BIJLAGE 12

Summary Notification Information Format

A. General information

A1. Details of notification

Notification Number
B/BE/13/V1

Member State
Belgium

Date of Acknowledgement
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Title of the Project
Field evaluation of poplars with a modified wood composition

Proposed period of release:
01/05/2014 to 31/04/2021

A2. Notifier

Name of the Institute
VIB

A3. Is the same GMPt release planned elsewhere in the Community?
No.

A4. Has the same GMPt been notified elsewhere by the same notifier?
No

B. Information on the genetically modified plant

B1. Identity of the recipient or parental plant

a) family name:	Salicaceae
b) genus:	*Populus,* sectie *Populus*. Subsectie *Albidea*
c) species:	*Populus tremula x Populus alba* (*Populus x canescens)*
d) subspecies:	-
e) cultivar / breeding line:	717-1B4, vrouwelijke kloon
f) gangbare naam:	Grey poplar

B2. Description of the traits and characteristics which have been introduced or modified, including marker genes and previous modifications
The genetically modified trees have an altered lignin composition resulting from the

downregulation of the Cinnamyl Alcohol Dehydrogenase (CAD) enzyme through RNAi. CAD catalyzes the last step in the monolignol synthesis. The remaining CAD activity in the modified trees is about 15% of that in wildtype trees. The altered lignin composition has a positive effect on the ease with which the lignin can be broken down to gain access to the valuable sugar content in the cellulose and hemicellulose in the wood of the trees.
A lowered CAD activity has been found to naturally occur in the U.S. in loblolly pine (*Pinus taeda*) and in wild black poplar in Europe with comparable effects on the wood composition.
The modified trees also carry the NPT-II selection marker gene allowing an easy selection of transformed plants.

B3. Type of genetic modification
Insertion of genetic material.

B4. In case of insertion of genetic material, give the source and intended function of each constituent fragment of the region to be inserted
The region which has been inserted, and which is flanked by the T-DNA borders from the Ti-plasmid of Agrobacterium tumefaciens contains the following elements:

Element	Function	Origin
Right T-DNA-border	T-DNA insert border	*Agrobacterium tumefaciens*
CaMV 35S	Transcription promotor	cauliflowermosaicvirus
attB1	Recombination site*	E.coli
scad (sense CAD)	Coding sequence of part of the enzyme cinnamyl alcohol dehydrogenase	Poplar
attB2	Recombination site*	E.coli
intron	Leads, together with the (A)SCAD sequences, to the formation of a hairpin RNA molecule	
attB2	Recombination site*	E.coli
scad (in opposite direction)	Coding sequence of part of the enzyme cinnamyl alcohol dehydrogenase	Poplar
attB1	Recombination site*	E.coli
OCS terminator	Transcription terminator of the octopine synthase gene	*Agrobacterium tumefaciens*
NOS promotor	Transcripton promotor of the nopaline synthase gene	*Agrobacterium tumefaciens*
nptII	Neomycine phosphotransferase	Tn5
NOS terminator	Transcription terminator of the nopaline synthase gene	*Agrobacterium tumefaciens*
Left T-DNA-border	T-DNA insert border	*Agrobacterium tumefaciens*

*the AttB1 and -2 recombination sites are synthetically altered versions of a recombination site originally isolated from E.coli.

B6. Brief description of the method used for the genetic modification
The method used for the genetic transformation is based on Agrobacterium tumefaciens cocultivation of excised internodes from in vitro grown poplar plantlets (Leplé et al., 1992). After this cocultivation step where the gene transfer takes place, the transformed cells are selected using a positive screen (based on antibiotic resistance) and induced to regenerate a whole plant.

B7. If the recipient or parental plant is a forest tree species, describe ways and extent of dissemination and specific factors affecting dissemination
Grey poplar (*P. x canescens*) can disseminate vegetatively through the production of suckers from superficial roots. Pollen and seed are disseminated by the wind, possibly on rather long distance. The seed is very small and devoid of albumen: for this reason the seed viability in the wild is rather short (between 2 and 4 weeks). In fact, seed regeneration is not often observed as ecological conditions necessary to seed germination and plantlet development are seldom met:

naked soil, no competition at all with any other species, full light, permanent humidity, but not in excess…

C. Experimental Release

C1. Purpose of the release
As already specified, the genetically modified poplars are modified in their lignin content. Lignin is very important for both tree growth and development, particularly for water conduction and mechanical support. Different transgenic lines of poplars with a modified lignin content have already been evaluated in previous field trials in the UK and France, for agricultural performances and for evaluation of the technological properties of wood for pulp and paper making. This release has the purposes to test the performance of these new CAD-downregulated poplar lines under real life conditions and to produce enough wood from lignin modified poplars in order to evaluate its properties to serve as a good biomass source for extracting sugars and other valuable compounds. Lignin composition, lignin/cellulose ratio and the accessibility to cellulose are critical for the extraction of sugars from ligno-cellulosic feedstock. The poplar trees will be grown as a short rotation intensive culture on a low-grade soil using sustainable low-input conditions. The release also intends to take advantage of the developments in the Ghent-BioEnergy-Valley, where a number of bio-energy initiatives have taken ground, including a bioprocess pilot plant for bio-energy production.

C2. Geographical location of the site
On grounds belonging to the ILVO research institute on the border of the municipalities of Wetteren and Melle.

C3. Size of the site (m2)
The trial site is in total about 1300 m2, of which about 810 m2 will be planted with transgenic poplars.

C4. Relevant data regarding previous releases carried out with the same GM-plant, if any, specifically related to the potential environmental and human health impacts from the release
The genetically modified plants have not been released before.

D. Summary of the potential environmental impact from the release of the GMPts

The environmental impact from the release is expected to be zero, since the GM poplars are not going to flower and any suckers from superficial roots will be destroyed. Spontaneous regrowing of trees from fallen branches is considered to be extremely unlikely, as it is known that *P.x canescens* and the clone 717-1-B4 does not easily shoot. Only under ideal conditions in the laboratory with the application of shooting powder, *P. x canescens* is able to shoot. This means that there will be no transfer of transgenes to native or cultivated poplars, or spread of the GM poplars themselves. When poplar is grown in short rotation intensive culture the trunks and branches will not become older than three years, and therefore they will not flower. Grey poplar normally starts to flower between 5 – 8 years of age, only in some cases after 4 years. But anyhow, if monitoring would reveal any flowering, these flowers will be removed. For information: The clone used as a recipient is a female clone, unable to produce male flowers and therefore also unable to produce pollen.

The modification of the trees is not expected to have significant effects on non target species. In former trials no effects on non target species were identified. From scientific literature it can be

deduced that lignin modified trees do not have an effect on the interaction with pathogens, that there is no or very limited effect on leaf-eating insects, and that for the decay of lignin-modified wood other factors like environmental conditions, the chosen poplar species and clone have more significant effects than the lignin modification.

And as outlined above, there is no expected selective advantage of the GM poplar. It is more likely that the GM poplar will have a selective disadvantage.

With regard to possible toxic and allergenic effects we state that any possible toxic effects of these specific lines has not been tested. With regard to allergenicity it can be stated that for these transgenic lines there is not a concern for an altered allergenicity of the transgenic pollen (pollen form poplar is known as a moderate allergen), as we are working with a female clone that does not produce pollen.

It is also known that trees with comparable alterations in the lignin content already exist in nature (in loblolly pine in the U.S. and in black poplar in Europe). If there would be any alteration of the way the modified trees interact with nature and in particular with non-target organisms, this altered interaction would be comparable with the interactions of those wilde type trees. Also, there are no indications from the loblolly pine and black poplar mutants that the modified wood, would have any negative impact on the health of humans or animals.

E. Brief description of any measures taken for the management of risks

Grey poplar (P. x canescens) is dioecious (every tree is either male or female). The 717-1B4 clone is female. In consequence, there is no risk of dissemination through pollen. Moreover, as flower development occurs before vegetative bud burst and leaf development, it is very easy to identify and eliminate female catkins, before their full development. But as the modified poplars will be grown as short rotation intensive culture with a harvest of all trunks and branches after 3 years of growing, the GM poplars will not flower. Suckers are also regularly monitored and destroyed once a year using a contact herbicide. After a storm the site will be inspected for possible fallen branches and these will be removed. The site is designed in such a manner that fallen branches will not disperse by wind from the plot and will remain within the boundaries of a fence surrounding the trial.
At the end of the trial, the rootstock will be mechanically removed and the soil will be worked with a rotary cultivator. The plot will be monitored for at least two years for suckers, which will be destroyed using a suitable contact herbicide. If necessary monitoring will be extended until there has been one year without any suckers.
The field trial plot will be surrounded by a 1.80 m high wire fence to prevent accidental trespassing and accidental removal or spread of GM material.

F. Summary of foreseen field trial studies focused to gain new data on environmental and human health impact from the release

There will be monitoring of certain insects and of endofytes.

G. Final report

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H. European Commission administrative information

I. Consent given by the Competent Authority:

Not know