using Visual Basic. NET as front-end and Microsoft Access as back-end.

Information System on Cotton Cultivars (InsCOT- ver 1.1) will provide information on all the cultivars released by various agencies so far in India. The CD was created with Visual Basic. NET as front-end and Microsoft Access as back-end. Digital Cotton Photo Library (DigiCOT – ver 1.1) contains collection of around 1000 photographs with thumb view as well as full view on various features of cotton including production, protection, wild species collection, cotton disease, biotechnology, post harvest, extension activities, etc.

Package of practice for cotton production system and ITKs related to cotton production system were documented.

4.19 Pest Scenario

Nagpur

During 2004-05, jassids, thrips and mirids (*Ragmus spp.)* attained pest status during mid-August, 1st week of September and mid-September, respectively. Aphid incidence was very low. Three peaks of fruit damage during mid-September, October last and November-December caused mainly by *H. armigera* to squares, *E. vitella* and *P. gossypiella* to bolls, respectively were observed. Damage peaks in respect of bollworms occurred with the simultaneous presence of all larval instars. With the occurrence of all fruiting structures simultaneously preference by *H. armigera* to squares followed by bolls and *vice-versa* by *E. vittella* was observed. Emerging pest status of thrips and mirids among sucking pests, and pink bollworm attaining the status of key pest in cotton were established. Considering larval diapause and off season moth emergence patterns in pink bollworm it is highly essential to focus on off-season pink bollworm management.

Aphidophagous coccinellids and syrphids were meager on account of low aphid abundance. Chrysopid oviposition coincided with both the peaks of *H. armigera* oviposition. Spiders and predatory mirids were regular in occurrence between September and November. Estimated egg mortality of *H. armigera* was 47.2 %. Seasonal mean parasitisation of *Earias* by *Rogas aligarhensis* and *H. armigera* by *Campoletis chlorideae* was 16.3 and 13.9 %, respectively.

A population of reniform nematode (*Rotylenchulus reniformis*) ranged from 20-150 nematodes per 250 cc soil at the time of sowing CICR experimental farm. At mid cropping season, the population varied from 200-360 nematodes/ 250 cc soil. Reniform nematode population dipped to 10-30 nematodes/ 250 cc soil with onset of winter. Soil solarisation reduced the population of root-knot nematode (Meloidaogyne incognita significantly.

Three plant species viz. marigold, custard apple and bitter gourd were found to repel phyto-nematodes from their rhizosphere up to one meter. There was reduction in nematode population involving crops as Sorghum while in cropping systems with Soybean, there was four fold increase in population of reniform nematode.

Soil with different nutrient status was surveyed to explore the possibility of use of nematodes as indicators of soil health. It was observed that free living nematodes as Cephalobids and Rhabditids were associated with high organic matter soil. Soil with high incidence of root-rot was found to have corresponding high presence of fungal feeding nematodes as *Aphelenchus spp.* and *Aphelenchoides spp*. High population levels of plant parasitic nematodes were associated with low population levels of free living nematodes and *vice versa.*

Coimbatore

During the year 2004-05, the pest infestation was low. Aphids appeared in the month of September and persisted all through peaking during October and January. Leafhopper incidence started in the month of September and the peak activity was in the month of December and the hopper population was 5-8 per plant during this period. Infestation of white fly and spotted bollworm was very low. Incidence of

Helicoverpa armigera was seen in the month of December. Bollworm damage to reproductive parts was highest in the month of January (13.38%).

Observation on aphid infestation on Bt cotton (RCH 2) indicated that peak infestation was in the month of December. Leafhopper on Bt cotton persisted throughout the crop period and the peak infestation was in the month of December. Natural enemy population (coccinellids and spiders) was more during November –December.

Expert System on Cotton pest/insect

Basic information on cotton pests was collated and a Network Diagram was formulated. Initially attempt was made to develop the system in Visual Basic, but it has been reoriented to develop in ASP.NET, which is useful to float the developed software online in CICR web site. Preliminary shell for the expert system was developed in visual basic and the images of pests and their symptoms of damage and control measure information were collected from entomologist.

4.20 Resistance to Insect Pests and Diseases in *Gossypium* Spp.

Insect Pests

Nagpur

Out of 229 genotypes involving 131 crosses and 98 single plant selections evaluated for genotypic tolerance using phenological trait of compensation respectively 14, 32 and 21 were susceptible, moderately tolerant and tolerant to early season bollworm damage and had higher compensation.

$BC_1 F_1$ plants of three crosses, Bikaneri Nerma x (Bikaneri Nerma x Pee Dee0695), G Cot 10 x (G Cot 10 x Pee Dee 0695) and Mahalaxmi x (Mahalaxmi x Pee Dee 0695) raised under unprotected condition in the field. Plants were screened for the identification of high protease inhibitor (PI) inducible lines in the seedling and boll formation stages. Out of 17, ten plants of the cross G Cot 10 x (G Cot 10 x Pee Dee 0695) produced protease inhibitors. Of the 10 plants, six were tagged and have been used in the generation of $BC_2 F_1$ plants. Out of the 17 plants of the cross Bikaneri Nerma x (Bikaneri Nerma x Pee Dee 0695), seven plant demonstrated the presence of PI in the seedling stage. Of these, three demonstrating the highest PI activity were carried forward for the generation of $BC_2 F_1$. Of the thirty-three plants of G Cot 10 x (G Cot 10 x Pee Dee 0695) screened, 9 demonstrated the presence of inducible PIs. Of the 25 plants screened of Bikaneri Nerma x (Bikaneri Nerma x Pee Dee 0695) 14 demonstrated the presence of inducible PI and their inhibitory activity ranged from 28- 67%. The same plants of both crosses demonstrated the presence of PI in the seedling as well as boll stage and their PI values are presented in Table 10.

Table 10 : Protease inhibitory properties of $BC_1 F_1$

	G Cot 10 x (G Cot 10 x Pee Dee 0695)		Bikaneri Nerma x (Bikaneri Nerma x Pee Dee 0695)		
Plant no	**% PI activity in seedling stage**	**% PI activity in the boll stage**	**Plant no**	**% PI activity in seedling stage**	**% PI activity in the boll stage**
P2	57.14	33.33	P1	13.33	28.06
P4	42.85	0.00	P2	33.33	43.00
P8	16.66	40.00	P4	40.00	40.00
P9	25.00	40.00	P5	72.22	60.00
P10	25.00	60.00	P7	53.33	60.00
P11	16.66	-	P 17	11.11	37.05
P13	41.66	-	-	-	-

The donor parent (Pee Dee 0695) demonstrated an inducible seedling protein PI activity of 60 % and the boll protein demonstrated an inhibitory activity of 55 %.

Three MDR cultures CNH 911, CNH 2713 and CNH 4736 were maintained and two alternaria resistant cultures were evaluated.

Coimbatore

Screening of genotypes and advanced cultures of breeders' materials to bollworms

Of the 110 genotypes field screened against bollworms with Abadhita (bollworm tolerant variety) and Surabhi as check variety, four *viz.*, (VRS x V112) 3-2-4, 5 (1x2) 724-2, (V22 x V112) (LxM55)-443 and LS 3 were found to be resistant to pink bollworm and had less loculi damage, ranging from 8.5 to 10.9 per cent as against 8.8 per cent in Abadhita and 25.0 per cent in Surabhi. Six others *viz.*, (VRS x V112) 3-2-4, (VRS x V113) 6-3-4, Sumangala, LS 1, TK 32, TK 43 were found tolerant to pink bollworm and recorded loculi damage ranging from 10.9 to 18.3 per cent as against 25.0 per cent in Surabhi. They also recorded higher seed cotton yield than Surabhi. Only one entry, BRS 5 (L x BRS) 355 was found to be tolerant to all the three bollworms. It had 18.3 and 15.7 per cent damage in bolls and loculi, respectively as against 42.4 and 25.0 per cent damage in Surabhi. It also recorded significantly higher number of good opened bolls per plant and higher seed cotton yield over Surabhi.

Performance of selected genotypes with field tolerance against bollworms

Five selected genotypes *viz.*, 5 (1 x 2) 714-7, 5 (1 x 2) 718 -2, BRS-5 (L x BRS 3-3), IRH-1-10 and L (Paiyur x RCH 2-5-2) were screened for bollworm resistance in the field for the second year. The damage caused by *H. armigera* in squares, bolls and loculi were relatively less in all the five entries as compared to IC-472, which is a susceptible check. Three out of five entries recorded significantly higher seed cotton yield as compared to the susceptible check. From the two years study, the two entries, 5 (2x2) 714-7 and (1x2) 718-29 were seen consistently tolerant against pink bollworm.

Identification of resistant lines against jassids

Among 120 cultures screened for their reaction to jassid under unprotected condition, three *viz.*, L (RCH x T13) 5-2-11, L (RCH x T13) 74- 4, L (RCH x T13) HS 52-116 were found to possess resistance to while, twenty six recorded moderate resistance.

Evaluation of germplasm and AICCIP entries for resistance to foliar diseases

All *Gossypium arboreum* germplasm lines (100) and AICCIP entries (159) screened following inoculation separately in pot culture under polyhouse conditions against alternaria leaf spot, grey mildew and bacterial blight diseases were found to be susceptible to grey mildew.

Development of resistant lines to foliar diseases

Fifty-three single plant selections having resistance to grey mildew (27), alternaria leaf spot (3) and MAR lines (23) with resistance to more than one disease were advanced for further testing. Thirteen advanced lines having resistance to grey mildew (4), alternaria leaf spot (5) and MAR (4) were evaluated for yield in comparison with LRA 5166 and Sumangala. Seven lines had a yield potential on par with the test line Sumangala and significantly better than LRA 5166. Forty-one progenies involving 101-102 B, Badnawar-1, CBR 1 and CBR 3 were screened for bacterial blight disease.

Studies on soil borne diseases of cotton

There was no serious out break of Verticillium wilt. Under artificial conditions, 34 selected Verticillium wilt resistant progenies were once again screened and the resistance was confirmed. Ninety-four single plants involving six crosses were selected based on yield and ginning out turn.

Studies on biochemical mechanisms of resistance to bollworms of cotton

Advanced genotypes such as BRS 3, BRS 5,

BRS 22, and BRS 23 were noticed to possess higher levels of defensive biochemical constituents *viz.*, gossypol, tannin and phenols with higher bollworm tolerance as compared to susceptible genotypes. The yielding ability was seen in the range of 37 to 50g / plant under protected conditions, while it ranged between 34 and 45 g/plant under unprotected conditions (Fig. 9).

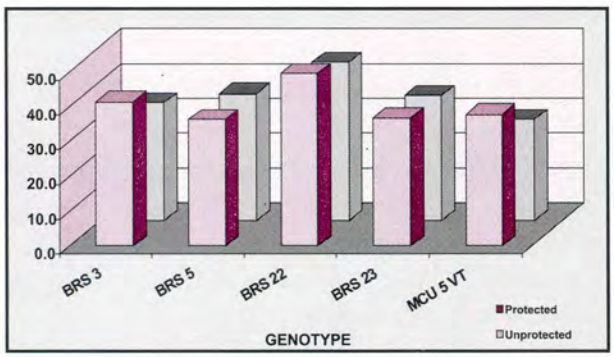


Fig. 9 : Yield of bollworm tolerant cotton genotypes under unprotected and protected conditions

Molecular evaluation through RAPD profiling revealed polymorphism between tolerant and susceptible cultivars (Fig. 10 a&b). Derivatives of tolerant introgressed lines possessing elevated levels of metabolic process intermediates have also been subjected to RAPD analysis and found to be polymorphic.

Fig. 10a.

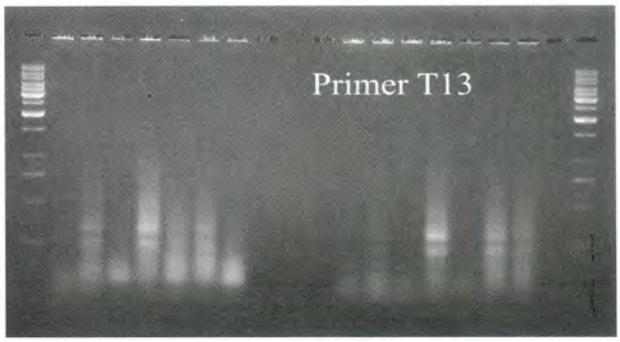
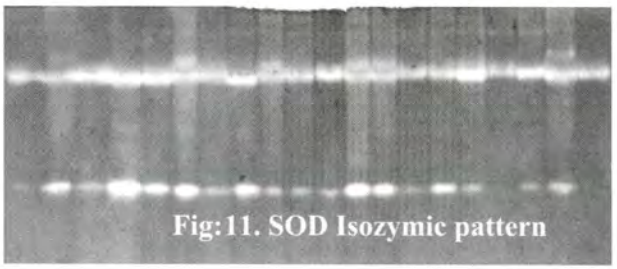
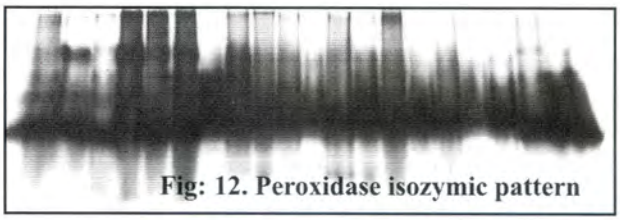


Fig. 10 a & b : RAPD profile of BRS Cultures studies on developmental biochemistry of cotton – pest / disease interaction

The effect of *Pseudomonas fluorescens* pf1 application was found superior with enhanced peroxidase activity of 102 -115 units, while the control plants possessed activity between 36-51 units. Similarly, enhanced superoxide dismutase (SOD) activity could be seen following *P. fluorescens* application in cotton. Characteristic isozymic variation could be noticed during host plant – *R. areola* interaction (Fig. 11 & 12).





Biochemical metabolic status due to interaction of pesticides was studied in field in Sumangala using Clothianidine, Methyl–o–dementon and four dosage levels of Imidacloprid. Imidacloprid @75ml/ha resulted in maximum peroxidase activity of 250 units, followed by Clothianidine, while control plants

exhibited 181 units of activity. A similar trend could be seen regarding accumulation of soluble protein (53, 54 & 44 mg/g) and nitrate reductase activity (5.2, 4.5 & 3.5 µmol NO_2 released/hr/g fr.wt), respectively.

Significant enhancement in photosynthetic rate and nitrate reductase activity could be observed following initial application of *Kamdhenu kitniyantrak* (natural insect repellent). However, subsequent rounds of application did not bring about changes in photosynthetic rate and nitrate reductase activity.

Sirsa

Studies on resistance to insect pest and diseases

On two years evaluation; genotypes CSH 3047, CSH 3051, CSH 3088 and CSH 3123 were found free from jassids and bollworms and CSH 3047 was free from jassids, bollworms and CLCuV disease. Study indicated that CSH 3047 had good fibre quality traits and yield also.

Screening of around 2000 lines of *G. hirsutum* against cotton leaf curl virus disease has led to the identification of forty resistant lines which are being used by the plant breeders.

4.21 Variability in Insect Pests and Pathogens

Nagpur

Helicoverpa

In India two species of *Helicoverpa* and one species of *Heliothis* have been recorded, viz: *Helicoverpa armigera* (Hubner), *Helicoverpa assulta* Guenee and *Heliothis peltigera* (Denis and Schiffermuller).

The primers used in the study were designed to amplify a 598 bp fragment corresponding to mid to near terminal region of CO I. Sequence of the amplified fragment was subjected to multiple sequence alignment using Clustal X. Nucleotide sequence and translated amino acid sequences, with the invertebrate mitochondrial genetic code in each of the strains resulted in high level of consensus between the two *Helicoverpa* species.

The region sequenced in this study was capable of being selectively restricted in *H. armigera* with eight restriction enzymes of which *Rsa* I was one. Its recognition site is masked by a mutation- GT¯AC in *H. armigera* that is replaced with GT¯TC in *H. assulta*, which is responsible for the absence of restriction digestion (Fig. 13).

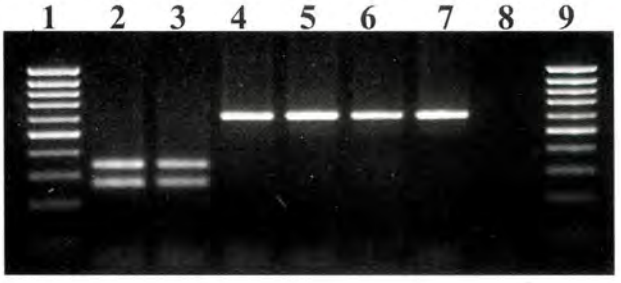


Fig. 13: Restriction digestion of PCR amplified partial COI region of *H. armigera* and *H. assulta* with *Rsa I.* L1&L9: 100 bp MW ladder, L2, L3: *H. armigera* PCR product, digested with *Rsa I*, L4: *H. armigera* PCR product undigested control, L5, L6: *H. assulta* PCR product digested with *Rsa I*, L7: *H. assulta* PCR product undigested control, L8: negative control

Molecular tools for distinguishing two haplotypes of *Helicoverpa* that differ in their ability to utilize cotton squares for the growth

PCR – RFLP using enzymes *Nci*1 for the cotton strain and *Bst* 2 UI for the non- cotton strain is proposed for the identification of the two haplotypes of *H. armigera*. Point mutations (Table 11) observed in the CO1 region have been exploited for designing this tool.

Reproductive compatibility and feeding preferences of interstrain crosses

Of the 109 single pair crosses made 48 were inter strain crosses and 61 were intra strain crosses. From the table it is clearly evident that the two strains mate and inter breed freely under laboratory conditions. Of the 109 crosses, fertile crosses ranged between 22-38% .

Table 11: Mutations in partial nucleotide sequences and the deduced amino acid sequences of Cytochrome Oxidase I (COI) gene of cotton and Non-cotton strains of *Helicoverpa armigera*

	Nucleotide sequence	Corresponding amino acid sequence
Ha Cotton	(2206)5'-ATTTTACC***G***GGA-3' (2217)	(245)ILPG (248)
Ha Non-Cotton	(2206)5'-ATTTTACC***A***GGA-3' (2217)	(245)ILPG (248)
Ha Cotton	(2377)5'-TATTT***T***ACATCAGCT-3' (2392)	(302)YFTSA (306)
Ha Non-Cotton	(2377)5'-TATTT***C***ACATCAGCT-3' (2392)	(302)YFTSA (306)

It was amply clear from this study that progeny of cotton strain feed on both cotton squares and redgram pods while progeny of non- cotton strains prefer to feed more on redgram pods than on cotton squares. In inter strain crosses where the mother was a cotton strain female, the progeny preferred to feed on both redgram pods and cotton squares. In inter strain crosses where the mother was non- cotton female the progeny preferred to feed on redgram pods when compared to cotton squares

Values in brackets indicate position of nucleotides with reference to *Drosophila yakuba* mitochondrial genome and that of amino acids in the deduced protein sequence. Letters in bold and italics indicate base substitutions.

Temporal variation in the cornutal spine numbers of pheromone trapped male moths (Fig.14).

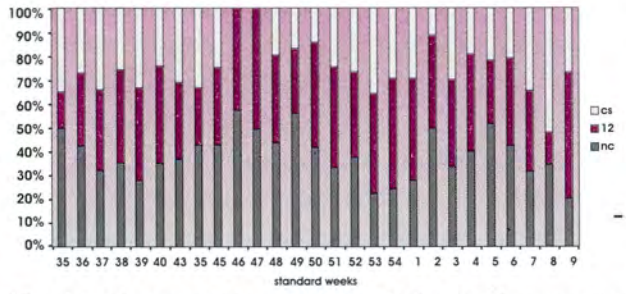


Fig. 14 : Cotton and non cotton strains in pheromone trapped male moths

Xanthomonas axonopodis pv malvacearum (Xam)

For knowing the pathogenic variability, 150 isolates were made from the bacterial blight infected leaf samples of five susceptible cultivars *viz.* Ganganagar ageti, LRA 5166, LRK 516, PKV 081 and Rajat having varying degree of susceptibility. Five races viz. 3, 7, 10, 15 and 18 were identified of which race 18 was predominant and 66.67 – 83.33 per cent isolates belonged to this race.

Races 3 and 18 were able to maintain their specific virulent nature when re-isolated from susceptible reactions of Stoneville 20 inoculated with race 3 and from hypersensitive reactions of S 295 inoculated with race 18. Inoculation of resistant host with race 3 was able to improve its virulence from single gene (B_{In}) to two genes (B_{In}, B_N) of race 5. However, dilution of virulence from five genes to three genes (B_2, B_{In}, B_N) of race 7 was observed in inoculation of resistant host with race 18. Association of susceptible or resistant hosts appeared to be responsible for dilution or increase of virulence.

Seventy one isolates made from the bacterial blight infected leaves collected from Marathwada and Vidarbha cotton growing areas of Maharashtra revealed the presence of five races *viz.* 4,7,10,15 and 18. Race 18 was most predominant and 81.69 per cent isolates belonged to this race.

Susceptible Stoneville 20 and resistant S 295 were inoculated with races 3 and 18 of *Xam* having virulence against one major gene B_{In} and five major genes B_7, B_2, B_{In}, B_N, B_4, respectively for knowing their virulence specificity after passing through

resistant and susceptible hosts. Races 3 and 18 were able to maintain their specific virulent nature when re-isolated from susceptible reactions of Stoneville 20 inoculated with race 3 and hypersensitive reactions of S 295 inoculated with race 18. Inoculation of resistant host with race 3 could be able to improve its virulence from single gene (B_{In}) to two genes (B_{In}, B_n).of race 5. However, dilution of virulence from five genes to three genes (B_2, B_{In}, B_N) of race 7 was observed with race 18 inoculation of susceptible host.

Biotypes variability in race 18 of *Xam*

Thirty new isolates of *Xam* collected from farmers' fields in districts of Wardha, Nagpur and Amravati were found to belong to race 18. Genomic and plasmid DNA were extracted from all 30 isolates in order to delineate biotype variability based on their native plasmid profiles, RAPD, Rep-PCR and RFLP. Based on the BOX-PCR fingerprinting pattern 30 isolates were grouped in 10 major groups with group V, accommodating 13 isolates. Six groups were unique having one isolate each. However, ERIC-PCR was more sensitive as it further delineated variability within three isolates that were accommodated within group V, based on BOX-PCR. Based on ERIC PCR, the isolates formed 11 groups. The isolates 19, 20 and 35 grouped together based on BOX-PCR fingerprinting formed separate groups on the basis of ERIC-PCR. RAPD analysis with OPA 13 primer further found variabilities among some group members that were grouped together based on Rep-PCR. Based on RAPD fingerprinting, 30 isolates were grouped in 16 groups. The primer generated amplicons ranging in size from 0.25 to 4.0 kb with minimum number of amplicons of one and maximum 10. Plasmid profiles of *Xam* isolates categorised 30 isolates in 12 groups with numbers ranging 1-3.

RFLP analysis of 30 isolates of *Xam* showed scorable hybridising bands in 15 isolates. These 15 isolates formed 7 groups based on their RFLP patterns. Most of the race 18 isolates possessed 2 pthN hybridising fragments. Accordingly group I is the biggest and is comprised of 5 isolates each having two hybridising bands of ca. 14 and 5 kb. Maximum of 6 hybridising bands were present in a lone isolate that belonged to group VI.

rDNA PCR analysis

In order to differentiate biotypes of race 18 based on rDNA sequence, PCR analysis of 6 representative isolates, one from each RFLP group, was done with conserved primers. The primers amplified approximately 0.65 kb DNA fragment from 5 out of 6 isolates. The rDNA fragment amplified from isolate 6 was slightly bigger compared to rest of the 5 isolates.

Ramularia areola

New synthetic media alone or in combination with either of cotton leaf decoction of carrot juice or combination of these was observed to be better for the growth of *R. areola*. The pathogen was successfully cultured with well method, inoculation of healthy leaf tissue and inoculation of conidia in broth. The method of inoculation of healthy leaf tissue was found more advantageous for isolation.

The growth pattern and mycelial dry weight of 30 isolates of *R. areola* grown on new synthetic media/ broth indicated that the isolates from the cultivars of *G. arboreum* and *G. herbaceum* were fast in growth as compared to the isolates from the varieties/hybrids of *G. hirsutum*.

The size of conidiophores of *R. areola* from freshly infected leaves of cultivars/germplasm lines of *G. herbaceum* and *G. arboreum* was comparatively smaller than the conidiophores from the varieties/ hybrids of *G. hirsutum*.

Thirteen isolates, four from the cultivars of *G. herbaceum*, six from *G. arboreum* and three from *G. hirsutum* were inoculated on 26 different cultivars belonging to four cultivated species of cotton. The isolates from *G. arboreum*, *G. herbaceum* and *G. hirsutum* were able to easily infect the cultivars/lines of these species respectively. However, the lines of *G. barbadense* were almost free to all thirteen isolates. The variability in host reaction of thirteen isolates to

26 different cultivars of four species indicate the existence of races/biotypes in *R areola*.

Twenty arbitrary primers from kit OPA were tested for their RAPD pattern by using genomic DNA from isolates of *R. areola*. Primer OPA-3 successfully amplified most of the isolates. RAPD-PCR pattern of amplification with primer OPA-3 from the isolates of *G. arboreum*, *G. herbaceum* and *G. hirsutum* gives clear indication of variation among the isolates at species level.

Fusarium oxysporum f.* sp. *vasinfectum

Thirteen cultures of *F. o.* f. sp. *vasinfectum* were isolated from different cotton growing areas of Maharashtra. Growth pattern, influence of salt concentration on growth and pigmentation of 13 isolates indicated the variability among the isolates. Pathogenic variability of these cultures was tested on susceptible cultivar G-27. The mortality varied between 30 to 100 per cent within 30 days of germination indicating the variability among the isolates.

The protocol for isolation of DNA from *F. o.* f. sp. *vasinfectum* was standardized. A PCR assay was developed for the detection of *F. o.* f. sp. *vasinfectum*. Twenty arbitrary primers OPF (Operon Technologies) were tested for RAPD pattern. Out of these, primer OPA-5 successfully amplified most of the isolates.

Coimbatore

Development of diagnostic tools for differentiation of biotypes/races of pathogens and insect pests

Insect pests: *Helicoverpa armigera*

Larvae were collected from cotton, bengal gram and pigeon pea from different locations and were reared in 12 well multicell well plates, on semi synthetic diet at 27±1°C and 75% RH. The larvae were kept for starvation for 14-18 h to avoid the interference of host DNA and used for isolation of genomic DNA of the larvae. A total number of 19 samples of DNA were isolated for subsequent PCR studies.

Morphological markers

Cornutal spine numbers varied from 9-14 in cotton and from 10-12 in pigeon pea and 12-13 in chickpea. Maximum number of individuals collected from cotton recorded a spine number of 12 followed by 11. Differentiation of cotton and non cotton strains was also attempted by measuring the weight gain after feeding for seven days with 10 day old squares of cotton cv. LRA 5166.

Pathogens

***Ramularia areola* Atk**

During 2004 - 05, heavy incidence of Grey mildew was noticed on all four cultivated *Gossypium* spp. For the first time, many germplasm lines of *G. barbadense* were found affected by this disease. However, the damage to the foliage was not to the extent as on other three cultivated species. In addition, Grey mildew like symptoms were also observed extensively on the common weed (*Euphorbia heterophylla*) found near the cotton fields.

Diagnosis through disease symptoms and morphology

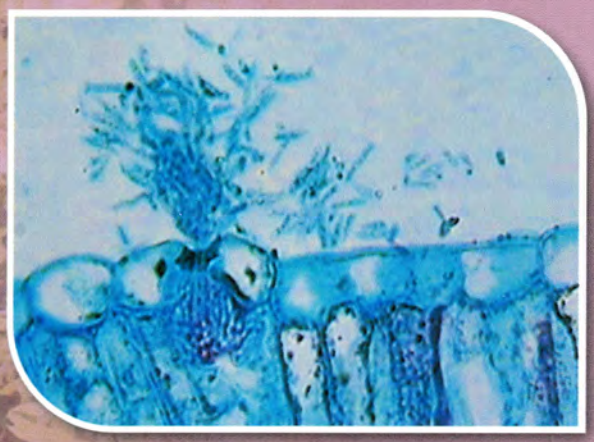
The *R. areola* isolates have been categorized into four groups based on the disease symptoms and the morphology of the fungus on the hosts.

***Arboreum and Herbaceum* isolate:** Irregular, angular/areolate spots with powdery growth appear on the under surface of the leaves with corresponding yellowish green lesions on the upper surface. In severe cases, the mildew also appears on the upper surface. The conidiophores short, emerging through epidermal layer in clusters from the sub-epidermal stroma bearing 0 – 3 septate, cylindrical/ oblong conidia singly on each conidiophore.

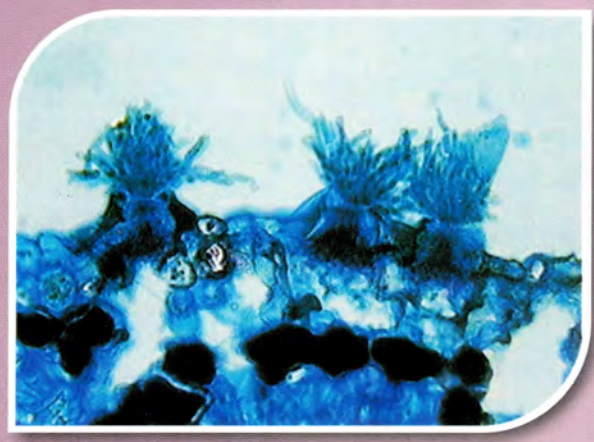
***Hirsutum* isolate:** Initially scattered greyish white powdery growth on the under surface of older leaves at the base of the plant with corresponding yellowish green spots on the upper surface. As the disease develops, the powdery growth spreads on the entire leaf and also on the upper surface. The conidiophores long, emerging through the epidermis in clusters from

Ramularia areola on different hosts

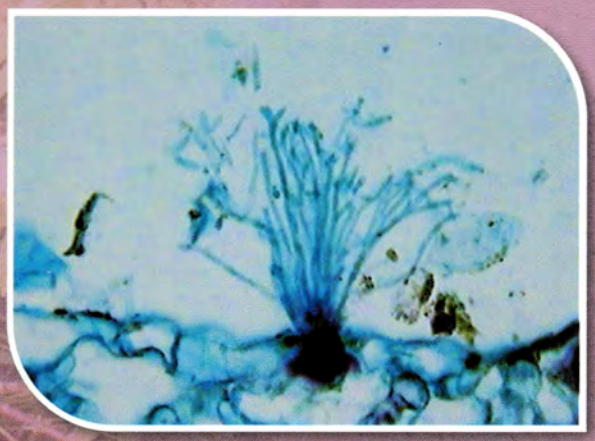
G. arboreum



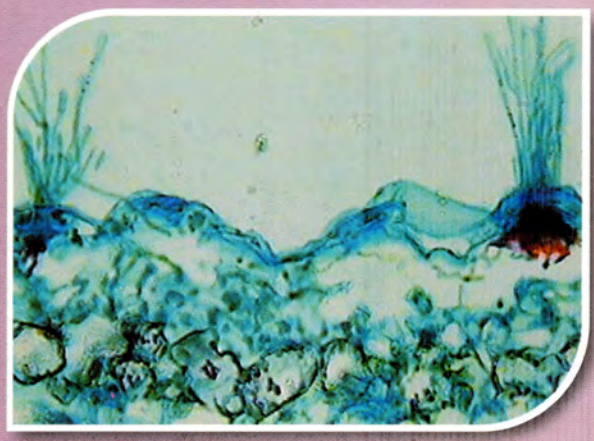
G. herbaceum



G. hirsutum

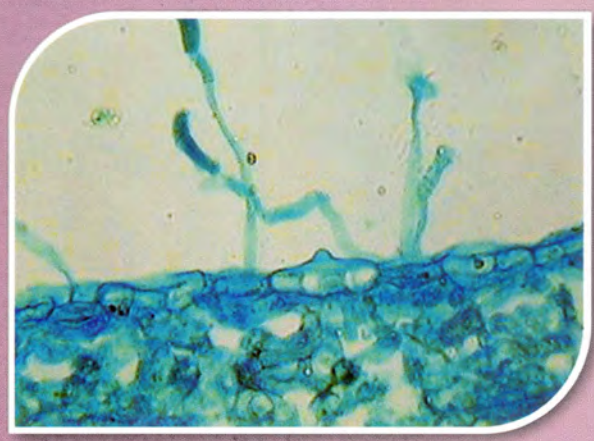


G. barbadense



E. heterophylla

E. heterophylla



a sub-epidermal stroma-bearing single 0 – 3 septate cylindrical/ oblong conidium on each conidiophore.

***Barbadense* isolate:** Scattered greyish powdery growth mainly on the under surface of the leaves with yellowish/brownish spots seen on the upper surface necrotic spots are also seen on the upper side of leaves. Hyaline long conidiophores borne singly or in bunches emerging through epidermis from a sub-epidermal stroma bearing single celled or multi-septate, cylindrical or oblong conidia singly on each conidiophore.

***Euphorbia* isolate:** Large areolate powdery spots seen on the under surface of the leaves with visible chlorotic (yellowish green discolouration) on the upper surface. Under severe disease development, powdery growth seen on the upper surface also. The conidiophores hyaline, single, long and flexuous; emerging through epidermal layer and bearing a single cylindrical, club or spear shaped conidia; the conidia are single celled.

Pathogenic variability on different hosts

Six genotypes of *G. hirsutum*, nine of *G. arboreum*, four of *G. herbaceum*, five of *G. barbadense* (identified from earlier studies) and the weed host (*E. heterophylla*) were raised in pots in polyhouse condition and inoculated with spore suspensions of *R. areola* collected from the various hosts.

The weed (*Euphorbia heterophylla*) has been identified as an alternate host for *R. areola*. The isolate from *G. barbadense* was the most virulent followed by the isolate from *G. herbaceum*. Similarly the lines LRA 5166 (*G. hirsutum*), Cernuum, Chandrolla and G. 27 (*G. arboreum*) and Jayadhar (*G. herbaceum*) and the weed host have been identified as the universal suscepts. All *G. arboreum* lines except AC 36 and *G. herbaceum* lines have been found highly susceptible to all isolates of *R. areola*. There was only hypersensitive reaction or few spots to the weed isolate on IC 629 (*G. hirsutum*), AC 36 (*G. arboreum*), GB 119 and ERB 3758 (*G. barbadense*). Similarly among the *G. barbadense* lines, GB 119 expressed hypersensitive reaction to all isolates; GB 124 resistant (no symptoms) to both *hirsutum* and *arboreum* isolates and ERB 3758 resistant to the *arboreum* isolate and hypersensitive or few spots to *hirsutum*, *herbaceum* and weed isolates. Suvin was resistant to the *arboreum* isolate.

Biochemical basis of variation

Eventhough there were minor differences among the cotton leaf constituents estimated (phenol, protein, proline and gossypol contents), these might not serve as a tool for differentiation of *R. areola* isolates. However, there were differences in the defense enzyme activities.

- The *barbadense* isolate when inoculated on the four *Gossypium* spp. exhibited higher catalase activity and low super oxide dismutase (SOD) and Ascorbic acid oxidase (AAO) activities except on LRA 5166 (*G. hirsutum*).
- The *herbaceum* isolate when inoculated showed lower catalase activity (except on Cernuum – *G. arboreum*) and very high AAO activity (except on Suvin – *G. barbadense*)
- The *hirsutum* isolate led to high SOD activity upon interaction with all hosts.
- The *arboreum* isolate had exhibited high polyphenol oxidase (PPO) activity on RAHS 14 (*G. herbaceum*) and LRA 5166 and low PPO activity on Cernuum and Suvin.

The results indicate that host defensive enzymes can be used for the differentiation of various isolates of *R. areola*.

4.22 Development of Molecular Tools

Rapid, PCR protocol was deployed for detection of CLCuV infection in *G. hirsutum* cotton, weed hosts as well as from diploid cotton grown in the vicinity of CLCuV infected *G. hirsutum*. Besides, detecting infection in plants showing typical symptoms of disease, primers also detected infection in several asymptomatic cotton by amplification of a 0.7 kb D.

Three genomes of 0.7, 1.2 and 2.7 kb were amplified and cloned. These were used to detect leaf curl virus from symptomless cotton and weed plants using dot blot method.

NA fragment.

Diploid cotton also generated some non-specific amplicons of size less than 0.7 kb. Southern hybridisation of these PCR amplicons with CP gene as DNA probe showed that the probe did not hybridise to the amplified fragments ruling out any possibility of diploid cotton serving the collateral host to the pathogen.

The conditions for expression of CLCuV coat protein in *E. coli* were standardized. Protein expression and western blotting of several recombinant clones were done to evaluate the level of coat protein expression. In an effort to further improve expression of recombinant coat protein in *E. coli*, the gene was swapped from pCaln (Stratagene) expression vector. The new vector pET 27b (Novagen) was transformed in *E.coli* and prepared for cloning the gene for expression.

Sirsa

Development of diagnostic tools for differentiation of isolates of root rot of cotton

The isolates of *R. bataticola* and *R. solani* were amplified using different OPM and OPN series primers. On the basis of dendrogram, the *R. solani* isolates were classified into four and *R. bataticola* isolates into two broad groups. The Group II was further subdivided into five sub groups. The mortality in case of group I in *R. solani* isolates was maximum and it was minimum in group IV. Groups II and III showed mortality in between group I and IV. In case of *R bataticola* isolates, no clear cut trend with respect to mortality and molecular groupings was noted (Table 12).

Table 12 : Grouping of isolates on the basis of RAPD data and pathogenicity

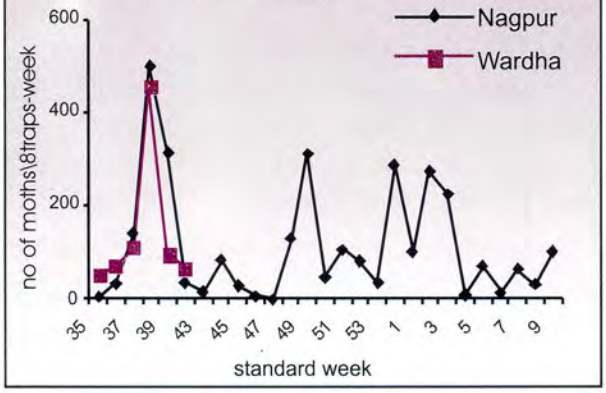
Group	*R. solani* isolates	Mortality (%)	Group	Mortality (%)	*R. bataticola* isolates
I	2, 3, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15 & 16	75.0-100.0	I	41.6	16
II	19, 20, 22 & 23	41.6-100	II IIa	25.0,58.3	1 & 11
III	17, 18 & 21	58.3-91.6	IIb	16.6-58.3	2, 3, 4, 5, 6, 7, 8, 9 & 19
IV	1, 4 & 11	15.8-75.0	IIc	16.6-75.0	10, 13, 17, 18, 20, 21, 23, 24 & 25
			IId	33.3,83.3	14 & 15
			IIe	58.3,66.6	12 & 22

4.23 Epidemiology

Analytical approaches were developed to arrive at forecast models predicting pest abundance as well as their outbreaks. During the season, the oviposition *H.armigera* on cotton occurred at 2450.5 degree/days from the calendar year. The rainy days of more than two in each of 35 & 41 standard weeks (SW) led to higher incidence of *H.armigera* in cotton ecosystem. The critical periods of weather influence were SWs 35, 41, 45 & 47 corresponding to rainy days, maximum temperature and rainfall, respectively. Higher levels of humidity (> 70%) throughout the day during August-September months, with high rainfall during the season distributed on many rainy days, and rainfall amount more than 50 mm during October resulted in outbreak of *H.armigera*. Greater than 33 °C maximum temperature, less than 70 % morning relative humidity, greater than 40 % evening relative humidity and less than 12 °C minimum temperature during standard weeks of 40, 41, 43 onwards, 48 and 49, respectively were evolved as criteria for *P. gossypiella* severity in Central zone. At South zone greater than 50 mm total rainfall during 43rd and 44th standard weeks, fall in evening relative humidity from 43rd SW with < 65% during 45th standard week would result in *P. gossypiella* severity.

The distribution of male moths that differ in their cornutal spine numbers caught in pheromone traps was studied across the season in both Nagpur and Wardha (Fig.15).

Fig. 15 : Pheromone trap catches of *H.armigera*



The 39th week, and the 3rd week comprised of *H. armigera* peaks on cotton and chickpea respectively. The 49th week corresponded to a peak on cotton overlapping with tur and it comprised of more numbers of non-cotton individuals. The 46th and 47th week did not record moth trap catches on cotton.

Coimbatore

Survey for the incidence of grey mildew and alternaria leaf spot

Severe incidence of *Alternaria* leaf spot was observed on cotton at all places in Tamil Nadu during late September and early October when there was continuous wet spell due to southwest as well as northeast monsoons. Further spread was restricted due to dry spell that prevailed after October 2004. During winter season of 2004-05, grey mildew incidence was severe at all places in Tamil Nadu as well as at Dharwad and Siruguppa in Karnataka. Earliest incidence of the disease was noticed during the first week of October, 2004.

Yield loss assessment due to *Ramularia areola*

Spore inoculum of *Ramularia areola* was applied on cotton cv. LRA 5166 in the field on 35 and 50 DAS followed by continuous spray through sprinkler to create epiphytotic conditions. The fungicide carbendazim 50 WP @ 0.1% was sprayed as per schedule at 35, 50, 65, 80 and 95 DAS depending on the treatments which reduced the grey mildew incidence and also increased the seed cotton yield to an extent of 25 % over the check (water sprayed).

Yield loss assessment due to bollworms

Sumangala followed by LRA 5166, Anjali and Surabhi recorded 8.9, 15.5, 16.3 and 44.2 per cent yield loss in varieties while Bunny followed by DCH-32 and NHH 44 recorded 6.9, 18.7 and 21.1 per cent yield loss among hybrids.

Sirsa

Epidemiological studies of cotton leaf curl virus disease

In epidemiological studies of cotton leaf curl

virus disease, ordinary runs, doublet and corrected doublet analysis was carried out for determining the pattern of CLCuV infected plants in two one acre fields selected at CICR farm and village Khairekan. The pooled data suggested mostly clustering of infected plants showing that a part of inoculum comes from outside and then the disease is spreading from plant to plant within a field.

Based on the incidence of cotton leaf curl disease (CLCuD) in different years, the data of two years i.e. years with maximum (Season 2001-Disease incidence- 98%) and minimum (Season 2004-Disease incidence- 19%) disease incidence on *G. hirsutum* variety HS-6 grown in screening nursery was compared with respect to weather parameters to pin point the role of weather factors on disease development. It was observed that higher maximum temperature (41.54°C 2004 & 36.11°C 2001), lower maximum (68.57% 2004 & 81.71% 2001) and minimum (51.00% 2004 & 61.88% 2001) relative humidities and lower rainfall (0.0 mm 2004 & 17.88 mm 2001) upto 30th meteorological week (23rd-29th July) led to lower incidence of cotton leaf curl virus disease during 2004 crop season as compared to 2001 season.

Further data on the incidence of CLCuD for six years (1999-2004) from 27th to 33rd meteorological week (MW) was regressed using step down regression procedure against six weather factors (maximum and minimum temperature, morning and evening RH, rainfall and sunshine hours) for one to three lag weeks to develop prediction equations. Based on the equations it was found that the incidence was mainly influenced by minimum temperature, evening RH and sunshine hours. It was also noted that these weather factors of two lag weeks are having maximum influence on the disease incidence, as they were represented in many of the equations. However, on 28th and 31st MW the minimum temperature and sunshine hours respectively of 3rd lag week influenced the disease incidence.

4.24 Management of Pests

Studies on the role of insecticides in cotton ecosystem

Nagpur

Testing of S1812, individually and in combination with Meothrin

S 1812 (Pyradyl) was tested in three doses, 50 g, 75 g and 100 g a.i per hectare, individually. It was also tested in combination with Meothrin (fenpropathrin) in 3 combinations (S1812 + Meothrin 50+50, 50+ 75, 75 +75 g a.i./ha). Indoxacarb, Endosulfan and Metasystox were also included as treatments at the recommended doses in the replicated experiment.

S 1812 (100 g a.i/ha was the most effective against aphids followed by S 1812 (50 g a.i/ha) and S 1812+ Meothrin (75+75 g a.i/ha), especially during the first spray. S 1812+ meothrin was on par with indoxacarb against jassids. S 1812 at the three doses tested and S 1812+ meothrin at the 3 combinations tested were effective against thrips. The least square damage was recorded in S 1812 + Meothrin (75 +75 g a.i./ha) followed by S 1812 + meothrin (50 + 75 g a.i/ha). The new molecules at the doses tested were on par with Indoxacarb against damage by bollworm both during the first and second sprays. There were no significant differences between the treatments against the whiteflies. No phytotoxic symptoms were observed at the doses tested. There were no significant differences between the yields obtained from different treatments

Testing of Servo Agrospray oil S

Servo Agrospray oil S, a product of the Indian Oil Corporation Limited is being used as foliar sprays in orchard crops. It was tested for the first time on cotton at CICR, Nagpur in the year 2004-05.

Servo Agrospray oil S was superior to control i.e. a plot wherein no chemical intervention was resorted to after the first and second spray. Servo Agrospray oil S at the lowest concentration of 0.5%

was effective against aphids and jassids, thrips especially during the first spray and was on par with the insecticidal treatment endosulfan. No square damage was recorded in the aforesaid Servo oil treatments as was observed with endosulfan. Indian Oil's Servo Agrospray oil S was effective against the sucking pest complex, especially, aphids, jassids and thrips and was found to be on par with the recommended dose of endosulfan on CNH 120MB.

Coimbatore

Efficacy of newer insecticide molecules against cotton pests and their natural enemies

Effect on fruiting body damage: Spinosad (50, 75, 100 g), NNI 0001(48, 60 g) and RIL 038 (50, 60 g) were effective in reducting the fruiting body damage during 93 to 123 DAS.

Larval incidence of Bollworms: (a) ***H. armigera***: Spinosad 45 SC all the three doses, NNI 0001 @ 48 and 60 g, KN 128 and RIL 038 @ 50 and 60 g and Spinosad 45 SC (Standard) were found effective in reducing the *H. armigera* larval incidence during 93 to 110 DAS. (b) ***Earias* spp.**: Occurrence of spotted bollworm was very low and ranged from 0.0 to 3.0 larvae per 5 plants in the various treatments. There was no significant difference among the treatments. (c) **Pink bollworm:** Pink bollworm incidence was significantly low in Spinosad at 100 g, Karate Zion @ 20 and 25 g, Karate 5 EC @ 25 g and Endosulfan @ 700 g treated plots as compared to control. The larval population ranged from 0.7 to 2.7 per 20 green bolls in the above treatments as against 6.3 in control.

Pink bollworm damage in green bolls: Spinosad @ 100 g alone was most effective in reducing green boll damage. Karate Zion 5 CS @ 25 g and Karate 5 EC @ 25 g were also effective in significantly reducing the loculi damage in green bolls.

Boll damage and seed cotton yield: There was no significant difference among the treatments in reducing the damage to bolls and loculi and seed cotton yield.

Impact on sucking pests and natural enemies: There was no much difference among the treatments in recording the sucking pests (aphids, jassids and whitefly) and predators (coccinellids and spiders). However, pyrethroid treatments Karate Zion 5 CS @ 20, 25 g and Karate 5 EC @ 25 g treatments harboured higher number of aphid population as compared to other treatments including control.

Control of Sucking Pests

Imidacloprid 350 SC 60 ml/ha, 75 ml/ha and imidacloprid 70 WG @ 40 g/ha and 35 g/ha and Clothianidin 50 WG @ 40 g/ha were sprayed on 35, 55 and 70 days after sowing. Clothianidin had the minimum incidence of 0.24 aphids per plant and remained on par with other insecticides, but superior to methyl-o-demeton and untreated check. With regard to jassid also, Clothianidin recorded the minimum incidence of 0.58 and remained on par with other insecticides and superior to methyl-o-demeton. Imidacloprid 70WG @ 30 g/ha recorded the maximum seed cotton yield of 2174 kg/ha and was superior to methyl-o-demeton and untreated check.

Carbosulfan 25 EC was tested against aphid and jassid at two dosages (1 lit/ha and 1.2 lit/ha) and compared with imidacloprid 200SL @ 100 ml/ha. Among the insecticides tested, imidacloprid recorded the minimum aphid population of 0.68/plant as compared to 6.41 in untreated check and 2.11/plant in Carbosulfan @ 1.2 lit/ha. With regard to jassid, imidacloprid 100 ml/ha recorded the minimum population of 2.33/plant as compared to 4.38 and 4.69 recorded in untreated check and carbosulfan 1.2 lit/ha, indicating the ineffectiveness of carbosulfan against jassid. There was no significant difference with regard to seed cotton yield.

Studies on bio ecology and management of cotton stem weevil *Pempherulus affinis* Faust

Evaluation of new neem products and insecticides

Three dose of Neemazal granules namely 7.5, 10 and 12.5 kgs /ha, carbofuran 33.33 kgs/ha and phorate 10 kgs/ha were evaluated against stem weevil. The treatments Phorate, carbofuran and Neemazal 12.5 kgs

/ha recorded significantly minimum percentage of incidence on par with each other and superior than the control.

Impact of organic manures

To study the impact of organic manures on the incidence of stem weevil *P. affinis*, five organic manures namely farmyard manure 12.5 t/ha, farm boon 1.25 t/ha, cell rich 1.25 t/ha, vermi-compost 1.25 t/ha and neem cake 150 kg/ha were applied twice at 30 DAS and 45 DAS. The different treatments were replicated four times in RBD design. Neem cake recorded significantly minimum percentage of incidence and on par with Farm boon.

Field trial against stem weevil at farmers' field

Two field trials were conducted against stem weevil with the variety Surabhi at Kanjapalli and Allapalayam villages of Annur block, Coimbatore Among the treatments, application of Neemcake (150 kgs/ha) + Carbofuran (1.0 kg a.i/ha) and application of neem cake (150 kgs/ha) +carbofuran (1.0 kg ai /ha), followed by stem drenching with neem seed kernel extract 5% were on par with each other and superior than the other treatments. There was no incidence of grubs when treated with above insecticides; however in control more numbers of grubs were recorded.(Table 13).

In another trial, it was seen that Carbofuran (1.0 kg a.i/ha) + chlorpyriphos (0.08%) recorded significantly minimum percentage of infestation and superior than the other treatments.

Bio Control Studies

Nagpur

Characterization of biocontrol agents

Antagonist

Soil samples were collected from cotton rhizosphere and CICR microbes were isolated by serial dilution method. Morphological and biochemical studies showed that eight out of nine microbes were the members of fluorescent *Pseudomonas* while one was identified as a strain of *Bacillus firmus*. All strains produced inhibition zone ranging between 10-50 mm in dense lawn of *Xanthomonas* grown in YGCA. The microbes were analysed for their ability to liquify gelatin and produce antimicrobial biochemicals like H_2S, levan, protease, siderophore, fluorescin and pyocyanin. Although all of them effectively inhibited the growth of *Xam*, the bacterial strains exhibited variability in their mode of antagonism. *P.fluorescens* strain CICR which caused highest inhibition of *Xam in vitro* was also the most efficient to liquify gelatin and produced highest concentrations of levan and protease but was poor in siderophore production. *Pseudomonas* strain H1a and *Bacillus firmus* strain CD1 were efficient in production of H_2S. Strains P1a, P1b and H1a efficiently produced H_2S, which was at par with CICR strain. Protease production was highest in *B. firmus* strain CD1 and *Pseudomonas* strains H1a and CICR. Strain K1a produced highest concentration of

Table 13 :Evaluation of neem cake with insecticides against stem weevil – Field trial at Farmers' fields

Treatments	Stem Weevil incidence (%)	Grubs (%)
Neem cake (150 kg/ha) + Carbofuran (1.0 kg a.i ha)	7.00 (15.15)	0.00 (2.87)
Neem cake (150 kg/ha) + Carbofuran (1.0 kg a.i ha) + NSKE 5%	8.80 (17.18)	0.00 (2.87)
Neem cake + Carbofuran (1.0 kg ai ha) + Confidor (0.04%)	17.40 (24.62)	0.00 (2.87)
Control	59.40 (50.49)	47.80 (43.74)
CD (p=0.05)	3.67	4.32

siderophore, an iron chelator while F1b and P1a produced highest concentration of fluorescin. P1a also produced highest concentration of pyocyanin.

The presence of genes governing production of DAPG was detected in strains K1a, CICR, H1a and CHAO by amplification of a 0.7 kb DNA fragment using primers conserved to phloroglucinol gene. The PCR amplified fragment was cloned. PCR and RFLP based DNA fingerprinting showed patterns unique to each strain of antagonists.

Out of 148 phylloplane rhigosphere bacterial cultures, 17 were observed to be promising inhibitors showing the inhibition zones ranging from 9-15 mm against the virulent race 18 of *Xam.*

Coimbatore

Biological control for management of grey mildew

Talc formulations of three fungal bioagents *viz., Trichoderma viride, T. harzianum* and *T. virens* and two bacterial bioagents *viz., Pseudomonas fluorescens* strains Pf1 and CHAO and combinations of the above fungal bioagents with *P. fluorescens* Pf1 strain and the standard fungicide propiconazole were sprayed four times in the field at 10 and 15 days intervals following the appearance of grey mildew on cv. Sumangala. The fungicide propiconazole (6.04 PDI) gave the best control. However, spraying of talc preparations of *Trichoderma harzianum* (26.39 PDI) and *Pseudomonas fluorescens* CHAO (27.42 PDI) at 10 day intervals greatly reduced the grey mildew incidence, when compared to the check (41.16 PDI).

Entomopathogenic nematodes (EPN)

Nagpur

Modification of media for *Metarhizium* and *Nomurea* culture.

Protocol was standardized using soaked grains of rice and jowar fortified with 1% yeast granules for development of *N. rileyi* mycelia and sporulation and metarhizium.

Results indicated that sufficient variability exists in temperature tolerance of different EPNs. Isolates of EPN from hot cotton growing areas were better adapted to higher temperature regimes whereas those isolated from comparatively cooler areas were better suited for lower temperature regimes. *S.glaseri* was better adapted for searching the host. The isolates, which showed better vertical host finding ability, were also better at horizontal host searching ability.

Induction of tolerance to high temperature and moisture stress in EPNs

The results indicated that HI5 isolate of *H.indica* was more amenable to selection for all *viz.*, the three attributes temperature stress, moisture stress and host finding ability recording maximum percent increase in tolerance for these factors. One isolate of indica isolated from cotton field of CICR, Nagpur could be made to tolerate high temperatures. Besides *H. armigera*, this isolate was found to be effective against other cotton insect pests.

Two Photorhabdus isolates symbiont of Entomopathogenic nematode which were earlier recorded to be antagonistic towards sucking insect pests of cotton, were tested in preliminary field trials at Sirsa, Nagpur and Nanded for control of sucking pests of cotton. During preliminary laboratory work, the bacterial symbiont Photorhabdus sp. broth when sprayed was found to cause mortality of nymphs of sucking pests, *Aphis gossypii, Amrasca devastans, Bemisia tabaci* and *Thrips tabaci*. Field studies indicated that spray of *Photorhabdus* sp. broth as well as toxin extracted is effective against sucking pests. Repeating the spray after three days was found to substantially further enhance mortality of sucking pests.

An experiment set up to evaluate parameters influencing storage viability of *H.indica* isolates and evaluate anti-desiccants to see if they can enhance viability of EPN under storage conditions, indicated that *H.indica* having origin from hot dry cotton ecosystem store better at higher temperature of 28°C. Of the various anti-desiccants tested, A.V.gel at 1 and 10% was found to enhance storage viability.

Integrated Pest Management

Nagpur

Implementation of two pest management approaches viz., IPM with biocontrol options including ETL based insecticidal sprays and need based chemical sprays were undertaken in the farmers fields in addition to IPM on Bt cotton. The differences for incidence of sucking pests, bollworms, native predators and bollworm damage between IPM and NBC farms were non significant. Sucking insects had increased pest status on Bt-cotton. The IPM practices had higher CB ratio over NBC in all the three villages.

Field experiment was conducted to quantify the efficacy of individual components of IPM at research station using cotton cultivar NHH 44. Based on crop phenology and seasonal occurrence of insect pests in relation to pest management options the scheme of measures ideal under rainfed IPM system are: avoidance of prophylactic measure of seed treatment, early season sucking pest management fully based on host plant resistance, avoidance of insecticidal application during the first peak of *H. armigera* occurring on first flush when more than 90 % of fruiting structures are squares, use of boll damage based ETLs, *Trichogramma* release coinciding with second peak of *H. armigera* oviposition alone, focusing bollworm management during October to November months and proper selection of insecticide considering the gain threshold. Rainfed cotton IPM proved to be cost effective and profitable with focus on conservation of native natural enemies and strict adherence to judicious application of insecticides.

Coimbatore

Integrated pest management (IPM) at village level to produce cost effective quality fibre

The success of the location specific IPM developed at this centre has been evaluated in five villages in Annur block of Coimbatore District. Encouraging results were obtained in terms of reduction in the quantity of insecticides applied from 2607 to 1086 g a.i./ ha, savings on plant protection cost by Rs. 688/ha and increase in yield by 350 kg/ ha. Efficient pest management including resistant pest *H. armigera* led to higher income of Rs.9003/ha to the project farmers as against Rs. 2482/ha to the control farmers (Fig.16).

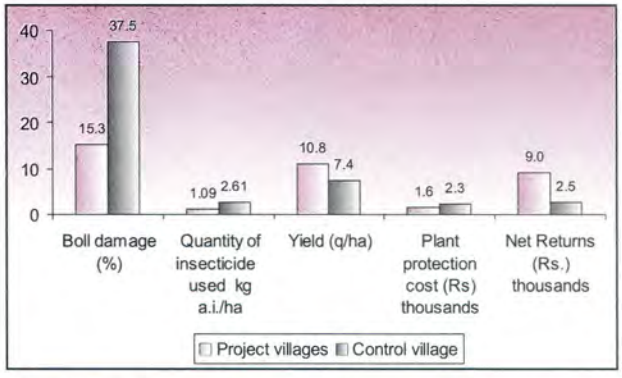


Fig. 16 : Economics and other attributes in IPM and Control Villages (2004-05)

Sirsa

The village Rangri in Sirsa district was selected for demonstrating and refining the following IPM tool kits.

- Farm yard manure application was done in the preceeding wheat crop. Emphasis was made to reduce the synthetic fertilizers.
- Emphasis was given for the selection of released varieties / hybrids.
- Bird perches were erected @ 3-5 sticks / acre.
- The pheromone traps for American, spotted and pink bollworms were installed.
- Sprays of NSKE 5 % and neem oil 1% in alternation with synthetic insecticides to scare away bollworm adults.
- Besides, the insecticides such as profenophos, and spinosad were sprayed. In general the total number of insecticidal sprays amounted to two in IPM fields

Among all the entries the maximum yield of 30.8 q / ha was noted in *desi* cotton in IPM plot followed by 29.73 q/ha in hybrids and 27.30 q/ha in American

cotton. In *desi*, the cost : benefit ratio was more in IPM (1 : 3.61) than non IPM (1 : 2.65). The C : B ratio of 1 : 3.60 was obtained in IPM plots of American cotton followed by 1 : 3.42 in the hybrids IPM. The increase in C : B ratio was mainly obtained because of 14.28 % reduction in spray and that too 4 out of 6 sprays consisted of low cost neem products.

The yield in RCH 134 Bt was 33 % more than its non Bt counterpart, and in MRC 6301 and 6304 it was 17 and 7 % respectively than non Bt LHH 144. However, the yield was slightly less in RCH 317 Bt cotton than its counterpart, which may be because of poor plant stand in this Bt cotton plot. The cost : benefit ratio in Bt cotton ranged from 1 : 3.52 to 1:2.11 and in non Bt it was from 1 : 2.64 to 2.08.

Insecticide Resistance Management

Nagpur

Dissemination of Insecticide Resistance Management programme

The IRM strategies were implemented in an area of 59,233 ha in fields of 20,525 farmers of 444 villages in 30 districts of 10 cotton growing states.

Studies on toxicity of Bt (*cry*) toxins to cotton pests, assessment of impact of Bt transgenic cotton plants on the ecosystem and development of resistance to Bt toxins in cotton bollworm *Helicoverpa armigera.*

Monitoring resistance in *H. armigera* to *cry*1Ac

Field populations of the cotton bollworm *Helicoverpa armigera* were collected from 22 locations. Log dose probit assays with *cry*1Ac showed that there has been a slight shift in the baseline toxicity values in Vadodara and Surendranagar of Gujarat and Abohar district of Punjab.

A novel bioassay on *H. armigera* utilizing Bt-cotton seed

A simple bioassay on *Helicoverpa armigera*, utilizing Bt-cotton seed as a source of *cry*1Ac toxin is described. The *cry*1Ac content in seeds was found to be 1.77 + 0.23 µg/g and the variability between individual seeds and seed lots was minimal. Bioassays on *H. armigera* using Bt-seeds stored at room temperature for two years showed that there was no significant reduction in bioactivity of the toxin present in the seeds. A discrimination dose assay utilizing 160 g Bt seeds in 1.3 L diet is proposed for detection and monitoring of *H. armigera* resistance to *cry*1Ac based Bt-cotton.

Inheritance of *H. armigera* resistance to *cry*1Ac

The mode of inheritance of the cotton bollworm, *Helicoverpa armigera* (Hübner) resistance to *cry*1Ac toxin of *Bacillus thuringiensis*, was elucidated through bioassay analysis of the response of resistant, susceptible, F_1 hybrid and backcross *H. armigera* progeny to *cry*1Ac in semisynthetic diet and transgenic Bt-cotton plants. The values of estimates of dominance were found to range between 0.40 – 0.57. Resistance was found to be monogenic, autosomal and inherited as a semi-dominant trait. The genetic studies of response of *H. armigera* to transgenic Bt-cotton showed that the effective dominance was also inherited as a semi-dominant trait.

Development of a SCAR marker to detect the frequency of *cry*1Ac resistant alleles in field populations of *Helicoverpa armigera*

A Cry1Ac near-isogenic *H. armigera* line was developed and subjected to RAPD analysis using recurrent parent as control. A total of 120 primers were used. Nine primers were found to clearly distinguish the isogenic line from the parent strain. The primers were tested with resistant individuals of *cry*1Ac resistant and susceptible populations. Four bands, which co-segregated with resistance were isolated, cloned and sequenced. Based on the unique sequences obtained four pairs of SCAR markers were designed. The markers are being validated with field populations.

Estimation of *cry*1Ac and its toxicity to *H. armigera* in Bt-cotton

The survival of *H. armigera* was correlated to the variable expression of *cry*1Ac in leaves and other

fruiting structures. Increasing levels of *H. armigera* survival were correlated with the toxin levels decreasing below 1.8 µg/g in the plant parts. Genotype independent seasonal decline of the *cry*1Ac toxin levels was observed in all the hybrids. *cry*1Ac expression decreased consistently as the plant aged. The choice of parental background appeared to be crucial for sustainable expression of the *cry*1Ac transgene.

*cry*1Ac Resistant allele frequency in *H. armigera*

The initial frequency of resistant alleles was estimated using an F_2 screen test on 180,195 and 210 isofemale lines of *H. armigera* collected from north, central and southern parts of the country. A Bayesian analysis of the data indicated the respective frequency of resistance alleles as 0.0023, 0.0025 and 0.0013 with 95% probability, and a detection probability of >80%.

Coimbatore

Dissemination of IRM strategies in Coimbatore and Theni Districts of Tamil Nadu

Through farmers participatory approach, IRM strategy was successfully disseminated in 16 villages in Avinashi block of Coimbatore District. The project covered 448 farmers and an area of 385 ha. The pest damage, number of sprays and cost on plant protection was reduced substantially in the project villages as compared to control villages. Further, seed cotton yield increased by 22.9 per cent over control villages. The project farmers obtained higher net return of Rs. 8289 / ha (Figure 17) over control farmers (Rs. 1117 / ha).

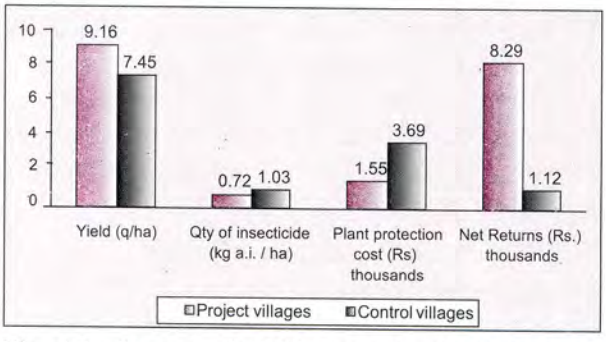


Fig. 17 : Impact of IRM Strategies in Coimbatore District (2004-05)

Discriminating dose bio-assay tests were carried out for assessing the resistance levels to different insecticides viz., synthetic pyrethroids (fenvalerate), organophosphorus compound (quinalphos) and cyclodine compound (endosulfan). The resistance levels were very high to fenvalerate (82.88), moderate level to endosulfan (40.59 %) and low level to quinalphos (21.57 %) (Table 14).

Table 14 : Resistance level of *H. armigera* to different insecticides (2004-05)

Insecticide	Dose (µg /µl)	Percent survival + standard error
Quinalphos	0.75	21.57 ± 4.09
Endosulfan	10.0	40.59±4.91
Fenvalerate	0.2	82.88±3.59

Sirsa

Basic studies on insecticide resistance

The insecticide resistance in general was slightly low in *Helicoverpa armigera* collected from IRM villages than non-IRM villages for all the six insecticide doses tested. The maximum resistance of 81.14% was observed for cypermethrin 0.1µg/µl in non-IRM villages compared to 77.64% in IRM villages, however at higher doses of cypermethrin (1.0 µg/µl) also the resistance was more in both non-IRM (70.39%)and IRM (65.88%). This was followed by methomyl 1.2 µg/µl (55.0 and 57.1% in IRM and Non-IRM villages respectively). The resistance was very low (18.18 and 20.13%) against endosulfan 10.0 µg/µl in IRM and non-IRM villages respectively. The resistance against organophosphate insecticide (Chlorpyriphos and Quinalphos) was ranging from 25 to 30 % in Sirsa district. In Hisar and Fatehabad districts also the same trend of insecticide resistance was observed in both the doses of cypermethrin. However the resistance was low in methomyl (20.83 to 34.66%) compared to Sirsa. In Hisar the resistance

to organophosphate insecticide such as Chlorpyriphos (50.66 and 52.00%) and Quinalphos (38.66 and 42.66%) in IRM and non-IRM villages respectively was more than Sirsa district. In Fatehabad the resistance against endosulfan (33.33 and 41.67%) was more than methomyl (20.83 and 26.67%), Chlorpyriphos (10.00 and 13.33%) and Quinalphos (33.33 and 40.00%) in IRM and non-IRM villages respectively.

Based on three years data of implementation of IRM strategies in Haryana, 32.80 to 47.98% reduction in number of sprays was achieved in IRM villages. The number of sprays in IRM villages was 4.39 to 5.10 as compared to 7.64 to 8.44 sprays in non IRM villages. Accordingly the cost of plant protection ranged between Rs. 1944 to 2686 in IRM villages as compared to that of Rs. 4420 to 4906 in non IRM villages.The average seed cotton yield ranged from 17.25 to 21.65 q / ha in IRM villages compared to that of 16.43 to 19.84 q / ha in non-IRM villages. The percent increase in yield ranged from 4.75 to 8.36 percent over non-IRM villages. This has led to increase in B:C ratio (1:2.93-4.14 in IRM village then 1:2.39-3.14 in non –IRM village) which subsequently yielded Rs.3760.0 to 6073.20 more net profit in IRM villages over non-IRM villages in the districts of Sirsa, Fatehabad and Hisar in Haryana.