Rational deployment of *Bacillus thuringiensis* strains for control of insect pests in India

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Is the fear of silkworm epizootics due to use of a well-known biological insecticide unfounded?

Bacillus thuringiensis (BT) is a gram-positive soil bacterium which produces proteinaceous crystals containing one or more insecticidal proteins during sporulation. The crystal proteins, when ingested by susceptible insects, interact with and lyse their midgut epithelial cells, eventually causing death. Literally thousands of BT strains have been isolated and characterized. They are grouped into approximately 30 serotypes or subspecies on the basis of their flagellar antigens. Most of the strains are active against larvae of certain members of the Lepidoptera (moths and butterflies), but some affect dipteran (flies) and coleopteran (beetles) species also[1].

The insecticidal activity of BT has been commercially exploited to control pests for more than twenty years. BT formulations under at least a dozen brand names are marketed all over the world. However, their use in India is banned because of the fear that BT, once applied in the field, might spread and cause epizootics in silkworms. The majority of the commercial BT formulations are based on subspecies *kurstaki* strain HD-1 and are highly toxic to silkworm It would be better to choose BT strains that are not toxic to silkworm but to insect pests alone. Such strains are available and I propose here that they can be rationally deployed to control insect pests. Some background information is provided to appreciate the diversity of crystal proteins.

The insecticidal spectrum (i.e. the range of species affected) of any particular BT strain is dependent on the type, amount and specific toxicity of the crystal proteins it produces Since many BT strains produce more than one crystal protein, it is difficult to determine the specific toxicity of individual crystal proteins. However, recent experiments in cloning, sequencing and expression of crystal-protein genes in *E. coli* have now made it possible to study the structural similarity and insecticidal spectrum of individual crystal proteins. On the basis of the available information, 44 sequenced crystal proteins are grouped into four major classes· Lepidoptera-specific, Diptera-specific, Coleoptera-specific, and Lepidoptera-and-Diptera-specific. Among the four classes, the Lepidoptera-specific crystal proteins are the best studied and have been grouped into seven different types by comparing their amino-acid sequence homology[2,3]. Sensitivities of silkworm and five insect pests of crops in India to seven Lepidoptera-specific crystal proteins are shown in the Table. Silkworm (*Bombyx mori*) is most sensitive to CryIA(a) whereas pod borer (*Heliothis armigera*) and tobacco caterpillar (*Spodoptera litura*), the most important pests attacking many crop plants all over India, are most sensitive to CryIA(c) and CryIC, respectively. BT strains that produce CryIA(c) but not CryIA(a) could be used to control pod borer effectively. The same strains will also be effective against white butterfly (*Pieris brassicae*) and cabbage looper (*Trichoplusia ni*) (see Table). Alternatively, plants could be genetically engineered to express CryIA(c). It is imperative that such strains, which are available[4,5], be deployed without further delay. Similarly, BT strains producing CryIC but not CryIA(a) could be used to control the tobacco caterpillar. The sensitivity of silkworm to CryIB, IC, ID and IE has not been determined yet, but there are indications that it would not be affected. Any BT strain that is highly active against a serious pest does not produce CryIA(a) would be useful in India.

The deployment of a single, highly active crystal protein might favour the emergence of resistant insect populations. The emergence of resistant populations could be delayed by simultaneously deploying a second crystal protein. If the two crystal proteins bind to two different receptors on the midgut epithelial cells, each receptor has to mutate independently to render the insect insensitive. For control of pod borer CryIA(c) and CryIA(b) could be deployed simultaneously, and for tobacco caterpillar, CryIC and CryIE (see Table) Receptor-binding studies show heterogeneity among binding sites in Indian meal moth (*Plodia interpunctella*)[6] and Egyptian cotton leaf worm (*Spodoptera littoralis*)[7]. This might

Sensitivity of silkworm and five Indian insect pests to crystal proteins of *Bacillus thuringiensis*

Crystal protein	Silkworm (*Bombyx mori*)[8,9]	Pod borer (*Heliothis armigera*)[5]	Tobacco caterpillar (*Spodoptera litura*)[10]	Cutworm (*Spodoptera exigua*)[3,11]	White butterfly (*Pieris brassicae*)[2,3]	Cabbage looper (*Trichoplusia ni*)[3,12]
CryIA(a)	+ + +	−	+	+	+ + +	+ +
CryIA(b)	+	+ +	−	−	+ + +	+ + +
CryIA(c)	−	+ + +	−	−	+ + +	+ + +
CryIB	ND	−	−	−	+ + +	ND
CryIC	ND	−	+ +	+ +	+	ND
CryID	ND	−	−	−	−	ND
CryIE	ND	ND	ND	+ +	−	−

+ + +, Highly sensitive, + +, moderately sensitive; +, least sensitive, −, not sensitive, ND, not determined explain why field resistance to BT has not been observed so far despite the relatively widespread use of BT. However, for some insects such as pod borer, there is no evidence yet that CryIA(c) and CryIA(b) bind at two different sites on the midgut epithelial cells.

The concern that widespread application of BT in the field might be detrimental to sericulture is ill-founded. BT is a ubiquitous soil microorganism and yet, not a single epizootic in silkworm populations (or, for that matter, in any insect population) has so far been reported. The persistence of applied BT on plants and in soil is limited to a few weeks because the ultraviolet in sunlight effectively inactivates it. Moreover, drift experiments conducted in Japan, a major silk-producing country, showed that silkworms reared on mulberry leaves collected 70 metres away from BT-sprayed plots developed normally. Japan has no restrictions on use of BT formulations, most of which are toxic to silkworm. It should be pointed out here that chemical pesticides that are in use in India are highly toxic to silkworm

BT offers a number of advantages over chemical pesticides: lack of polluting residues, high levels of safety to non-target organisms (including beneficial insects), lower development costs, and a lower likelihood of pest resistance. Additionally, all the necessary technology to deploy BT successfully is available in India. Therefore the use of BT formulations should be undertaken immediately.

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A caveat

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BT must be allowed, but with caution and after carefully controlled trials.

Most of the information here is well documented in several internationally published reviews. It is now well known that BT strains differ in their action spectrum. However, most of the agricultural scientists are not aware of the molecular mechanisms governing this diversity. This article does not supply the details either. I therefore feel that Padidam has passed an opinion without adequate scientific data appropriately summarized.

Secondly, while I concur with the author that BT must be brought to India, the arguments levied are emotional rather than scientific. To date I am of the opinion that, no matter what the arguments are about safety to silkworms, unless trials are conducted in a controlled manner in the neighbourhood of a silkworm colony, either a non-sporulating strain of BT or the extracted toxin material alone has to be used as insecticidal application. Verification is required of the statement that BT is applied extensively in Japan. Perhaps, there, emphasis on sericulture is declining owing to the manpower intensity of the programme, while in India the reverse is true.

Such opinions may be misquoted and misused by firms interested in importing the old-fashioned BT preparations that multinational companies are willing to dump in India.

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Padidam replies:

My intention was to present the fact that BT strains that are safe to silkworm are available and can be used to control insect pests in India. I have stated that there are no restrictions on the use of BT formulations in Japan, and not that BT is applied extensively in Japan. It is the Indian farmer who would gain if an alternative [to chemical pesticides] and safe method of controlling insect pests is permitted.