ANNEX 13

2011 MONITORING FOR VOLUNTEER POTATOES AT 2010 STARCH POTATO PRODUCTION FIELDS

BASF
The Chemical Company

BASF Plant Science

REPORT # AMF-11-003

2011 MONITORING FOR VOLUNTEER POTATOES AT 2010 STARCH POTATO PRODUCTION FIELDS

DATA REQUIREMENT: N/A

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[*name deleted*]

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TEST FACILITIES:

FIELD STUDY CONDUCTED IN THE CZECH REPUBLIC ON AMFLORA STARCH PRODUCTION FIELDS FROM 2010, AND

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BASF Reg. Doc. No. 2012/7000382

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d) (1) (A), (B), or (C).

Company: BASF Plant Science Company GmbH

Company Agent: [*name deleted*] Date: _____

Title: _____

Signature: [*signature deleted*]

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The study described in this volume was not conducted in compliance with the OECD Principles of Good Laboratory Practice or the GLP Principles of German Chemikaliengesetz (Chemicals Act) and does not meet the United States Environmental Protection Agency Good Laboratory Practice Standards [40 CFR Part 150 (FIFRA)]. The data generated by BASF Plant Science Company GmbH in support of product safety comply with generally accepted scientific procedures. Record keeping is consistent with procedures used throughout the research community. This report accurately presents the raw data developed during the studies.

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CERTIFICATION OF AUTHENTICITY

I, the undersigned, hereby declare that this study was performed under our supervison according to the procedures described herein, and that this report provides a true and accurate record of the results obtained.

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ABBREVIATIONS AND DEFINITIONS

CFR	Code of Federal Regulations (USA)
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act (USA)
PCR	Polymerase chain reaction

STUDY INFORMATION PAGE

BASF Plant Science Report #:	AMF-11-003
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Study Title:	2011 Monitoring for Volunteer Potatoes at 2010 Starch Potato Production Fields
Sponsor:	BASF Plant Science Company GmbH Carl-Bosch-Str. 38 D-67056 Ludwigshafen Germany
Testing Facilities	Field study conducted in the Czech Republic on Amflora starch production fields, and SunGene GmbH, a BASF Plant Science Company Corrensstrasse 3 D-06466 Gatersleben Germany
Study Completion Date:	See date of author's signature on page 3

2011 MONITORING FOR VOLUNTEER POTATOES AT 2010 STARCH POTATO PRODUCTION FIELDS

SUMMARY

The amylopectin potato EH92-527-1, variety Amflora, has been genetically modified for increased amylopectin content in the tuber starch via transformation with a gene fragment encoding granule bound starch synthase (*gbss*) from potato in antisense orientation. This modification leads to the silencing of the amylose synthesizing enzyme in the potato tuber. In March 2010, Amflora was approved for commercial cultivation in the European Union and was grown for starch potato production at locations in the Czech Republic in 2010.

As part of the Amflora post-market environmental monitoring plan the purpose of this study was to evaluate the presence and persistence of Amflora volunteer plants and their frequency in the years following the Amflora cultivation for starch production.

Out of seven fields monitored in the Czech Republic only at one field planted with maize in the season following the Amflora cultivation potato volunteer plants were detected. All potato volunteers were confirmed as being Amflora potato plants.

INTRODUCTION

The amylopectin potato EH92-527-1, variety Amflora, has been genetically modified for increased amylopectin content in the tuber starch and was approved for commercial cultivation in the European Union in March 2010. Cultivation for starch potato production took place in the Czech Republic in 2010.

According to the Amflora post-market environmental monitoring plan the purpose of this volunteer monitoring study was to evaluate the presence and persistence of Amflora volunteer plants and their frequency in the years following the Amflora cultivation for starch production.

At harvest of potatoes always a certain portion of potato tubers remain in the field. The survival of these remaining potato tubers depends on soil management practices and low temperature during the winter period. Tubers which survive under winter

conditions can give rise to potato plants, also known as tuber-borne volunteer potatoes, in subsequent crops.

The purpose of this study was to demonstrate that amylopectin potato EH92-527-1 is comparable to conventional potatoes with regard to its competitive behavior and the capacity to survive or tolerate environmental conditions like frost. Evidence should be given that amylopectin potato EH92-527-1 does fit in the management scheme of conventional starch potatoes and that possible volunteer potatoes will be controlled effectively by the applied cultural practices.

Annex 2 of the Amflora post-market environmental monitoring plan (EU Register, 2010) outlines the number of production sites to be selected for surveillance should be 20. Therefore all Amflora starch production fields from 2010 were included in this volunteer monitoring study which relates to seven fields in the Czech Republic.

In addition to this volunteer monitoring study the volunteer monitoring according to the requirements set out by the Amflora Identity Preservation (IP) System was conducted.

MATERIALS AND METHODS

Potato volunteer monitoring. Monitoring was performed at all fields which were cultivated for Amflora starch production in 2010. This comprised a total of seven fields in the Czech Republic (Table 1). The monitoring was conducted at two time points during the cultivation period in 2011. The method described has been adapted from field inspection procedures and has already been used successfully for monitoring releases of genetically modified plants (MacDonald and Rouan, 2000).

Two different methods were applied to perform the volunteer monitoring: monitoring within the fields and outside the fields.

Volunteer monitoring within the field. Per field three or four points were chosen along one side of the field and marker stakes were driven into the ground to mark these points. Points were chosen e.g. along tractor lines passing the field to facilitate walking through the field for the observer. GPS-coordinates of the points were taken and recorded. Vertical lines through the field were mapped out and the observer walked along these lines across the field (blue lines in Figures 1 to 6). Per mapped vertical line six or seven plots were selected randomly to a total number of 20

observation plots per field. The individual selected plots were at least 5 m apart from each other and the 6 to 7 plots were distributed randomly across the field along the mapped vertical line. As recommended by Roberts-Pichette and Gillespie (1999) for each plot an area of 1 m x 1 m was measured and this square meter represented the area to be monitored for the occurrence of potato volunteers. The number of observed volunteers within the 1 m^2 plots was recorded. For any volunteer potatoes found, leaf samples were taken and analyzed for identity via PCR analysis. After recording the volunteer occurrence and in order to destroy the observed volunteers according to the requirements of the Amflora IP System, they were either dug out of the soil or the area of volunteer occurrence was treated with herbicides.

Volunteer monitoring outside the field. The observer monitoring for the occurrence of tuber-borne Amflora volunteers walked around the circumfence of the field at a distance of 1 m to the outer edge of the area cultivated with Amflora the previous year. Thereby, an area of 1 m left and 1 m to the right (resulting in a 2 m wide stripe directly surrounding Amflora area) was monitored for the presence of potato volunteers. The number of volunteers was recorded and the area where the volunteers were found marked in the map. For any volunteer potatoes found, leaf samples were taken and analyzed for identity via PCR analysis. Following the Amflora IP System, observed volunteers were either dug out of soil or the area of volunteer occurrence was treated with herbicides in order to destroy the potato volunteers.

PCR Analysis. Leaf samples from all volunteer potatoes detected were taken in the field, put in bags and transported to Sungene GmbH, Gatersleben, Germany. At Sungene a validated real-time PCR measurement was conducted according to the method described in Annex 9 of the Post-market monitoring report for the cultivation and monitoring of amylopectin potato EH92-527-1 variety Amflora in 2011. The first run was done with Amflora-specific primers (Annex 9). If these analyses would not have been positive further tests would have been performed.

RESULTS AND DISCUSSION

Number of volunteers observed within and outside the field. A total of seven Amflora production fields in the Czech Republic were monitored for the occurrence of potato volunteers. During the first monitoring conducted in June 2011 only at one field (CZ04) volunteer potato plants were observed. They were found within as well as outside the cultivated area (Table 2). Within the field three potato plants were detected growing within close proximity. These plants were found while passing the field (Figure 8). Therefore it was decided to position a plot at this location. A reason for the volunteers found within that field might be that growing conditions for potato seedlings are more favorable in a maize field than within a wheat or barley field where the potato seedling would get less light compared to a late developing maize field. It remains uncertain if the occurrence of Amflora volunteer plants was dependent on the crop chosen after the Amflora cultivation or on field cultivation measures which have been slightly different for the maize field compared to the wheat or barley field. During the second monitoring end of August no potato plants were found at the location within field CZ04 where the three plants were found in June.

When monitoring the outside of the field area at the first monitoring time point along the field border 61 small and non-flowering potato plants were observed in one area between the last and pre-last row of the maize crop (Figures 9 and 10). These potatoes were found within a stretch of around 50 meters along the border of the field. At the same location two potato plants were detected during the second monitoring time point end of August.

According to the information from the farmer the place where the potato volunteers were detected at the field border was a place where potato tubers fell from the harvester in 2010. Most likely not all tubers were recovered well, so that during the soil treatment the tubers were transferred to a depth where they could survive the frost period. This hypothesis is supported by the fact that the mother tuber from the volunteers found was buried in deep soil (Figure 10).

PCR Analysis. The PCR analysis from leaf samples taken from all volunteer potatoes found confirmed that all samples taken derived from Amflora potatoes.

CONCLUSIONS

The current study provides evidence that in most cases (six out of seven fields) the potato volunteer plants have been destroyed completely by soil management practices and by frost following the cultivation in 2010. At the first monitoring time point the only potato volunteer plants found were detected within and at the border of the field CZ04 which was cultivated with maize in 2011. However, the number of volunteer plants was reduced to zero within the field at the second monitoring time point. Therefore, the standard cultivation measures applied in the first year following the Amflora starch potato production were appropriate to control potato volunteers. It can be concluded that Amflora does not differ in its persistence from any other potato variety.

These findings confirm the information presented in Amflora Notification C/SE/96/3501 according to Directive 2001/18/EC (EFSA, 2006) and verify the assumption made in the environmental risk assessment.

REFERENCES

EU Register (2010). Post-market monitoring plan for Notification C/SE/96/3501. Available at: http://ec.europa.eu/food/dyna/gm_register/monitoringplan_eh92-527-1.pdf

MacDonald Robert and Rouan Dominique (2000). Monitoring Commercial Releases of Genetically Modified Crops: InVigor Hybrid Canola. The 6th International Symposium on The Biosafety Of Genetically Modified Organisms, July 2000, Saskatoon, Canada.

Roberts-Pichette, Patricia, and Lynn Gillespie (1999). Terrestrial vegetation biodiversity monitoring protocols. EMAN Occasional Paper Series, Report No. 9. Ecological Monitoring Coordinating Office,Burlington, Ontario.

Table 1. Amflora starch potato production fields 2010

Field code	Region	Size [ha]	Harvesting period [dd.mm.yyyy]
CZ01	Olešná	33.6	16.10. – 21.10.2010
CZ02	Olešná	1.0	21.10.2010
CZ03	Olešná	11.5	21.10. – 23.10.2010
CZ04	Bohdalec	18.4	01.10. – 27.10.2010
CZ05	Bohdalec	28.4	05.10. – 24.10.2010
CZ06	Nové Dvory	2.0	23.10.2010
CZ07	Nové Dvory	44.1	18.10. – 23.10.2010

Table 2. Number of volunteers found

		Number of volunteers observed in June 2011		Number of volunteers observed in August 2011	
Field code	Crop planted in 2011	within the field	outside the field	within the field	outside the field
CZ01	spring barley	0	0	0	0
CZ02	spring barley	0	0	0	0
CZ03	spring barley	0	0	0	0
CZ04	maize	3	61	0	2
CZ05	spring wheat	0	0	0	0
CZ06	spring barley	0	0	0	0
CZ07	spring barley	0	0	0	0

Figure 1: Fields CZ01

The yellow line indicates a length of 0.1 km; red lines represent the outer border of the area planted with Amflora in 2010; blue lines indicate the paths taken through the fields (within the red outer border) to select the observation plots.

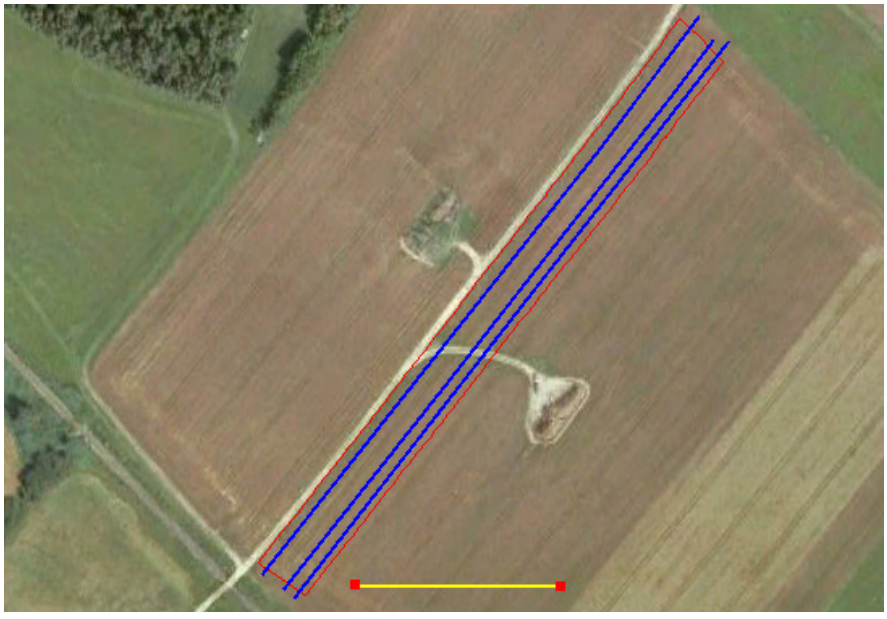

Figure 2: Field CZ02

The yellow line indicates a length of 0.1 km; red lines represent the outer border of the area planted with Amflora in 2010; blue lines indicate the paths taken through the field (within the red outer border) to select the observation plots.

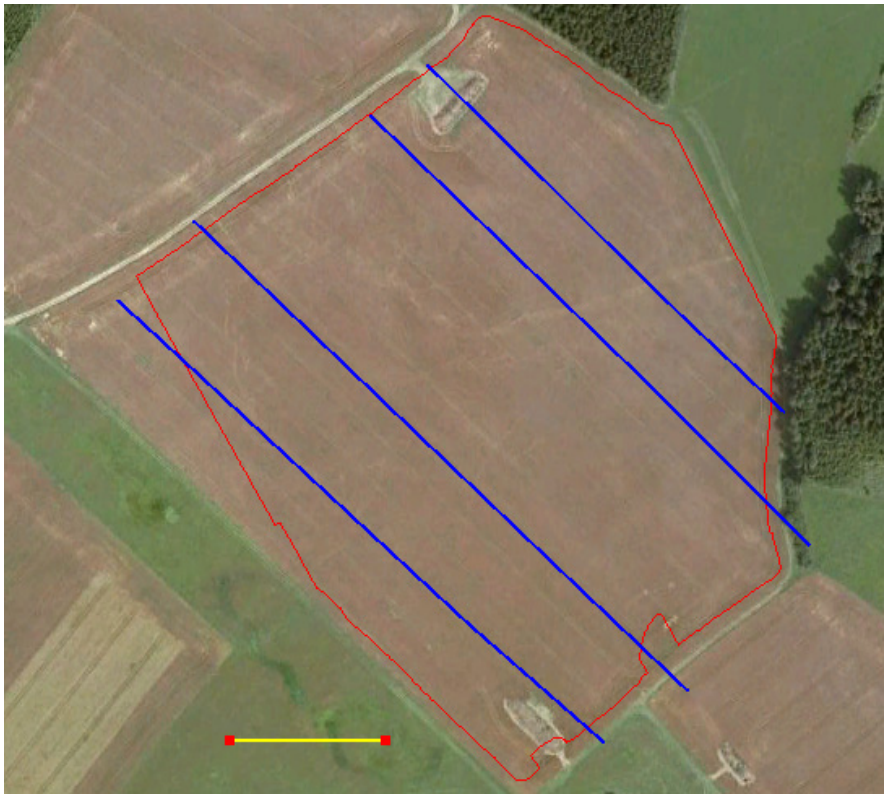

Figure 3: Field CZ03

The yellow line indicates a length of 0.1 km; red lines represent the outer border of the area planted with Amflora in 2010; blue lines indicate the paths taken through the field (within the red outer border) to select the observation plots.

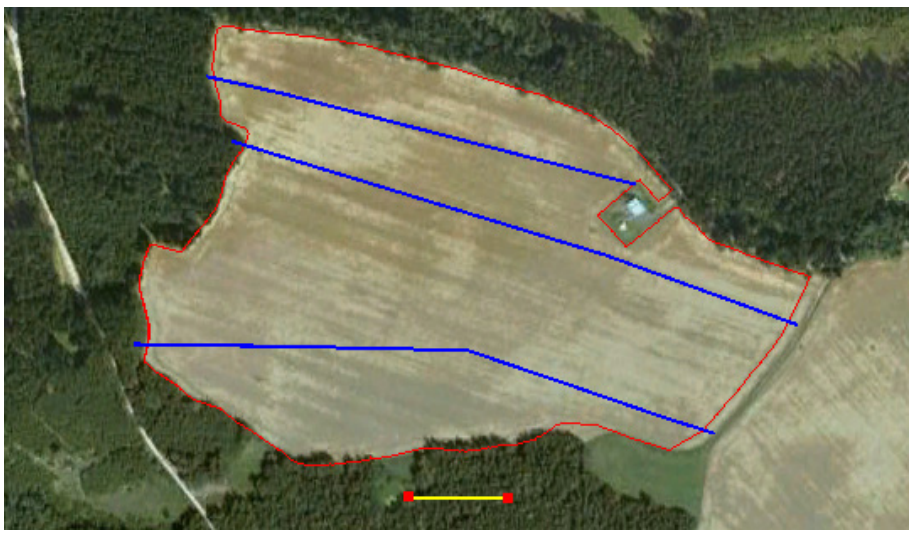

Figure 4: Field CZ04

The yellow line indicates a length of 0.1 km; red lines represent the outer border of the area planted with Amflora in 2010; blue lines indicate the paths taken through the field (within the red outer border) to select the observation plots.

Figure 5: Field CZ05

The yellow line indicates a length of 0.1 km; red lines represent the outer border of the area planted with Amflora in 2010; blue lines indicate the paths taken through the field (within the red outer border) to select the observation plots.

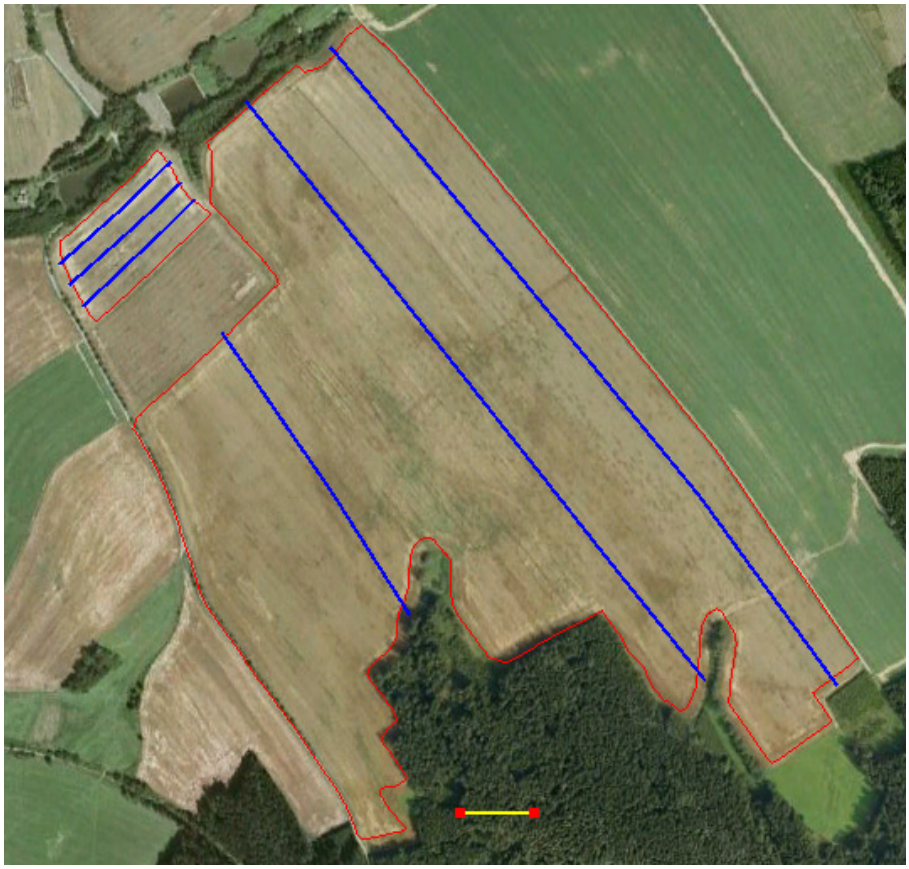

Figure 6: Fields CZ06 and CZ07

(CZ06 small field on top, left hand, CZ07 big field)
The yellow line indicates a length of 0.1 km; red lines represent the outer border of the area planted with Amflora in 2010; blue lines indicate the paths taken through the fields (within the red outer border) to select the observation plots.

Figure 7: Volunteer Monitoring June 2011, field CZ05
Field CZ05 planted with spring wheat, walking path marked with stakes

Figure 8: Volunteer Monitoring June 2011, field CZ04
Potato volunteer plant within the field CZ04

Figure 9: Volunteer Monitoring June 2011, field CZ04
Potato volunteer plants at the field border of field CZ04

Figure 10: Volunteer Monitoring June 2011, field CZ04
Potato volunteer plants at the border of field CZ04

Figure 11: Volunteer Monitoring August 2011, field CZ04
Potato volunteer plants at the border of field CZ04

Figure 12: Volunteer Monitoring August 2011, field CZ04
Potato volunteer plant at the border of field CZ04

Figure 13: Volunteer Monitoring August 2011, field CZ01