Learn the Truth

About Big Quality Differences Between Bts

DiPel® DF
BIOLOGICAL INSECTICIDE

VALENT BIOSCIENCES CORPORATION

Biorationals Bring Unique Value

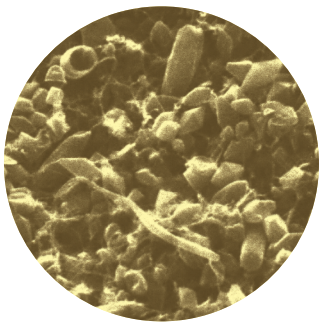
The need for highly effective and sustainable farming solutions has growers around the world adopting integrated programs using biorationals. Easy on beneficials while featuring residue management, resistance management, and environmental and worker safety benefits, biorationals meet the needs of today's food value chain.

Despite the important attributes that biorationals provide, not all biorational products are created equal. As you consider your biorational choices, it's important to understand that biorational benefits are dependent upon inherent quality differences between products – and **those differences can have a dramatic impact on your crop and your bottom line.**

Mode of Action: Biorational vs. Conventional Chemicals

Understanding quality differences in biorationals begins with a fundamental difference between biorationals and conventional chemicals. Conventional products have a single mode of action, but biorational insecticides - such as *Bacillus thuringiensis* (Bt) - are more complex. Bt has multiple modes of action – mutiple protein toxins and Bt spores that all contribute to insect mortality.

Multiple modes of action with a Bt mean that quality is multifaceted as well. The strength of a single mode of action, conventional product is often expressed by "% Active Ingredient." But % Active Ingredient does not provide a good basis for comparisons between Bt products. For a product with multiple modes of action, it is the ***levels*** of the insecticidal components ***within*** the active ingredient that determine Bt product efficacy.



Bt kurstaki (Btk) is the top biorational product in the world. Btk contains four different insecticidal protiens: the Cry1 protoxins – Cry1Aa, Cry1Ab, Cry1Ac – and the Cry2 toxin. When ingested, these toxins bind to the midgut of pest larvae and causes cell death, rapidly inhibiting further insect feeding. Bt products also include Bt spores, which germinate inside the insect and contribute to mortality.

Each batch of **DiPel®** is tested on over 2000 lepidopteran larvae at various stages in manufacturing.

Factors that Determine Bt Quality

The relative amounts of insecticidal proteins and spores within a finished Bt product are what determine its field efficacy, and field efficacy equates to value.

Several factors combine to determine insecticidal protein (protoxin) levels in Bt products, and most are related to manufacturing. Bt quality can be thought of as the sum of the following parts:

1. The Bt **strain** being used
2. The Bt **manufacturing** process
3. In-process **testing** and monitoring procedures

Differences between Bt strains

There are thousands of strains of Bt, but a relatively small number of strains are used commercially for insect control. The strains have been selected for toxicity on insect pests, driven by the strain's Cry toxin profile. **Every Bt manufacturer uses different strains.** The level of protoxins produced in fermentation are unique to each strain and each manufacturing process.

Variables that Contribute to Bt Quality

Bt manufacturing is a delicate and complex process that Valent BioSciences has been perfecting for more than forty years.

It begins with the culture of the Bt strain being used and continues through fermentation and formulation. Results are delivered in the field. Here's a list of important factors that figure into Bt quality.

- ☑ Strain and culture purity
- ☑ Type and concentration of raw materials
- ☑ Method of feed
- ☑ Sterilization of lines and tanks
- ☑ Sterilization of growth media
- ☑ Continuous monitoring of:
  - Pressurization
  - Aeration
  - pH
  - Temperature
- ☑ Cycle time
- ☑ Frequency of in-process sampling
- ☑ Quality of intentionally-added inerts (surfactants, buffers, speaders, stickers)
- ☑ Stability through formulation
- ☑ Stablity in packaging
- ☑ Stability on foliage

The Role of Manufacturing

The Bt strain determines protoxin and spore producing ***potential.*** The proprietary manufacturing process of each producer is what converts that potential into available levels in the end-product. Table 1 identifies several important aspects of Bt manufacturing that impact the quality of the finished product and establish in-field performance.

In-Process Testing and Monitoring

Bt products are cultured, fermented, and formulated. The quality of the finished Bt product is closely tied to how stringent in-process testing and monitoring methods are employed throughout the production cycle. The more sophisticated the manufacturing process, the more frequent and concisely samples can be extracted and tested. In the end, **product strength and consistency in the field reflect the rigor of in-process testing.**

Chemical Analysis of Finished Bt Products

Assessing Bt Quality

There are hundreds of Bt insecticides in the agricultural market. How can growers evaluate quality if % Active Ingredient is not a good indicator?

Manufactured under pharmaceutical-grade standards, **DiPel® DF** is the top brand of Btk in the world. Quality in manufacturing is why **DiPel** is regarded by many as the industry standard among biorationals for efficacy and consistent, in-field performance.

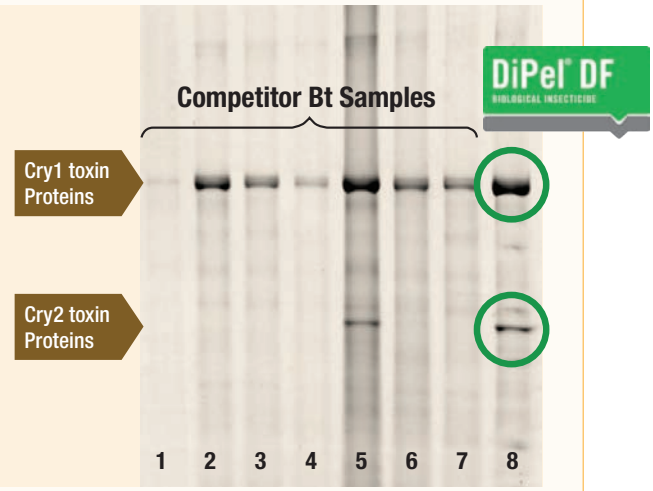
Bt Quality: Analytical Methods

SDS-PAGE - Sodium dodecyl sulfate polyacrylamide gel electrophoresis, or SDS-PAGE, is an analytical technique used in biochemistry, genetics, molecular biology, and forensics. Using SDS-PAGE analysis, scientists are able to separate individual proteins in a gel matrix based on their reactions under the influence of an electric field.

Inside Bt: Laboratory Analyses of Bt Products

Quality differences in Bt are easily revealed in the lab. SDS-PAGE analysis (see sidebar) is a commonly used tool to identify levels of protein toxins in **DiPel** and other Bt products.

Figure 1



SDS-PAGE: Cry1 and Cry2 Toxin Levels in DiPel DF and Competitor Bt Samples

DiPel DF displayed the most intense concentration of Cry1 toxins among any of the Btk products sampled. While Btk is characterized by having Cry2 toxin as well, most of the samples analyzed showed little, if any Cry2 toxin present.

Source: Valent BioSciences Corporation

The results of Bt chemical analyses are quite clear. Figure 1 shows an SDS-PAGE gel image where several competitor Bt products were compared to the industry standard. Notice the presence and intensity (more intensity means higher protein levels) of Cry1 and Cry2 toxins in the **DiPel DF** column, as compared to the weakness (or absence) of the toxins in the Competitor samples.

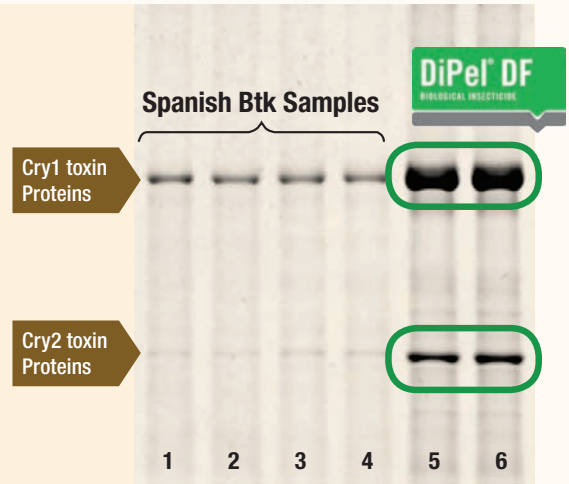
Table 1 shows the quantitative results of another SDS-PAGE analysis that measured the total concentration of Cry1 + Cry2 protoxins in **DiPel** and three different competitor Bt product samples. In this analysis, the level of protoxins available in **DiPel exceeded the amounts in the competitor samples by ratios of 8-1, 13-to-1, and 14-to-1.**

Table 1

Bt Product	Concentrations of Protoxins Cry1 + Cry2 (mg/g)	Protoxins Ratio: DiPel® DF to Sample
DiPel® DF	76.3	1/1
Competitor Sample A	8.9	8/1
Competitor Sample B	5.9	13/1
Competitor Sample C	5.3	14/1

Source: Valent BioSciences Corporation

Figure 2



SDS-PAGE: Cry1 and Cry2 Toxin Levels in DiPel DF and Spanish Bt Samples

Four samples of a Spanish Btk product (lanes 1-4) revealed low Cry1 toxin levels and extremely low levels of Cry2 toxin. Strong levels of each were visible in the **DiPel DF** samples (lanes 5-6).

Source: Valent BioSciences Corporation

Similar results emerged from analyses comparing a Spanish-manufactured Btk product. An SDS-PAGE gel image (Figure 2) provides a dramatic visual evidence of the difference in toxin levels between products. In a study conducted by an independent European lab comparing

Table 2

Concentrations of Protoxins Cry1 + Cry2

	Content of Active Ingredient		
	Cry1 %	Cry2 %	Total % toxin CRY1 + CRY2
DiPel® DF	24.36%	6.74%	**31.10%**
Spanish Btk Sample A	5.52%	0.38%	**5.90%**
Spanish Btk Sample B	5.39%	0.43%	**5.82%**

Source: Valent BioSciences via work performed by BioTecnologie BT S.r.l., Perugia, It

three blind Bt samples (Table 2), **DiPel registered at more than 31% Cry1 + Cry2 toxin content while the samples of the Spanish Btk registered below 6%** each case.

These analytical test results translate into practical differences in leaf protection, which can also be seen in the lab. Figure 3 shows the differences between **DiPel DF**, the Spanish-manufactured Btk product, and an untreated control. Leaf protection tests demonstrate that a significant amount of plant damage occurs even after perfect application of an instrument laboratory analyzed, lower toxin-content product.

Figure 3: **Leaf Protection Assay: Untreated Control, DiPel DF, and Spanish Btk Sample**

Cabbage leaf damage sustained from *Trichoplusia ni* larvae three days after treatment.

Bt Quality: Effects on Crop Quality & Yield

Laboratory analysis is one method to quantify Bt quality, but there's no better measure than how it translates to the field.

Field trials demonstrate the impact of Bt quality on crop quality and yield focused on comparing **DiPel® DF** versus the same Spanish Btk product analyzed in the lab. The first trial was conducted in Grenada, Spain on organic industrial tomatoes, targeting the pest *Helicoverpa armigera.*

An organic trial was chosen to limit control measures to Bt-only applications, and eliminate potential interactions with other chemistries.

In this trial, five Bt applications were made on each plot. The yield from the **DiPel** plot delivered more than 30% more commercial grade tomatoes (9244 kg/ha) than the Spanish Btk product (see Figure 4).

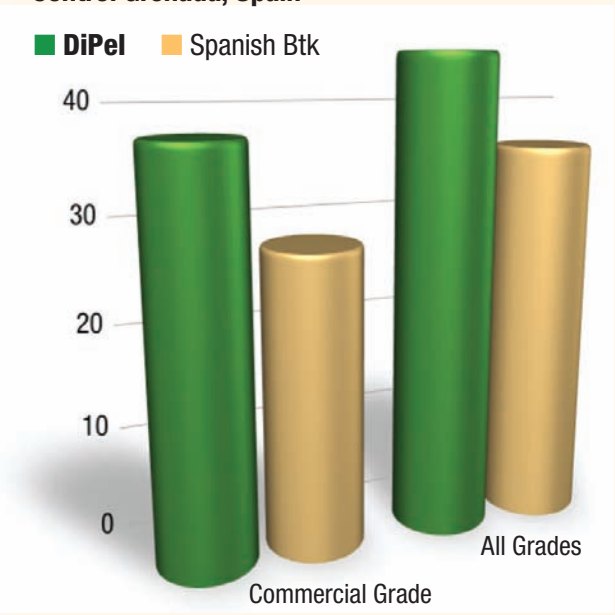
Tomato Trial: Bottom Line Impact

Taking this trial data one step further, we can extrapolate the value of using the higher quality Bt product (see Table 3). **The DiPel treated plot produced approximately 9244 more kg/ha of commercial grade tomatoes than the plot treated with the Spanish Bt.** Using an average industrial tomato price of € 0.15/kg, **revenue generated from the DiPel plot was € 1386/ha more than was generated using the Spanish product**.

Figure 4

Field Trial: Industial Organic Tomato

DiPel and Spanish Btk for *Helicoverpa amigera* Control Grenada, Spain



Yield from the **DiPel**-treated plot delivered more than 30% more commercial grade tomatoes (9244 kg/ha) than did the plot treated with the Spanish Btk product.

Source: Valent BioSciences Corporation

Helicoverpa armigera

All *Helicoverpa armigera* photos - Gyorgy Csoka, Hungary Forest Research Institute, Bugwood.org

Table 3

Bt Tomato Trial: Bottom Line Impact

Profit Indicator	DiPel®	Spanish Btk
Commercial Grade Yield	37,450 kg/ha	28,206 kg/ha
x Average Crop Price/kg	€ 0.15	€ 0.15
Total Revenue / ha	€ 5617	€ 4231
Net Gain / ha	€ 1386	--

Lobesia Botrana

Lobesia Botrana photo: Jack K. Clark, courtesy of University of California IPM Statewide Program

Wine Grape Trial

A similar trial was conducted in Murcia, Spain on Monastrell wine grapes to control the second generation of the pest *Lobesia botrana.* Once again, the insecticide applications were limited to Bt, and in this trial, just a single application was made at egghatch.

Two measures of effectiveness were evaluated in this trial. First, post application larval counts were conducted to examine the effects of the Bt application on each plot. The survival rate for the *Lobesia* larvae was less than 8% in the plot treated with the industry standard, **DiPel**. In the plot treated with the Spanish Btk product, the *Lobesia* survival rate was about three times greater - at more than 25% (see Table 4).

Table 4

Field Trial: Monastrell Wine Grapes

Post-Application Larval Count: DiPel vs. Spanish Bt
Murcia, Spain

Bt Treatment	% Efficacy
DiPel® DF (1x)	92.45%
Spanish Btk (1x)	74.53%

Source: Servicio de Sanidad Vegetal

Wine Grape Trial: Bottom Line Impact

The second measure of efficacy in this trial was how much of the fruit was protected. In the **DiPel** treated plot, nearly 85% of the clusters sustained no damage. Of the 15% that sustained damage, more than 87% of the grapes per cluster remained unharmed. Levels of protection were much lower in the Spanish Bt plot, where only half of the clusters were undamaged. And for those that sustained damage, nearly half of the grapes were affected (Table 5).

Bottom line, it is **estimated the harvest from the DiPel treated plot would have earned as much as €1500 more per hectare than the grapes harvested from the plot treated with Spanish Btk.**

Tank Mix Value

It's common knowledge that Bt is often used as a tank mix or rotation partner with conventional chemicals because its multiple modes of action provide resistance management benefits.

That said, experts suggest it's common for the activity from a conventional to mask the poor efficacy of a low-quality Bt partner. Most recommend comparing competitive Bt products in a stand alone trial before tank mixing, ensuring you get the benefits you pay for with a Bt.

Learn the Truth – What have we learned?

Now that we've explored the importance of using a high quality Bt in your crop protection program, take a moment to review:

- There is a growing trend toward using biorational products, and **DiPel** is the top Btk product in the world. **It's important to make informed decisions as you prepare to incorporate biorationals into your control program.**
- Bt quality can be highly variable, and it all begins with the manufacturing process. **This variability can easily be seen through chemical analysis and in field trials** limited to Bt.
- Low cost products, while less expensive, often deliver inferior results. **The Bt you select can have a dramatic effect on your bottom line.**
- **DiPel delivers consistent results both in the lab and in the field, and has been bringing value to its customers around the world for more than 40 years.**

Table 5

Wine Grape Trial: Bottom Line Impact

Profit Indicator	DiPel®	Spanish Btk
% Control (larval count)	92.45%	74.53%
% Clusters Protected	84.70%	50.80%
% Grapes Protected per Damaged Cluster	87.30%	52.10%
Estimated Increased in Crop Value (per ha)	€ 1,500	--

Source: Servicio de Sanidad Vegetal

Valent BioSciences Corporation is a worldwide leader in the research, development and commercialization of highly effective low-risk, environmentally compatible technologies and products for the agricultural, public health, forestry, and household markets. Through the power of technology, Valent BioSciences develops biorational products that create value and provide innovative solutions for its customers around the world.

Valent BioSciences Corporation is an ISO 9001:2008 Certified Company

Read and follow the label instructions before using.

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