**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 Cry3Ba1, Cry3Bb2 (formerly CryIIIa & CryIIIb3). Laboratory and turf plug tests reported in 1992 and 1993 by Ecogen, cooperating with Chemlawn Research and Development, showed Cry3Bb2 toxin to cause mortality of two main U.S. grub pests, Japanese beetle and northern masked chafer *Cyclocephala borealis*. Tests by Mycogen Corp. (currently Continued on page 66
No *Bt* candidate has yet made the long trek into commercialization as an option for white grub control.

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

*Bt* toluworthi had no toxicity against other U.S. pests Oriental beetle *Anoplophora chinensis*, Black turfgrass ataenius (*Ataenius sp.*), green fruit beetle (*Cotinis sp.*), and June beetle (*Phyllophaga sp.*).

Mycogen reported in 1993 that *Bt* kumamotoensis, which expresses a protein classified as Cry8Ba (formerly CryIIIIE), 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 CryIIIIF) to be toxic to white grub Cupreus chafer (*Anoplophora chinensis*), 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 Buibui hunter in Japan intended for use against chafers in sweet potatoes.

Sumitomo Chemical has the intention to expand the Buibui hunter label to turf. However, at this writing, the plan on whether to develop 

*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 Dow AgroSciences 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.