Table 1. The SSA at Kanpur during the week of the Diwali festival

	Single scattering albedo (SSA)							
	Year 2001				Year 2002			
Days from Diwali	440 nm	675 nm	875 nm	1020 nm	440 nm	675 nm	875 nm	1020 nm
–1	0.89	0.883	0.866	0.861	0.909	0.861	0.855	0.85
0	0.917	0.913	0.912	0.91	0.869	0.867	0.85	0.874
1	0.902	0.88	0.872	0.865	0.85	0.843	0.805	0.855
2	0.875	0.874	0.852	0.846	0.852	0.804	0.849	0.859
3	0.872	0.87	0.865	0.85	0.855	0.771	0.828	0.842
4	0.90	0.899	0.894	0.891	0.863	0.81	0.853	0.861

absorbing characteristics[5]. During 2001, the SSA is found to decrease by 0.05 after the Diwali festival and during 2002 the SSA is found to decrease by 0.07. The decrease of SSA is found from 15 November in 2001 (one day after the main Diwali day) and from 5 November in 2002 (one day after the main Diwali day), which is due to the higher concentrations of absorbing aerosols. The higher change in SSA after the Diwali festival during the year 2002 compared to those from the Diwali festival of the year 2001 may be attributed to the prevailing meteorological conditions and the background aerosol concentrations. The values of SSA at 440, 675, 875 and 1020 nm wavelengths are shown in Table 1. The decrease of SSA with higher wavelengths (Table 1) is due to the higher absorption by smaller particles. But during the year of 2002, SSA at 1020 nm is found to be higher compared to those at other wavelengths which is attributed to the large increase in the volume concentrations of the aerosols around 1 μm size range (Figure 2 *b*) compared to the year 2001. The maximum decrease in SSA after the Diwali festival is found at 675 nm wavelength for both the years.

The increase of aerosols during the Diwali festival period, especially in urban areas is very common due to fireworks and crackers. Such increase in concentration of the aerosols has been found[3] over Delhi. The present results show that the AOD increase during Diwali festival period is reflected in the size distribution curves. In view of the increasing population and the use of fireworks and crackers in major urban areas, the routine measurements of aerosols using sunphotometers will help in guiding people and local administrators for the use of fireworks and crackers during Diwali festival period and also in understanding the changing climatology of Indo-Gangetic basin.

1. Parameswaran, K., *Proc. Indian National Science Academy,* 1998, **64**, 245–266.
2. Suresh Babu, S. and Krishna Moorthy, K., *Curr. Sci.*, 2001, **81**, 1208–1214.
3. Jain, S. L., Arya, B. C., Kumar, A., Singh, P., Tiwari, M. K. and Garg, S. C., *IASTA Bull.*, 2002, **14**, 146–148.
4. http://aeronet.gsfc.nasa.gov:8080/.
5. IPCC, *Climate Change: A Scientific Basis* (eds Houghton, T. *et al.*), Cambridge University Press, UK, 2001, p. 944.

ACKNOWLEDGEMENT. We thank the Indian Space Research Organization, Bangalore for financial support through ISRO-GBP. We also thank to the anonymous reviewer for useful comments.

Received 25 November 2002; revised accepted 3 April 2003

RAMESH P. SINGH*
SAGNIK DEY
BRENT HOLBEN†

Department of Civil Engineering, Indian Institute of Technology, Kanpur 208 016, India
†*NASA Goddard Space Flight Center, Maryland 20771, USA*
**For correspondence.*
e-mail: ramesh@iitk.ac.in

Baseline resistance to Cry1Ac toxin in cotton bollworm, *Helicoverpa armigera* (Hubner) in south Indian cotton ecosystem

Insecticidal protein genes coding for crystal (Cry) toxins of Cry1A group from the bacterium *Bacillus thuringiensis* (*Bt*) have been transferred to and expressed in a number of crops in order to confer resistance against lepidopteron insect pests[1–3]. Several transgenic crop species incorporating *cry1A* genes have been commercialized and cultivated in a number of countries over the past few years. *Bt* transgenic cotton was cleared by the Department of Biotechnology, Government of India for commercial cultivation from the year 2002, after a long debate and discussion. The primary target pest of this technology in India and several other countries is the cotton bollworm, *Helicoverpa armigera* (Hubner). It is a polyphagous pest with a wide host range of 181 plant species including cotton, pigeonpea, tomato, chickpea, maize, sun-

flower and several vegetable crops which is currently estimated to cause economic losses up to about Rs 250 billion in India[3,4]. Lately, the problems of pest management in cotton and other crops have been compounded by the development of resistance to insecticides in *H. armigera*[5]. This led to excessive and indiscriminate use of insecticides by desperate farmers in many parts of the country. Outbreak of *H. armigera* in south Indian cotton and pigeonpea ecosystem usually leads to severe socio-economical disturbances, including several cases of suicide by farmers. Introduction of insect-resistant transgenic crops, especially *Bt* transgenics, is expected to be useful in management and effective control of lepidopteron pests with a significant reduction in the overall use of insecticides. However, long-term exposure to *Bt* transgenic crops is likely to render lepidopteron pests resistant to the Cry toxins due to continuous selection pressure[6]. Moreover, the introduction of transgenic plants expressing a Cry toxin under the influence of constitutive promoters, is likely to hasten this process. The development of resistance to *Bt* toxins can be quite distinct, depending upon the species, selection regime or geographical origin of the founder colony[7]. Hence, initial survey to assess the susceptibility of test insect to the Cry toxins will establish a baseline that can be used in monitoring resistance development in future. We report the resistance of *H. armigera* to Cry1Ac toxin in 11 distinct geographic populations representing the entire south Indian cotton ecosystem.

The Cry1Ac protein was produced according to the method in Albert *et al.*[8] from *E. coli* strain containing hyper-expressivity recombinant plasmid vector pKK223, kindly provided by Daniel R. Zeigler, Ohio State University, USA. The toxin was purified from over-expressing cells by sonication and extensive washing with sodium bromide. Proteins were quantified according to Lowry *et al*[9]. and the toxin was quantified by SDS–PAGE densitometry before preparing dilutions (ranging from 10 to 20000 fold) in distilled water[10]. Forty per cent of the protein extracted from the recombinant *E. coli* cultures was found to comprise Cry1Ac toxin. LC_{50} values were determined for the toxin.

Laboratory strains of *H. armigera* were established from those collected in cotton fields during cropping season of 2001–02 from major cotton-growing regions of south Indian cotton ecosystem: Nagpur and Nanded (Maharastra); Guntur, Madhira and Nalgonda (Andhra Pradesh); Dharwad, Raichur and Mysore (Karnataka); Coimbatore, Madurai and Kovilpatti (Tamil Nadu). These 11 sampling locations represent the cotton growing ecosystems of south India (Figure 1). An insecticide-susceptible *H. armigera* obtained from ICRISAT, Patancheru, Hyderabad was used as baseline susceptible strain for comparison. Larvae were reared individually on chickpea-based semi-synthetic diet[11], in 32-well multicavity trays till pupation. Moths were kept in glass jar at 27°C ± 1°C and 70% RH and fed with 10% honey solution. A layer of muslin cloth was placed on the inner surface of jar for oviposition.

Laboratory cultures were established for each population of 500–650 moths and reared to get homogenous F_1 populations before conducting bioassays. Bioassays were carried out in 32-well multicavity culture trays. Six-day-old juvenile larvae (ca 30–40 mg) were tested, one per well, on cotton leaves dipped in different concentrations of the toxin. In all, 30 larvae in three replicates were tested for each treatment. Mortality was recorded daily for six days. All assays were repeated three times and pooled data were subjected to statistical analysis. Assays were performed in the laboratory at 27°C ± 1°C and 70% RH. Median lethal concentrations (LC_{50}) presented in Table 1 were derived from log dose probit calculations[12] using MLP 0.38 statistical package[13].

Cry1Ac protein was found to be toxic to all geographic populations tested (Table 1). The insecticide-susceptible ICRISAT laboratory strain was the most susceptible. Compared with the others, geographic populations from Nagpur, Nanded, Guntur, Nalgonda, Madhira and Raichur were found tolerant to the toxin. Mortality of different populations is presented in Table 1. LC_{50} values for Cry1Ac ranged from 0.147 to 1.095 µg/ml. The fiducial limits (at $P = 0.95$) of the probit assay data indicated that there was a good deal of variability in response of different populations to Cry1Ac. Kovilpatti (Tamil Nadu; southern most part of south Indian cotton ecosystem) population was found to be as susceptible as the laboratory strain for Cry1Ac. Coimbatore and Dharwad populations were similar to each other at a resistance factor (RF) of 1.5. Geographic populations of Guntur and Nanded recorded highest RF 8.03 and 8.42, respectively. The LC_{50} values of the test populations can be considered as the baseline susceptibility LC_{50} values for these individual populations and used for monitoring resistance in future.

For resistance management programmes to be effective, monitoring, surveillance and early detection of resistant pheno-

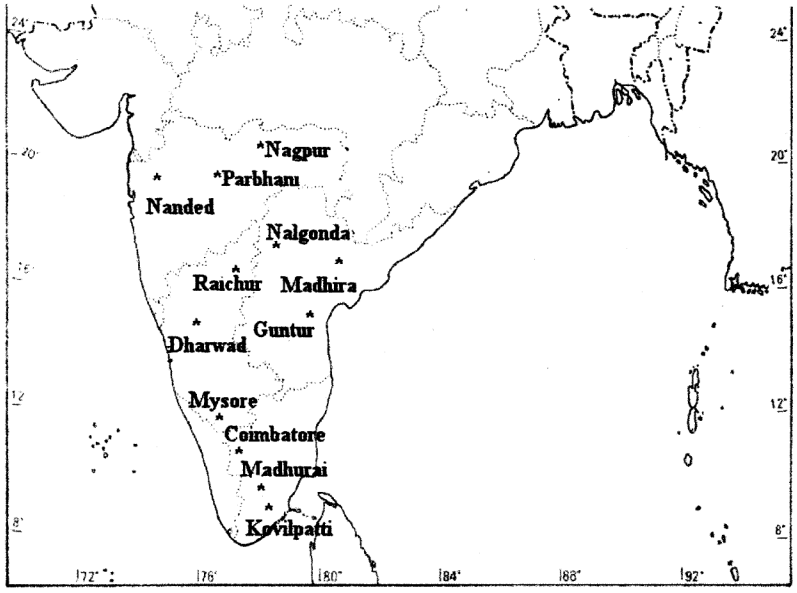

Figure 1. Geographic distribution of cotton bollworm populations in four states of south Indian cotton ecosystem used in the present investigation.

Table 1. Log dose probit response of south Indian cotton ecosystem field populations of *H. armigera* to Cry1Ac toxin

Location	LC_{50} (µg/ml)	95% FL		Slope	RF
		Lower	Upper		
ICRISAT†	0.130[f]	0.080	0.180	1.15	1.000
Nagpur	0.927[bc]	0.688	1.087	4.43	7.131
Nanded	1.095[a]	1.032	1.479	4.13	8.423
Guntur	1.044[ab]	0.980	1.154	8.67	8.031
Nalgonda	1.001[ab]	0.909	1.204	4.99	7.700
Madhira	0.927[bc]	0.688	1.087	4.43	7.131
Raichur	0.884[c]	0.647	0.975	4.57	6.800
Dharwad†	0.191[e]	0.094	0.277	1.07	1.469
Mysore†	0.260[d]	0.121	0.408	0.87	2.000
Coimbatore†	0.191[e]	0.094	0.277	1.07	1.469
Madurai†	0.177[ef]	0.061	0.302	0.77	1.362
Kovilpatti†	0.147[ef]	0.057	0.306	0.54	1.131

†Test for heterogeneity; $\div^2$ significant at 5% level of significance. LC_{50} values designated by different letters are significantly different from each other through non-overlap of 95% fiducial limits[25].

LC_{50}, Median lethal concentrations expressed in µg/ml diet; FL, Fiducial Limits; RF, Resistant Factors calculated using ICRISAT strain as susceptible reference.

types in the field populations are important pre-requisites in order to initiate timely remedial measures and to evaluate the effectiveness of resistance management strategies. Traditionally, log dose probit assays and recently diagnostic dose assays, have been routinely used to monitor development of resistance to insecticides[3,14,15]. Diagnostic or discriminatory dose assays are normally employed to identify individuals in a population resistant to the toxin[16], whereas, log dose probit assays are useful to assess the level of resistance of a population as a ratio over a reference strain or a population, usually a susceptible check. Therefore, for monitoring resistance built up in a population, diagnostic dose assay and log dose probit assay is the most appropriate[10,16,17]. The results of the present analysis, showing significant differences in susceptibility to Cry1Ac toxin among geographical locations of south Indian cotton ecosystem are consistent with the studies of Gujar *et al.*[18] in Maharastra and Karnataka. Geographical variation in susceptibility to *Bt* toxins was reported earlier for the related species *H. virescense* and *H. zea*[10].

An important exercise in the success of *Bt* transgenics is to assess and monitor baseline resistance levels in representative geographical populations of the target insect and ensure that they do not cross the present values. It is obvious that this value would vary for each location/area. Data shows that even before the use of Cry1Ac transgenics, the level of resistance was 8.4-fold in Nanded population followed by 8.03, 7.70, 7.13, 6.80, respectively for Guntur, Nalgonda, Madhira and Raichur. It was as low as 1.131-fold in Kovilpatti population located in the extreme south. This is difficult to explain. Even where *Bt* sprays are used to some extent as a component of Integrated Pest Management (IPM) programs carried out in Andhra Pradesh and Tamil Nadu by the state department agencies, RF values are not indicative of a definite trend. Apparently, there is some relationship in slope and RF value indicative of heterogeneity and levels of resistance, respectively. Heterogeneity within a geographical location is expected due to migratory nature of the *H. armigera* and lack of selection history for Cry1Ac toxin in these geographic populations. Inter-population variation is difficult to explain in a species like *Heliothis*[19]. Notably, variability for response to Cry1Ac toxin does exist in the target population, whether or not previously exposed to the toxin. Except for Raichur district where *Bt* constitutes 9.03% of total insecticides used (unpublished data), *Bt* sprays hardly constitute 0.1% of the total insecticides used on cotton in other districts.

The introduction of *Bt* transgenic crops is an important addition to the existing components of Integrated Pest Management. The technology is perceived to be effective and eco-friendly. However, much of its success will depend on the sustained susceptibility of the target pests to the *Bt* toxins used in transgenic crops. *Bt* transgenic crops, which express Cry1Ac, were found to cause 100% and 75–90% mortality in susceptible *H. virescens* and *H. zea* respectively, in the US[20]. The same level of expression caused less than 90% mortality of *H. armigera* and *H. punctigera* in Australia[21], indicating that *Helicoverpa* species appears to have certain level of tolerance to the *Bt* toxin, Cry1Ac, whether or not previously exposed to *Bt* toxin, when compared with the *Heliothis* species. It is important to note in this study that a few individuals of *H. armigera* in almost all the populations tested were found to survive even the highest concentrations of Cry1Ac. Under field conditions, tolerant individuals are likely to persist despite high expression of the Cry1A toxins and may subsequently contribute to the resistant gene pool. Daly[22] reported that transgenic cotton plants in Australia killed susceptible larvae early in the season but the effect significantly declined later (95–100 days after sowing), when an increasing proportion of first instar larvae placed on transgenic leaves survived to late instars. The studies on *Bt* cotton in the USA and Australia have shown that Cry1Ac protein production decreased over the growing season and that the bio-efficacy of the protein was reduced by interaction with increasing levels of secondary plant metabolites[23,24]. Differential expression in plant tissues may contribute towards a reduced efficacy of the *Bt* transgenic crops. If proper resistance management strategies are not implemented, the efficacy of pest management through *Bt* transgenic crops will be seriously diminished due to widespread development of resistance. Such strategies have not yet been developed for the small farmer and predominantly non-irrigated cotton growing systems found in India and elsewhere.

1. Schuler, T. H., Poppy, G. M., Kerry, B. R. and Denholm, I., *TIBTECH*, 1998, **16**, 168–175.
2. Jouanin, L., Michel, B., Girard, C., Morrot, G. and Giband, M., *Plant Sci.*, 1998, **131**, 1–11.
3. Kranthi, K. R., Kranthi, S. and Wanjari, R. R., *Int. J. Pest Manage.*, 2001, **47**, 141–145.
4. Manjunatha, T. M., Bhatnagar, V. S., Pawar, C. S. and Sithanatham, S., Pro-

ceedings of the International Workshop on Biological Control of Heliothis, New Delhi, 1985, pp. 197–228.
5. Armes, N. J., Jadhav, D. R. and Lonergan, P. A., Proceedings of the World Cotton Research Conference-I, Brisbane, Australia, 1994, pp. 533–552.
6. Kranthi, K. R., Kranthi, S., Ali, S. and Banarjee, S. K., *Curr. Sci.*, 2000, **78**, 1001–1004.
7. Heckel, D., *Biol. Technol.*, 1994, **4**, 405–408.
8. Albert, Z. G., Pfister, R. M. and Dean, D. H., *Gene*, 1990, **93**, 49–54.
9. Lowry, D. H., Roseborough, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265–275.
10. Sims, S. R., Berberich, S. A., Nida, D. L., Segalini, L. L., Leach, J. N., Ebert, C. C. and Fuchs, R. L., *Crop Sci.*, 1996, **36**, 1212–1216.
11. Armes, N. J., Bond, G. S. and Cooter, R. J., *Nat. Res. Inst. Bull.*, 1992, 57.
12. Finney, D. J., *Probit Anal.*, Cambridge University Press, Cambridge, 1971, 3rd edn, p. 318.
13. Ross, G. E. S., *Maximum Likelihood Program: The Numerical Algorithms Group*, Rothamsted Experiment Station, Harpenden, UK, 1977.
14. Forrester, N. W., Cahill, M., Bird, L. J. and Layland, J. K., *Bull. Entomol. Res.*, 1993, **1**, 1–132.
15. Kranthi, K. R., Armes, N. J., Rao, N. G. V., Raj, S. and Sundaramurthy, V. T., *Pesticide Sci.*, 1997, **50**, 91–98.
16. Roush, R. T. and Miller, G. L., *J. Econ. Entomol.*, 1986, **79**, 293–298.
17. Georghiou, G. P. and Taylor, C. E., Proceedings of the 15th International Congress of Entomology, Washington DC, 1976, pp. 759–785.
18. Gujar, G. T., Archana Kumari, Vinay Kalia and Chandrashekar, K., *Curr. Sci.*, 2000, **78**, 995–1001.
19. Fitt, G. P., *Annu. Rev. Entomol.*, 1989, **34**, 17–52.
20. Mahaffey, J. S., Bradley, J. R. and Van Duyn, J. W., Proceedings of the Belt-wide Cotton Conference, 1995, vol. 4, pp. 563–571.
21. Forrester, N. and Pyke, B., *Aust. Cotton Grower*, 1997, **18**, 23.
22. Daly, J. C., *Biocontrol Sci. Technol.*, 1994, **4**, 563–571.
23. Daly, J. C. and Fitt, G. P., World Cotton Research Conference-I, Athens, Greece, 1998, p. 182.
24. Federici, B. A., *Calif. Agric.*, 1998, **52**, 14–20.
25. Litchfield, D. H. and Wilcoxin, F., *J. Pharmacol. Exp. Ther.*, 1949, **96**, 99–103.

ACKNOWLEDGEMENT. We thank Dr Daniel R. Zeigler, Ohio State University, US for providing the Cry1Ac over-expressing clone. This research work was supported by the DBT, GOI project grants to BF. Thanks are also due to Department of Agricultural Entomology, UAS Dharwad for providing infrastructure facilities and all those scientists who helped us during the visit to different locations across south India for *H. armigera* collection. We thank Dr G. T. Gujar, IARI, New Delhi, for his useful suggestions during the study and encouragement.

Received 23 October 2002; revised accepted 18 March 2003

B. FAKRUDIN*
P. R. BADARI PRASAD
S. H. PRAKASH
K. B. KRISHNAREDDY
B. V. PATIL†
M. S. KURUVINASHETTI

Department of Biotechnology, University of Agricultural Sciences, Dharwad, Krishinagar, Dharwad 580 005, India
†*Department of Agricultural Entomology, University of Agricultural Science, College of Agriculture, Raichur 584 101, India*
**For correspondence.*
e-mail: fakru_b@yahoo.com

Restoration of *Eremostachys superba* Royle ex Benth. – a critically endangered species

The Botanical Survey of India was the first to report the threatened status of *Eremostachys superba*, a member of Lamiaceae in the *Indian Plant Red Data Book*[1]. This report may have gone unnoticed if Rao and Garg[2] had not raised an alarm regarding the further deteriorating status of the species at Mohand (Dehradun), its type locality in 1994 and highlighted the need for conservation measures. The highly endangered nature of *Eremostachys superba* was also highlighted by the press[3,4].

As a follow-up of Rao and Garg's report[2], we initiated extensive explorations of Jammu Shiwaliks in 1996, as the species was also reported from this area[5]. Up to 2001 we located five populations of the species containing about 1,300 (ref 6) individuals. In April 2003, one large population of approximately 2,000 individuals was discovered in Sunderbani (District Rajouri) area about 80 km in the north-west of Jammu. The latest discovery has swollen the number to a total of 3,300 plants in nature. This report reviews the present status of the species to indicate its restoration.

One of the reasons for reduction in plant number is the use of root tubers for curing mastitis and restoration of milching process in cattle, including cows and buffaloes (Figure 1 *a*, *b*)[7]. The other cause is low fruit and seed set largely due to pollinator limitation.

In view of the magnitude of threat which the species is facing and its status, a number of measures were tried for its conservation. We have been able to raise plants *ex situ* from seeds and tubers collected from their natural habitat in the University Conservatory. At present more than 1000 plants have been raised (Figure 1 *c*).

Ex situ plants perform well and produce well-filled seeds (35%). Seeds have been distributed to several National Institutes and Universities in India including Departments of Botany at the Guru Nanak Dev University (GNDU), Amritsar; Delhi University (DU), Delhi; Osmania University (OU), Hyderabad; DBS College, Dehradun; Regional Research Laboratory, Jorhat; Botanical Survey of India (BSI) Northern Circle, Dehradun and Indian Botanic Garden, Howrah (Kolkata); State Forest Research Institute (SFRI), Janipura (Jammu) and National Bureau of Plant Genetic Resources (NBPGR), Delhi for cryopreservation.

The species is reported to be doing well at GNDU, DU, DBS College, BSI northern circle, Dehradun and SFRI, Janipura (Jammu). The species has been multiplied *in vitro* by Sunnichen and Shivanna[8] out of a seed sample provided