

## Non-Homogenised Crop Harvests and the GM Threshold.

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### Introduction

The interpretation of current EU GM labelling thresholds (0.9% GM DNA for food and feed) is not clear for some foodstuffs. Grains, flour and other ingredients which consist of small particles can be tested and examined statistically with existing approaches such as those described by ISO and CEN documents. This is because their small particle size ensures that each batch or packet or sack consists of a sufficiently large sample to ensure that the risk of exceeding the threshold is constant. For larger foods, such as large fruits or fruit sold in bunches, each sold unit represents a single sample unit with an individual probability of greatly exceeding the threshold (i.e. being ~50% GM).

For example, in an open pollinated field of maize grain, 0.3% GM may have been present in the seed and this may translate to a similar percentage in the harvested grain because the grain is reasonably well homogenised (by the pollination and by mechanical mixing during harvest), each sample of grain can be expected to have 0.3% GM with a relatively small margin of error. However, if the maize is sold as whole cobs, e.g. as sweet corn, then individual cobs (i.e. those which were near the GM plants) could greatly exceed the threshold despite the overall crop being below the threshold. Such cases we refer to here as 'non-homogenised' crops.

We have chosen sweet corn as an example in this study because:

- It is a crop species in which many GM lines have been commercialised.
- It is an out-crossing species and therefore has an increased chance AGP.
- There is likely to be a continuing demand for 'non-GM' sweet corn
- Individual cobs which exceed the threshold have been observed by CSL in non-UK field data (not shown).

We therefore need to improve our understanding of the probability that such units of non-homogenised crops will individually exceed the EU GM food threshold. Several factors are likely to affect this probability. We have used a computer model of the spatial distribution of adventitious GM presence (AGP) to identify and quantify the

impact of five factors: planting density; seed AGP heterogeneity; seed AGP threshold and within-crop pollen flow.

We have based the following modelling on empirical data where possible:

- FSE maize gene flow data (Weekes *et al.*, 2006). This allows the estimation of the spread of GM through the growing crop as a function of distance from sown AGP seed.
- SIGMEA maize gene flow data, collected with spatial information allowing estimation of field GM heterogeneity.

EU AGP thresholds for seed have yet to be decided or incorporated in legislation. The EU Scientific Committee on Plants (European Commission, 2001) has suggested thresholds of 0.3% and 0.5% depending on the species' out-crossing ability. The objective of this study is to provide an estimate of the probability of sweet corn cobs exceeding the EU 0.9% GM threshold under a variety of conditions given three key seed AGP thresholds: 0.1%, 0.3% and 0.5%. The source of AGP in this study is considered in the seed only. The effect of additional AGP from pollen flow or downstream mixing is not considered, but this could relatively easily be added to future versions of the model, drawing on existing gene flow and processing models used at CSL.

## Methods

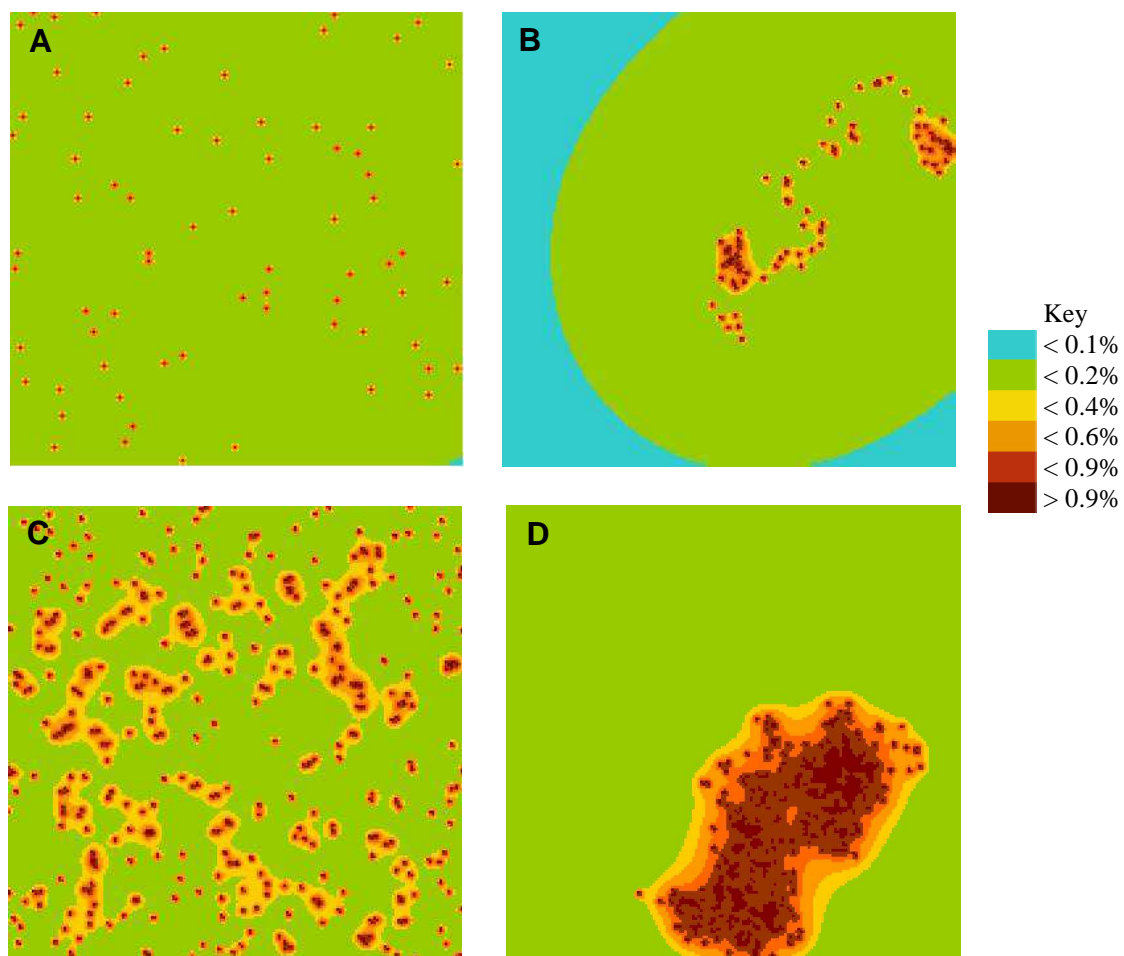
Before construction of a model we must consider the appropriate units of AGP to be used. EU advisory documents and the European Network of GMO Laboratories (ENGL) recommend the use of %GM DNA for measurement of GM in foods (European Commission, 2004). In order to obtain consistent results through supply chains and different raw materials, it is likely that %GM DNA will also be adopted for seed in future. The thresholds considered below are therefore expressed as %GM DNA.

A simple computer model has been constructed, written in Microsoft Excel VBA, which simulates a square 1 ha field. The field is populated with maize plants which have a probability of being heterozygous GM, based on the seed AGP threshold under examination. However, because the seed AGP is expressed in %GM DNA, it is not equivalent to the % of GM plants in the field. The potential % of GM plants is higher because the %GM DNA *per seed* is less than one. Approximately 2.4% maize plants will be present in a field with 1% GM DNA AGP in the sown seed, i.e. if each AGP seed has arisen from cross-pollination at the seed production stage the genotype of the AGP seed will be: Tt | Ttt (embryo| endosperm), where T = GM allele and t = wt allele. Therefore each seed having an overall of 41.5% GM DNA (Allnutt *et al.*, 2005; Papazova *et al.*, 2005). This equates to  $1 / 0.415 = 2.4$  %GM plants *per* %GM DNA.

The distribution of seed AGP in fields is unlikely to be randomly homogeneous. We have therefore modelled the AGP spatial distribution with varying heterogeneity. This has been done by introducing a variable for frequency of AGP clusters and the density of clusters. In the most homogeneous case, each AGP derived plant is independent with a random probability of being at its location. In the most heterogeneous case, all AGP is within a single cluster with a low probability of other clusters existing in the

field. Heterogeneity is also dependent on the % of AGP plants. A higher %AGP field has a lower probability of being heterogeneous and this is reflected in the model, where higher seed AGP values give lower heterogeneity when all other factors are equal. Figure 1 illustrates simulated fields of varying heterogeneity. We have expressed heterogeneity as 'H<sub>95</sub>', the proportion of the population which contains 95% of the total %GM DNA. High H<sub>95</sub> is therefore a *low* heterogeneity and *vice versa*.. Under ISTA rules, certified seed is required to pass tests for homogeneity (approximately >60% H<sub>95</sub>). The lower level of heterogeneity examined here (H<sub>95</sub> ≈ 40%) is therefore a worst-case scenario, and the upper level (H<sub>95</sub> ≈ 70%) more likely in practice.

Figure 1. Examples of simulated 1 ha maize fields with AGP shown in kernel content (following pollination). All at 30,000 plants / ha: A = 0.1% GM DNA AGP, 80% H<sub>95</sub>; B = 0.1% GM DNA AGP, 48% H<sub>95</sub>; C = 0.5% GM DNA AGP, 78% H<sub>95</sub>; D = 0.5% GM DNA AGP, 40% H<sub>95</sub>.



The probability of AGP on any cob in the crop is calculated from the frequency of GM pollination at that point from the simulated AGP maternal plants (in units of %GM DNA). The cobs from the AGP maternal plants themselves is also included. The seed yield and amount of pollen from all plants is assumed to be equal.

The dispersal of GM pollen from the AGP plants in the growing crop has been modelled using the equations for maize FSE GM gene flow described in Weekes *et al.*, 2006. However an important modification has been included. The FSE model describes gene flow between equally sized fields of GM and non-GM maize. Pollination from the individual plants in the current simulation is therefore assumed to follow the same rate of decline with distance as the FSE model, but diluted by a factor equal to the circumference of the circle at the distance in question multiplied by the plant density:

$$f(r) = f(fse) / (2\pi.r \times d) \quad \text{Equation 1.}$$

where  $f(r)$  is the frequency of pollination given distance,  $r$  (m);  $f(fse)$  is the frequency of pollination at distance  $r$  using the FSE model and  $d$  is the planting density in  $m^{-2}$ . Also, below 1.7 m, the FSE model is incorrect (curve decreases) and a simple exponential model was used below this distance, which fitted observed data well (not shown):

$$f(fse) = 0.2731 \times r^{-1.3718} \quad \text{Equation 2}$$

Four types of cross involving the AGP parent plants (41.5% GM DNA *per kernel*) contribute to the total %GM DNA in the sweet corn crop:

1. AGP♂ (Tt Ttt) × wt♀ (tt ttt) total determined by Equation 1.

f2	t	t
T	Tt Ttt	Tt Ttt
t	tt ttt	tt ttt

mean = 20.83% GM DNA

2. AGP♂ (Tt Ttt) × AGP♀ (Tt Ttt) total determined by Equation 1.

f2	T	t
T	TT TTt	Tt Ttt
t	Tt TTt	tt ttt

mean = 41.7% GM DNA

3. Selfing - same as 2., assumed at 5%

4. . wt♂ (tt ttt) × AGP♀ (Tt Ttt) total determined by 1 - Equation 1 - selfing.

f2	T	t
t	Tt TTt	tt ttt
t	Tt TTt	tt ttt

mean = 29% GM DNA

Therefore only crosses between AGP plants (type 2) cause a slight increase in the total %GM DNA from f1 (seed) to f2 (sweet corn crop). Due to the contribution of the maternal endosperm being two chromosomes, all other crosses reduce the total % GM DNA. However, it is important to note that the proportion of GM pollen is greater than the %GM DNA in the seed because there are 2.4% heterozygous GM plants for every 1% GM DNA seed, as mentioned previously.

Considering the distribution of f2 sweet corn due to AGP plants and their pollen dispersal the prime output of the simulation is the frequency of cobs that contain over the EU food threshold of 0.9% GM DNA, based on the maternal genotype and the frequency of crosses 1-3 at a point in the field. The total %, 95th and 98th percentile of >0.9% cobs is then calculated. Ten replicate simulations were carried out for each scenario (below) and the s.d. also calculated among replicates, for five values of % GM DNA seed AGP. The potential thresholds for particular consideration are 0.1%, 0.3% and 0.5%, higher values have been included to better illustrate trends.

Scenarios:

- A. Random heterogeneity; planting density 30,000 / ha.
- B. Random heterogeneity; planting density 50,000 / ha.
- C. High heterogeneity; planting density 30,000 / ha.
- D. High heterogeneity; planting density 50,000 / ha.

A further consideration when calculating the %GM DNA on sweet corn cobs in the method by which the DNA will be extracted. If kernels are stripped from the maternal genotype 'core' then the total %GM DNA will be different from when the entire cob is analysed as one. A simple experiment was performed to determine the proportion of DNA present in the core and kernels from sweet corn. Four sweet corn cobs were stripped of their kernels, weighed and DNA was extracted (Qiagen plant mini kit) from equal masses of the core and kernels. The concentration of DNA was accurately determined for core and cob samples using the fluorometric Pico Green method (Molecular Probes Inc.)

## Results and Discussion.

Sweet corn kernels were found to contain  $1.88 \times$  the mass of the core. Kernels also contained  $8 \times$  the concentration of extractable DNA than the core. The proportion of cobs exceeding 0.9% GM DNA is also shown as attenuated by  $0.067 \times$  the proportion due to kernels alone.

Tables 1, A-D show the results for the f2 sweet corn field simulations and Figure 1, A and B the corresponding graphs. As expected, increasing f1 seed AGP causes increased probability of crop sweet corn cobs exceeding the 0.9% GM DNA threshold ( $P_{>0.9}$ ). The rate of this increase also increases with AGP, up to the limit of 0.9% examined in this study.  $P_{>0.9}$  was also higher for a given seed AGP with higher planting density. This is most likely due to increased density increasing the frequency of cross type 2, as described above, which increases the net proportion of GM from f1 to f2. The forming of GM plant clusters is also increased with higher planting density and the number of recipient plants receiving AGP pollen increased.

Table 1 A-D. Result of the f2 sweet corn field simulations. Each row of values was obtained from ten replicates. %AGP is equivalent to the % GMDNA of a 'seed threshold'. % GM plants is that actual proportion of f1 GM plants growing in the field given the genotypes outlined above. The f2 mean %GM DNA is the mean GM content of f2 sweet corn crop as a whole (98th percentile of this value also shown). '% > 0.9%' is the proportion of cobs in the crop over the 0.9% GM DNA threshold, equivalent to the probability of the threshold being exceeded for a randomly chosen cob from the crop. '% > 0.9% as cob' is the same value when DNA from the cob 'core' is also included in analysis.

A

Random heterogeneity

Density = 30,000 / ha

%AGP	% GM plants	f2 Mean %GM DNA	s.d	%GM DNA 98th	s.d.	H95	% >0.9%	s.d.	% >0.9% as cob
0.10	0.24	0.13	0.00131	0.38	0.00239	76.00	0.25	0.01	0.23
0.30	0.72	0.39	0.00224	0.72	0.00641	77.00	0.92	0.07	0.86
0.50	1.20	0.65	0.00343	0.94	0.03712	80.00	2.28	0.21	2.13
0.70	1.69	0.91	0.00407	1.38	0.07061	81.00	6.28	0.40	5.89
0.90	2.17	1.17	0.00539	2.07	0.06734	81.00	11.18	0.37	10.49

B

Random heterogeneity

Density = 50,000 / ha

%AGP	% GM plants	f2 Mean %GM DNA	s.d	%GM DNA 98th	s.d.	H95	% >0.9%	s.d.	% >0.9% as cob
0.10	0.24	0.14	0.00124	0.54	0.00189	73.00	0.41	0.05	0.38
0.30	0.72	0.42	0.00123	0.98	0.00515	75.00	3.55	0.02	3.33
0.50	1.20	0.70	0.00266	1.24	0.05250	78.00	6.45	0.10	6.05
0.70	1.69	0.98	0.00154	1.82	0.07640	78.00	11.15	0.17	10.46
0.90	2.17	1.26	0.00364	30.34	0.01089	80.00	18.40	0.17	17.26

E

high heterogeneity

Density = 30,000 / ha

%AGP	% GM plants	f2 Mean %GM DNA	s.d	%GM DNA 98th	s.d.	H95	% >0.9%	s.d.	% >0.9% as cob
0.10	0.24	0.14	0.00760	0.72	0.09423	48.60	1.47	0.37	1.38
0.30	0.72	0.42	0.01222	1.76	0.29172	30.40	5.51	1.03	5.17
0.50	1.20	0.71	0.01536	2.82	0.70658	42.00	9.09	0.79	8.52
0.70	1.69	1.03	0.10332	4.76	0.69092	52.00	14.87	2.15	13.95
0.90	2.17	1.27	0.01642	31.21	0.07897	39.50	21.37	1.93	20.04

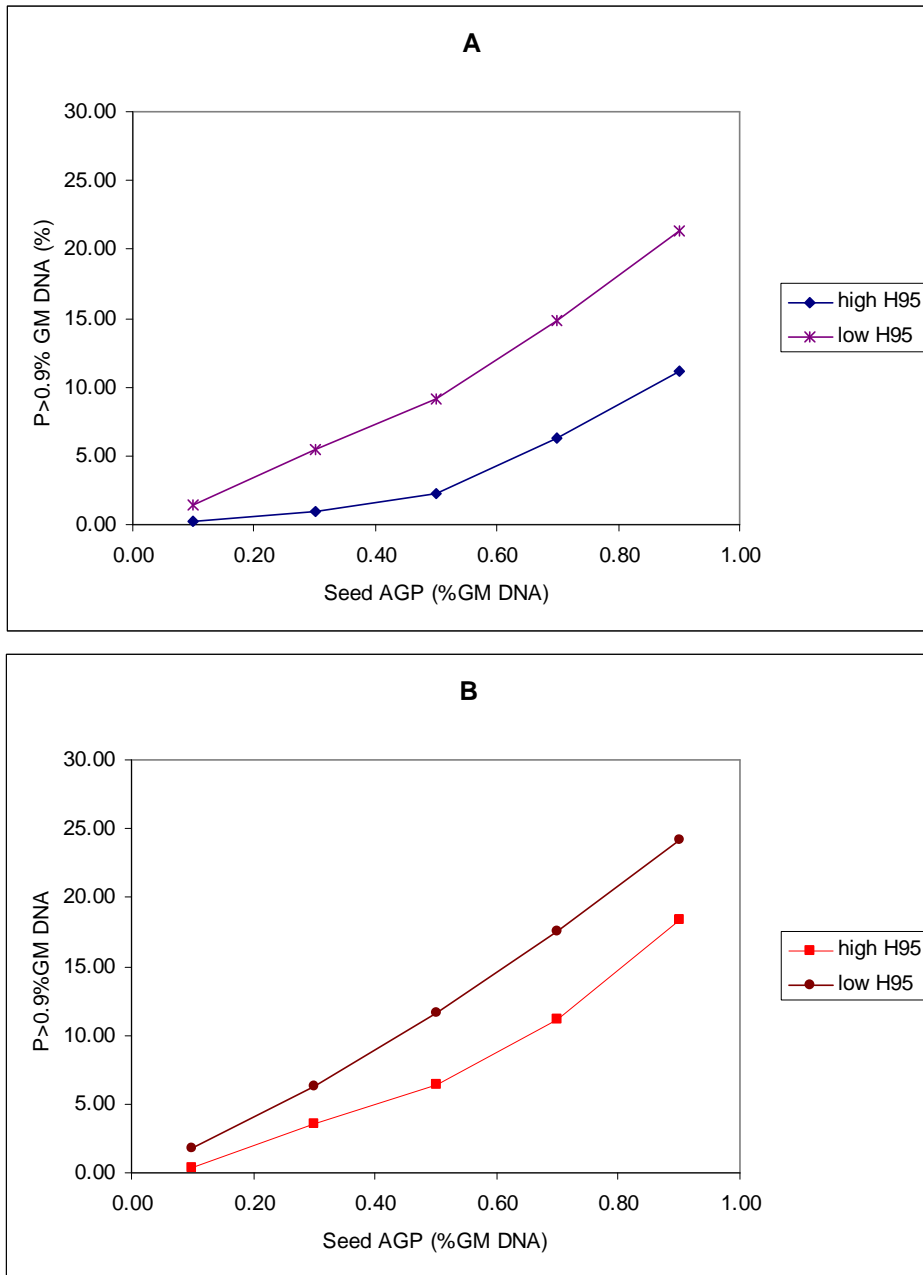
F

high heterogeneity

Density = 50,000 / ha

%AGP	% GM plants	f2 Mean %GM DNA	s.d	%GM DNA 98th	s.d.	H95	% >0.9%	s.d.	% >0.9% as cob
0.10	0.24	0.15	0.00495	0.82	0.10657	49.54	1.72	0.33	1.61
0.30	0.72	0.48	0.00478	1.89	0.07433	39.06	6.33	0.35	5.93
0.50	1.20	0.77	0.00980	3.81	0.71764	54.14	11.67	1.19	10.94
0.70	1.69	1.11	0.03807	6.02	0.39105	44.88	17.55	1.80	16.46
0.90	2.17	1.40	0.01174	31.48	0.13612	57.99	24.19	2.12	22.69

Figure 2 A and B. Graphs showing probability of a crop sweet corn cob exceeding the 0.9% GM DNA threshold vs. the fl seeds AGP at low heterogeneity (high  $H_{95}$ ) and high heterogeneity (low  $H_{95}$ ). A = 30,000 plants / ha; B = 50,000 plants / ha.



Heterogeneity increases  $P_{>0.9}$ , as shown in Figure 2. As heterogeneity increases, more dense clusters of AGP plants are formed and their combined pollen increases the probability that intervening plants will exceed  $P_{>0.9}$ .

Tables 1, A and B, with low heterogeneity represent the most likely situation for certified seed, given its requirement for homogeneity and under these conditions the  $P_{>0.9}$  for a seed AGP threshold of 0.5% GM DNA is low (2.13% and 6.05% for 30,000 and 50,000 plants / ha respectively). If a worst-case of heterogeneity is considered (Table 1, C and D) then these  $P_{>0.9}$  values rise to 8.52% and 10.94% respectively. If, following such examples as rules for seed purity, we require a less than 5%  $P_{>0.9}$

(equivalent to 5% beta risk, Banyai & Barabas, 2002) then a 0.3% GM DNA seed AGP threshold would be required, based on the result of this simulation. A 0.1% GM DNA seed AGP threshold would reduce the beta risk to 2%, however, the uncertainty of GM quantification at 0.1% is high and alpha risk (Banyai & Barabas, 2002) at this level would be likely to be unacceptably high.

## References

Allnut, T.R., Roper, K., Thomas, C., Hugo, S. and Kerins, G. (2005) Detection and Traceability Technologies to Underpin the GM Inspectorate. Research Report to Defra: [http://www.csl.gov.uk/prodserv/rds/gmo/detect\\_trace011205.pdf](http://www.csl.gov.uk/prodserv/rds/gmo/detect_trace011205.pdf)

Banyai, J. & Barabas, J. (2002). Handbook on Statistics in Seed Testing. ISTA.

European Commission (2001). Scientific Committee on Plants: Opinion of the Scientific Committee on Plants Concerning the Adventitious presence of GM seeds in conventional seeds.

European Commission (2004). Commission Recommendation on technical guidance for sampling and detection of genetically modified organisms and material produced from genetically modified organisms as or in products in the context of Regulation (EC) No 1830/2003. *Official Journal of the European Union* 348:18-26.

Papazova, N; Malef, A; Degrieck, I; Van Bockstaele, E; De Loose, M. 2005. DNA extractability from the maize embryo and endosperm - relevance to GMO assessment in seed samples. *Seed Science and Technology* 33(3): 533-542.

Weekes, R., Allnut, T. R., Boffey, C., Morgan, S., Bilton, M., Daniels, R. Henry, H. (2006) A study of crop-to-crop gene flow using farm scale sites of fodder maize (*Zea mays* L.) in the UK. *Transgenic Research. In Press.*