Deliberate releases B/BE/07/V2

Final report

1. General information

1.1. European notification number
B/BE/07/V2.

1.2. Member state of notification
Belgium

1.3. Date of consent and consent number
Consent of 17 February 2009 and of 2 May 2016

2. Report status
This is a post-release monitoring end report.

3. Characteristics of the release

3.1. Scientific name of recipient organism
Grey poplar: *Populus tremula x Populus alba* (*Populus x canescens)*.
Line 717-1-B4 female line

3.2. Transformation event(s) (acronym(s)) or vectors used

N° of the event	Recipient	Vector used
WT/52-3 (FS3)	717-1-B4	pBIBHygro/S-CCR
WT/52-40 (FS40)	717-1-B4	pBIBHygro/S-CCR

The trees were modified by introduction of a sense-copy of the Cinnamoyl-CoA Reductase gene, leading to co-suppression. The co-suppression results in a significant lowering of the production of the CCR enzyme, which then leads to lower amounts of the polymer lignin being produced, which is one of the three main components of wood, next to cellulose and hemicellulose.

3.3. Unique identifier
The lines do not have a unique identifier.

3.4. Please provide the following information as well as the field(s) layout:
The release took place on a site on the Technologiepark in the municipality of Ghent-Zwijnaarde, Belgium. The size of the field trial was 14.25 x 23.25m.

The number of GM trees released was 120 per event, making a total of 240 released GM trees. The total number of trees in the field trial, including reference line and border row was 448. The plant density was 1.19 trees per m^2.

The field trial plot design is given in annex.

4. Any kind of product that the notifier intends to notify at a later stage

4.1. Does the notifier intend to notify the released transformation event(s) as product(s) for placing on the market under Community legislation(s) at a later stage?
There is no intention to notify the released events as products for placing on the market under Community legislation.

5. Type of deliberate release
Deliberate release for research purposes.

6. Method(s), result(s) of the release, management and monitoring measure(s) in respect of any risk to human health or the environment

6.1. Risk management measures

6.1.1. Before planting
Prior to the release the trees were grown in pots in an adjacent greenhouse and were carefully labeled to avoid mistakes regarding their identity.

6.1.2. During planting activities
Carefully labeled trees were transported from the greenhouse to the adjacent field site using a chain of persons receiving the labeled tree and handing it over to the next person in the chain. Personnel involved in the planting of the GM trees received a compliance training. Trees were very carefully planted manually with verification of event number and plot location. There was no left-over planting material. All delivered GM trees were planted.

6.1.3. During the period of release
The field trial plot was surrounded by a fence to prevent any unwanted trespassing. A locked gate gave entrance to the plot, but was only accessible to the trained researchers involved and compliance personnel. The trees were grown in so-called short-rotation which meant that the branches of the trees did not get older than 3 years, thus avoiding the production of flowers. Grey poplars only start producing flowers when they are 5 to 8 years old. Each year during springtime monitoring took place to check for any flowers. During the whole of the release period no flowers were detected. If a flower would have been formed, it would have been a female flower that could not spread pollen. During the whole of the release period also monitoring took place to check on the emergence of root suckers. Quite a large number of root suckers were detected (several hundreds over the course of the release), which were all removed and destroyed. Especially in the growing season after a harvest the number of root suckers that emerged was quite high. This is logical, because cutting down the trees generates a lot of stress that promotes the emergence of root suckers. The trees were cut down three times: after year 1, after year 4 and after year 7 at the end of the release. This was always done manually. There was strict monitoring to avoid unwanted spread of GM material. Material that was not

used for further research was destroyed by chipping. Stems of trees that were kept for further research were debarked, and the bark destroyed, which makes that the stems are no longer viable.

6.1.4. At the end of the release

Also the final harvest of the trees was performed manually. All stems were removed close to the ground. All material that was not used for further research was destroyed by chipping. The trunks and root systems of the trees were taken out of the soil using a crane. These trunks and root systems were taken through a crusher twice to tear them into very small pieces, which were then left to die and decompose together with the tree chips under a plastic cover.

6.1.5. Post-harvest measures

Pieces of root that had remained in the soil after the harvest of the trunks and root systems, were removed six months later (during the dry summer season) by digging out the soil of the field trial plot and a surrounding strip to a depth of 1.5 m (rooting was proven to be no deeper than about 1 m) and taking that soil through a soil sieve, thus taking out all of the remaining pieces of root. The clean soil was then put back into the trial plot, leaving behind a completely clean field trial plot in which no GM material was left anymore. The pieces of root that came out of the soil were left to decompose under plastic to make sure that they were not viable anymore.

All equipment and machinery used during harvests and sieving was cleaned before leaving the field trial plot, thus preventing any viable GM material leaving the site.

6.1.6. Other measures

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6.1.7. Emergency plan

After having had a destruction on a GM potato field trial in 2011 on another location in Flanders, a number of additional safety measures were taken to protect the GM poplar field trial in Zwijnaarde from actions by anti-GM protestors. An extra wired fence and an electronic alarm system were installed to prevent any harm to the field trial.

6.2. Post-release monitoring measures

In the six month period between the final harvest of the trees and the sieving of the soil the location was monitored for root suckers, a number of which were found and which were removed and destroyed. But after having sieved the soil and thus having removed all remaining pieces of root, no monitoring is obligatory anymore.

6.3. Plan for observation(s)method(s) involved

During the field trial the plot was visited at least once a month and during the period of flowering twice a week. Visual observations took place to score the general growth and health of the plants. Additionally also an environmental research protocol was performed to check on any differences between the GM trees and their conventional counterparts with regard to sensitivity to certain pests and diseases, sensitivity to climate stress, growth and morphology, and endophytes.

6.4. Observed effects

There were no observations providing any indications of risks for human health or the environment.

6.4.1. Expected effects

The genetically modified poplar trees had an altered wood composition, with as expected a lower amount of lignin, but the effect of the modification was not very uniform. Some trees were more affected in their amount of lignin than others. Trees that did have a significantly lower amount of lignin also showed a significant improvement in the enzymatic breakdown of the wood and a significant increase in ethanol yield after fermentation of the wood[1].

6.4.2. Unexpected effects

The GM trees with an altered wood composition were proven to also have an altered endosphere bacterial microbiome[2]. The composition of the bacterial flora living in the trees differed somewhat from that of the conventional counterpart. As such this may even not be that unexpected because the wood is the main component that these micro-organisms are confronted with, and if that changes somewhat it is not illogical that the composition of the bacterial flora also changes somewhat.

6.4.3. Other information

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7. Conclusion

The field trial confirmed that these GM poplar trees produce wood with an altered lignin content when they are grown under normal field conditions, and that this wood can be more easily broken down to give a higher ethanol yield upon fermentation. The GM trees did however grow somewhat slower than their conventional counterpart.

The GM trees did not differ from their conventional counterpart in their sensitivity to pests and diseases, climate stress, or endophyte composition. But there was a difference in the composition of the endosphere bacterial microbiome as a result of the altered wood composition.

There are no indications for any negative impacts on human health or the environment resulting from the field trial. The monitoring of the trial confirmed that these poplars do not flower in their first years and that this type of poplar is prone to making root suckers like many types of poplar do. Consistent removal and destruction of such root suckers prevents any spread of trees beyond the field trial plot. The sieving of soil of the whole of the field trial plot has removed all GM material leaving behind a clean plot.

Date: 30 December 2016

[1] See: Van Acker et al, Improved saccharification and ethanol yield from field-grown transgenic poplar deficient in cinnamoyl-CoA reductase, PNAS, 2013.

[2] See: Beckers et al, Lignin engineering in field-grown poplar trees affects the endosphere bacterial microbiome, PNAS, 2016.

Annex
Field trial B/BE/07/V2 Field trial design

Pink = CCR FS 03
Blue = CCR FS 40
Green = wild type (717-1-B4)
Black = Buffer row