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PRODUCTION AND USE OF *BACILLUS THURINGIENSIS* – PERSPECTIVE FROM 1989

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The potential of *Bt* is being recognized more and more around the world. Industries in several countries – China, Japan, India, Germany, Norway, Switzerland, the USSR, and the United States are producing one or more formulations of different subspecies of this *Bacillus*, primarily subsp. *kurstaki* (HD-1) (used in the control of many lepidopterous insect species), and subsp. *israelensis* (used in the control of many vector mosquitoes and blackflies). Companies that are producing formulations of one of these subspecies usually produce both, since the same equipment can be used for both. Interest extends beyond the commercial: Taking my own experiences as an example, I have participated in international workshops and symposia in The Philippines, Israel, Mexico, China, and now, of course, Brazil. In addition to these programs, I correspond with scientists in at least 15 other countries. My project is just one of many. For example, Dr Huguette de Barjac, who is also participating in this symposium, has a very large program in cooperation with the World Health Organization (WHO) involving several laboratories, as does Dr Anatole Dubitski in the USSR. Also, Japan has an active project under the leadership of Dr Keio Aizawa.

The participants in the development of *Bt* have widely diverse backgrounds and interests, ranging from purely basic research on strains and microbial genetics of this *Bacillus*, through a combination of basic and applied research in fermentation and recovery studies with various strains and isolates of *Bt;* and finally, primarily applied research on the formulation and application of selected isolates. All this makes it difficult to design a talk that will interest and be useful to the various types of scientists involved in studies of *Bt*. Rather than try to cover all the differing kinds of research carried on with *Bt*, I thought it would be interesting to select two distinctly different programs of *Bt* research and show the contributions that these two programs have made in developing potent, economical, formulations of *Bt*. Therefore, I will restrict my talk to (1) a review of the needs for fermentation research, including a discussion of the importance of the spectra of activities in these fermentation studies; and (2) an explanation why *Bt*'s that are highly active in the laboratory are sometimes not active in the field. Examining the problems involved in the development of these two completely different phases of *Bt* will, I hope, make it easier for entomologists and microbiologists to understand each other's problems as they conduct research and development experimentation with *Bt*. Only when we can integrate all the aspects of the fermentation/recovery/and utilization/of *Bt* will *Bt* research proceed efficiently.

Based in part on a series of lectures held in The Philippines, Mexico, and China.

STRAINS AND INSECTICIDAL ACTIVITIES

It is important to remember that there is not just one *Bt* – or one *Bt*-delta-endotoxin. The species is composed of groups of microbes, each group of which has its own insecticidal characteristics. First, the species can be divided into subspecies or variants according to the serological reactions of the thread-like flagellae present in young, motile, cells of the *Bacillus*. These serological characteristics are based on the "H-antigens" and assigned an "H-number" in consecutive order. The discoverer of a subspecies also assigns a subspecies name to the isolate. Three of the better known subspecies are: H-3a, 3b *kurstaki*, (Dulmage, 1970); H-7 *aizawa* (de Barjac & Bonnefoi, 1962), and H-14 *israelensis* (de Barjac & Bonnefoi, 1973).

In addition to the classifications that can be made by use of flagellar antigens, Krywienczyk & Angus (1967) and Krywienczyk et al. (1978) proposed a serological classification of the crystals present in *Bt* cells. One might think that this classification would be definitive, since the insecticidal activities of the isolates are so

closely associated with the crystals. However, while Krywienczyk's procedures do contribute to sorting the activities in the isolates, the associations are not total. For example, crystals associated with subsp. *kurstaki* (the *"k-1"* crystals) can be found in some isolates of subsp. *thuringiensis* (Krywienczyk et al., 1978, 1981; Dulmage and cooperators, 1981). These sorting procedures are valuable, however, and the Krywienczyk classifications are frequently appended to the flagellar classification. Recently, Dulmage (in press) has compared the activity ratios described in Dulmage and cooperators (1981) and found that these ratios could be used to further subdivide the flagellar and crystal serology of the different *Bt*'s. I summarized these three sorting procedures and found that they could be used in a three-stage sort, with the H-antigen as the primary sort, the crystal antigen as the secondary sort, and the relative activity against *Trichoplusia ni* and *H. virescens* – i. e., the Tn/Hv activity ratio – as a third, or tertiary sort.

This leads us to the key aspect of these *B. thuringiensis* isolates: What makes the species so fascinating and so important is that the toxins produced by different isolates of *B. thuringiensis* can kill different insects. Why this is so, and what the interrelationships are between the different delta-endotoxins are poorly understood. All these toxins are high-molecular-weight proteins with similar, but not identical, molecular weights. (The delta-endotoxin of subsp. *israelensis* is an exception. The molecular weight of this toxin is about half the molecular weights of the toxins produced by other isolates of *Bt*). The toxins all must be eaten to be effective, and there is some relationship between the subspecies and the types of delta-endotoxin that they produce.

It is important to understand that the quantity of delta-endotoxin is independent of the number of spores. Spore counts only measure the growth of the *Bacillus:* They do not indicate the amount of toxin produced or recovered in formulations or beers of *Bt.* The only way to measure the quantity of delta-endotoxin is through bioassay. There are several procedures for *Bt* bioassays that have been proposed, depending on the insect species involved (Dulmage et al., 1971, 1976, 1985; De Barjac & Larget, 1979; McLaughlin et al., 1984). Based on the methods described in those publications, the samples are then assigned potencies in either IU's or ITU's.

IU's or ITU's are calculated by comparing the LC_{50}'s of the test samples with the LC_{50} of a standard formulation, and multiplying by the assigned potencies of the standard in IU or ITU, and the units expressed derive from this comparison. The potencies determined will depend, not only on the toxins tested in a bioassay, but also on the different susceptibilities of the insect species being tested. Thus the units determined in a bioassay will differ depending upon the insect used in the assay. By running bioassays against several insect species, one can develop spectra of insecticidal activity for each delta-endotoxin. These can then be used to compare the spectra of other formulations of *Bt* to see whether or not the toxins are identical (Dulmage and cooperators, 1981).

Table I illustrates the importance of learning the spectra of activities produced by the various isolates of *Bt.* The table shows typical insecticidal activities produced by six subspecies of *B. thuringiensis.* The table shows that the kind of delta-endotoxins produced by different subspecies of *Bt* kill different insects or differ in the degree of their activities toward them. The table lists examples from only 6 subspecies out of 25 subspecies so far discovered, but as can be seen from the tables, there are patterns in the spectra of the different subspecies that could help us design a screening program to find higher potency isolates. For example, as the table shows, the toxins produced by typical isolates of subsp. *alesti* have little or no activity vs. *H. virescens* and *T. ni,* so it would probably be wasted effort to search for isolates producing high activity against these two insects by screening isolates of subsp. *alesti.* On the other hand, typical isolates of subsp. *kurstaki* are active against these two insects, and isolates of this subspecies would be good candidates for a screening program designed to search for higly active strains.

Sometimes a new isolate or subspecies will have some desirable characteristics that will warrant its replacing a currently used isolate. For example, Table II also shows that HD-1 and HD-73 both control the cabbage looper, *T. ni.* However, HD-1 is very active against the silk worm, *Bombyx mori.* This is a cause for some concern within countries depending upon the production of silk. This characteristic has slowed or prevented registration of HD-1 in some silk-producing countries.

TABLE I

Comparison of "typical" spectra of activities of seven isolates of six subspecies of *Bacillus thuringiensis*[a]

Subspecies	Crystal type	Trichoplusia ni	Typical potency – iu/mμ			
			Heliothis virescens	Hyphantria cunea	Bombyx mori	Mosquito
thuringiensis	*thu*	4,000	1,000	30,000	1,200	na
alesti	*ale*	na	na	51,000	30,000	na
kurstaki	*k-1*	18,000	18,000	60,000	40,000	+
kurstaki	*k-73*	18,000	35,000	15,000	na	na
galleriae	gal	5,000	600	48,000	7,000	±
aizawai	*aiz*	11,000	900	80,000	46,000	±
israelensis	*isr*	na	na	na	na	+++

[a] Potencies shown are approximate values, typical of the subspecies.
na = Not active.
± = Weakly active against one or two mosquito species.
+ = Measurable, but low, activity against three mosquito species.
+++ = High activity against a wide range of mosquito species.

TABLE II

Comparison of the activities of 9 isolates of *Bacillus thuringiensis* against 4 lepidopterous and 5 mosquito species[a]

Culture No.	Subspecies	Crystal Type	Potency – IU/mg				
			T. ni	H. virescens	H. cunea	B. mori	Mosquito activity
HD-59	*thuringiensis*	*thu* -	7,960	1,440	13,900	1,300	[b]
HD-83	*alesti*	*ale*	Inactive	Inactive	93,200	27,500	Inactive
HD-1	*kurstaki*	*k-1*	39,800	15,400	47,200	50,500	[c]
HD-263	*kurstaki*	*k-1*	39,600	54,600	18,100	18,400	[c]
HD-73	*kurstaki*	*k-73*	29,300	34,500	15,400	Inactive	Inactive
HD-184	*galleriae*	*G-1*	21,900	15,600	93,300	5,500	[d]
HD-154	*galleriae*	*G-9*	2,250	394	12,100	4,300	[d]
HD-135	*aizawai*	*aiz*	18,000	1,460	134,100	75,900	[e]
HD-567	*israelensis*	*isr*	Inactive	Inactive	Inactive	Inactive	High act.

[a] Potencies of lepidopterous powders are based on a comparison with the HD-1-S-1971 standard.
[b] HD-59 had borderline activity against *Ae. aegypti*, but none against the other mosquito species tested.
[c] HD-1 and HD-263, both isolates of subsp. *kurstaki* with *k-1* crystals, were active against *Ae. aegypt*, *Ae. triseriatis*, and *C. tarsalis*. Note that all powders containing the *k-1* crystals were active against these three mosquito species. Cells of the same subspecies, but containing *k-73* crystals had no mosquito activity.
[d] HD-154 and HD-184 were active against *C. tarsalis*, but not against any of the other subspecies.
[e] Mosquito activity of subsp. *aizawai* was similar to the activities observed in powders of subsp. *galleriae* with the principal mosquito activity being against *C. tarsalis*. However, potencies of subsp. *aizawai* were greater than of subsp. *galleria*, and perhaps because of this, many of the powders of *aizawai*, but not of *galleriae*, were active vs. *Ae. aegypti*.

One can see from Table I that there is a possible solution to this problem – at least in silk-producing countries: HD-73, which is similar to HD-1 in its activity vs. *T. ni*, is not active vs. *B. mori*, and one might consider substituting HD-73 for HD-1 in the fermentation. However, there would be unexpected ramifications from such a change. For example, while HD-1 and HD-73 may have similar activity against *T. ni*, a comparison of the potencies of HD-1 and HD-73 against the gypsy moth, *Lymantria dispar*, gives a different picture. Studies conducted by N. DuBois have shown that HD-1 isolates are much more active against *L. dispar* than are HD-73 isolates (DuBois, personal communication). If the primary use of the *Bt* being produced is for protection against *L. dispar*, then HD-1 should be used. If the primary use will be against *T. ni* but safety to *B. mori* is also important, then HD-73 should be selected for use. Thus, the choice will depend upon need. Also, it should be remembered that this has been a highly limited example. In actual practice, it will be necessary to examine the spectra of activities of as many *Bt* isolates as possible in a screening program before one decides which isolates should be retained for further study.

FERMENTATION PARAMETERS

All of my discussions so far have been restricted to the delta-endotoxins produced by the various isolates of *B. thuringiensis* and to their spectra of insecticidal activities. As shown in Tables II and III, studies on fermentation nutrients and conditions can also improve yields of delta-endotoxins in *Bt* fermentations. As can also be seen in Table III, there are considerable differences in yield of toxin in the various fermentations, depending on the medium on which the isolate was grown – and on the fermentation conditions under which the *Bt* was produced.

The fermentations of the different isolates of *B. thuringiensis*, regardless of subspecies, have some general characteristics in common. They all utilize dextrose, molasses, and starch. They all produce acid from dextrose. In general, they are similar in their use of proteins and protein hydrolysates, they can utilize NH_4^+ salts, and they are similar in their requirements for minerals. Results of routine biochemical diagnostic tests are similar and, where they differ, their differences do not parallel insecticidal activities. *Bt* isolates are all very closely related to *B. cereus*. Except for their various insecticidal activities, they are more or less all alike. However, the individual isolates are unique in themselves, and a medium that may increase toxin yields of one isolate may not do well as a substrate for delta-endotoxin production by another isolate. The fact that changes in medium can affect *Bt* yields is very important to remember, but it is too often forgotten, in spite of the large number of papers showing the value of fermentatation research. Papers by Dulmage (1970), Dulmage & de Barjac (1973), Dulmage and cooperators (1981), and Smith (1982), all show the value of media studies.

TABLE III

Comparison of nitrogen sources for the production of delta-endotoxin by *Bacillus thuringiensis* subsp. *kurstaki*, HD-263[a]

Expt.	Nutrient	Level nutrient gm/liter[b]	Yield of toxin kIU/ml
1	Cottonseed flour	10.0	1,180
	Soy peptone	9.53	409
	Peptonized milk	5.50	759
2	Cottonseed flour	10.0	1,250
	Soybean flour	10.0	1,050
	Peptone (Difco)	10.0	779
	Corn steep[c]	20.0	724

a Measured in bioassays vs. *H. virescens*. Bioassay standard: HD-1-S-1981 – 18,000 IU/mg.
b Adjusted to equal N levels.
c Medium adjusted to pH 7.0 before autoclaving.

Table I showed that different subspecies of *Bt* may produce delta-endotoxins with different spectra of insecticidal activity. The increases in yield shown in Table I were partly due to proper selection of strain of *Bt*. The increases in yield shown in Table II, on the other hand, were due to changes in fermentation nutrients and conditions and showed that the *quantity* of toxin in a fermentation beer can also be increased by studying the various parameters used to produce the delta-endotoxin involved. This is important if producers are to have hope of improving their formulations or lowering their production costs.

Table IV illustrates another important factor in the production of *B. thuringiensis*. It demonstrates that the type of toxin produced by a given isolate of *B. thuringiensis* will always

be the same in spite of changes in media or fermentation conditions. This is a very important – and vital – characteristic of *Bt* fermentations: Earlier in this paper, I discussed the "activity ratio" and showed that the ratio could be used to distinguish whether different toxins might be identical. To accomplish this, the average activity ratios of the individual powders of a proposed set are calculated, along with the averages and the CV's of the ratios in the sets. As earlier work has shown, if the CV's of the activity ratios of a set of *Bt* powders are > 0.32, then some of the toxins in the set are different. If the CV's determined for the set are < 0.32, then the powders are probably identical.

TABLE IV

Reproducibility of the activity ratios of formulations derived from fermentations of 5 isolates of *Bacillus thuringiensis* in 14-liter fermentors[a]

Culture number	Number of formulations tested	Average Tn/Hv ratio[b]	Coefficient of variations[c]
HD-1	60	2.40	0.25
HD-129	59	5.63	0.32
HD-241	26	0.63	0.24
HD-244	26	1.54	0.23
HD-263	136	0.44	0.29

a Includes formulations from different media or fermentation conditions and from different ages of beer at harvest.

b Ratio determined by dividing the IU/mg as measured in bioassays against *Trichoplusia ni* by the IU/mg as measured in bioassays against *Heliothis virescens*, using HD-1-S-1971 as the standard in both assays. HD-1-S-1971 has a potency of 18,000 IU/mg.

c If the average coefficient of variation (CV) between the formulations within a set is > 0.32, this indicates that the set is not homologous – that more than one kind of formulation was used in assembling the set. If the CV within the set is < 0.32, the presumption is that the set is homologous – that the formulations with the set are all the same. This is a presumption, not a conclusion, more assays against other species of insects will be needed to confirm this.

When this rule is applied to the powders of the five subspecies shown in Table IV, they can easily be sorted into a few different sets of toxins. Furthermore, examination of the activity ratios of these powders showed that activity ratios of powders of individual isolates were the same, even though the various powders studied in these sets were derived from a wide variety of sources, including studies on the types of fermentation equipment, temperature of incubation, and age of the beer at harvest. Thus the *yield* of delta-endotoxins varied depending upon fermentation conditions, but the Tn/Hv activity ratios (showing the *type* of toxin) was always the same, no matter how the isolate was grown. This is a very important observation because a successful production process requires such reproducibility. The producer must always be able to rely on the fact that the product produced in his fermentors will always be the same.

I have spent a considerable portion of my time discussing strains of *Bt*. This is because I believe that our greatest hope for more effective and more potent *Bt* formulations lies in as yet undiscovered isolates of subspecies of this *Bacillus*. Yet there have been only a few systematic searches for more potent or more useful isolates of *B. thuringiensis*, and these have had a remarkably high level of success. Some of these are listed in Table IV. This list is by no means complete. Each of the isolates in the table was found after a systematic search, and two of them (subsp. *kurstaki* and subsp. *israelensis*) are being marketed and widely used around the world. There is no reason to doubt that still better strains than those we have today can be found.

FERMENTATION STUDIES

Fermentations of *B. thuringiensis* do not require exotic media or ingredient or any unusual conditions for growth and production of the delta-endotoxin. However, this does not mean that the yields of toxin in fermentations cannot be improved by a careful attention to the needs of the isolate under study. Also, as discussed in Dulmage and cooperators (1981) not only can different subspecies of *Bt* have different spectra of insecticidal activity, different isolates of the same subspecies may also have different spectra.

Carbohydrate – There is an obvious relationship between carbohydrate metabolism and the production of delta-endotoxin. However, interpretations of this relationship must be made carefully. As discussed earlier, isolates of *B. thuringiensis* produce considerable amounts of acid from dextrose (The usual source of carbon in *Bt* fermentations). If this is not corrected, the acid produced can cause the pH of the beer to drop below pH 5.6. If this occurs,

the medium will be too acid to support the growth of the *B. thuringiensis*, and the fermentation will cease. The easiest way to avoid the pH problem is to balance the fermentation ingredients between acid-producing carbohydrate and alkali-producing protein or protein hydrolysate. A typical fermentation with the carbohydrate and protein buffering each other will start at pH 6.8-7.0, drop rapidly to 5.8-6.0, and rise steadily thereafter to pH 8.0. Alternatively, the pH of the medium can be regulated mechanically by monitoring the pH of the medium and adding sterile alkali whenever the pH drops close to pH 5.8.

The role of carbohydrate in a *Bt* fermentation is more complex than the pH alone. Dextrose stimulates cell growth and can increase cell yields. However, dextrose can also inhibit spore formation. A careful balance is needed between these two propertes of carbohydrate metabolism. The picture may be further complicated by a more direct effect of carbohydrate on crystal formation: Scherrer et al. (1973) showed that when the dextrose levels in their basal media were increased, the crystals of the delta-endotoxin were increased in size and potency.

The formation of the crystal and the formation of delta-endotoxin are obviously related. Also, the spore and the sporulation process are obviously deeply intertwined in the behavior of the cells of the *Bacilli*. Since the spore and the crystal are closely related, the crystal must have some influence in the metabolic activities within the cells of *B. thuringiensis*. Yet, of course, the cell can survive without producing spores or delta-endotoxin.

Aeration – Work in my laboratory (and elsewhere) has shown that adequate aeration is very important to the *Bt* fermentation. For example, yields from my laboratory fermentations of subsp. *israelensis* were about 50% higher when grown in 14-liter fermentors (high aeration) as compared with yields from 500-ml flasks (low aeration).

Potassium ions – Other factors may play an important direct role in the *Bt*-fermentation. For example, Wakisaka et al. (1982) and Foda et al. (1985) showed the necessity for K^+ for the production of crystals of *Bt*. Also, Foda showed that there was some interaction between the production and utilization of polyhydroxy-butyric acid and the activity of the K^+ ion. It would be very interesting, and possibly very important, to find out what role K^+ plays in endotoxin formation (Foda et al., 1985).

Particle size and Bt *formulation* – In contrast to most fermentations, the particle size of the nutrients used in *Bt* fermentations must be controlled so that the particle sizes of any solid used in the fermentation are $<$ 200 mesh. This requirement derives from the necessity of controlling the cost of the *Bt* formulation. The delta-endotoxin is, of course, an agricultural chemical and the farmer will not use it if its cost is greater than that of a chemical. To be competitive, each step of the fermentation and recovery processes must be designed to be as inexpensive as possible. Thus the purification of the delta-endotoxin from a fermentation beer is minimal (Table V): If we strip the process to its bare essentials, we can see that the only real purification of the toxin is in the first step, when the beer is centrifuged. In this step, we rid ourselves of water and dissolved solubles, but we retain, in addition to the delta-endotoxin, a large quantity of unused nutrients or waste products, all contained in a thick cream.

This cream is essentially the final product. The only additional treatment given to this cream is to add various agents to make it more suitable for use in the field. We add to the cream, we do not purify it. Thus if there are any coarse particles in the fermentation beers, they will be carried over into the final product. If the formulation is a flowable or wettable powder, the particles that it contains must be small enough to pass through a 200-mesh screen in the user's spray apparatus in order to avoid plugging up the pipes and nozzles of the application equipment. This limits the choice of nutrients used in the fermentation. For example, soybean meal and soybean flour support the growth of *B. thuringiensis* about equally well. However, soybean meal, which is almost entirely composed of coarse particles $>$ 200 mesh in size, is unsatisfactory as a fermentation ingredient because the residual flakes of bran in the medium are too coarse to pass through the screens in the usual spray apparatus. Soybean flour, on the other hand, which is almost entirely $<$ 200 mesh, is highly satisfactory.

FIELD APPLICATIONS

Influence of crop on field activity of Bt – The discussions so far have shown the

TABLE V

Sources and uses of 7 commercially valuable isolates of *Bacillus thuringiensis*

Subspecies	Where found	Year found	Principal target insects	Principal uses
kurstaki (HD-1)	Texas	1968	Lepidoptera	Leafy vegetables and forests.
kurstaki (HD-12)	Connecticut	1980	Lepidoptera	2-4X more potent than HD-1 against gypsy moths.
aizawai	England	1977	Lepidoptera	Control of *Galleria mellonella* in apiaries.
israelensis	Israel	1977	Lepidoptera	Control of mosquitoes and aquatic blackflies.
* *morrisoni*	Japan	1985	Diptera	Spectrum of insecticidal activity similar to that subsp. *israelensis*.
* *tenebrionis*	Germany	1983	Coleoptera	Colorado potato beetle and some armyworms.
* *sandiego*	California	1985	Coleoptera	Similar to subsp. *tenebrionis* and may be identical.

* Not being marketed in the United States, but formulations of subsp. *morrisoni*, *tenebrionis*, and *sandiego* are either in advance stages of field testing or with an Experimental Use Permit.

progress that has been made in developing highly active formulations of *B. thuringiensis*-delta-endotoxins. As active as some of these formulations are in the laboratory, they do not always perform well in the field. To answer the question why the applications of some *Bt* succeed while others fail, one must look at the interrelations between the plant, the habits of the insect pest, and the microbe or its product. Where these are effectively integrated, the treatment will probably be effective. When they are not effectively integrated, the treatment will probably fail. The following sections illustrate some of these interrelationships.

Mode of Action of Bt *Formulations* – Critical to the use and effectiveness of *Bt* is its action on the insect. The *Bt*-toxin is a stomach poison. Like all stomach poisons, the insect must find the toxin and eat it. Good coverage of the plant by the *Bt* formulation is therefore necessary. There is less room for error than with the fuming types of chemicals commonly used.

One of the first and most sucessful uses of *Bt* was in the control of leaf-feeding pests on cole crops and other leafy vegetables. The *Bt*-toxins are particularly effective on these crops because it is easy to get the toxin to the insect. Our application methods, whether by air or by ground equipment, primarily deposit the *Bt* on the leaves of the plants where the insect feeds. As a result, the application places the *Bt* in close proximity to the target insect. The habits of the insects – i. e. feeding on the leaves – draw the insect to where the toxin is so that the insect has every opportunity to feed on the toxin, and the result is effective control.

TABLE VI

Recovery process for the spore-crystal complex of *Bacillus thuringiensis:* Flow-sheet for pilot-plant and production-scale recovery[a]

Spray drying

Whole beer – pH 8.4 – 9.0
|
Centrifuge
(tubular bowl continuous flow centrifuge)
|
Residue (weigh cream) — Supernate (discard)
|
Prepare slurry
(based on weight of cream)
|
Add 10% lactose
1% wetting agent
|
1% dispersant
|
Mix thoroughly
|
Adjust total solids
to approximately 20%
|
Spray dry
Centrifugal atomization feed
|
Inlet temperature: 125-150°C
|
Outlet temperature: 75-100°C

[a] From Dulmage, 1983.

Bt *and the tobacco budworm*, Heliothis virescens – One of the best examples of the importance of proper formulations and application procedures can be found in the difference of effectiveness of HD-1 formulations against the tobacco budworm, *Heliothis virescens*, on tobacco and on cotton, as shown in the following sections.

Bt *on tobacco* – The use of HD-1 on tobacco illustrates the problems of using *Bt* on a crop where good application procedures are difficult to achieve. *H. virescens* is a vigorous pest of tobacco, attacking both the buds and the leaves of tobacco, where it can cause serious damage to the crop. The leaves of the tobacco plants are large and derive from a central bud. On the plant, the arrangement of the leaves forms a "V-shape" with the bud at the bottom of the "V". While tobacco is a leafy plant, the budworm feeds principally on the buds that give rise to the next set of stems and leaves. Coverage of the plant with sprays or dusts of *Bt* could control the budworm, but the costs were higher than the farmer could afford. Little *Bt* was used. Then a granular form of the *Bt* was developed. Those granules that fell on any portion of the leaves would tend to roll down to the base of the "V" where the budworm was feeding. Once the formulation was designed to get the toxin to the *Bt*, HD-1 became an effective and economical agent against the budworm.

Bt *on cotton* – Cotton is also a very leafy plant. In this case, however, the budworm feeds on the developing fruiting bodies – the terminals, squares, buds, and developing bolls – only occasionally on the leaves. These fruiting bodies are located toward the interior of the plant and are, to a large extent, protected from the *Bt* by the shielding action of the leaves. Thus only a small percentage of the *Bt* that is applied to a field reaches the larvae. One would have to apply a very large amount of *Bt* to get enough *Bt* to the fruiting bodies to be effective. However, as shown by McGarr et al. (1970), if sufficient toxin were gotten past the leaves, HD-1 could protect cotton from the budworm.

At the present time, I know of no way to use *Bt* on cotton. Costs would be much too high. For example, about 0.5 kg/ha of a granular formulation will protect tobacco from the budworm. To achieve the same degree of control on cotton, we would need, according to my estimates, 5-10 kg/ha to protect cotton from this insect.

It is possible to develop practical methods of control of the budworm on cotton. The most obvious answer is to find a more potent formulation of the *Bt*, and this can be done. New strains of *B. thuringiensis* can be found that are more active than HD-1 against the budworm, and progress is being made toward achieving this. However, if we could develop procedures that would get a bigger percentage of the *Bt* being applied to a cotton field, to the insect, we could achieve the same goal. One way to accomplish this would be to induce the insect to come to the leaf. This might be achieved by incorporating a feeding stimulant or a bait into the formulation being applied. This type of approach has not been adequately explored, but research by Bell in Arizona and Mississippi has given some indication that a bait-stimulant process that they have developed can attract the insect to the cotton leaf and reduce the level of *Bt* needed to effect economical control.

Bt *and the pink bollworm*, Pectinophora gossypiella – Sometimes the habits of the insect itself protect the insect from the *Bt*-delta-endotoxin. For example, laboratory bioassays indicate that HD-1 is highly active against the pink bollworm, *Pectinophora gossypiella*. Yet field tests show that HD-1 usually fails to control this insect. The reason in this case is that the habits of the insect allow little chance for the *Bt* to come in contact with the insect. The eggs of *P. gossypiella* are laid near the terminals and squares of the cotton plant. The newly-hatched larvae need travel only a short distance before reaching their goal – to burrow into a developing square, bud, or terminal, where it can feed and mature out of reach of the *Bt* toxin. The exposure time of the insect to the *Bt* is very short, so that the chance of a *Bt* application coming in contact with the insect is minimal, and the application usually fails. There is only a short window in the time between hatch and entry into the fruiting bodies where he escapes from the toxin. If the application of the *Bt* is close to the beginning of that window, the larvae may be controlled. However, the timing of exposure of larvae to the toxin in that window is too short for any reproducible control, and, so far, the use of *Bt* against *P. gossypiella* has not been practical.

It is very important to remember that, in any case where the target insect burrows into the host plant shortly after hatch, it will be difficult to control the insect with any stomach poison, including the *Bt* toxin. This must be considered when evaluating any *Bt* against a particular insect species.

Potential of Bt *in insect control* — This discussion has been designed to emphasize the importance of understanding the interrelationships between insect, plant, and *Bt*. There are, of course, other reasons why a farmer might elect not to use *Bt*. For example, one of the virtues in the use of *Bt* is the narrow spectrum of insecticidal activity of these delta-endotoxins which allow us to attack the pest insect but spare beneficial insects that normally would help in protecting the crop. However, this same characteristic (narrow spectrum) can also mean that in those cases where there are more than one type of pest attacking a crop, that the *Bt* would not be able to attack all the pests, where a broad spectrum chemical insecticide could. This can induce a farmer to treat subsequent crops only with chemicals, just "to be on the safe side", even though *Bt* might have given him equally as effective control at a lower cost.

I do not want to end this discussion on a pessimistic note. Users -- farmers, applicators, mosquito control districts — need to be educated about the potential and the effectiveness of *Bt*. This is natural because these *Bt*'s are new agents with differing properties and new application techniques. But they can be educated, and they will use *Bt* once they understand what this delta-endotoxin is. Sales of *Bt* formulations are growing sharply, and the potential for its further development are great.

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