the early stages of the crop, beneficial insects such as ladybird grubs and beetles, *Chrysoperla* spp., syrphid flies, *Geocoris* spp., *Aenasius* spp., *Aphilinus* spp., predatory mirid bugs and spiders start getting established in the ecosystem. Several minor lepidopteran insects such as leaf folders, semiloopers, leaf eating caterpillars and hairy caterpillars occur during the peak vegetative phase of the crop. It is important to understand that such insects are easier to control with many eco-friendly botanical and biological pesticides. They generally do not cause significant damage and occur as one or two short cycles in the season. If the crop is not subjected to broad spectrum chemical insecticides, these minor lepidopteran insect pests serve as excellent hosts for parasitoids such as *Trichogramma* spp., *Apanteles* spp. and *Sysiropa formosa*, that attack *H. armigera* and other bollworms. Several insecticides cause a resurgence of insect pests when applied repeatedly, or indiscriminately or used in mixtures. As far as possible, ecofriendly pesticides must be preferred especially as early season sprays, if necessary at economic threshold levels of sucking pests or bollworms, since most of them are relatively less toxic to parasitoids and predators.
- **Ecosystem engineering of crops** (Bender *et al*., 2016): A few crops when grown together in the same ecosystem work in tandem to favour naturally occurring parasitoids and predators that keep insect pests under check. Cultivation of legume intercrops should be preferred wherever possible. Crops such as soybean, clusterbean, cowpea or blackgram, help in proliferation of a wide range of generalist predators and parasitoids of insect pests. Borders (2-3 rows) of pigeonpea or bajra or maize or sorghum around cotton fields was found to reduce infestation of sap sucking insects such as whiteflies, mealy bugs etc. Such crops also serve as refuge in *Bt*-cotton fields for *Helicoverpa armigera*. These recommendations are easier to implement in small scale farming systems.

Specific strategies for bollworm management

Integrated pest management (IPM), biotech cotton and Insecticide resistante management (IRM) strategies together can play a crucial role in controlling pest populations and also in delaying insect resistance development to *Bt*-cotton and insecticides. IPM strategies comprising of tools to conserve naturally occurring biological control coupled with ecologically compatible tools such as botanical insecticides, mainly neem based pesticides, biological control with endophytic fungi, *Trichogramma* spp., *Ha*NPV (*Helicoverpa armigera* Nuclear Polyhedrosis Virus), pheromone traps, light traps etc., can help in ecologically sound pest management and mitigating insect resistance to insecticides and *Bt*-cotton. Insecticide resistance management can be effective with careful choice and regulated use of insecticides by alternating or rotating chemical groups selectively according to the presence of pest-predator-parasitoid ratios at a specific crop-stage, only when necessary at economic thresholds of insect pests, after exhausting options of biological pesticides or botanical pesticides. When used within the ambit of IPM, *Bt*-cotton can strengthen IPM and IRM by reducing the need for chemical insecticides.

Primarily, resistance management principles are based on use of a rational and sensible sequence or rotation of insecticides that are effective on the target species, cause less disturbance to beneficial fauna, minimize selection pressure and have unrelated resistance mechanisms or modes of action. The sequence of insecticides suggested under IRM has been developed based on the resistance risk assessment, pest control efficacy, ecological selectivity (based on International organization of biological control, IOBC rating) and environmental risk assessment (based on environmental impact quotient, EIQ rating) (Russell and Kranthi, 2006). When implemented on a large scale, the strategies were found to reduce insecticide resistance levels; stabilize eco-systems so as to encourage entomophage insect populations; reduce pest populations and improve socio-economic conditions of farmers. In general, the most successful integrated pest management practices are the ones that support the role of naturally occurring predators and parasitoids, instead of relying in the application of inoculative or inundative releases.

The choice of insecticides plays a crucial role in IPM and IRM. Insecticides categorized by the World Health Organization

(WHO) as Class-I (Extremely Harzardous category), such as phosphamidon, methyl parathion, phorate, monocrotophos, dichlorvos, carbofuran, methomyl, triazophos and metasystox and all insecticide-mixtures must be avoided. Application of synthetic pyrethroids must be avoided during the first 3-4 months after sowing and insecticide mixtures must be avoided all through the crop phase to prevent whitefly and other pest outbreaks. Synthetic pyrethroids may be used only late in the season as one or at the most two sprays for the control of pink bollworm. Research publications show that a few relatively recent insecticides belonging to spinosyns, amides, avermectins, pyrroles, chitin synthesis inhibitors, oxadiazines and diamides are compatible with IPM.

Recent Exciting Research Advances

Efficient resistance management holds the key for sustainable pest management. In an interesting study, Ives *et al*. (2017) addressed the effects of spatio-temporal variation in a management setting for two bollworm pests of cotton, *Helicoverpa armigera* and *H. punctigera*, and field data on landscape crop distributions from Australia. They showed that even a small proportion of *Bt* fields available to egg-laying females when refuges are sparse may result in high exposure to *Bt* for just a single generation per year and cause a surge in selection. Therefore, rapid resistance evolution can occur when *Bt* crops are rare rather than common in the landscape. The authors suggest that their results highlight the need to understand spatio-temporal fluctuations in the landscape composition of *Bt* crops and non-*Bt* habitats in order to design effective resistance magement strategies. Wei *et al*. (2017) showed that four laboratory strains of *H. armigera* with high levels of resistance to Cry1Ac or Cry2Ab had no cross-resistance to Vip3Aa protein thereby enabling the deployment of all the three toxins for sustainable management of *H. armigera.* Wei *et al*. (2015) conducted studies to understand cross-resistance of *H. armigera* for Cry1Ac and Cry2Ab. They selected *H. armigera* with Cry1Ac for 125 generations to produce 1000-fold resistance to Cry1Ac and 6.8-fold cross-resistance to Cry2Ab. Selection with Cry2Ab for 29 generations caused 5.6-fold resistance to Cry2Ab and 61-fold cross-resistance to Cry1Ac. Without exposure to *Bt* proteins, resistance to both proteins decreased. Results showed minor cross-resistance to Cry2Ab caused by selection with Cry1Ac and synergism between the two proteins against resistant insects suggesting that plants producing both proteins could prolong the efficacy of *Bt* cotton.

The Whitefly Menace

During the 2015-16 cropping season, a massive whitefly outbreak caused up to 50-60% crop loss in north India (Singh *et al*., 2016) and Pakistan. A press report in the Times of India 8th October 2015, claimed "whitefly destroys 2/3rd of Punjab's cotton crop, 15 farmers commit suicide"

http://timesofindia.indiatimes.com/india/Whitefly-destroys-2/3rd-of-Punjabs-cotton-crop-15-farmers-commit-suicide/articleshow/49265083.cms

The report also stated that the pest caused losses worth US$ 636 million despite insecticide usage worth US$ 22 million. Government of India announced a financial compensation of US$ 97 million to farmers. Whiteflies also caused serious crop losses in Pakistan.

During the 2015-16 cotton season, a severe epidemic of whitefly incidence was noticed in the cotton growing zone of north India and Pakistan, beginning early August. Whitefly infestation levels and cotton leaf curl virus (CLCuV) disease in July-August were higher than the previous three years in both countries. Whitefly infestation and the CLCuV disease were first noticed in early June in all the three states of north India. A combination of late sowing of susceptible hybrids coupled with favourable weather factors for whitefly development and multiplication were the probable factors for outbreak (Singh *et al*., 2016). Deficient rainfall of less than 100 mm up to July in the cotton growing areas in the region is believed to have led to severe incidence of white fly during June and July thereby escalating leaf curl virus disease (Kranthi, 2015). Late sown crop suffered the most. In India and Pakistan, increased whitefly infestation levels were related to high temperatures above 30^0 C, sparse rainfall, drier spells in the monsoon and high humidity in the range of 80-90%. Indiscriminate use of insecticides, especially excessive pyrethroids and increased use of nitrogenous fertilizers were also associated with resurgence of the whitefly. Whitefly incidence ranged from 1.6 to 90 adults /3 leaves during July-August. By the end of September severe damage was caused to cotton. Fields sprayed repeatedly with insecticides, insecticide mixtures and pyrethroids had the highest levels of whitefly infestation (Rishi Kumar, CICR personal communication).

The whitefly is a small insect of 1.0 mm in length with a waxy coating over its wings. Insecticides are less effective because of the waxy coating. Whiteflies cause significant damage to crops through direct feeding, induction of host plant phytotoxic disorders, transmission of viruses and excretion of honeydew leading to fungal growths (Inbar and

Fig. 3 Whiteflies *Bemisia tabaci* feeding on cotton leaf

Gerling, 2008). Whiteflies suck sap from the under-surface of leaves causes yellowing and distortion in leaf shape causing upward curling mostly. Whiteflies excrete honey dew which causes stickiness in the fibre thereby reducing the quality and commercial value of the fibre. Whiteflies cause sticky cotton fibre especially during late season infestation. The honey dew also attracts a fungal sooty mould and spoils leaves and bolls. The black sooty mould blocks photosynthesis in leaves to reduce yield. Though, the ideal conditions for growth are 27°C and 71% relative humidity, hot and humid conditions favour the insect. In recent times, hot and dry conditions also favoured survival and multiplication in India. Outbreaks in India and Pakistan after 1984 were a result of weather factors aggravated by indiscriminate use of insecticides.

The whitefly is scientifically known as *Bemisia tabaci.* It was first reported in Greece 125 years ago and subsequently spread all across the globe. A total number of 1556 whitefly species are documented in 161 genera (Martin and Mound, 2007). Amongst these, *Bemisia tabaci* is the most damaging with an ability to infest more than 1000 plant species (Abd-Rabou *et al*., 2010) and transmit more than 300 plant pathogenic viruses (Navas-Castillo *et al*., 2011). *Bemisia tabaci* feeds on more than 600 plant species (Quintela *et al*., 2016) including a wide spectrum of hosts such as vegetables, pulses, floriculture, horticultural crops and most notably cotton and citrus. Losses to cotton crop were estimated to be in the range of 15-60% and more under severe infestation levels. Whiteflies transmit a range of viral diseases in plants. *B. tabaci* is the vector of several genera of plant viruses, of which the most widespread and damaging are those in the genus Begomovirus (family, Geminiviridae) (Quintela *et al*., 2016). *B. tabaci* transmits Cotton leaf curl virus (CLCuV) to cause the dreaded cotton leaf curl disease (CLCuD) in north India and Pakistan.

The taxonomy of the whitefly traversed through complex debates to further get complicated and categorized into a complexity of cryptic species. Recent studies regrouped *Bemisia tabaci* into a complex of 34 morphometric species

Close-up of a whitefly. Two eggs can be seen of the stem.
Photograph: Dr Vishlesh Nagrare, ICAR-CICR Nagpur, India

(De Barro *et al*., 2011, Cuthbertson and Vanninen, 2015). The whitefly *Bemisia tabaci* is now considered as a cryptic species complex that is comprised of at least eleven well defined genetic groups and at least 34 morpho-cryptic species, which are indistinguishable morphologically but can be differentiated at the molecular level (De Barro *et al*., 2011, Powell and Cuthbertson, 2013. Lee *et al*., 2013, Boykin and De Barro, 2014). Until recently, populations of *Bemisia tabaci* were grouped into biotypes based on their biochemical polymorphism and biological characteristics, such as insecticide-resistance, 'vector competence for disease transmission, vulnerability to predators and parasitoids, host range, invasiveness etc. (Brown *et al*., 1995; Perring, 2001; De Barrow *et al*., 2005; Xu *et al*., 2010). Reports also suggest that a few biotypes were characterized by the presence of certain bacterial endo-symbionts (Gottlieb *et al*., 2006; Chiel *et al.*, 2007) one of which conferred resistance to insecticides (Kontsedalov *et al*., 2008). These biotypes were distinguished by clear esterase profiles (Brownet *et al*., 1992; 1995). The two most invasive and damaging *Bemisia tabaci* biotypes are B and Q. The B biotype was first identified in the late 1980s (Costa and Brown, 1991) and the Q biotype was presumed to have originated in the Iberian Peninsula (Guirao *et al.*, 1997). Though debatable, in 1994, a new aggressive biotype 'B biotype' was separated into a new species *Bemisia argentifolii* and subsequently based on the mitochondrial DNA CO-1 markers, the B biotype was rechristened as Middle East-Asia Minor 1 (MEAM1) species, while the Q biotype was re-designated as the Mediterranean species (Cuthbertson and Vanninen, 2015). Subsequently, biotype-specific mitochondrial cytochrome oxidase-1 (CO1) based DNA bar-coding methods were developed to distinguish different biotypes (Shatters *et al*., 2009). Recently, Hadjistylli *et al*., (2016) characterized the global diversity of *Bemisia tabaci* sibling species group using microsatellite markers to compare with species profiles derived from mitochondrial markers. Their results with microsatellite markers were in broad agreement with the published data of mitochondrial markers. Studies conducted in Pakistan using cytochrome oxidase-1 (CO-1) with *Bemisia tabaci* populations collected in Pakistan indicated the presence of 15 deeply divergent lineages, including 12 from the Indo-Pakistan region comprising of six species, Asia II 1, Asia II 5, Asia II 7, Asia 1, MEAM 1, and a new species "Pakistan" (Ashfaq *et al*., 2014). The species Asia II 7 was not collected on cotton crop in Pakistan. Studies conducted in India using CO-1 showed the presence of five species, Asia I, Asia II-1, Asia II-7, Asia II-8, and Asia II-11 on cotton (Ellango *et al*., 2015).

The following factors were found to have contributed to the whitefly outbreak in 2015 (Kranthi 2015):

- Hot and humid conditions at early stage of the crop
- Approval of varieties and hybrids that were susceptible to CLCuD and whiteflies
- Late sowing

- Excess urea & irrigation at early stage of the crop
- Indiscriminate use of insecticides and mixtures
- Whitefly resistance to insecticides
- Other host crops and weeds
- Improper spray application methods
- Non-compliance of ecologically consonant pest management strategies

Management Strategies

Global experimental data (Naranjo *et al*., 2015) affirm that majority of insecticides disrupt naturally occurring biological control of whiteflies thereby leading to whitefly outbreaks in cotton across the world. Therefore it is important to be as diligent as possible when insecticide recommendations are made to ensure that the chemicals have as much selective toxicity to whiteflies without disrupting naturally occurring biological control. In brief, indiscriminate use of insecticides, especially chemicals with broad spectrum toxicity such as organophosphates, pyrethroids, neonicotinoids and mixtures must be avoided. Excessive nitrogenous fertilizers must be avoided. Use Nitrogen (N), Phosphorus (P) and Potash (P) as mixed fertilizers in split doses based on soil testing results to ensure balanced nutrition to the crop. Yellow sticky traps may be used at 500 traps per hectare. For best long term results, neem-oil and castor oil based insecticides, soap sprays and insect growth regulators are recommended. Initially vacuum suction traps may be used followed by a sequential use of water sprays, soap sprays and neem-oil based neem seed kernel extracts. If needed insect growth regulators such as difenthiauron, buprofezin, spiromesifen, and pyriproxifen can be used during the peak vegetative and early boll formation stage of the crop at recommended economic thresholds of the whitefly. These insecticides are effective on whiteflies and are less disruptive to predators and parasitoids of the whitefly. Following are a set of whitefly management strategies derived from published experimental results and are applicable more for India and Pakistan.

Pest monitoring and IPM

Whitefly management depends on proper sampling and subsequent decision support systems that involve proper selection of pest management tools in consonance with ecology of the crop to keep the pest under check with least disturbance to the ecosystem.

Gossypium arboreum

The native diploid cotton species *Gossypium arboreum* is cultivated in India and Paksitan. It is a potential source of genes for resistance to whiteflies, cotton leaf curl virus and other insect pests and diseases (Vij *et al*., 2016). Several *G. arboreum* varieties are known to be tolerant to the whiteflies and highly resistant (almost immune) to the Gemini virus CLCuD. Recently long staple *G. arboreum* varieties with 32mm fiber length with good strength and micronaire, were developed in India. The varieties and traits if harnessed properly, present good prospects for a long term solution to the whitefly-virus problem.

Tolerant Varieties

A few *Bt*-cotton hybrids and non-*Bt* varieties/hybrids from the public sector were identified to be tolerant to whiteflies and the leaf curl virus in India and Pakistan. Based on intensive screening, at least five *Gossypium hirsutum* genotypes, NIBGE-207, NIBGE-115, NN-3, VH-289, and MNH-886 were considered to be CLCuD-resistant (Abbas *et al*., 2015). The following CLCuD tolerant varieties were identified in India: LH 1556, RS 875, Maru Vikas, RS 810, HHH 223, RS 2013, F 1861, H 1226, CSHH 243, LH 2076, H 1236, H1098-improved, H 1300, RG 542 and MR 786. The following varieties were found to be tolerant to whiteflies: Supriya, Aravinda, G.Cot.Hy MDH-11 (GSGDH-2), NDLHH-240, NDLH 1938 (Sri Rama) and MR 786. Interestingly, MR 786 was found to be tolerant to both whitefly and the leaf curl virus.

Short duration varieties

Early maturing short season varieties escape whiteflies especially when sown early or in time. Additionally, termination of cotton crop in time before the onset of winter facilitates timely sowing of wheat in the cotton-wheat rotation system of India and Pakistan.

Timely sowing

When compared to late sown crop, cotton sown in April or before mid-May was found to be significantly more tolerant to whiteflies and the leaf curl virus (Singh *et al*., 2016; Ghazanfar *et al.*, 2007). Therefore timely sowing is an important factor in managing the whiteflies and the virus.

Balanced nutrient management

Application of N (nitrogen), P (phosphorus) and K (potassium) in a balanced ratio based on soil analysis to ensure adequate N with appropriate P and K assist the crop to combat whiteflies and CLCuD. Knowledge about K nutrition on association between plants and pests may help in developing strategies to set up high yielding production system by reducing disease incidence (Zafar and Athar, 2013). Excessive nitrogen application during vegetative phase of the crop makes the crop vulnerable to sap-sucking insects (Bharati *et al*., 2012, Anusha *et al*., 2017) and warrants insecticide usage, which may disrupt ecological balance to cause insecticide-induced resurgence of whiteflies.

Ecological balance

Three species of *Aphelinid* parasites*, Encarsia lutea* (Masi), *Encarsia sophia* (giarualt & Dodd) and *Eretmocerus mundus* (Mercet) and predators such as *Chrysopa* spp., *Geocoris* spp., Coccinellid spp., *Orius* spp. and spiders were found to be important in naturally occurring biological control of the whitefly in Pakistan (Naveed *et al*., 2008). At least

three whitefly predators, *Serangium parcesetosum* (Sicard), *Cheilomenes sexmaculata* (Fabricius) and *Brumoides suturalis* (Fabricius) were most commonly in cotton ecosystems in addition to *Coccinella septempunctata* (Linnaeus)., *Chrysoperla zastrowi sillemi* (Esben-Petersen) in north India (Kedar *et al*., 2014). The parasiotid *Encarsia lutea* (Masi) and *Eretmocerus* spp., were also found to be important components of biological control of whiteflies in north India. Naturally occuring *Aphelinid* parasitoids, *Encarsia formosa* (Gahan) and *Eretmocerus mundus* (Mercet) and predators such as *Chrysoperla carnea* (Stephens) and *Chilomenes sexmaculatus* (Fabricius) were observed to reduce the pest populations. Naturally occurring biological control in the field is reported to have been effective to the extent of 65.0%. Therefore care must be exercised to ensure that the natural ecosystems are not disrupted with inappropriate choice and indiscriminate use of insecticides.

Clean cultivation

Whiteflies have a wide host range that includes weeds on which they can survive all through the year. Therefore it is important to keep fields and the vicinity free of weeds to the best possible extent.

Yellow sticky traps and vacuum suction traps

Yellow sticky traps (Murugan and Uthamasamy, 2001, Bantewad and Thakare, 2017) were found to be useful in reducing pest populations. Yellow sticky traps and vacuum suction traps may be encouraged during the early phase of infestation.

Botanicals

Application of Neem oil, castor oil, cotton seed oil, fish oil rosin soap etc., are very effective in keeping the pest populations under check in the initial stage of whitefly infestation (Puri *et al*., 1998).

Entomopathogenic fungus

More than 20 species of entomopathogenic fungi are recorded to cause whitefly mortality (Lacey *et al*., 1996; Steenberg and Humber, 1999). The most effective amongst these were *Isaria fumosorosea, Isaria farinosa*, *Metarhizium anisopliae, Lecanicillium muscarium, Beauveria bassiana and Verticillium lecanii* (Cuthbertson, 2013*;* Islam *et al*., 2014) which caused 70 to 100% mortality in lab, green house and field conditions (Batta, 2003; Faria and Wraight, 2001; Liu and Stansly, 2000; Zaki, 1998; Quesada-Moraga *et al.* 2006). The use of entomopathogenic endophytic fungus must be explored further and ecouraged.

Insecticides

For effective management of whitefly, selective insect growth regulating (IGR) chemicals such as buprofezin and pyriproxifen should be preferred (Naranjo and Ellsworth, 2009) because they are less toxic to natural enemies of whiteflies. Excessive and indiscriminate use of synthetic pyrethroids and all kinds of insecticide mixtures must be avoided during the initial phase of whitefly infestation because of the possible resurgence of whiteflies when used indiscriminately (Jeyakumar and Gupta 2007)

Recent Exciting Research Advances

Recent promising results with endophytic entomopathogenic fungi have opened up new avenues in integrated pest management. The work of Cuthbertson, (2013) and Islam *et al*. (2014) demonstrated the practical value of entomopathigenic fungi in controlling whiteflies. They identified effective fungal species such as *Isaria fumosorosea, Isaria farinosa*, *Metarhizium anisopliae, Lecanicillium muscarium, Beauveria bassiana and Verticillium lecanii.* Garrido-Jurado *et al*. (2016) showed that foliar applications of myco-insecticide strains *Beauveria bassiana* and *Metarhizium brunneum* lead to transient endophytic colonization of crop tissues and whiteflies were infected with entomopathogenic fungi via ingestion of hyphae growing as endophytes. They also showed that secondary metabolites produced by the endophytic fungi contributed to the control of whitefly pests. The research opens up new avenues for testing different entomopathogenic fungi for endophytic properties and efficacy for whitefly control.

Genome sequencing studies have enabled the identification of several useful gene sequences that can serve as a reference for resolving the *B. tabaci* cryptic species complex, understanding fundamental biological novelties, and providing valuable genetic information to assist the development of novel strategies for controlling whiteflies and the viruses they transmit. Chen *et al*. (2016) reported a 615-Mb high-quality genome sequence of *B. tabaci* Middle East-Asia Minor 1 (MEAM1), comprising of 15,664 protein-coding genes. The genome comprised of other expanded gene families, including cathepsins, large clusters of tandemly duplicated *B. tabaci*-specific genes, and phosphatidyl-ethanolamine-binding proteins (PEBPs), which were found to be associated with virus acquisition and transmission and/ or insecticide resistance, and possibly contributing to the global invasiveness and efficient virus transmission capacity of the species. In a novel approach, microsatellite markers were used to study genetic diversity to compare results with mitochondrial DNA taxonomy. Hadjistylli *et al*. (2016) used nuclear data from variable microsatellite markers to explore global population structures of *B. tabaci* representing most of the available diversity, including known monophagous, polyphagous, invasive, and indigenous haplotypes. The study showed that the invasive B and Q biotypes exhibited moderate to high levels of genetic diversity, suggesting that they stemmed from large founding populations that have maintained ancestral variation, despite homogenizing effects, possibly due to human-mediated among-population gene flow. The study with its application of microsatellite markers in the characterization of global diversity, adds a new dimension to the existing mitochondrial DNA markers that could be used to unravel the cryptic diversity of the species.

Several new sources of genes were explored and a few most effective genes were identified to be deployed for the development of biotech cotton. Javaid *et al*. (2016) developed biotech tobacco (*Nicotiana tabacum*) as a model system using phloem-specific promoters isolated from Banana bunchy top virus (BBTV) for the expression of two insecticidal proteins derived from *Hadronyche versuta* (Blue Mountains funnel-web spider) neurotoxin (Hvt) and onion leaf lectin. They demonstrated that transgenic plants expressing Hvt alone or in combination with onion leaf lectin were resistant to *Phenacoccus solenopsis* (cotton mealybug), *Myzus persicae* (green peach aphids) and *Bemisia tabaci* (silver leaf whitefly). The expression of both proteins under different phloem-specific promoters resulted in close to 100% mortality and provided robust protection. Raza *et al*. (2016) showed that osmoregulation of feeding in whiteflies could be disrupted through RNA interference in biotech tobacco plants by expressing double stranded RNA (dsRNA) to silence aquaporin (AQP) and a *sucrase* gene and *alpha glucosidase* (AGLU) which are involved in maintaining osmotic pressure in whiteflies. More than 70% mortality was observed in *Bemisia tabaci* after six days of feeding on plants expressing dsRNA. The study clearly demonstrated that downregulation of genes related to osmoregulation may find practical applications for the control of whiteflies on cotton. Shukla *et al*. (2016) developed biotech cotton plants expressing a protein (Tma12) from an edible fern, *Tectaria macrodonta* (Fee) that was insecticidal to whitefly. Results of contained field trials showed that the biotech cotton lines expressing *Tma*12 at ~0.01% of total soluble leaf protein were resistant to whitefly infestation and the whitefly transmitted cotton leaf curl disease with no detectable yield penalty.

In an important discovery of genes mediating cotton host plant resistance to whiteflies, Li *et al*. (2016) used transcriptome studies to identify WRKY40 and copper transport protein as hub genes that are likely to regulate cotton defenses to whitefly infestation. Silencing of *G. hirsutum* mitogen activated protein kinase-3 (GhMPK3) by virus-induced gene silencing (VIGS) resulted in suppression of the jasmonic acid and ethylene pathways leading to enhanced whitefly susceptibility, thereby suggesting that the candidate insect resistant genes identified in the study are credible and offer significant utility.

Exogenous application of botanically derived repellents and natural products to induce host plant resistance to whiteflies were also found to be promising. Geraniol and citronellol were found to be most effective repellents against *Bemisia tabaci* (Deletre *et al*. 2016). The authors also found that 1.0% (w/w or v/v) of the essential oils from lemongrass (*Cymbopogon citratus*), cinnamon (*Cinnamomum zeylanicum*), cumin (*Cuminum cyminum*) and citronella (*Cymbopogon winternarius*) caused 96.3% mortality for cinnamon oil, 64.7% for citronella oil, 61.0% for lemongrass oil and 30.0% for cumin oil after 4 hours of exposure. Exogenous application of salicylic acid caused plants to repel non-viruliferous B and Q biotype whiteflies, but the effect was reduced when the salicylic acid concentration was low and when the whiteflies were viruliferous. Exogenous salicylic acid increased the number and quantity of plant volatiles, especially the quantity of methyl salicylate and δ–limonene (Shi *et al*., 2016)

Cotton Leaf Curl Virus, Disease (CLCuV and CLCuD)

Cotton leaf curl virus (CLCuV) is a major pathogen in India and Pakistan and is a threat to China and several other cotton growing countries. The Cotton leaf curl disease (CLCuD) has been causing severe economic losses in the indo-Pak region intermittently in epidemic cycles over the past twenty years. Unfortunately, there is no cure for the ailment after it strikes. The best method to combat the disease is to develop resistant varieties. Managing the insect vector whitefly, *Bemisia tabaci* may partly reduce the intensity of disease. Scientific efforts in India and Pakistan over the recent past have been commendable. The disease can be effectively mitigated with combined efforts of the two countries.

Cotton leaf curl viral disease (CLCuD) is transmitted by viruliferous whitefly, *Bemisia tabaci*. The B-biotype, now re-designated as Middle Easy Asia Minor -1 (MEAM1) is known as the most viruliferous. The whitefly takes about 30 minutes for virus-acquisition and 10 minutes for transmission. The virus complex infects plant cells and multiplies using the cell DNA for its survival and replication. Inter-cellular movement occurs through the plasmodesmata to spread in cells and other plant parts of the plant. Typically the symptoms in leaves start with vein thickening followed by leaf thickening, finally resulting in deformed cup shaped curled leaf, sometimes with enations as outgrowth from the midrib of the lower leaf surface. In severe cases of infection, the virus causes complete distortion of plant parts and stunting, thereby resulting in total yield losses.

CLCuD outbreaks were first reported from Nigeria in 1912 and 1924 and subsequently in 1926 from Tanzania. The disease caused persistent problems for several years in various parts of Africa. During the 1950s, the disease caused estimated losses of 30-40% in Gezira, Sudan. Though the Egyptian cotton species *Gossypium barbadense* is most susceptible, the disease incidence has been at low levels in Egypt. Reports of leaf curl virus in Guangdong and Guangxi provinces of China (Cai *et al*., 2010) indicate the worrying possibility of long range accidental spread, which could not rule out the possibility of the disease invading the main cotton growing regions of central and south India. In the Indo-pak region, CLCuD was first noticed in 1967 near Multan in Pakistan (Hussain and Ali, 1975), but turned into an epidemic in Multan only in 1988. The introduction and cultivation of the highly susceptible varieties S12 and CIM-70 in 1988 is presumed to have triggered the infection into an epidemic form. The area under S12 reached about 46% area in Punjab-Pakistan (Briddon and Markham, 2000) and the disease started gaining hold. Symptoms were noticed from 1973 onwards in

popular cotton varieties such as 149-F and B-557. More and more new hairy varieties that were tolerant to leaf hoppers but susceptible to whiteflies were replacing the glabrous (smooth leaf) varieties that were susceptible to leaf hoppers but were tolerant to whiteflies. The change effectively replaced leaf hoppers with whiteflies in the cotton eco-system of Pakistan. The disease gradually spread over Multan, Khanewal and Vehari to reach an epidemic level in 1993 to an area of 0.89 million hectares that accounted for about one-third of Pakistan's total cotton area. Losses due to CLCuD 'Multan epidemic' (Zhou *et al*., 1998) were estimated to be 30-40% at a production of 1.34 million tons in an epidemic year in 1994-95 as compared to 2.17 million tons in 1991-92. Yield losses to the extent of 80.0% were being recorded. The disease caused an estimated loss of US$ 5.0 Billion in five years from 1992 to 1997 (Briddon and Markham, 2000).

The, Indo-Pak region is known to have the following six species of Begomoviruses that may occur individually or in combination in infected plants. 1. Cotton leaf curl Burewala virus (CLCuBuV), 2. Cotton leaf curl Alabad virus (CLCuAlV), 3. Cotton leaf curl Kokhran virus (CLCuKoV), 4. Cotton leaf curl Multan virus (CLCuMuV), 5. Cotton leaf curl Rajasthan virus (CLCuRaV) and 6. Papaya leaf curl virus (PaLCuV). Additionally the tomato leaf curl Bangalore virus (ToLCBaV) which was reported to be present in southern India is an impending threat for cotton and is a possible source for the evolution of new highly virulent recombinant viruses. The CLCuD in Pakistan is caused by a complex of five single-stranded DNA viruses belonging to genus Begomovirus (family Geminiviridae) along with their DNA satellites, viz. betasatellite and alphasatellite (Brown *et al*., 2015). CLCuD in the Indo-Pak region is caused mainly by single stranded monopartite DNA Begomoviruses (genus *Begomovirus*: family, *Geminiviridae*) that are encapsulated in twin quasi-icosahedral capsid geminate particles. Thus far two major species, the cotton leaf curl Multan virus (CLCuMuV) and the cotton leaf curl Burewala virus (CLCuBuV) have been most damaging. The first epidemic was caused by the Multan virus in 1998 and the subsequent epidemics after 2002 in India and Pakistan were dominated by the Burewala virus. Both the species operate through a monopartite Begomovirus complex that is comprised of a circular single stranded DNA (ssDNA) molecule supported by two smaller circular non-viral single stranded DNA molecules called alpha-satellite and beta-satellite. The two molecules play a crucial role for disease expression. They suppress the host defense systems to enhance virulence thus accelerating the disease. The beta satellite CLCuMuB plays a role in disease transmission through replication, systemic movement in plants and by trans-encapsidation in the helper virus' coat protein. The alpha satellite replicates independently. It also enables a continuous virus transmission by the whitefly vector by sustaining the presence of the CLCuBuV and the beta-satellite in the host plants for a longer time.

Cotton leaves damaged due to CLCuV.
Photograph: Dr D. Monga, ICAR-CICR, Nagpur (Regional Station, Sirsa) , India

The Multan epidemic was found to have been caused by three species of Begomoviruses, namely Cotton leaf curl Alabad virus (CLCuAlV), Cotton leaf curl Kokhran virus (CLCuKoV), and CLCuMuV (Zhou *et al*., 1998) which were associated with a single species of betasatellite Cotton leaf curl Multan betasatellite (CLCuMuB) and satellite-like component Cotton leaf curl Multan alphasatellite (CLCuMuA). Subsequent reports showed that Tomato leaf curl Bangalore virus (ToLCBaV) and Papaya leaf curl virus (PaLCuV) were also infecting cotton (Mansoor *et al*., 2003; Kirthi *et al*., 2004). Plant breeders responded quickly to identify CLCuD tolerant varieties to stabilize cotton production for about 10 years. The Indian variety LRA 5166 developed by ICAR-CICR was widely used as a resistant donor in India and Pakistan to develop new varieties that could withstand the virus. With sustained efforts, the disease declined until the year 2000, with only sporadic instances of infection. However, a new strain named as Cotton leaf curl Burewala virus (CLCuBuV) appeared in 2001-02 season in the Punjab province of Pakistan and caused the 'Burewala epidemic' by infecting all cotton varieties of Pakistan. Cotton production was reduced to 1.7 million tons in the years 2002 and 2003. The new 'Burewala CLCuBuV species' had infected all the varieties that were resistant to the 'Multan CLCuMuV species'. (Amrao *et al*., 2010; Sattar *et al*., 2013). The Burewala strain was supposed to have formed from the recombination of two virulent species of Pakistan called 'Multan species' and Kokhran species'. Further, a new strain named CLCuMuBBur evolved from the recombination of CLCuMuB and Tomato leaf curl betasatellite (Amin *et al*., 2006). Recently, two new strains were identified from the Sindh province of Pakistan. One of them was the African strain Cotton leaf curl Gezira virus (CLCuGeV) and the other was Shahdadpur virus (CLCuShV) (Amrao *et al*., 2010; Tahir *et al*., 2011). The origins of the most damaging virus strains have so far been traced to CLCuKoV and CLCuMuV. The presence of new strains such as the African CLCuGeV (Cotton Leaf Curl Gezira Virus) and the Shahdadpur virus (CLCuShV)

pose a new threat for possibilities of not only direct damage but also for the possible evolution of new recombinant viruses that can play havoc in virulence.

While so far, bipartite begomo viruses were reported only from Africa, and CLCuD in Indo-Pak region was caused by mono-partite viruses, recently bipartite Begomoviruses such as the Tomato leaf curl virus (ToLCV) (Zaidi *et al*., 2015) and Tomato leaf curl New Delhi virus (ToLCNDV) were also reported from cotton plants over a vast area in Punjab in Pakistan. Concerns were enhanced with the synergistic action of ToLCNDV in enhancing the infection of Burewala virus (Zaidi *et al*., 2016).

In India CLCuD was first noticed in 1989 in Sriganganagar district of Rajasthan along the Pakistan border (Ajmera,1994) and on *G. barbadense* at Indian Agricultural Research Institute, New Delhi in 1989 (Anonymous, 1990; Varma *et al*., 1993). Over the subsequent few years, CLCuD spread to the cotton growing districts in north Indian states to assume serious proportions of almost an epidemic form in 1996 and 1997 affecting 0.2 million hectares, especially in north Rajasthan and adjoining regions of Punjab and Haryana. The disease soon spread to the interior cotton growing regions of Haryana and Punjab in north India (Rishi and Chauhan,1994) and established itself as a major problem in cotton production in north India. Surprisingly, India experienced the leaf curl virus outbreaks in 1993 and 1996 just concurrent to the occurrence of epidemics in Pakistan during 1992 and 1995 (Kranthi 2015a). A new virus called Cotton leaf curl Rajasthan virus (CLCuRV) that evolved from recombination of Multan (CLCuMV) & Kokhran (CLCuKV) viruses, was reported to occur in Rajasthan (Malathi *et al*. 2004). Until 2003, the 'Multan CLCuMuV' and the 'Rajasthan CLCuRaV' were the main species that caused the CLCuD in India, after which the 'Burewala CLCuBuV' virus replaced the two viruses and became the main dominant species. The Burewala strain could infect all the varieties that were resistant to other strains of the virus, mainly the 'Multan species'. Recent studies by Rajagopalan *et al*., (2012), showed that CLCuD in north India was mainly caused by the cotton leaf curl Burewala virus (CLCuBuV) and three of its variants which displaced CLCuRV and CLCuKoV isolates. However, CLCuD in Delhi was found to be caused by CLCuRV (Rajasthan), CLCuMuV (Multan), and Burewala CLCuBuV species (Godara *et al.,* 2015; 2017).

In India, for about 15-16 years after 1990, the cotton research net-work and the varietal approval committees in India had made it mandatory that only varieties or hybrids resistant to the CLCuD would be approved for identification, notification and cultivation in north India. Plant breeding and evaluation efforts were intensified through the All India Coordinated Cotton Improvement Program (AICCIP). Several resistant varieties such as RST9, RS875, RS810, RS2013, F1861, LH2076, H117, H1126 and resistant hybrids LHH144, CSH198, CSHH238 and CSHH243 were developed and cultivated until 2007 to combat the disease effectively. Clearly the efforts paid off and damage due to CLCuD declined significantly in India during the years 1998 to 2006 due to the cultivation of resistant varieties, extensive weed management and implementation of stringent IPM measures to manage whiteflies and the leaf curl virus. However, the complete replacement of public sector varieties with new *Bt* cotton hybrids/varieties by the private companies after 2007 changed the scenario completely in India and Pakistan. CLCuD started showing up in again in north India due to the progressive replacement of the CLCuD tolerant varieties with commercial *Bt*-cotton hybrids. Staring from 2009 within 3-4 years, the disease established itself firmly in almost all the main cotton growing districts of Punjab and Haryana with high levels of severity in Ferozepur, Muktsar, Faridkot, Abohar and Fazilka, Jind, Fatehabad, Hisar and Sirsa districts. The disease intensity was less severe in Rajasthan because, unlike Punjab and Haryana, Rajasthan did not adopt the new *Bt* cotton hybrids so readily. *Bt* cotton hybrid area in Punjab and Haryana reached 70% by 2008, whereas in Rajasthan only 25% of the area in 2008 was under *Bt*-cotton hybrids. Data suggest that indiscriminate introduction and cultivation of new hybrids could have been one of the major factors in creating congenial conditions for the CLCuD in north India.

Causes for CLCuD Epidemics in India and Pakistan

A combination of factors such as long duration bushy hybrids, susceptibility to whiteflies and CLCuD, late sowing, excessive application of nitrogenous fertilizers and indiscriminate use of insecticides and insecticide mixtures with broad spectrum action caused frequent outbreaks of whiteflies and the leaf curl disease in India.

Following are some of the main causal factors for outbreaks:

- Late sown crop is more vulnerable to whiteflies and CLCuD. *Bt*-cotton hybrids sown late showed significantly higher disease at all locations in north India (Monga, 2016). Similar observation were recorded in Pakistan and other countries (Iqbal *et al.*, 2008; Iqbal and Khan., 2010; Tanveer and Mirza, 1996; James *et al.,* 2004).
- Increase in nitrogenous fertilizers to harness hybrid vigour. Excessive canopy provided congenial micro-climate for sucking pests and whiteflies to thrive.
- The mandatory requirement of varietal tolerance to CLCuD was diluted in India for the private sector *Bt*-cotton hybrids during the period 2007 to 2013. Thus a large number of varieties & hybrids that were susceptible to CLCuD and the whiteflies were approved, which led to rapid spread of the disease across north India and Pakistan. Seed-cotton yields were reduced by 15.7% to 56.7% (Monga, 2014).
- Majority of the commercial hybrids were susceptible to sap-sucking insects. Indiscriminate use of insecticides and insecticide mixtures during the early vegetative stage of the crop to control sucking pests disrupted naturally

occurring biological control, thereby causing resurgence of whiteflies.

- Excessive use of insecticides with broad spectrum toxicity aggravated whitefly infestation.

Management Strategies

The most important management strategies are related to early sowing of CLCuD-tolerant varieties. balanced nutrition by avoiding excessive nitrogenous ferilizers and conserving naturally occurring biological control by diligent selection and use of selective eco-friendly insecticides to manage whitefly populations. The following strategies were recommended in both India and Pakistan, based on research results from the two countries.

Timely or early sowing

Government efforts should be streamlined to ensure that canal water is released in time to avoid late sowing. Late sown crop creates congenial conditions for the disease to get aggravated and thus creates more viral and whitefly inoculum in the farm ecosystems. Early sowing must be encouraged to provide conditions for the crop to escape pest and disease infestation.

Tolerant varieties

Regulatory authorities should enforce a strict ban on the approval of varieties and hybrids that are susceptible to CLCuD in India and Pakistan, to ensure that only tolerant/ resistant genotypes are approved, released and cultivated. Plant breeders must identify resistant sources of germplasm lines on priority and attempts must be made to pyramid resistance genes. CLCuD resistant varieties such as FH-682, CIM-240, CRIS-9, BH-36, CIM-109 and CIM-1100 in Pakistan and RST9, RS875, RS810, RS2013, F1861, LH2076, H117, H1126 in India must be used as sources of resistance to be used in breeding programmes to develop new varieties that can tolerate Burewala virus.

Native desi species

Cultivation of the native diploid species *Gossypium arboreum* and Gossypium *herbaceum* must be promoted, especially in the districts bordering India and Pakistan. The two species are almost immune to all the species of leaf curl viruses.

Reducing the load of virus inoculum in the crop environment

Infected plants must be destroyed after harvest. Crop rotation plans must be devised with alternate crops that are not host plants for whiteflies. Cultivation of American cotton, *Gossypium hirsutum* must be avoided in orchards and near malvaceous crops such as okra (bhendi) or tomato especially in disease prone areas. Simultaneous cultivation of cotton with alternate hosts such as vegetable and citrus intensifies the disease inoculum. It was observed that whitefly picks virus from egg plant, okra, *Ablemoschus esculentus* and *Hibiscus rosa-sinensis* and primary sites of infection are formed in cotton plants (Gusain *et al.,* 1991; Muhammad *et al*., 1998; Farooq *et al.*, 2011; Briddon and Markham, 2000). Clean cultivation should be practised. Off-season weeds must be destroyed and clean cultivation should be followed scrupulously during the season to minimize sources of virus inoculums. Several weed species were found to harbour and sustain the 'Burewala species' inoculum all across north India. Notable amongst these are *Althea rosea*, *Achyranthus aspera*, *Chenopodium album, Convolvulus arvensis, Croton sperciflorus*, *Clerodeadron eneansi, Corchorus acutangularis*, *Eclipta alba*, *Parthenium hysterophorus, Lantana camara, Sida spinosa*, *Trianthema monogyna* and *Tribulus terrestris.*

Monitoring and management

Regular pest and disease monitoring and initiation of appropriate IPM measures based on economic thresholds, are important steps for effective management of whiteflies and the virus. The whitefly species *Bemisia tabaci* in North India has been recently showing high level of resistance to the recommended insecticides, thus enhancing insect survival and thereby increased transmission of virus.

Recent Exciting Research Advances

Commendable efforts were made in India and Pakistan to identify gene resources to be deployed for the development of biotech cotton varieties that can withstand the virus. Biotech cotton expressing a double stranded RNA-insert homologous to the intergenic region (IR) of Cotton leaf curl Rajasthan virus (CLCuRV) was developed using an intron hairpin (ihp) RNAi construct. Transformed cotton plants showed the presence of siRNAs and upon inoculation with viruliferous whiteflies, none of the nine plants with independent events displayed any CLCuD symptoms even after 90 days post inoculation (Khatoon *et al*., 2016). Sohrab *et al*. (2016) developed biotech cotton plants by using βC1 gene in antisense orientation gene to control CLCuD. Molecular studies confirmed a single copy integration in the genome. The plants were tested in green house and open field conditions and were found to confer resistance through reduced infection and delayed symptom appearance. Iqbal *et al*. (2016) proposed a broad spectrum CLCuD controlling method based on multiplexed clustered regulatory interspaced short palindromic repeats CRISPR/Cas9 system where a cassette of synthetic guide RNA (sgRNA) is designed to target not only the whole CLCuD-associated Begomovirus complex but also the associated satellite molecules. They devised a multiplex sgRNA cassette targeting IR and replication-associated protein (Rep) of the CLCuD-associated Begomoviruses (CABs), non-coding intergenic regions (IR) of Cotton leafcurl Burewala virus associated with recombinant Cotton leaf curl Multan betasatellite (CLCuMuB) and *rep* gene of diverse alphasatellites. This system offers a flexible approach for stacking multiple nucleases as one transgene, thereby offering targeted cleavage of mixed infections by multiple viruses and associated DNA-satellites, such as CLCuD-complex.

Interesting studies were conducted to unravel the association and interactions between whiteflies, viruses and plants. Wang

et al. (2016) reported a gene from the MEAM-1 (B biotype) *Bemisia tabaci BtPGRP*, encoding a PGRP that binds and kills bacteria *in vitro*. Begomovirus infection led to increased expression of *BtPGRP*. The authors detected *in vitro* interaction between *BtPGRP* and TYLCV and recorded the co-localization of TYLCV and *BtPGRP* in whitefly mid-gut. The study addresses a visible gap in understanding whitefly immunity and provides insight into how the whitefly immunity acts in complex mechanisms of Begomovirus transmission among plants. Peptidoglycan recognition proteins (PGRPs) are multifunctional pattern recognition proteins. Further, Shalev *et al*. (2016) idenfied a *knottin* gene, *knot-1* which was found to play a role in restricting the quantity of virions an insect may acquire and transmit. The authors suggest that *knot-1* protects *B. tabaci* against deleterious effects caused by TYLCV by limiting the amount of virus associated with the whitefly vector. In a path breaking study, the phenomenon of 'autophagy' was reported in cotton plants (Haxim *et al*., 2017) as a novel anti-pathogenic mechanism that plays an important role in antiviral immunity in plants. The authors reported that the cotton plant autophagic machinery mainly comprising of the key autophagy protein ATG8 targets the virulence factor βC1 of Cotton leaf curl Multan virus (CLCuMuV) to degrade it, thus conferring resistance in plants. Haxim *et al*. (2017) also showed that a V32A mutation in βC1 abolished its interaction with NbATG8f, and virus carrying βC1V32A showed increased symptoms and viral DNA accumulation in plants. Furthermore, silencing of autophagy-related genes ATG5 and ATG7 reduced plant resistance to the DNA viruses CLCuMuV. The study opens up a new scope for the exploration of autophagy genes, which must be identified and used to develop biotech plants for enhanced resistance to viruses.

Conclusion

The four insect pests, bollweevil, pink bollworm, cotton bollworm and whitefly are global pests. The whitefly and pink bollworms are prevalent everywhere in the world. The boll weevil is currently restricted to the Western Hemisphere. The cotton bollworm was confined so far to Africa, Asia, Europe and Australasia. But the recent discovery of the cotton bollworm *Helicoverpa armigera* in South America signals new agri-concerns for the continent. The leaf curl virus disease is limited to Pakistan and north India. These problems are serious. They have been causing enormous economic losses to cotton in various parts of the world. Through commendable efforts, the boll weevil problem was managed scientifically in the US through a 'bollweevil eradication program' and recently in Mexico with the help of the United States Department of Agriculture (USDA). The recent discovery of *H. armigera* in Brazil, Argentina, Uruguay and Paraguay and the damaging presence of boll weevils in these countries in South America are worrisome. The two pests not only cause crop losses but also lead to progressive increase in the use of insecticide applications. In an impressive development, researchers in Brazil have claimed to have made a new biotech *Bt*-cotton technology which in combination with the existing 'bollworm-controlling' *Bt*-cotton technologies and the boll weevil eradication program could herald a new beginning for sustainable pest management in South America. The whiteflies and leaf curl viruses are significant threats to cotton in Pakistan and north India. Researchers in both countries found that the best way to combat both problems was through four simple strategies. 1. Early sowing, 2. Varieties tolerant to CLCuD, 3. Balanced NPK nutrition, and 4. Diligent choice of insecticides. Non-adherence to any of the four strategies could result in serious problems with the pest and disease. Biotech cotton technologies were developed for resistance to whiteflies and the CLCuD in India and Pakistan. Collaborative efforts between the two countries to pyramid the new biotech events into elite varieties for resistance to the two problems can tackle the menace in a sustainable manner. The problem of *Bt*-resistant pink bollworm is serious, but manageable. Scientists in the US showed how a combination of three technologies 1. *Bt*-cotton 2. Gossyplure pheromones for mass trapping and mating disruption and 3. Mass release of biotech-male sterile moths, could effectively diminish the problem. Additionally proper compliance of refuge strategy strengthened the 'pink bollworm eradication' program. There is a lot to learn from the technological and administrative essence of the success stories to combat the problems efficiently.

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