Bt Bacteria Might Form Basis for Future Biological Insecticide

By Tracy Ellis, Greg Bradfisch and Joel Coats

Will U.S. turf markets ever see the benefit of a *Bacillus thuringiensis (Bt)*-based biological insecticide for white grub control? Insecticides based on *Bt* may have advantages over current biologicals by having easier application parameters than nematode bioinsecticides or faster action than milky spore (Bacillus popilliae) disease. But the environmental benefit of *Bt*-based insecticides may never outweigh the spectrum and other advantages offered by some current chemical insecticides such as Merit and Mach 2 for grub control.

Hundreds of diverse crystalline proteins are known to be produced by *Bt* and many of these have insecticidal activity against a variety of pests. The name of the subspecies often has its origin with the discoverers or geographic regions of discovery such as *Bt tolworthi, kumamotoensis and japonensis*. Gene and amino acid sequencing often determine that a subspecies harbors genes for several insecticidal proteins. Also, members of different subspecies may have identical genes and express identical proteins.

Key knowledge of the gene sequence and the resulting expressed proteins are already known for many *Bt*. For more information on *Bt* toxin diversity and nomenclature please visit N. Crickmore, D.R. Zeigler, E. Schnepf, J. Van Rie, D. Lereclus, J. Baum, A. Bravo and D.H. Dean's "Bacillus thuringiensis toxin nomenclature" (2004) *http://www.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/index.html*.

Specific details on the mode of action differ with each insect and *Bt* toxin interaction, but a general model for *Bt* mode of action is widely accepted by scientists (for mode of action information please visit Dr. D.J. Ellar's Web site at *http://www.bioc.cam.ac.uk/UTOs/Ellar.html#anchor736270*).

After being eaten, the *Bt* protein in the insect midgut is dissolved and enzymatically digested to render a solubilized and truncated protein toxin. The toxin then must bind to specific receptors in the gut where it causes pore formation and membrane disruption. The insect dies by septicemia and/or starvation. The efficiency of protein solubilization, truncation and toxin attachment for each insect and toxin combination accounts for the specificity and lethality of certain *Bt* proteins against different insect pests.

If any one of the steps in the mode of action fails to occur, the protein will not show toxicity against the insect. For example, the *Bt* protein will not be toxic if the insect gut has a high pH and the protein dissolves only at low pH, or if the insect digestive enzymes do not cleave to expose the active site, or the insect lacks specific gut receptors to bind the truncated toxin. One researcher (Sharpe) observed *Bt* toxicity differed between fall-collected grubs preparing for winter diapause and actively feeding spring-collected grubs. Presumably, the midgut biochemical pH of spring-collected grubs was more suitable for solubilizing *Bt* inclusions, the first step in *Bt* mode of action.

The physiological age of the pest may be an important consideration the optimal timing of *Bt* product application, adding to possible use complications of a *Bt* product.

Other subspecies of *Bt* which express proteins known to have toxicity against one or more U.S. white grub pests: *Bt tolworthi* that expresses proteins of lengths 61, 68 and 75 kilo dalton (kDa — a unit used to measure the molecular weight of the protein); *Bt kumamotoensis* that expresses doublets at 130 kDa; and *Bt japonensis* at 131 kDa as measured by polyacrylamide gel electrophoresis.

Bt tolworthi and other subspecies of *Bt* express proteins classified as Cry3Ba$_1$, Cry3Bb$_2$ (formerly CryIIIB & CryIIIB3). Laboratory and turf plug tests reported in 1992 and 1993 by Ecogen, cooperating with Chemlawn Research and Development, showed Cry3Bb$_2$ toxin to cause mortality of two main U.S. grub pests, Japanese beetle and northern masked chafer *Cyclocephala borealis.* Tests by Mycogen Corp. (currently

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TABLE 1

Host range spectra of Bt subspecies against U.S. grub pests[1]: (+) indicates susceptibility, (-) indicates no observed effect, (x) not reported.

	Oriental Beetle	Black Turfgrass Ataenius	Green Fruit Beetle	Northern, Southern & Pasadena Masked Chafer	May/June Beetles	Japanese Beetle
Bt subspecies						
kumamotoensis	X	-	+	-	-	-
tolworthi	-	-	-	+	-	+
japonensis	+	-	+	+	-	+

[1]results compiled from Michaels et al. 1993, 1996, MacIntosh et al.1990, Grossman 1992, Suzuki et al. 1992, & Robert el al. 1994.

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Dow AgroSciences LLC) confirmed *Bt tolworthi* to cause mortality of Japanese beetle and Northern masked chafers and concluded *Bt tolworthi* was also toxic to Southern masked chafer *C. lurida*, and Pasadena masked chafer (*C. pasadenae*).

Bt tolworthi had no toxicity against other U.S. pests Oriental beetle *Anomala orientalis*, Black turfgrass ataenius (*Ataenius sp.*), green fruit beetle (*Cotinis sp.*), nor June beetle (*Phyllophaga sp.*).

Mycogen reported in 1993 that *Bt kumamotoensis*, which expresses a protein classified as $Cry8Ba_1$ (formerly CryIIIE), was toxic to green fruit beetle grub (*Cotinis sp.*) *Bt kumamotoensis* was not toxic to black turfgrass ataenius, masked chafers, June beetles nor Japanese beetles.

By 1992, Ohba discovered subspecies *japonensis* (*Btj*) which expresses toxin Cry8C (formerly CryIIIF) to be toxic to white grub Cupreus chafer (*Anomala cuprea*), hence the nickname "buibui" after the insect. The host range of *Btj* buibui was well-defined for several insect pests of economic importance in Japan by Suzuki et. al., and shown to be highly toxic to genera in the subfamily Rutelinae, which includes pests also of U.S. economic significance, such as Japanese beetle and Oriental beetle. Mycogen later reported high levels of toxicity of *Btj* against the native U.S. grubs green fruit beetle and the Southern, Northern and Pasadena masked chafers.

There was a brief effort in the United States to develop a *Btj*-based product by Mycogen and Kubota Corp. in the mid-1990s. However, the limited activity against black turfgrass ataenius and May/June beetles discouraged further investment. *Btj* is now owned by Sumitomo Chemical Co. which, in late 2003, acquired Kubota's biological pesticide business and is the basis of registered flowable and granular product Buihunter in Japan intended for use against chafers in sweet potatoes.

Sumitomo Chemical has the intention to expand the Buihunter label to turf. However, at this writing, the plan on whether to develop *Btj* in the United States by subsidiary Valent BioSciences is unclear.

No *Bt* candidate has yet successfully made the long trek into commercialization to be available as an option for white grub control in turf despite candidates like *Btj*, which have activity against main U.S. grub pests of Japanese beetle, Oriental beetle and masked chafers.

One weight against the usefulness of *Bt* subspecies active against grubs may be apparent lack of toxicity by known *Bt* candidates against grubs in the subfamilies Melolonthinae and Aphodinae that include May or June beetles, European chafer, Asiatic garden beetle, black turfgrass ataenius and *Aphodius* sp., while some chemical insecticides have a broader spectrum. However, industrial players holding ownership and knowledge of the *Bt* strains may decide to further develop some of these biological candidates and discover new ones.

In the future, market forces may turn to give *Bt* products a place in the biological product line-up for grub control.

Ellis, a turfgrass pest consultant, performed Bt grub research with guidance from Bradfisch while at Mycogen (now DowAgroSciences LLC in Indianapolis) as well as at Iowa State University with direction from Coats, a professor and chair at its Department of Entomology in Ames.