MIR604 Maize
Syngenta Agrisure™ RW Maize
for Corn Rootworm Control

EU approval for import, food and feed use

December 2009
Maize: an Important Crop

Maize, also known as corn, is one of the three most important grains in the world. Some 155 million hectares of maize are harvested annually around the world. Over two-thirds of this is used directly as animal feed; the rest is used for food, processed food, feed additives and even industrial uses such as ethanol and starch production. The European Union imports approximately three million tonnes of maize grain each year.

Insect-Protected Maize

Many different insect species feed on maize, including corn borers, earworms, and rootworms. These pests are estimated to decrease global production by more than 50 million metric tons each year, with severe economic consequences for growers. As a global leader in agriculture, Syngenta is committed to developing a range of solutions, including those that utilize biotechnology, to prevent crop losses from such devastating pests.

Syngenta has developed insect-resistant maize varieties which incorporate a trait event called MIR604 that has specific activity to corn rootworms and related species, without affecting non-target organisms. This rootworm-protected maize offers a powerful, convenient and competitive solution to pest control because it protects the plant from inside the roots, where the corn rootworms eat.

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1 USDA 2008 Figures, access August 2009
2 USDA (August 12, 2009, WASDE) and International Grains Council (July 30, 2009) – note: this figure does not include intra-EU trade
In regions with high levels of infestation, MIR604 maize is an effective tool in integrated pest management strategies (IPM) and offers growers a safe and environmentally sustainable solution to control infestations while delivering full yield potential.

MIR604 maize helps farmers improve productivity, secure yields, produce higher quality crops and reduce the environmental footprint of modern agriculture. Food and feed products made from MIR604 maize are safe to humans, animals and the environment.

**MIR604 Maize: Mode of Action**

Event MIR604 (marketed under the brand name Syngenta Agrisure™ RW) is genetically modified (GM) maize that expresses a protein which safely and effectively controls corn rootworm *(Coleopteran, Diabrotica sp)* infestations. This protein (modified Cry3A or mCry3A), is produced from the *mCry3a* gene, a synthetic gene that is related to the *cry* gene found in the naturally-occurring soil bacterium, *Bacillus thuringiensis (Bt)*. The gene is inserted into maize plants using modern biotechnology techniques and its effect is safe, targeted and reliable. Bt proteins have been used safely and successfully for insect control for nearly 50 years by farmers and gardeners.

State-of-the-art scientific methods have enabled Syngenta scientists to maximize the production of mCry3A protein in maize roots, where rootworms feed, while minimizing the presence of the protein in other parts of the plant, such as the pollen.

Additionally, a marker gene (*pmi*) has been added to allow the selection of transformed plants during the research and developmental stage. The *pmi* gene was isolated from the common bacterium *Escherichia coli*. The protein produced by this gene is phosphomannose isomerase (PMI). PMI allows transformed maize cells to utilize mannose (a simple sugar) as a sole carbon source, while maize cells lacking PMI cannot grow in this type of media. The PMI protein is already found naturally in many animal and plant species, and it is a safe selectable marker for plant transformation.
Benefits of MIR604 Maize

MIR604 represents a highly effective way to control the devastating corn rootworm pest, as well as to secure and increase yields and improve the environmental footprint of modern agriculture.

Corn rootworms are beetles (Coleoptera) from the genus *Diabrotica*. Western corn rootworm (*WCRW*, *Diabrotica virgifera virgifera*), is a major maize pest in North America. Although not as widespread, Northern corn rootworm (*NCRW*, *Diabrotica barberi*), is also a serious pest for maize in the USA. Together, these two rootworm species are frequently referred to as “billion dollar beetles” because the US Department of Agriculture (USDA) estimates that their combined cost to US corn growers is about $1 billion a year.

In Europe, WCRW is an exotic invasive species. This pest was first observed in 1992 near the city of Belgrade (former YU)\(^1\), and since this time it has dispersed in all directions. There have been outbreaks of this insect near the airports of Venezia (1998) and Milano (2000) in Italy, and near the airport of Lugano-Agno (2000) in Switzerland as well as Mulhouse airport (2002) in France\(^2\). WCRW is currently found in Austria, Bosnia & Herzegovina, Bulgaria, Croatia, Czech Republic, France, Italy, Germany, Hungary, Romania, Slovakia, Switzerland, and Ukraine. Stringent eradication efforts are underway.

\(^1\) Baca et al., 1993  
\(^2\) EPPO, 2004
How do these insects cause so much damage? The answer relies on two simple factors: location and number. Adult corn rootworm females lay their eggs in the soil near maize plants. A single female can lay up to 1000 eggs in her lifetime, so the number of pests present in a crop area can add up quickly and lead to extensive damage. After hatching, the young rootworms, called larvae, burrow through the ground to maize roots, where they feed. The larvae eat the fine root hairs and often burrow into roots. This removal of root tissues affects a plant’s survival by inhibiting its ability to take up water and soil nutrients. Roots also serve as an important anchoring system, and damage to them can lead to lodging and what is commonly known as a “goose neck” effect on the maize stalk.

After several weeks of eating the plant from within, the larvae pupate, or form a cocoon. They emerge from the cocoon as winged adults, and can fly from one plant to the next, eating maize silks and pollen, and colonizing nearby fields. Corn rootworms do not have natural enemies in many areas where they infest maize, and once they move into a field, they can be very difficult to control. Usually a variety of different methods are needed in order to be effective. Crop rotation can help in some cases, however, WCRW and NCRW have shown to adapt to rotational control methods in the US.
Event MIR604 maize has been specifically developed to combat attack from corn rootworms. Because the protection is within the plant’s roots where the corn rootworms feed, it can be more effective than traditional control methods alone. Combined with other approved Syngenta insect protection products, such as Cruiser® seed treatment, chemical insecticides, good farming practices and integrated pest management systems, Event MIR604 maize will offer growers a full range of options to protect their maize from corn rootworm damage, and thus help secure and increase yields in a targeted and sustainable way.

Source: Syngenta Agronomy Research, Third Party Contractors, and University Cooperators
Safety of MIR604 Maize

The safety of Syngenta’s products for humans, animals and the environment is of paramount importance. Even before any regulatory submissions were made, many years of research have been conducted with MIR604 maize and the mCry3A protein. MIR604 has been comprehensively and exhaustively analyzed for human health effects, digestibility, allergenicity and toxicity, environmental impact, and effects on mammals and non-target organisms. MIR604 has been assessed and endorsed by numerous independent scientific committees around the world. In all countries where MIR604 has been approved for food, feed and/or planting, the regulatory authorities have concluded, after extensive evaluation, that MIR604 maize is as safe as conventional varieties of maize. These conclusions have been based on a full range of scientific studies, including tests which examined the potential for human and animal health effects of the product, nutritional equivalency, the effects of the introduced protein and marker gene, and the potential impacts of the maize on the environment.
Human and Animal Safety

Cry proteins have been used in agriculture for many years and their safety has been well-established. Studies have been performed to specifically assess the safety of the mCry3A protein and Event MIR604 maize. Event MIR604 maize has been shown to have nutritional value equivalent to conventional maize and no adverse effects were observed from human or animal consumption of Event MIR604 maize, even at high doses.

The allergenicity potential of mCry3A was also assessed. Data indicate that the mCry3A protein, as expressed in Event MIR604 maize, will be readily digested as conventional dietary proteins. The mCry3A protein is labile to heat, meaning it is substantially eliminated in food and feed products by standard processing and cooking methods. The amino acid sequence of the mCry3A protein was compared to the sequences of known protein toxins and food allergens and it was determined that there were no similarities suggestive of toxic or allergenic potential for mCry3A. The results of these, and other, thorough assessments indicate that Event MIR604 maize is as safe as conventional maize.

Similar studies have been performed with PMI marker, also demonstrating that crops containing this protein are as safe as conventional crops.
Environmental Safety

Environmental risk assessments were conducted on the impacts of Event MIR604 maize for mammals and non-target organisms, such as birds, fish, earthworms, honeybees, ladybird beetles (ladybugs), soil beetles, and insects. These assessments, as well as numerous studies published by independent scientists, show MIR604 maize to be as safe as conventional maize.

Data demonstrate that mCry3A has specific activity toward the target pest insects, and that it does not have activity against other non-target organisms. This specific activity occurs because there are several barriers that must be overcome in order to make the mCry3A protein work: first, the target organism must feed on maize tissue containing the mCry3A protein. Second, once the mCry3A protein is ingested, the gut pH must be just right so that the protein is made soluble. Third, the right combination of gut digestive enzymes (proteases) must be present in order to process mCry3A to its smaller, active form. The modification in the mCry3A protein allows it to be activated by specific proteases in the corn rootworm’s gut, leading to improper salt balance and ultimately starvation. If any step in the process is missing, then the protein simply cannot perform its function. Because living organisms are so complex, only very specific target organisms have exactly the right combination of characteristics for mCry3A to be effective.
Molecular Biology Studies

Thorough studies have been undertaken to demonstrate that the inserted mcry3A gene is expressed as intended, and that the level of mCry3A protein is appropriate to have the desired effect on the corn rootworms. The data demonstrate that the mCry3A protein is not detectable in the pollen of Event MIR604 maize plants, and that the expression levels and CRW activity are stable across many generations. Similar studies have been performed with PMI, also demonstrating that crops containing this protein are as safe as conventional maize.

Global Regulatory Status of MIR604 Maize

Syngenta’s Agrisure® RW now has full food, feed and environmental approval in the United States, Canada, and Japan, and import approval in Mexico, Taiwan, Korea, China, Philippines, Russia, Australia, New Zealand. With the EU approval for food, feed and import, Agrisure® RW grain can now be accepted by elevators exporting grain to the EU.
Regulatory Framework for GM Crops in the EU

The EU has in place strict legislation covering GM food, feed and crops, including Directive 2001/18/EC for deliberate release of GMOs into the environment (repealing Directive 90/220/EEC) and Regulation (EC) No. 1829/2003 on GM food and feed. Additionally, the labeling and traceability Regulation (EC) 1830/2003 for GM food and feed requires labeling for food and feed containing GM material above 0.9%.

EU Approval of MIR604 for Food and Feed Imports

On 23 December 2004, Syngenta Seeds S.A.S. on behalf of Syngenta Crop Protection AG, submitted an application to the competent authority of the United Kingdom for the import approval of MIR604 maize, in accordance with Articles 5 and 17 of Regulation (EC) No. 1829/2003, for the placing on the market of foods, food ingredients, and feed containing, consisting of, or produced from MIR604 maize. The application also covers the placing on the market of other products containing or consisting of MIR604 maize for the same uses as any other maize with the exception of cultivation. The application also includes a monitoring plan for environmental effects conforming with Annex VII to Directive 2001/18/EC consisting of a general surveillance plan, in line with the intended use of the products.
On 21 July 2009, the European Food Safety Authority (EFSA) gave a positive opinion in accordance with Articles 6 and 18 of Regulation (EC) No. 1829/2003 and concluded that it is unlikely that the placing on the market of the products containing, consisting of, or produced from MIR604 maize will have any adverse effects on human or animal health or the environment in the context of their intended uses.

In addition, the EFSA opinion states:

“There are no indications of an increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of maize MIR604 viable grains during transportation and processing for food and feed uses. Taking into account the scope of the application, both the rare occurrence of feral plants and the low levels of exposure through other routes indicate that the risk to non-target organisms is negligible.”

After consideration by the Standing Committee on the Food Chain and Animal Health (SCFCAH) on 19 October 2009, and the Council of Agriculture Ministers on 20 November 2009, MIR604 was approved by the European Commission on 30 November 2009 for food, feed, import and processing in accordance with Regulation (EC) No. 1829/2003 on GM food and feed (Commission Decision of 30 November 2009 authorising the placing on the market of products containing, consisting of, or produced from genetically modified maize MIR604 (SYN-IR6Ø4-5) pursuant to Regulation (EC) No. 1829/2003 of the European Parliament and of the Council which adopted the proposals for 10 years authorisation.

1 http://www.efsa.europa.eu/EFSA/efsaloctime-1178620753812_1211902691168.htm
Detection Method

An event-specific method for the quantification of MIR604 using real-time PCR has been validated by the European Commission Joint Research Centre (JRC). It was published on the Community Reference Laboratory (CRL) website on 3 April 2007³.

Reference Material (ERM®-BF423) is accessible at the Institute of Reference Materials and Measurements (IRMM)⁴.

Traceability, Labelling, Unique Identifier

Operators importing, handling or using MIR604 grain and derived foods and feeds in the EU are informed of the legal obligations regarding traceability and labelling, laid down in Regulation (EC) No. 1830/2003 and in the conditions of placing on the market of the consent.

No specific labelling requirements other than those provided for in Articles 13⁵ and 25⁶ of Regulation (EC) No. 1829/2003 are necessary for foods, food ingredients and feed containing, consisting of, or produced from MIR604 maize. However, in order to ensure the use of the products within the limits of the authorisation, the Commission Decision request that the words ‘not for cultivation’ shall appear on the label of and in documents accompanying products containing or consisting of MIR604 maize. The unique identifier of MIR604 is SYN-IR6Ø4-5.
