A:355 *Bacillus thuringiensis crylA* endotoxin gene

Introduces resistance to insects

NOMENCLATURE: Approved name: *Bacillus thuringiensis* Berliner *crylA* endotoxin gene. **Other names:** *Bt* gene; *crylA(b)*; *crylA(c)*. **Promoter:** Most commonly the cauliflower mosaic virus (CaMV) 35S.

SOURCE: *Bacillus thuringiensis* produces parasporal, protein, crystalline inclusion bodies during sporulation (see the *Bacillus thuringiensis* main entry in Section 1 (1:17) for a full description of the mode of action). Upon ingestion, these are insecticidal. Different delta-endotoxins have different biological spectra and different genes (that are located on plasmids) are being used to transform different crops to afford protection from attack by different insects. The *crylA* genes produce lepidopteran-specific endotoxins (see *Bacillus thuringiensis* entries in Section 1). Monsanto has used *crylA(c)* genes in cotton and tomatoes and *crylA(b)* genes (all from *Btk*) in maize (corn) and Novartis (now Syngenta) and Mycogen have used *crylA(b)* genes from *Btk*, also in maize. In the USA, the EPA must register events before they can be used in commercial seed production. EPA has registered several unique *Bt crylA* events for maize, including: 176 (Novartis Seeds (now Syngenta Seeds) and Mycogen Seeds), BT11 (Northrup King/Novartis Seeds), Mon 810 (Monsanto) and DBT418 (DeKalb). The Mon 810 and BT11 events are used in production of 'YieldGard' corn. Event 176 is sold as 'KnockOut' by Novartis and 'NatureGard' by Mycogen. The DBT418 event is sold as 'BT-Xtra'.

PRODUCTION: The gene is isolated from *Bacillus thuringiensis*, is often truncated and introduced into the crop associated with the promoter, usually cauliflower mosaic virus 35S promoter. Before introduction into crops, it is common to increase the number of constructs in bacterial fermentation. The transgenic bacteria may be identified by use of a selectable marker such as antibiotic-resistance linked to a bacterium-specific promoter. The plants are produced by insertion of the *Bt* nucleic acid using transformed and disabled *Agrobacterium tumefaciens*, or by bombardment using a particle gun or other accepted transformation techniques. Transgenic crops are identified using a selectable marker such as a herbicide-tolerance or an antibiotic-resistance gene. Elite varieties are bred from these transgenic crops. Novartis launched transgenic insect-resistant maize that contained two selectable markers, glufosinate-tolerance and ampicillin-resistance, *Bt* 176. More recently, it has developed a second maize line, *Bt* 11, that contains no antibiotic-resistance genes.

TARGET PESTS: European corn borer (*Ostrinia nubilalis* Hübner), pink corn borer (*Sesamia cretica* Lederer) and noctuids (*Heliothis* spp. and *Helicoverpa* spp.).

TARGET CROPS: The main target crops are cotton and maize (corn).

BIOLOGICAL ACTIVITY: The gene is expressed constitutively throughout the plant such that susceptible insects that feed upon the crop succumb. (For a full description of the

mode of action and insect specificity of *Bt* endotoxins, see the *Bacillus thuringiensis* Berliner entries in Section 1: Micro-organisms). **Biology:** The *crylA(b)* gene codes for the full endotoxin that is hydrolysed by the gut digestive enzymes to form the toxin. It is active against a wide variety of Lepidoptera and is used to transform maize to give resistance to European corn borer (*Ostrinia nubilalis*). The *crylA(c)* gene codes for the endotoxin that is particularly active against tobacco budworm (*Heliothis virescens* (Fabricius)), a major pest of cotton. Many crops are now being introduced that contain both *Bt* genes and herbicide-tolerance genes. **Mode of action:** Once in the insect, the crystal proteins are solubilised and the insect gut proteases convert the original pro-toxin into a smaller toxin. These hydrolysed toxins bind to the insect's midgut at high-affinity, specific receptor binding sites, where they interfere with the potassium ion dependent active amino acid symport mechanism. This disruption causes the formation of a large cation-selective pore that increases the water permeability of the membrane. A large uptake of water causes epithelial cell swelling and eventual rupture with the subsequent disintegration of the midgut lining. Different toxins bind to different receptors and this explains the selectivity of different *Bt* strains. (This differential binding is described by some of those working in the field as different modes of action.) Different genes coding for these protein toxins have been isolated and used to transform various crop plants such that the crops produce insecticidal proteins throughout their life. In some cases, the truncated genes have been used so that the insect-active toxin is produced in the crop rather than the pro-toxin that requires hydrolysis in the insect gut. In 'KnockOut' and 'NatureGard', the *Bt*-toxin gene is *crylA(b)* and tissue that produces the toxin is green pollen and the stalk. The toxin level is relatively high, early season, in green tissue, but late season, the toxin levels decline rapidly. 'KnockOut' was developed by Ciba Seeds (then Novartis and now Syngenta) and Mycogen. The genetic event name is 176. In 'Bt-Xtra', the *Bt*-toxin gene is also *crylA(b)*, but the tissue that produces the toxin is leaf, kernel, stalk and silk. The toxin level is high in the leaves full season, but with much lower levels in kernel, stalk and silk. 'Bt-Xtra' was developed by DeKalb. The genetic event names are DeKalBt and DBT418. In 'YieldGard', the *Bt*-toxin gene is *crylA(c)*. The tissue that produces the pollen is leaf, pollen, tassel, silk and kernel. The toxin level is high in leaves, pollen, tassel, silk and kernel full season. 'YieldGard' was developed by Monsanto and Northrup King (Novartis) and is marketed by Pioneer Hi-Bred, Cargill, DeKalb and Golden Harvest. The genetic event names are Mon 810 and BT11. **Efficacy:** Field studies have shown good control of the target insects in the absence of additional chemical applications. Where other insect pests have appeared in the crop, additional chemical treatment is usually recommended.

COMMERCIALISATION: **Formulation:** Approval for Monsanto's transgenic cotton seed (line 531) was granted in the USA in late 1995, with the first introduction of 'BollGard' cotton in 1996 by Delta and Pineland as NUCOTN 33B and NUCOTN 35B. Monsanto's DBT418 maize, containing the *Bacillus thuringiensis* var *kurstaki crylA*(*c*) gene for resistance to European corn borers and the *phosphinothricin acetyl transferase* (*pat*) gene for tolerance to the herbicide glufosinate-ammonium, was introduced in 2001. Ciba (now Syngenta) was granted approval to sell *Bt*-transformed maize in the USA in 1995 and in Canada in 1996. **Trade names:** 'BollGard Cotton' (*CrylA(c)*) (Deltapine Seed), (Paymaster Cottonseed) and

(Stoneville Pedigreed Seed), 'BollGard II' (*CrylA(c)* and *CrylIA(b2)*) (Monsanto), 'InGard Cotton' (*CrylA(c)*) (Deltapine Australia), 'BollGard plus Roundup Ready Cotton' (*CrylA(c)*) (Deltapine Seed) and (Paymaster Cottonseed), 'Bt plus Buctril BXN System Cotton' (*CrylA(c)*) (Stoneville Pedigreed Seed), 'YieldGard Corn' (*Bt* MON 810) (Asgrow Seed), (Beck's Hybrids), (Cargill Hybrid Seeds), (Countrymark Cooperative), (Croplan Genetics), (DeKalb), (Golden Harvest Seeds), (Growmark), (Hoffman Seeds) and (Pioneer Hi-Bred International), 'NatureGard' (Mycogen Seeds), 'Maximizer Corn' (*CrylA(b)*), 'KnockOut', 'MaizeGard Corn' and 'MaisGard Corn' (Syngenta Seeds), 'Bt plus Liberty Link Corn' (Garst Seed), 'Bt Corn plus IMI Tolerance' (Garst Seed), (Mycogen Seeds) and (Syngenta Seeds), 'NatureGuard' (Mycogen Seeds), 'Bt-Xtra Corn' (DeKalb).

APPLICATION: Seed expressing the endotoxins from *Bacillus thuringiensis* shows tolerance to phytophagous insects, particularly from the order Lepidoptera. Its use often results in reduced applications of conventional insecticides.

COMPATIBILITY: Compatible with most crop protection agents. Cotton growers are recommended to plant 75% of their crop to *Bt*-cotton and to apply conventional chemicals to the remaining 25%, a refuge, which should not be treated with *Bacillus thuringiensis*-based products. Alternatively, they may sow 96% of their crop to *Bt*-cotton and leave the remaining 4% completely untreated. Refugia are designed to reduce the possibility of the onset of insect resistance to *Bt* endotoxins. In maize, a refuge of 20% of the planted area is required in non-cotton growing regions. This refuge should be located within half a mile of the *Bt*-maize. The refuge may be treated if insect damage reaches the economic threshold, but *Bt*-based insecticides may not be used. In potatoes, Monsanto has recommended to farmers that they plant at least 20% of their land to non-*Bt*-potatoes and that these must be planted adjacent, or as close as possible, to the *Bt*-crop. These refugia may be treated as the farmer deems appropriate.

MAMMALIAN TOXICITY: The truncated *Bt* CrylA toxin extracted from maize leaf tissue displayed activity that was similar to that produced in *E. coli* modified to produce CrylA endotoxins. The acute oral toxicity test with bacterially-derived CrylA protein caused no test substance-related deaths at a dose of 4 000 mg/kg. In addition, the endotoxin caused no allergic responses in any animal. There is minimal chance of dermal exposure or exposure by inhalation, as the endotoxins are enclosed within plant cells.

ENVIRONMENTAL IMPACT AND NON-TARGET TOXICITY: A review of the studies submitted for the registration of the *B. thuringiensis* CrylA protein produced in maize demonstrated a lack of adverse effects on birds, aquatic invertebrates, honeybee larvae, coccinellid predators and earthworms. However, *Bt* toxin was toxic to a collembolan species (*Folsomia candida*), which was selected as one of the soil invertebrate test organisms. If senescent post-harvest maize plants containing the CrylA(b) delta-endotoxin, were tilled into the top 6 inches (15 cm) of soil, a maximum of 4.2×10^{-4} mg toxin/kg of soil would be present. If a deeper disking or ploughing depth were used, then the concentration of toxin would be less. The NOEC level for the collembolan test species is 8.8×10^{-2} mg toxin/kg of soil, thus a safety factor of approximately 200-fold exists and an observable deleterious effect on the soil ecosystem is not expected to result from the

growing of CryIA delta-endotoxin-containing maize plants. **Bird toxicity:** When administered by oral gavage at a dose of 2 000 mg protein/kg b.w., *Bt*-maize had no apparent effect upon bobwhite quail after 14 days. Thus, the acute toxicity LD_{50} value for quail was determined to be >2 000 mg protein/kg b.w. In view of the lack of acute toxicity with *Bt*-maize, no avian hazard is expected from the proposed uses of this plant-pesticide. **Fish toxicity:** The requirement for a static renewal toxicity study has been waived, based on a lack of exposure of fish to the *Bt* endotoxin CryIA protein produced in maize. **Other aquatic toxicity:** In a 48-hour static renewal toxicity study of maize pollen containing *Bt* CryIA delta-endotoxin, *Daphnia magna* was treated at five concentration levels, including a maximum hazard dosage of 150 mg/litre (nominal) of water. No mortalities were observed at any of the treatment levels tested. Thus, the 48-hour EC_{50} was determined to be >150 mg/litre. The LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration) were found to be 150 mg/litre. **Effects on beneficial insects:** Maize pollen containing the Cry1A toxin caused no significant adverse effects to lady beetle predators or to earthworms. **Monarch butterflies:** Reports of toxicity of high doses of *Bt* toxin in pollen falling onto milkweed plants to monarch butterfly larvae in the laboratory do not translate into exposure to toxic levels in the field. Further, the monarch butterfly is neither an endangered nor a threatened species. It is an abundant and widespread insect which, in North America, ranges from central Mexico to southern Canada. There are many factors that cause severe mortality of monarch butterflies, among these are predation, parasitism, destruction of the overwintering habitat and, most notably, climatic variations. Nevertheless, the US-EPA has issued the following warning to farmers – 'The potential for non-target species (e.g., monarch butterfly larvae) to be affected by *Bt* corn pollen remains under study. As an interim measure, the EPA is encouraging growers to place the non-*Bt* corn refuge between *Bt* corn and habitats such as prairies, forests, conservation areas, and roadsides.' **Persistence in the environment:** *Bacillus thuringiensis*-derived CryIA protein, when added to soil as a component of maize tissue, decreased, with an estimated half-life (DT_{50}) of 15 days. When incubated without soil, it had a DT_{50} of 25.6 days and a DT_{90} of 40.7 days. The bioactivity of purified CryIA protein in soil decreased, with an estimated DT_{50} of 8.3 days and a DT_{90} of 32.5 days.