



Service of Biosafety and Biotechnology

SECRETARIAT

Dr. W. Moens

O./ref. : IPH/1520/BAC/03-0202

**At the attention of the Competent
Authorities**

CONCERNS: DOSSIER B/BE/03/V1 OF THE FRUITTEELTCENTRUM 'A FIELD ASSESSMENT OF THE INTRODUCTION OF THE SELF-COMPATIBILITY TRAIT IN TRANSGENIC ELSTAR TREES: FLOWER BUD FORMATION, FRUIT SET, YIELDS, PRODUCTION EFFICIENCY AND FRUIT QUALITY'

ADVICE OF THE BIOSAFETY ADVISORY COUNCIL

Having honestly taken into account that:

- on December 23rd, 2002, the Fruitteeltcentrum of the Catholic University of Leuven submitted a notification to the Competent Authority under part B of Directive 2001/18/EC of March 12th, 2001 and chapter II of the Royal Decree of December 18th, 1998 in order to be authorised to carry out a deliberate release for experimental purposes with genetically modified higher plants described in notification B/BE/03/V1 'A field assessment of the introduction of the self-compatibility trait in transgenic Elstar trees: flower bud formation, fruit set, yields, production efficiency and fruit quality',
- upon questioning of by Secretariat of the Biosafety Advisory Council, the Flemish Competent Authority has declared that this type of notification is classified as a deliberate release upon the choice of the notifier, and that this is in accordance with that of the juridical services of the European Commission questioned by the SBB through the Federal Competent Authority on December 1st, 2000,
- the Directive 2001/18/EC and the Royal Decree of December 18th, 1998 foresee the evaluation of the risks for the environment and human health,
- the deliberate release is foreseen for a period of 4 years at the commune of Rillaar (Aarschot),
- the transgenic apple trees of the Elstar variety were modified to be self-fertile,
- the transgenic apple trees contain the neomycin phosphotransferase gene (*nptII*) rendering the transformed plant cells resistant to kanamycin (for *nptIII* see further),
- due to the lack of knowledge on the ecological consequences of vertical gene flow between transgenic and non transgenic apple trees, the experts of the Scientific Committee 'Transgenic plants' of the Biosafety Advisory Council were of opinion that containment measures should suppress or at the worst strongly reduce vertical gene flow,
- the containment measures proposed by the notifier and amended by the experts regarding vertical gene flow will fulfil the former statement,

- the experiment clearly aims to obtain more knowledge on the effect of self-compatibility in Elstar trees on the yield, the quality and the production efficiency of fruit,
- several experts had doubts about the experimental design of the proposed release due to the fact that the yield, the quality, the production efficiency of fruit and flower bud formation are dependent on climatic conditions and the activity of pollinators, that the climatic conditions within the tunnels might be strongly modified, that it might be difficult to extrapolate the obtained results to classical breeding and that the sample size and the time frame of the experiment might not be sufficient to get statistically significant results,
- the Scientific Committee identified missing data needed before the potential authorisation and this information was requested on February 21st, 2003 and acknowledged on March 13th, 2003 by the Federal Public Service Health, Food Chain Safety and Environment,

the juridical responsibility is borne by the KULeuven, in this case represented by the 'Algemeen Beheerder' Prof. V. Goedseels, that the 'Milieudienst' is the administrator and that the 'Fruitteeltcentrum' is the applicant of the consent,

the transgenic lines #42 and #103 for which no complete data sets on molecular characterisation were provided, should be omitted from the field trial and the additional space available be taken up using additional trees from lines #39, #53, #66, and #102, as well as a second wild type Elstar control derived from in vitro tissue culture,

the notifier provided the additional information on the emergency plans and emergency measures for the flowering and fruiting period of the transgenic apple trees,

- the Scientific Committee identified missing data that did not prevent the Scientific Committee to assess the risks for the human health and the environment of the proposal, but that could be, if available, useful to obtain **before** the release, and that this information was asked by the Secretariat of the Biosafety Advisory Council on demand of the experts,

the notifier provided the Southern blots of genomic DNA indicating the absence of the nptIII gene derived from the pBIN19 vector backbone in the transgenic lines,

the notifier provided the additional information on segregation patterns,

- the Scientific Committee identified missing data that did not prevent the Scientific Committee to assess the risks for the human health and the environment of the proposal, but that could be useful to obtain **during** the release, and that this information will be requested through the restrictions and conditions of this advice, and that the Scientific Committee has further defined how, when and which additional information and reports should be provided,
- the Scientific Committee 'Transgenic Plants' of the Biosafety Advisory Council, on basis of the expressed consensus of the 5 reporting experts and 18 other experts, except one, has reached the conclusion that there was no pertinent objection from the biosafety viewpoint against the deliberate release as proposed and described by notification B/BE/03/V1,
- the scientific parameters taken into account by the notifier for the proposed release have been fully checked by the experts and found to be complying with annexes II and IIIb of Directive 2001/18/EC,

Advice

The Service of Biosafety and Biotechnology (SBB),

- assuming the full competency of the Biosafety Advisory Council on basis of article 19 of the Cooperation Agreement of April 25th, 1997 between the Federal State and the Regions on the administrative and scientific co-ordination regarding biosafety,
- having controlled the independency of the committed reporting experts and of the experts gathered in a plenary session on February 17th, 2003,
- having carried out the plenary evaluation in the presence of observers delegated by the partners of the said Cooperation Agreement,
- having received and acknowledged the complementary data requested by the Federal Public Service Health, Food Chain Safety and Environment,
- having submitted the additional information to the experts by written procedure for evaluation,
- having obtained the written approval of the experts about the report of the meeting,
- considering further that the declaration of the single objecting expert is joined to the report of the evaluation of notification B/BE/03/V1 that was also addressed to the Competent Authorities,

delivers a positive advice for the envisaged trial described in notification B/BE/03/V1 with the restrictions and conditions outlined further.

Proposed restrictions and conditions

Restrictions

Within the framework of a deliberate release of GMOs for experimental purposes under part B of Directive 2001/18/EC of March 12th, 2001 and chapter II of the Royal Decree of December 18th, 1998:

- the trial should be carried out for research purposes only,
- the transgenic apples should not be allowed to be used for feed and/or food purposes,
- the non-transgenic apples of the control lines used in the tunnels should not be allowed to be used for feed and/or food purposes,
- the assessment of the taste of the apples from the transgenic apple trees should not be allowed,
- the assessment of the taste of the apples from the non-transgenic control apple trees used in the tunnels should not be allowed.

To avoid horizontal gene transfer all vegetal remains of the transgenic apple trees and non-transgenic controls used in the tunnels should be incinerated by a certified organisation instead of being composted.

At the end of the release the apple trees of the tunnels, being transgenic or non-transgenic, should be destroyed by incineration by a certified organisation.

If the commercial apples cultivated at the Fieldstation are found to be transgenic, they should be destroyed by incineration by a certified organisation.

The trial should be carried out as described in the proposed notification. The containment, follow-up and monitoring measures proposed by the notifier should be respected.

Data that should be provided in case of authorisation

public dossier

The notifier is invited to adapt the public dossier to the recommendations made by the experts of the Scientific Committee 'Transgenic plants', to the additionally provided information and to the proposed restrictions and conditions. In the case an authorisation is granted the revised public dossier should be published on the internet site of the Biosafety Advisory Council at latest one month after the authorisation date.

greenhouse data

In order to obtain an important indication on the scientific quality of the foreseen experiment the notifier should document the results obtained with the same transgenic trees under greenhouse conditions. The publication about these results was described as confidential at the Scientific Committee because the data are new and presently in press. However, they should be provided as soon as accepted for publication. Alternatively, a confidential copy thereof should be provided to the Secretariat of the Biosafety Advisory Council.

protein expression

The protein expression data should be completed with data on the expression of S-RNAses in fruit of the transgenic line (#102) carrying a sense copy of the S3-allele.

pollen traps

In order to estimate the level of transgenic pollen escape, the notifier should place rows of pollen traps outside the tunnels during the flowering period of the transgenic apple trees. The notifier should in the annual interim reports and final report indicate the amount and type of used pollen traps, the position of the pollen traps, the frequency at which the pollen traps were checked and pollen samples taken, the frequency at which the pollen traps were replaced, if transgenic apple tree pollen was detected and the methods used to determine the transgenic character of that pollen.

The Federal Public Service Health, Food Chain Safety and Environment should immediately be informed if transgenic apple tree pollen is detected outside the tunnels.

beehives

Since honeybees play an important role in mediating pollen dispersal and efficiently communicate the exact location of interesting food sources, the notifier should during the flowering of the apple trees foresee the absence of beehives in a radius of 3km, which corresponds with the average action radius of swarming bees. Beehives present around the GM field during the flowering period of the transgenic apple trees have to be delocalised. In the annual interim reports and final report the notifier should explain how the absence of beehives was guaranteed in radius of 3km.

insect traps

In order to monitor the possible intrusion of pollinators in the tunnels, the notifier should place insect traps in the tunnels during the flowering period of the transgenic apple trees. As the 2 tunnels are 46m long and 6m wide, it is proposed to place on each side of the tunnel a Bug-Scan Roll® over the length of the tunnel (at least of 44m). The Bug-Scan Rolls® should be placed at the flowers height, be checked daily and be replaced every 2 weeks¹. The number of trapped

¹ Further information on the Bug-Scan Rolls® is available on <http://207.5.71.37/biobest/en/producten/monitoring/Bug-Scan/Bug-ScanRol.htm>

insects, the frequency of checking and replacing the Bug-Scan Rolls® should be documented in the annual interim reports and final report.

The Federal Public Service Health, Food Chain Safety and Environment should immediately be informed if pollinators especially honeybees are detected inside the tunnels during bloom of the apple trees.

inventory of apple trees

The notifier should make a detailed inventory of all apple trees (wild apple trees, escaped edible cultivars and ornamental apple trees) lying in a radius of 1km around the trial. Not only the area of the Fieldstation should be checked, but also gardens and roadsides should be taken into account. The used methodology to make the inventory as well as the obtained results should be addressed in the annual interim reports and final report.

annually randomised sample of apples

The annually randomised sample of apples (approx. 100) taken from the plots closest to the test area in order to test the transgenic character of the seed should be enlarged to a 1km radius. Not only the apple trees used in the breeding programs of the Fruitteeltcentrum at the Fieldstation should be tested, but also the other inventoried apple trees. The used methodology to collect the requested data and the obtained results should be addressed in the annual interim reports and final report.

The Federal Public Service Health, Food Chain Safety and Environment should immediately be informed if transgenic seeds are detected.

inventory of surrounding cultivars

For each cultivation year the notifier should provide a map of the trial parcels and the surroundings of the field trial with the foreseen cultures. The way of achieving this goal should be documented in the annual interim reports and final report.

climatic conditions

Taking into account that the aim of the experiment is to quantitatively assess the effect of self-fertility on several agronomical traits, that the environmental factors might have an influence on the level of cross-pollination and that the experimental conditions (temperature, humidity, light intensity, etc.) inside the tunnels might be very different from the outdoor conditions, the notifier should monitor the climatic conditions inside the tunnels. In addition, the notifier is invited to use at least 1 row of non-transgenic control Elstar outside the tunnels ideally at a distance of 100m to avoid attracting honeybees to measure the effect of the experimental design on the different agronomical traits under evaluation. If the apple trees used in the breeding programs of the Fruitteeltcentrum at the Fieldstation are from the Elstar variety they could be used as controls. The climatic conditions as well should be documented in the annual interim reports and final report.

consent restrictions and conditions

In function of the obtained and annually reported data from the insect traps, pollen traps and randomised sample of apples, the consent restrictions and conditions can be modified.

netting

During the flowering period the netting covering the tunnels should be checked on a daily basis to see if it is undamaged.

In the event of a heavy storm, which might result in damage, nettings should immediately be inspected for possible damage. Any torn or damaged netting should be repaired or replaced as appropriate.

Spare supplies of netting should be available to immediately repair or replace any damage that may have occurred.

All trees should be checked to ensure that no loss of material has occurred.

The Federal Public Service Health, Food Chain Safety and Environment should be informed of any rips or tears in the netting that has been detected, repaired or replaced.

irregularities and unforeseen events

The Federal Public Service Health, Food Chain Safety and Environment should be immediately informed of any irregularities or unforeseen events that take place.

Annual interim reports and final report

At the end of each growing season and at the end of the release, the notifier should respectively submit an annual interim activity report and a final report. By the elaboration of these reports the notifier should take into account the 'format for the presentation of the report of deliberate release into the environment of genetically modified higher plants according to part B of Directive 2001/18/EC (article 10)' that is currently being drafted by the European Commission and should include the following topics:

- a copy of the logbook
- the site and period of release
- the precise nature of the actually released transformants
- the actual surface of the trial plot
- the aim(s) of the trial
- the frequency and nature of the observations on the trial plot
- the measures that were taken to prevent the unwanted release of transgenic material outside the trial plot
- the incineration certificates confirming the incineration of all the transgenic and non-transgenic vegetal material in the tunnels during and at the end of the release (harvested apples, tested apples, pruning material, apple trees, etc.)
- the results obtained during the trial
- an overview of the surveillance of the trial plot
- the quantity of apples tested in the laboratory
- the greenhouse data
- the data on the expression of S-RNAses in fruit of the transgenic line (#102) carrying a sense copy of the S3-allele
- the data on the pollen traps
- the data on the beehives
- the data on the insect traps
- the data on the inventory of apple trees
- the data on the annually randomised sample of apples
- the data on the inventory of surrounding cultivars
- the data on the climatic conditions
- the data on the netting
- the data on the irregularities and unforeseen events

Post-release follow-up

After the release the area should be followed up for 2 years to ensure that no seedlings have escaped. During the 2 years of follow-up no apple trees should be cultivated at the trial site. The notifier should report on the post-release follow-up.

In function of the results obtained in the post-release follow-up phase specific management measures could be requested.

Final provisions

The present advice of seven pages under reference IPH/1520/BAC/03-0202 is joined to the full report of the evaluation procedure of the risks delivered to the Federal and Regional Competent Ministers according the Royal Decree of December 18th, 1998.

The present advice is addressed by recommended letter to:

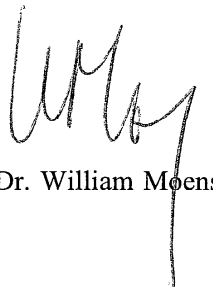
Dr. Dirk Cuypers
President of the Federal Public Service Health, Food Chain Safety and Environment
C/o Ir. Xavier Decuyper and Ir. Martine Delanoy
General direction Animals, Plants and Food
Division Raw materials and Plant protection

And to

Ir. Vera Dua
Flemish Minister for the Environment and Agriculture
C/o Ir. Sofie Luyten

Visa for release to the Competent Authority and to be published on the internet in Dutch, French and English respectively under the files 03V1A_N_BAC.pdf / 03V1A_F_BAC.pdf / 03V1A_E_BAC.pdf. on March 27th, 2003.

March 27th, 2003,
The Secretariat of the Biosafety Advisory Council



Dr. William Moens