

MON 531 × MON 1445 cotton

**Bollgard[®] with Genuity[®] Roundup
Ready[®] Cotton**

Insect protection and herbicide tolerance

Key facts



Monsanto EMEA

May 2015

MON 531 × MON 1445 - Bollgard® with Genuity® Roundup Ready® cotton

Cotton

Worldwide, four *Gossypium* species are collectively known as cotton and are grown commercially. These include two diploid species ($2n=2x=26$) *G. arboreum* L. and *G. herbaceum* L., which evolved in Africa and the Middle East, and two allotetraploid species ($2n=4x=52$) *G. barbadense* and *G. hirsutum*, which evolved in the Americas. The major type of cotton being grown commercially around the world is the upland cotton *G. hirsutum*. *G. herbaceum* and *G. arboreum*, are of regional agronomic importance, mostly in areas not suited for *G. hirsutum* or *G. barbadense* (Southeast Asia and the dry, unproductive areas of India and Pakistan) and they comprise less than 4% of the total cotton produced globally. There are no close wild relatives of cotton in the EU.

The fiber, or lint, is used to make cloth—for towels, clothes, sheets, etc. The cottonseeds from the plant are crushed into cottonseed oil, which can be used in everyday items such as cooking oil and salad dressing, and into hulls and meal, which are used for livestock feed.

In 2013/2014 season, the major cottonseed oil, meal and oilseed producing countries in the world were China, India, Pakistan, Brazil and the USA¹. In the EU, cotton is commercially grown in Italy, Spain and Greece.

What is MON 531 × MON 1445 cotton?

Monsanto Company has developed, via traditional breeding, Bollgard® with Genuity® Roundup Ready® cotton (hereafter referred to as MON 531 × MON 1445 cotton) which produces an insect control protein (Cry1Ac) derived from the naturally occurring soil bacterium, *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*) combined with glyphosate-tolerance.

Production of the Cry1Ac protein in the MON 531 cotton plant provides effective season-long protection against key Lepidopteran insect pests, while the glyphosate tolerance enables the application of a Roundup® agricultural herbicide over the top of the cotton crop.

MON 531 × MON 1445 cotton expresses the Cry1Ac protein, which provides protection against key lepidopteran insect pests, including tobacco budworm, pink bollworm and cotton bollworm. MON 531 × MON 1445 cotton expresses the 5-enolpyruvylshikimate-3 phosphate synthase protein from *Agrobacterium tumefaciens* sp. strain CP4 (CP4 EPSPS), which confers tolerance to glyphosate, which is the active ingredient in Roundup® agricultural herbicides. MON 531 × MON 1445 cotton also contains the *neomycin phosphotransferase II* (*nptII*) gene which provides a plant selectable marker and contains the 3'(9)-*O*-aminoglycoside adenyltransferase (*aad*) gene, a bacterial

selectable marker. The *cry1Ac*, *cp4 epsps* and *nptII* genes are expressed in the plant, while the *aad* gene-product is not produced in the plant as this gene is under the control of a bacterial promoter.

MON 531 × MON 1445 cotton: mode of action

Insecticidal activity of the Cry1Ac protein requires the protein to be ingested. In the insect gut, the protein is proteolytically cleaved to the active core of the protein. The core protein binds to a specific receptor on the mid-gut of lepidopteran insects, inserts into the membrane and forms ion-specific pores. This disrupts the digestive processes and causes the death of the insect. The digestive tract tissues of non-target insects, mammals, birds and fish do not contain receptors to bind the Cry1Ac protein. Therefore the Cry1Ac protein cannot disrupt digestion and is non-toxic to species other than lepidopteran insects.

Roundup® herbicides contain the active ingredient glyphosate, a broad spectrum herbicide that acts via inhibition of the protein EPSPS. EPSPS is found naturally in all plants, fungi and bacteria and is important in the production of essential aromatic amino acids. Inhibition of EPSPS by glyphosate blocks the production of these amino acids, interfering with growth and ultimately leading to plant death. MON 531 × MON 1445 cotton plants contain a glyphosate tolerant EPSPS, isolated from the CP4 strain of the common soil bacterium *A. tumefaciens*. The presence of the CP4 EPSPS ensures the continued function of the aromatic amino acid pathway, even in the presence of the herbicide glyphosate.

Worldwide plantings and regulatory status of MON 531 × MON 1445 cotton

In 2014, biotech cotton was planted to 25.1 million hectares, which is 68% of the 37 million hectares of global cotton (James, 2014). MON 531 × MON 1445 cotton has received regulatory approvals for cultivation in Argentina, Australia, Brazil, Colombia, Mexico, Paraguay, the Republic of South Africa. Some countries, for instance the USA, do not require approvals for stack products produced via traditional breeding. Additional import approvals are received in Colombia, EU, Korea, Mexico, New Zealand and the Philippines². The first commercial plantings of MON 531 × MON 1445 cotton were in the USA in 1997.

A stringent regulatory system for genetically modified crops in the EU

In the EU, the regulatory system for GM crops comprises several regulations and directives, including Directive 2001/18/EC for deliberate release of genetically modified organisms (GMOs) in the environment (repealing Directive 90/220/EEC) and Regulation (EC) No. 1829/2003 on genetically

¹Source: Foreign Agricultural Service, Official USDA Estimates. <http://apps.fas.usda.gov/psdonline/psdQuery.aspx>

² Bollgard, Genuity, Roundup and Roundup Ready are registered trademarks of Monsanto Technology LLC.

² <http://www.biotradestatus.com/> - Accessed 13 April 2015

modified food and feed (replacing Regulation (EC) No. 258/97 on novel foods and novel food ingredients for GM products).

Regulation (EC) No 1829/2003 includes procedures for the authorization of deliberate release (cultivation and/or import and processing), in addition to Food and Feed use, according to the “one door, one key” principle.

A regulation on traceability and labeling of GMOs and products produced from GMOs (Regulation (EC) No. 1830/2003) entered into force on 18 April 2004. A regulation laying down the methods of sampling and analysis for the official control of feed as regards presence of genetically modified material for which an authorization procedure is pending or the authorisation of which has expired (Commission regulation (EU) No. 619/2011) entered into force on 24 June 2011.

Regulatory status of MON 531 × MON 1445 cotton in the EU

Feed materials, feed additives and food additives

Feed materials, feed additives and food additives produced from MON 531 × MON 1445 cotton were first placed on the market in the EU in 1997, following the commercial introduction of MON 531 × MON 1445 cotton varieties in the US in 1997. Foods produced from MON 531 × MON 1445 cotton (food additives) were authorised under Directive 89/107/EEC, while feed produced from cotton MON 531 × MON 1445 (feed materials and feed additives) were subject to Directive 70/524/EEC.

After the date of entry into force of the Regulation (EC) 1829/2003, the products mentioned above were notified to the European Commission according to Articles 8(1)(a), 8(1)(b) or 20(1)(b) of this Regulation and subsequently included in the Community Register of GM food and feed.

Renewal application for use of food additives, feed material and feed additives

On 17 April 2007, Monsanto submitted a renewal application for use of food additives, feed material and feed additives produced from MON 531 × MON 1445 cotton as any other cotton under Regulation (EC) No 1829/2003 to European Food Safety Authority (EFSA) via the European Commission. The application received the reference number EFSA-GMO-RX-MON531×MON1445 and was declared valid on 12 March 2008.

food and feed products

On 30 November 2004 Monsanto submitted an application for food and feed products derived from MON 531 × MON 1445 cotton. The application received the reference number EFSA-GMO-UK-2005-09 and was declared valid on 12 July 2005.

Since both EFSA-GMO-RX-MON531×MON1445 and EFSA-GMO-UK-2005-09 cover products derived from MON 531 × MON 1445 cotton that do not contain viable plant parts, the EFSA GMO Panel provided a single scientific opinion, valid for both applications.

EFSA evaluated the application as well as Monsanto’s additional information, scientific comments submitted by the Member States and relevant scientific publications. The EFSA published a positive scientific opinion on 28 March 2012 (adopted 8 March 2012) (EFSA, 2012), in which the EFSA

concluded that “MON 531 × MON 1445 cotton-derived products are as safe as products derived from the conventional counterpart in the context of their intended uses”.

Finally, on 16 March 2015, the European Commission presented the Draft Commission Implementing Decision authorizing the placing on the market of products produced from genetically modified MON 531 × MON 1445 cotton, to the Standing Committee on Plants, Animals, Food and Feed (PAFF) for a vote. Since no qualifying majority was reached, the draft decision was passed to the Appeal Committee who met for a vote on 31 March 2015, again without reaching a qualified majority. The Appeal Committee forwarded the draft decision to the European Commission. The authorization was finally granted by the European Commission on 24 April 2015 (Commission Decision, 2015), 37 months after the EFSA opinion.

Traceability, labeling, unique identifier

Operators importing, handling or using MON 531 × MON 1445 cotton derived foods and feeds in the EU should be informed of the legal obligations regarding traceability and labeling, laid down in Regulation (EC) No. 1830/2003. The unique identifier of MON 531 × MON 1445 cotton is MON 00531-6 × MON 01445-2.

The validated methods, as well as the validation report for MON 531 and MON 1445, prepared by the CRL in collaboration with the European Network of GMO Laboratories (ENGL), were published on 18 June, 2008 and 10 June 2008 at the CRL website, respectively³. A report on the verification of the performance of the methods for MON 531 × MON 1445 cotton was published on 19 March 2009.

Food, feed and environmental safety of MON 531 × MON 1445 cotton

Food and feed safety

The food and feed safety of MON 531 × MON 1445 cotton was established through:

- A detailed molecular characterization of the inserted DNA,
- An assessment of the toxic and allergenic potential of Cry1Ac, CP4 EPSPS and NPTII, based upon their long history of safe use, their rapid digestibility and their lack of toxicity or allergenicity, as demonstrated with bioinformatics as well as *in vitro* and *in vivo* safety studies with the proteins,
- The compositional and nutritional analyses confirmed that MON 531 × MON 1445 cotton is compositionally and nutritionally equivalent to, and as safe as, those of conventional cotton,
- A large margin of safety resulted from the low dietary exposure,
- An assessment showed that the intake resulting from consumption of foods derived from MON 531 × MON 1445 cotton do not raise nutritional concerns. This was confirmed in a feeding study with catfish.

³ EU-RL GMFF - <http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx> - accessed February 26, 2015

Further details on the safety of MON 531 and MON 1445 used in the traditional breeding to make MON 531 × MON 1445 cotton are available in product safety summaries on Monsanto's website⁴.

Environmental safety

The environmental safety of MON 531 × MON 1445 cotton was established through extensive field trials conducted in 1998 and 1999 in the USA. All these field trials demonstrated that MON 531 × MON 1445 cotton poses negligible risk to human health or to the environment. Considering the scope of the application, potential interactions of MON 531 × MON 1445 cotton with non-target organisms and the abiotic environment were not considered to be an issue by the EFSA due to the low level of exposure. Moreover, there are no close wild relatives of cotton in the EU

In their scientific opinion on MON 531 × MON 1445 cotton, the EFSA concluded that "*crossing insect-resistant cotton MON 531 with glyphosate-tolerant cotton MON 1445 to produce the stacked MON 531 × MON 1445 cotton did not result in any consistent changes in phenotypic and agronomic characteristics compared with its conventional counterpart and the parental cotton lines*" (EFSA, 2012).

MON 531 × MON 1445 cotton, the benefits

In countries where MON 531 × MON 1445 cotton is grown, a number of benefits for both farmers and the environment are expected. These include:

- reduced insecticide use, improved control of target insect pests,
- improved yield, reduced production costs and improved opportunity to grow cotton, resulting in improved economics for the cotton growers,
- Improved flexibility in weed control compared to herbicide programs used in conventional cotton, as specific pre-emergent herbicides that are used for prevention are replaced by a broad-spectrum post-emergent herbicide that can be used on an 'as needed' basis.

Further reading

- Harrison, L., Bailey, M., Naylor, M., Ream, J., Hammond, B., Nida, D., Burnette, B., Nickson, T., Mitsky, T., Taylor, M., Fuchs, R., Padgett, S. 1996. The Expressed Protein in Glyphosate-Tolerant Soybean, 5-Enolpyruvylshikimate -3-Phosphate Synthase from *Agrobacterium* sp. Strain CP4, Is Rapidly Digested in Vitro and is Not Toxic to Acutely Gavage Mice. *Journal of Nutrition*. 126(3): 728-740..
- Perlak, F. J., Oppenhuizen, M., Gustafson, K., Voth, R., Sivasupramaniam, S., Heering, D., Carey, B., Ihring, R. A., Roberts, J. K. . 2001. Development and Commercial Use of Bollgard Cotton in the USA-early Promises Versus Today's Reality. *The Plant Journal*. 27: 489-501.
- Reddy, K. 2004. Weed Control and Species Shift in Bromoxynil- and Glyphosate-Resistant Cotton (*Gossypium hirsutum*) Rotation Systems. *Weed Technology*. 18: 1311-1319.

- Sims, S., Berberich, S., Nida, D., Segalini, L., Leach, J., Ebert, C., Fuchs, R. 1996. Crop Physiology and Metabolism: Analysis of Expressed Proteins in Fiber Fractions from Insect-Protected and Glyphosate-Tolerant Cotton Varieties. *Crop Science*. 5: 1212-1216.
- Carriere, Y., Dennehy, T., Pedersen, B., Haller, S., Eilers-Kirk, C., Antilla, L., Liu, Y., Willott, E., Tabashnik, B. 2001. Large-Scale Management of Insect Resistance to Transgenic Cotton in Arizona: Can Transgenic Insecticidal Crops be Sustained. *Journal of Economic Entomology*. 94(2): 315-325.
- Fuchs, R., Ream, J., Hammond, B., Naylor, M., Leimgruber, R., Berberich, S. 1993. Safety Assessment of the Neomycin Phosphotransferase II (NPTII) Protein. *Bio/Technology*. 11(13): 1543-1547.
- Macintosh, S., Stone, T., Sims, S., Hunst, P., Greenplate, J., Marrone, P., Perlak, F., Fischhoff, D., Fuchs, R. 1990. Specificity and Efficacy of Purified *Bacillus thuringiensis* Proteins Against Agronomically Important Insects. *Journal of Invertebrate Pathology*. 56(2): 258-266.
- York, A., Culpepper, A., Bowman, D., May, O. 2004. Performance of Glyphosate-tolerant Cotton Cultivars In Official Cultivar Trials. *Journal of Cotton Science*. 8(4): 261-270.

References

- Commission Decision, 2015. Commission Implementing Decision (EU) 2015/695 of 24 April 2015 renewing the authorisation for existing genetically modified cotton MON 531 × MON 1445 (MON-ØØ531-6 × MON-Ø1445-2) products and authorising the placing on the market of cottonseed oil produced from genetically modified cotton MON 531 × MON 1445 (MON-ØØ531-6 × MON-Ø1445-2) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council (notified under document C(2015) 2769). *Official Journal*, 1.
- EFSA, 2012. Scientific opinion on applications EFSA-GMO-UK-2005-09 and EFSA GMO-RX-MON 531×MON 1445 for the placing on the market of food and feed produced from or containing ingredients produced from insect-resistant and herbicide-tolerant genetically modified cotton MON 531 × MON 1445¹ and for the renewal of authorisation of existing products produced from cotton MON 531 × MON 1445² both under Regulation (EC) No 1829/2003 from Monsanto. *The EFSA Journal*, 10 (3), 1-31.
- James C, 2014. Global status of commercialized biotech/GM crops: 2014. ISAAA, 49, Executive Summary.

⁴<http://www.monsanto.com/products/pages/product-safety-summaries.aspx>. Accessed March 02, 2015.