Asynchronous Flowering or Buffer Zones: Technical Solutions for Small-scale Farming

Asynchrone Blütezeiten oder Pufferzonen: Technische Lösungen für landwirtschaftliche Kleinbetriebe

Floraison décalée ou zones tampon : des solutions techniques pour l'agriculture à petite échelle

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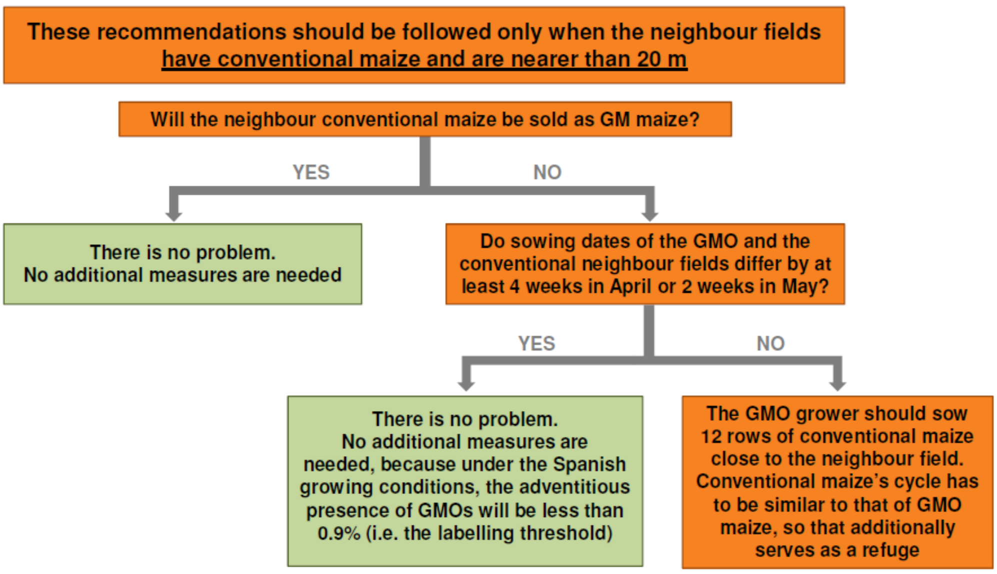
To assure consumers' free choice of conventional and genetically modified (GM) products, many countries such as those in the EU have established compulsory labeling of products containing or derived from GM crops, with a threshold for adventitious presence (e.g. Regulation (EC) 1829/2003, establishing a threshold at 0.9 per cent). The progressive implementation of GM plants has given rise to a situation of coexistence (see, for example, Regulation EC 1830/2003). Coexistence between conventional and genetically modified (GM) crop fields is understood as a principle according to which farmers should be able to freely cultivate the crops of their choice, whether GM, conventional or organic.

On the basis of scientific research the Spanish Seed Producers Association ANOVE compiled a series of recommendations to facilitate coexistence in small-scale farming zones. This guide for Good Agricultural Practice (GAP) (Figure 1) is provided to farmers. The EU-funded project PRICE (Practical Implementation of Coexistence in Europe) aimed at validating these recommendations in real agricultural sites. Here we report the experimental validation of these recommendations in the 'worst-case scenario', that is an agronomic region with small-scale fields (below 1 ha on average) and high GM pressure (about 75 per cent GM maize).

Figure 1: Coexistence recommendations according to the *Guide for Good Agricultural Practices* published by the Seed Producers Association ANOVE



The main factors determining the adventitious presence of GM material in the yield of a conventional field are accidental seed impurity, the presence of GM volunteer plants,[1] cross-pollination between GM and non-GM crops, and accidental mixture (admixture) of GM and conventional grain occurring during harvest, transport or storage processes. Of these, cross-pollination causes most concern to maize growers because it is difficult to control and depends on many factors, including the distance between the GM donor(s) and the conventional receptor fields, type of cultivars, farming practices and climatic conditions. The rate of cross-fertilisation is highest near the borders and decreases toward the centre of the conventional field (Palaudelmàs *et al*., 2012). Separation distances between GM and conventional fields have been proposed as a strategy to ensure coexistence (Bénétrix and Bloc, 2003; Henry *et al.*, 2003; Brookes *et al.*, 2004; Ma *et al.*, 2004; Melé *et al*., 2004; Sanvido *et al.*, 2005; Bannert, 2006; Della Porta *et al.*, 2006; Ortega Molina 2006; Pla *et al*., 2006; Weber *et al.*, 2007). From these studies it was concluded that a separation distance of 20–25 m will generally be enough to maintain the GM contents of conventional fields below the 0.9 per cent threshold, even though isolation distances required in different EU countries range from 25 to 800 m. In the particular case of farming regions based on small-scale fields the isolation distance is difficult to apply without disturbing the grower's freedom of choice. In these regions coexistence would benefit from alternative strategies.

Several field trials demonstrated that the GM cross-fertilisation rate is significantly lower when non-GM maize planting (i.e. a buffer zone) separates the GM donor and the conventional receptor field, compared with empty isolation distances (Pla *et al*., 2006). On the other hand, a strategy designed to reduce synchrony between male GM and female conventional flowering dates proved to be an efficient tool to minimise cross-fertilisation (Palaudelmàs *et al.*, 2008). The GAP guide recommendations are based on use of flowering asynchrony and buffer zones to guarantee coexistence.

Experimental validation of the GAP guide recommendations

A total of six field clusters were identified in the 2012 and 2013 seasons in the region of Baix Empordà, Catalonia, in which non-GM maize fields were surrounded by GM maize fields. Farmers cultivating these fields were asked to carefully follow the GAP recommendations. Growers in two sites opted to use flowering asynchrony while those in four sites chose to apply buffer zones to minimise cross-pollination. The GM and non-GM character of representative plants in all fields was confirmed using lateral flow Bt tests, including those in buffer zones when applicable. The progress of flowering of GM and conventional plants was monitored in all fields on 40 plants per field using a visual scale (Palaudelmàs *et al.*, 2008); and the date of flowering corresponded to that in which 50 per cent of monitored plants emitted mature pollen or were fertile. The possible adventitious presence of GM maize in conventional fields under study was experimentally determined using the standard sampling protocol (Messeguer *et al*., 2006) and real-time PCR-based DNA analysis[2] specifically targeting the MON810 transgene (3' flanking, Hernández *et al.*, 2003) and the *adh1* maize control (Hernández *et al.*, 2004). As an additional test, GM contents of conventional fields were in silico[3] predicted using the GIMI 2.0 software (freely available at http://gimi.phytonanywhere.com/, Melé *et al.*, 2014), using the geographical distribution of fields, GM or non-GM character of varieties and flowering dates obtained in this study (see below).

Contribution of flowering asynchrony to improving coexistence

A separation in the sowing dates of GM and non-GM maize in neighbouring fields is recommended to achieve asynchronous flowering periods. Lack of flowering coincidence, i.e. pollen emission in the GM donor field (male flower maturity) during a period in which the non-GM female flower is non receptive, should avoid cross-pollination. According to the GAP recommendation, sufficient flowering asynchrony can be achieved by means of a difference in the sowing dates of at least 4 weeks in April or 2 weeks in May (Figure 1). Fields 2A and 2B growers chose to use this strategy (Figure 2). Sowing date differences and the resulting gaps between conventional male and GM female flowering are shown in Table 1.

Figure 2: Geographical location of conventional and neighbouring GM maize fields where GAP recommendations based on flowering asynchrony were applied. (Fields sown with GM maize are shown in red and those sown with conventional varieties are indicated in blue).

Table 1: Main characteristics of field clusters using a flowering separation coexistence strategy, and adventitious GM contents.

field ID	GM/no-GM	Variety	Sowing date	Sowing asynchrony	Flowering date	Slowering asynchrony	Experimental %GM	In silico %GM
2A	**non-GM**	DKC6815	6th May	0	21st July	0	**0.07**	**0.88**
V	**GM**	PR33D48	18th April	18	17th July	4		
VI	**GM**	PR33Y72	20th April	16	13th July	8		
2B	**non-GM**	DKC6101	17th June	0	21st August	0	**0.01**	**0**
V	**GM**	PR33D48	18th April	60	17th July	35		
VI	**GM**	PR33Y72	20th April	58	13th July	39		

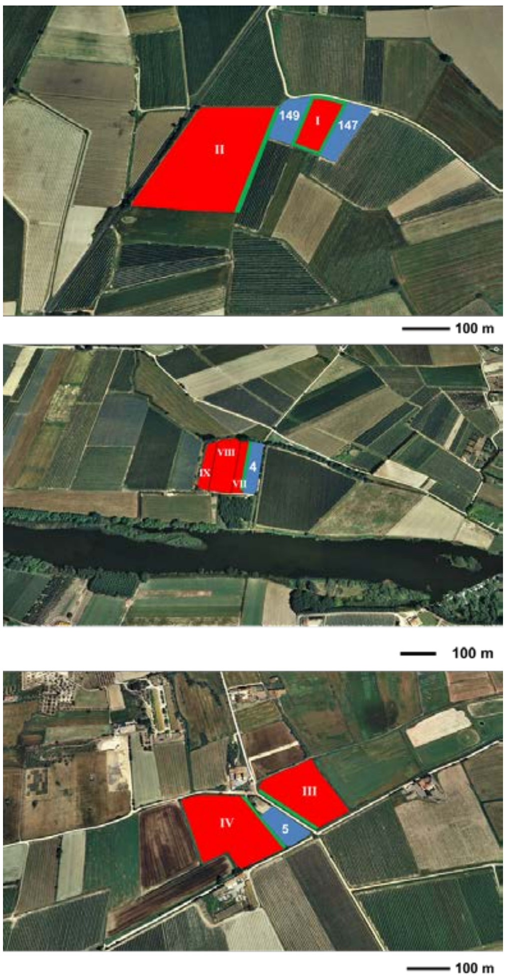
Note: Maize varieties, sowing and flowering dates in field clusters 2A and 2B and resulting asynchrony between non-GM male and GM female flowering (in days). GM percentages in conventional fields 2A and 2B, quantified using the standard sampling method and real-time PCR analysis (experimental) and in silico predicted using GIMI 2.0 software. Real-time PCR had a limit of detection (LOD) = 0.1 per cent and limit of quantification (LOQ) = 0.3 per cent and RSD<20 per cent. Note that per cent GM is calculated by weighted mean of up to 28 values (Messeguer *et al*., 2006), taking values between the LOD and the LOQ as approximate and those below the LOQ as 0).

The percentage of GM contents in the yields of conventional fields, as experimentally determined and in silico predicted, is shown in Table 1. Field 2B clearly shows that flowering asynchrony reduced cross-fertilisation down to background levels. Field 2A did not rigorously follow the GAP recommendations since there was a reduced sowing separation of 2–2.5 weeks in April (instead of 4). Taking into consideration the small size and particular shape of this field, in silico prediction indicated GM levels close to the 0.9 per cent threshold. This was higher than the experimental per cent GM values. There was a strong hailstorm 2 days after flowering of GM plants in field V. The damage suffered by masculine inflorescences most probably explains the discrepancy between real cross-pollination and that predicted (which did not take into account this extremely unusual meteorological condition). The differences between GM and conventional sowing dates recommended in the GAP manual are based on experimental observations (Palaudelmàs *et al.*, 2008). Normal photoperiod and temperatures in April result in slower rate of maize growth than those in May; and this is the basis for establishing longer sowing differences in April than in May. The results corresponding to field 2B demonstrate the GAP recommendations are accurate.

Contribution of buffer zones to improving coexistence

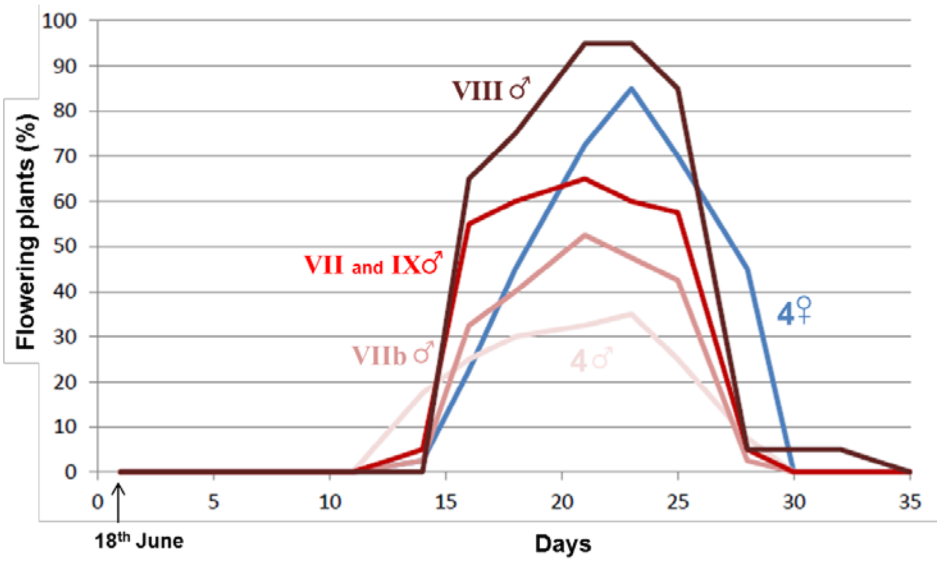
In case there was no flowering asynchrony, the GM fields were sown with 12 m wide buffer zones (i.e. 9 maize rows with conventional plants, according to the cultural practices in the region) immediately flanking the receptor conventional fields (Figure 3). In the worst case scenario, small conventional fields would be close to large GM fields with full flowering coincidence.

Figure 3: Geographical location of conventional and neighbouring GM maize fields where GAP recommendations based on buffer zones were applied. (Fields sown with GM maize are shown in red, those sown with conventional varieties are indicated in blue, and buffer zones in green).



Four field clusters were monitored in which there was strong flowering coincidence in the GM, non-GM and buffer plants (Figure 4); and conventional field sizes were at most 0.5 ha.

Figure 4: Example of flowering monitoring in a field cluster in which buffer zones were used to minimise GM to non-GM cross-pollination. (Only male flowering was monitored in donor GM and buffer plants; while both male and female flowering of conventional receptor plants are shown. Numbers indicate the different fields depicted in Figure 3. VIIb corresponds to the buffer zone of field VII. Visual analysis of 40 plants per field was carried out on a daily basis).



Even in this scenario all analysed conventional fields had adventitious GM contents below the 0.9 per cent threshold in their yields (Table 2). Interestingly, these experimental results were in agreement with those predicted using the GIMI 2.0 software. Moreover, this software allowed simulation of a situation in which the same fields were cultivated in exactly the same conditions except for the lack of buffer zone. In this hypothetical case the conventional fields would have had per cent GM values ≥ 0.9 per cent (i.e. 0.9, 1.3, 1.1 and 1.2 per cent for fields with ID 147, 149, 4 and 5, respectively). This validated the coexistence approach based on buffer zones.

Note that the buffer zone on field VII, adjacent to conventional field 4 (shown in green in Figure 3), was incomplete. This was due to a routine maneuver by the sowing machine on exiting the field. Even so, the field with ID=4 had 0.33 per cent adventitious GM in its yield. Importantly, GAP recommendations are robust enough to allow this type of unavoidable practice to be conducted without having a negative effect on coexistence.

Table 2. GM percentages in conventional fields 147, 149, 4 and 5, quantified using the standard sampling method and real-time PCR analysis (experimental) and in silico predicted using GIMI 2.0 software. (Real-time PCR had a limit of detection (LOD) = 0.1% and limit of quantification (LOQ) = 0.3% and RSD<20%. Note that % GM is calculated by weighted mean of up to 28 values (Messeguer *et al*., 2006), taking values between the LOD and the LOQ as approximate and those below the LOQ as 0).

field ID	Non-GM field area (Ha)	Experimental %GM	In silico %GM
147	0.40	0.24	0.10
149	0.50	0.41	0.26
4	0.41	0.33	0.29
5	0.47	0.12	0.34

The GAP is effective

The GAP provides adequate recommendations to ensure coexistence. In six agricultural field sites selected to represent the worst case scenario we experimentally proved that application of GAP recommendations resulted in adventitious GM contents below the 0.9 per cent threshold established in countries in the EU. We showed that it is possible to use the knowledge of specific agronomical practices and climatic conditions to improve coexistence through measures other than the high isolation distances currently set up by various European Member States. Buffer zones and, at least in temperate areas such as Mediterranean ones, sufficiently different sowing dates are capable of guaranteeing coexistence in the frame of the present EU regulation without any negative effect on the yields. These measures do not lead to unnecessary costs, are simple to apply and are compatible with small deviations resulting from common agricultural practices.

Notes

1. During harvesting, some maize grains may remain in the field. In temperate areas, it is common that these grains germinate in the next season, together with the new seeds. In the case where a field was sown with GM plants during one season, and with conventional plants the next season, GM volunteer plants (producing GM grain and GM pollen) can grow together with conventional plants.

2. Real-time Polymerase Chain Reaction (PCR) is the analytical method recommended to detect and quantify the GM contents in food/feed samples. It is based on detection of a DNA sequence; and in this case it specifically allows detection and quantification of the transgene present in MON810.

3. In silico, literally Latin for ‘in silicon’, is an expression used to mean ‘performed via computer simulation.

Further Reading

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Summary

To ensure consumers' and farmers' freedom of choice, many countries have regulated the labeling of products derived from genetically modified (GM) crops and coexistence of conventional and GM fields. On the basis of published research results a guide for Good Agricultural Practices (GAP) was compiled to facilitate maize coexistence in farming zones with temperate climate. In the frame of the PRICE EU-funded research project we validated

these recommended measures in real agricultural fields representing the worst case scenario, i.e. small-scale farming in a region with around 75 per cent commercial GM maize. The two alternative strategies proposed in the GAP, buffer zones and flowering asynchrony, successfully guaranteed coexistence in the frame of the present EU regulation. These measures do not lead to unnecessary costs, are simple to apply and are compatible with small deviations resulting from common agricultural practices. The results are applicable for temperate zones such as Southern Europe and cannot easily be transferred to other parts of Europe due to differences in climatic conditions which limit the use of different flowering times as a coexistence strategy.

Zahlreiche Länder haben die Kennzeichnung von Produkten, die aus genetisch veränderten Feldfrüchten erzeugt werden, und die Koexistenz von Anbauflächen für konventionelle und genetisch veränderte Feldfrüchte reguliert, um die Entscheidungsfreiheit für Verbraucher und Landwirte zu gewährleisten. Auf der Grundlage veröffentlichter Forschungsergebnisse wurde ein Leitfaden für die gute landwirtschaftliche Praxis zusammengestellt, um die Koexistenz bei Mais in Anbaugebieten der gemäßigten Zonen zu unterstützen. Im Rahmen des EU-finanzierten PRICE-Projekts überprüften wir diese empfohlenen Maßnahmen anhand von konkreten Anbauflächen für den schlimmsten Fall, d.h. für landwirtschaftliche Kleinbetriebe in einer Region, in der der Anteil an gewerblichem Anbau von genetisch verändertem Mais etwa 75 Prozent beträgt. Die beiden im Sinne der guten landwirtschaftlichen Praxis vorgeschlagenen Alternativstrategien – Pufferzonen und asynchrone Blütezeiten – gewährleisteten die Koexistenz im Rahmen der aktuellen EU-Regelung. Diese Maßnahmen verursachen keine unnötigen Kosten, sind einfach umzusetzen und, abgesehen von kleinen Abweichungen zur üblichen landwirtschaftlichen Praxis, kompatibel. Die Ergebnisse gelten für gemäßigte Klimazonen wie z.B. Südeuropa und lassen sich aufgrund der unterschiedlichen Klimaverhältnisse nicht ohne Weiteres auf andere Teile Europas abbilden, wodurch asynchrone Blütezeiten nur eingeschränkt als Koexistenzstrategie in Frage kommen.

Pour donner la liberté de choix aux agriculteurs et aux consommateurs, de nombreux pays ont réglementé l'étiquetage des produits dérivés de cultures génétiquement modifiées (GM) et la coexistence des champs en cultures conventionnelles et GM. À partir des résultats de recherches publiées, un guide de bonnes pratiques agricoles (BPA) a été préparé pour faciliter la coexistence du maïs dans des zones agricoles au climat tempéré. Dans le cadre du projet de recherche financé par l'Union européenne PRICE, nous avons validé les mesures recommandées en plein champ dans les conditions les pires : l'agriculture à petite échelle d'une région cultivant environ 75 pour cent de maïs GM. Les deux stratégies alternatives proposées dans le guide, les zones tampon et le décalage de la floraison, ont réussi à garantir la coexistence dans le cadre des réglementations européennes en vigueur. Ces mesures n'entraînent pas de coûts excessifs, sont faciles d'emploi et sont compatibles avec les faibles écarts résultant des pratiques agricoles communes. Les résultats s'appliquent aux zones tempérées comme le sud de l'Europe et ne peuvent pas facilement s'appliquer à d'autres parties de l'Europe du fait de différences de conditions climatiques limitant le recours à des périodes de floraison différentes comme stratégie de coexistence.

Pullquote

“The GAP provides adequate recommendations to ensure coexistence.”

“Die gute landwirtschaftliche Praxis bietet Empfehlungen zur Gewährleistung der Koexistenz.”

"Les BPA donnent des recommandations permettant d'assurer la coexistence."

IMAGE CAPTIONS

Image 1.
Separation in the sowing dates of GM (right field) and non-GM maize (left field) to achieve asynchronous flowering.

Image 2.
GM (left field) and non-GM (right field) maize fields separated by a buffer zone immediately flanking the conventional field.

Image 3.
Aerial image of GM and non-GM maize fields in the region of Baix Empordà, Catalonia.