

Cultivation of glyphosate tolerant GHB614 cotton

COGEM advisory report CGM/130321-01

Summary

The present application by Bayer CropScience (file EFSA/GMO/ES/2012/104) concerns the cultivation of cotton GHB614 in Europe. Cotton line GHB614 was produced by Rhizobium radiobacter mediated transformation of conventional cotton. GHB614 expresses the 2mepsps gene and is as a result tolerant to glyphosate based herbicides. In the past, COGEM advised positively on the import of cotton line GHB614.

In North West Europe, no wild relatives of cotton are present. Modern cotton cultivars do not possess any of the attributes commonly associated with problematic weeds. COGEM does not know any reasons to assume that the introduced traits will increase the potential of cotton to establish feral populations.

Most importantly, cotton cannot survive the current climatologic conditions in North West Europe, making cultivation in the Netherlands highly unlikely. Since cotton is not cultivated in the Netherlands, there will be no exposure of organisms above- or belowground to GHB614. COGEM concludes therefore that risks for non-target organisms (NTOs) or soil functions are absent in the Netherlands. Although the NTO and soil studies might not fulfil the standards of COGEM, COGEM deems an evaluation of these studies unnecessary because the Dutch environment is not likely to be exposed to GHB614 plants. The General Surveillance plan could be improved on several points.

In view of the above, COGEM is of the opinion that the risks of cultivation of cotton line GHB614 in Europe for the environment in the Netherlands are negligible.

Introduction

The scope of the present notification (EFSA/GMO/ES/2012/104) by Bayer CropScience AG concerns cultivation of the genetically modified cotton line GHB614 in Europe. This cotton line was produced by *Rhizobium radiobacter* (previously known as *Agrobacterium tumefaciens*¹) mediated transformation of conventional cotton and expresses the 2mepsps gene originally from maize. As a result, GHB614 cotton is tolerant to glyphosate based herbicides.

COGEM was asked by the Dutch State Secretary for Infrastructure and the Environment to evaluate the environmental risks of commercial cultivation of this cotton line. Market authorisation is a European centralised procedure and an eventual authorisation would be valid in all EU member states. However, as an advisory body to the Dutch government, COGEM primarily assesses the risks of cultivation of the GM crop for the environment in the Netherlands.

Previous COGEM advice

COGEM advised positively on the import and processing of cotton GHB614 in 2008.² The EFSA approved import of GHB614 cotton in June 2011. Furthermore, in 2011, COGEM advised positively on the import and processing of cotton line GHB614xLLCotton25 expressing the *2mepsps* and *bar* genes.³

Aspects of the crop

Cotton is a member of the genus *Gossypium* and belongs to the *Malvaceae* family. The majority of cultivated cotton (90%) is *Gossypium hirsutum*, but *Gossypium barbadense*, *Gossypium arboreum* and *Gossypium herbaceum* are cultivated as well.^{4,5,6} In the south of Europe *G. hirsutum* cotton is grown in Greece, Spain and Bulgaria.⁷

Cotton plants reproduce sexually.⁶ Cotton is predominantly a self-pollinating species, but crosspollination may occur. The pollen of cotton is large, heavy and somewhat sticky.^{5,6} The viability of *G. hirsutum* pollen decreases rapidly after eight hours.⁶ Outcrossing rates for cotton are strongly influenced by the prevalence of insects. Dissemination of pollen by wind is (almost) absent.^{5,6} Pollinators of cotton flowers include bumblebees (*Bombus*), honeybees (*Apis*) and other bee species (*Anthophora*, *Melissodes* and *Halictus*).⁵ Wild relatives of cotton (*G. hirsutum*) do not occur in North West Europe. Therefore, hybridisation with wild relatives cannot occur in North West Europe.⁵

Cotton is highly sensitive to temperature and susceptible to frost. Temperature is the main factor which determines the geographic range in which cotton can be grown. *G. hirsutum* seeds do not germinate below 15 °C.⁵ The optimum temperature for germination is 34 °C, for growth of seedlings 24-29 °C, and for later continuous growth 34 °C. Plant development ceases below a temperature of 12°C and delays when the temperature rises above 38 °C.^{5,6} Cotton needs a period of 180 to 200 frost-free days for normal maturation, with an average of 150 days of suitable temperatures (averaging 21-22 °C).^{4,5} When the crop is grown at lower temperatures, the production of vegetative branches increases and the cropping period is extended. In places where cotton is grown as a rain-fed crop the average rainfall is 800-1200 mm.⁴ In areas where the rainfall is less than 500 mm a year, irrigation should be applied.⁵ In the seedling stage cotton does not tolerate shady circumstances, and in later plant stages reduced light intensity affects flowering and fruiting.⁴

Cottonseed can be dispersed by wind, water, during transport or when feeding cattle.⁶ In addition, cottonseed can be transported by birds or rodents. Germination is less likely to occur in undisturbed sites than in disturbed sites.⁶ Seeds from cotton cultivars do not possess dormancy and will germinate in autumn if conditions are favourable.^{5,6} In addition, seeds will usually not survive in humid soil.⁶ In regions with mild and dry winters, cottonseeds may overwinter and germinate in spring. Seedlings are sensitive to competition from weeds.⁵

Modern cotton cultivars do not possess any of the attributes commonly associated with problematic weeds, such as dormancy, persistence in seed banks, germination under adverse environmental conditions, rapid vegetative growth, a short life cycle, very high seed output, high seed dispersal and long-distance dispersal of seeds. Cotton volunteers occur in cotton

growing areas and may occur when cottonseed is used as livestock feed. The presence of volunteer cotton is limited by soil moisture content and frost.⁶ There are reports that *G. hirsutum* and *G. herbaceum* cotton are naturalised in some Southern European countries, e.g. Greece and Spain.^{8,9,10,11}

Molecular characterisation

The molecular characterisation of cotton GHB614 was previously evaluated by COGEM.² COGEM concluded that the molecular characterisation of GHB614 is adequate. The dossier for GHB614 cultivation included an updated bioinformatic analysis of the inserted sequences and putative polypeptides spanning the genomic DNA-insert junctions, which did not reveal any new concerns. An overview of the construction of GHB614, the inserted genetic elements as well as the properties of the introduced genes is given below.

Cotton line GHB614 was produced by *R. radiobacter*-mediated transformation of tissue from conventional cotton variety Coker312, using the vector pTEM2. This vector contains the following elements:

- Right border region derived from the Ti-plasmid of *R. radiobacter* used for transfer of the T-DNA;
- Ph4a748At, constitutive promoter derived from *Arabidopsis thaliana*;
- Intron1 h3At, intron from *A. thaliana*;
- TPotpC, chloroplast transit peptide (CTP), based on CTP sequences from sunflower (*Helianthus annuus*) and maize (*Zea mays*);
- *2mepsps* gene, modified 5-enolpyruvylshikimate-3-phosphate synthase gene originally derived from *Z. mays*;
- 3'histon At, terminator derived from *A. thaliana*;
- Left border region derived from the Ti-plasmid of *R. radiobacter* used for transfer of the T-DNA.

Properties of the introduced gene conferring herbicide tolerance

Cotton line GHB614 expresses the *2mepsps* gene, which encodes a modified 5-enolpyruvylshikimate-3-phosphate synthase 2mEPSPS protein. The *epsps* gene was originally isolated from maize (*Zea mays* L.). The modified 2mEPSPS protein differs from the wild type EPSPS enzyme by two amino acid substitutions. EPSPS is a naturally occurring enzyme involved in the biosynthesis of aromatic amino acids. Glyphosate inhibits EPSPS, resulting in a lack of amino acids essential for growth and development of plants. In contrast to EPSPS, the 2mEPSPS protein is not inhibited by glyphosate and therefore the plant is tolerant to glyphosate containing herbicides.¹²

Environmental risk assessment

Cotton is predominantly a self-pollinating species, but crosspollination may occur. Wild relatives of cotton (*G. hirsutum*) are not present in North West Europe and therefore, hybridisation with wild relatives is excluded.⁵

Cotton plants are very sensitive to temperature and susceptible to frost. A reasonably high temperature (optimally between 24 °C and 34 °C) is required in all stages of development. For normal maturation, a period of 180 to 200 days without frost is necessary. In addition, this period needs to have on average 150 days of suitable temperatures, averaging 21-22 °C. In the Netherlands, the summer months of May, June, July, August and September (in total 153 days) have average monthly temperatures above 12 °C, but below 18 °C (average monthly temperatures estimated over a period of 30 years between 1981 and 2010).¹³ In addition, in areas where rainfall is less than 500 mm a year, irrigation should be applied for cotton growth. In the months of May through September in the Netherlands, the average monthly precipitation does not exceed 100 mm.¹³ Based on the above, the current Dutch climate is unsuited for cotton growth.

Climate conditions in other parts of the European Union e.g. Greece, Spain, Bulgaria and Portugal are more suitable for growing cotton.⁷ Seeds from cotton cultivars do not possess dormancy and will germinate in autumn if conditions are favourable.^{5,6} In addition, seeds will usually not survive in humid soil.⁶ In regions with mild and dry winters, cottonseeds may overwinter and germinate in spring if adequate moisture is available.⁴ The occurrence of volunteer cotton is limited by soil moisture content and frost.⁶

There is no indication that the introduced trait, which confers tolerance to glyphosate containing herbicides, will increase the ability of cotton to survive in the environment. The applicant carried out an agronomic assessment for GHB614. COGEM is of the opinion that the agronomic assessment does not give any indication to assume that GHB614 has an increased fitness compared to conventional cotton lines.

In conclusion, the current climate in North West Europe is not suited for cotton growth and the introduced trait will not alter the fitness of cotton GHB614 and its inability to grow in North West Europe.

Moreover, since cotton cannot survive the Dutch climate, it is highly unlikely that cotton will be cultivated in the Netherlands. Since cotton is not cultivated in the Netherlands, there will be no exposure of organisms above- or belowground to GHB614 plant tissues. Therefore, COGEM concludes that there will be no risks for non-target organisms (NTOs) or soil functions in the Netherlands.

Based on a preliminary assessment, COGEM has reservations about the quality of the NTO and soil studies supplied by the applicant. However, COGEM deems a thorough evaluation of these studies unnecessary, because the Dutch environment will not be exposed to GHB614. Therefore, COGEM abstains from an opinion on the methods and results of the NTO and soil studies.

General surveillance

General surveillance (GS) has been introduced to be able to observe unexpected adverse effects of genetically modified crops on the environment. The setting or population in which these effects might occur is either not, or hardly predictable.

It is very unlikely that GHB614 cotton will be cultivated in the Netherlands. Therefore, the monitoring plan is not relevant for the Netherlands. However, based on a preliminary assessment of the monitoring plan, COGEM sees several points for improvement. COGEM would like to point out that she has published a report on the principles that should be followed for general surveillance.¹⁴ Several of COGEMs principles were incorporated into the EFSA guidance on Post Market Environmental Monitoring (PMEM), which has recently been published.¹⁵ COGEM recommends for the PMEM of the cultivation of GHB614 to take into account the points for improvement mentioned in these opinions.

Advice

In view of the above, COGEM is of the opinion that the risks for the environment in the Netherlands associated with cultivation of genetically modified cotton line GHB614 in Europe are negligible.

References

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