lation, nodule weight and weight per nodule. Another advantage of working with *Bradyrhizobium* strain is the availability of better technical knowledge of inoculum production and application.

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Variability in response of *Helicoverpa armigera* males from different locations in India to varying blends of female sex pheromone suggests male sex pheromone response polymorphism

A. J. Tamhankar*[,‡], T. P. Rajendran, N. Hariprasad Rao[#], R. C. Lavekar[$], P. Jeyakumar[†], D. Monga[†] and O. M. Bambawale[# #]**

*Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400 085, India
**Crop Protection Division, Central Institute for Cotton Research, PO Box 2, Shankarnagar, Nagpur 440 010, India
[#]Regional Agricultural Research Station, Acharya N.G. Ranga Agricultural University, Lam, Guntur 522 034, India
[$]Cotton Research Station, Marathwada Agricultural University, Nanded 431 604, India
[†]Regional Research Station, Central Institute for Cotton Research, Sirsa 125 055, India
[# #]National Centre for Integrated Pest Management, New Delhi 110 012, India

Field trials were conducted at various locations in India to assess sex pheromone response of *Helicoverpa armigera* males to varying blends of its two sex pheromone components. In the pheromone septa that were used to bait the pheromone traps varying blends were impregnated and the ratio of Z-9 : hexadecenal to Z-11 : hexadecenal in them varied from 0 : 100 to 15 : 85. Results indicated geographical variation in response of males to varying blends of the two sex pheromone components, suggesting male sex pheromone response polymorphism.

THE phenomenon of polymorphism is exhibited in insects in various ways such as variation in forms, castes, phases, colours, etc. Among insects belonging to order Lepidoptera, pheromone polymorphism, particularly with respect to male sex pheromone response specificity, has been reported and in some cases is associated with insects having variation in host plants and/or in habitat environment[1–6]. Since the American bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is polyphagous and also exists in varying environmental conditions, it was of interest to investigate the existence of geographical variation in male sex pheromone response variability in the species.

The sex pheromone of *H. armigera* consists of two major components – Z-9 : hexadecenal and Z-11 : hexadecenal[7,8]. Kehat *et al.*[9] reported that in Israel, there was no significant difference in response of males to blends containing between 1 and 10% of Z-9 : hexadecenal. While confirming this under Indian conditions, we enlarged the range to blends containing between 0 and 15% of Z-9 : hexadecenal. (A blend designated as 0%

[‡]For correspondence. (e-mail: pherosit@apsara.barc.ernet.in)

included no Z-9 : hexadecenal and only Z-11 : hexadecenal, while a blend designated as 15% included 15% Z-9 : hexadecenal and 85% Z-11 : hexadecenal by weight). The blends of the 2 sex pheromone components of *H. armigera* were prepared using pheromone chemicals that were > 95% pure by GLC analysis. Pheromone septa were prepared representing each blend and two mg of each pheromone blend was impregnated per rubber septa. The pheromone-impregnated septa remained at – 4°C till the time they were actually used for experimentation.

For evaluation of response of *H. armigera* males to varying blends, experiments were conducted at various locations in India, namely Dadbi in Sirsa district, Haryana (29.32N 75.02E), Panjari in Nagpur district (21.09N 79.07E) and Nawandi in Nanded district (19.08N 77.20E) both in Maharashtra, and Lam in Guntur district (16.18N 80.27E), Andhra Pradesh. (The distance between Dadbi and Lam was 1550 km, and between Dadbi and Panjari, and Dadbi and Nawandi was 1000 and 1175 km respectively.) Two field trials were conducted at each place in cotton fields, in which the crop was in boll formation stage. Each trial lasted for a period of 15 days, except for the first trial at Dadbi, which was for nine days. There was a gap of one week between two trials and in general, this was expected to sample males of two successive generations. The pheromone septa tested at all the locations were from the same batch. Pheromone traps (plastic funnel traps – funnel dia. 11 cm) were baited with rubber septa impregnated with varying blends and two traps were employed for each blend. Traps baited with the same blend were never adjacent to each other in the field. The traps were deployed at a height of 1 m above average crop canopy. The distance between two traps was 40 ± 5 m (at this distance there is no attraction interference between two traps[10]). Observations on number of males trapped were taken twice a week; the number of males trapped was pooled over the experimental period for each treatment, and the mean number of males caught per day per trap was calculated. After taking every observation, traps were shifted one place each. By doing this, every treatment got a chance to be at varying locations, since under field conditions infestation levels can vary from one field to another. In all the experiments, new clean traps were used and inner surface of the insect-holding plastic bag of the traps was coated with insecticide dust (1 g Lindane–10% a.i.) to kill the trapped insects.

The data were transformed to $\sqrt{X} + 1$ and analysed by employing analysis of variance (ANOVA) and Duncan's multiple range test (DMRT).

Tables 1 and 2 show the response of *H. armigera* males to various blends of its sex pheromone components at different locations in India, representative of north, central and southern regions. (Tables show actual–non-transformed–means). In North India, in the first trial males responded to all the blends containing between 0 and 7% Z-9 : hexadecenal equally, and in the second trial, only blends containing between 2 and 4% Z-9 : hexadecenal elicited significantly higher response. Thus, there was a distinct difference in the behaviour of males in these two tests. The reason for this could be the following. The first trial sampled a population that had appeared unusually early for this region and had assumed typical epidemic proportions[11], for which *H. armigera* is infamous. The second trial was conducted at a time when the insect normally appears in the fields every year in this region. Thus, it is possible that the two tests sampled two different populations. However, it is also known that *H. armigera* occurs in overlapping generations; therefore, it is difficult to offer any proper explanation for the observed phenomenon at this stage, because there are still enough lacunae in the information available on various aspects of *H. armigera* behaviour.

In central India, in both the trials at Panjari, blends between 2 and 15% were equally attractive and attracted significantly high number of males than 1% and pure

Table 1. Male sex pheromone response profile of *H. armigera* at various locations in India – Trial I

Ratio of Z-11 : Z-9 hexadecenal	Mean males/trap/day			
	Location			
	Dadbi	Panjari	Nawandi	Lam
100 : 0	**6.25a**	0.00b	0.5b	0.0c
99 : 1	**4.32a**	0.71b	0.5b	0.5c
98 : 2	NT	**3.96a**	1.5b	7.0b
97 : 3	**5.06a**	**4.93a**	1.0b	6.5b
96 : 4	NT	**5.82a**	1.0b	2.0b
95 : 5	**5.34a**	**7.68a**	3.5b	**15.0a**
94 : 6	NT	**4.54a**	2.0b	2.5b
93 : 7	**5.80a**	**6.93a**	**10.0a**	0.5c
90 : 10	NT	**3.86a**	1.5b	6.0b
85 : 15	NT	**5.11a**	1.5b	2.5b

NT, Not tested.
Figures followed by the same letter are not significantly different at $P = 0.05$ (ANOVA and DMRT).

Table 2. Male sex pheromone response profile of *H. armigera* at various locations in India – Trial II

Ratio of Z-11 : Z-9 hexadecenal	Mean males/trap/day			
	Location			
	Dadbi	Panjari	Nawandi	Lam
100 : 0	0.16c	0.89c	1.5b	0.5c
99 : 1	4.25c	2.14c	1.5b	0.5c
98 : 2	**15.75a**	11.43b	1.5b	12.5b
97 : 3	**14.50a**	**15.68a**	0.0c	12.0c
96 : 4	**18.66a**	**14.79a**	0.0c	16.0b
95 : 5	11.33b	**14.86a**	0.5b	10.0c
94 : 6	11.00b	**16.79a**	**2.5a**	**22.5a**
93 : 7	7.75b	**14.14a**	0.5b	**22.5a**
90 : 10	NT	**20.82a**	1.5b	**25.0a**
85 : 15	NT	**19.07a**	**4.0a**	**21.0a**

NT, Not tested.
Figures followed by the same letter are not significantly different at $P = 0.05$ (ANOVA and DMRT).

Z-11 : hexadecenal. This population appeared to be uniform in its response profile. At Nawandi, in the first trial, it was the 7% blend alone and in the second trial 6 and 15% blends, which elicited significantly high response from males, and this was nearly 2–3 times more than the nearest competing blends. This population also appeared typical in its response in that, in general, blends containing more than 6% Z-9 : hexadecenal attracted maximum males. In the southern region, in the first trial at Lam the 5% blend alone attracted highest number of males, and this was 2–7 times higher than the next competing blends.

In the second trial, all the blends between 6 and 15% showed equal attraction. In this context, Nawandi and Lam populations had some similarities in that the males did not respond to blends with less than 5% Z-9 : hexadecenal. Kehat *et al.*[9] earlier evaluated the response of *H. armigera* males to various blends in Israel and found that there was no significant difference in the response of males to blends containing between 1 and 10% of Z-9 : hexadecenal. Our results suggest that there is a possibility of existence of sex-pheromone response polymorphism in males of *H. armigera* (this is probably associated with geographical location). For one more species of genus *Helicoverpa* (*H. assulta* (Gn)) geographical variation in response to blend composition is known, implying pheromone polymorphism[12]. In this species, the sex pheromone consists of a binary blend of Z-9 : hexadecenal and Z-11: hexadecenal, and the most attractive ratio of pheromone components varies with location. In Korea and Thailand, 20 : 1 and 7.5 : 1 blends of Z-9: hexadecenal and Z-11 : hexadecenal respectively are most attractive, while in China both blends are equally attractive and addition of hexadecenyl acetate to the 20 : 1 blend increases the trap catch.

In *Zeiraphera diniana* Gn. (Lepidoptera: Tortricidae) two host races exist – Larch form (LF) and Crembian pine form (AF). The sex pheromone of this species consists of two components – *trans* 9-dodecenyl acetate (E9–12 : AC) and *trans* 11-tetradecenyl acetate (E11–14 : AC). The E9–12 : AC sources attract only AF males, while E11–14 : AC attract only LF males, and mixtures attract both types[1,13]. In European corn borer, *Ostrinia nubilalis* (Hb), (Lepidoptera: Pyralidae) E and Z pheromone type races exist and the F1 males respond to a wide range of blends[14]. For this species, Frolov[15] hypothesized that variable climate stimulated pheromone polymorphism. In the light of this information, in case of *H. armigera*, which is polyphagous and has habitats varying in climatic conditions, there is a possibility of existence of both host and environment types. With continuity in time and space of both these types, there exists a possibility of interbreeding amongst various types. At any given time therefore, *H. armigera* males possess the potential of exhibiting at least some response to any blend, even if it is at the periphery of their response window. However, diligent experimentation needs to be done to obtain such information.

Presently, based on the results of Kehat *et al.*[9], *H. armigera* males are routinely monitored using the 3% blend (Z-9 : hexadecenal and Z-11 : hexadecenal in 3 : 97 ratio), possibly with a consideration that any of the blends between 1 and 10% should be good enough. Our results suggest that for monitoring and certainly for mass-trapping, a bouquet of blends of the two components be used rather than only one blend, because, if polymorphism indeed exists then, when only one blend is used, only a small proportion of males might be trapped. Thus, for example, if ten traps are being used, instead of baiting all of them with a blend containing 3% Z-9 : hexadecenal, it would be better if two of each were baited with blends containing 3, 5, 7, 10 and 15% Z-9 : hexadecenal. However, the blends and their numbers will depend on the predetermined location-specific pheromone profile of *H. armigera*.

Investigations need to be conducted on blend profile of females from various locations so as to assess and confirm pheromone polymorphism in *H. armigera*. Further studies also need to be taken up on correlation/association between insect habitat and pheromone blend profile, and utility of this information in improving monitoring and mass trapping of this species.

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