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Field cross-fertilization between GM and non-GM maize in Mallorca: a Mediterranean insular approach

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Abstract:

Under EU policy, Member States have developed national strategies or regulations for co-existence. Spain is the EU member state with largest area destined to grow genetically modified (GM) maize. However, still lacks of a national co-existence strategy and regional attempts to regulate this issue have been unfruitful. Several studies of real situation of coexistence in maize have been realized in a continental land context in Catalonia region, Spain, but Mediterranean insular microclimatic conditions such as land and sea breezes phenomenon, humidity or rains regime among other factors, might have an effect over flowering and pollination. The present study shows the first field trial on cross-fertilization between MON810 GM and non-GM maize in Mediterranean insular conditions. Our objective was to perform a trial that could be used as a scientific base for a future regional regulation on co-existence in Balearic Islands, Spain. To that end, we analyzed the adventitious presence of GM material in a non-GM maize adjacent field by cross-fertilization in a real situation in Mallorca; the size, distance and orientation (affecting the slope and wind direction) of GM and non-GM fields as well as the synchrony of flowering, were designed to favor maximum cross-fertilization. Our results suggest that, under these conditions, an isolation distance of 10-30 m between GM and non-GM maize fields might be sufficient to keep the adventitious presence below the EU labeling threshold of 0.9%.

Keywords: Corn / co-existence / cross-fertilization / GM-crop-free zones / isolation distances / maize / pollen barriers / pollen flow / thresholds / Zea mays L.

JEL codes:I1, K13, K2, K32, K33, L65, L66, O13, Q1.

1. Introduction

Despite latest trends in the European Union (EU), with strong regulations concerning genetically modified (GM) crops cultivation, co-existence between GM and non-GM crops remains yet a major goal of the EU policy, reflected in a multitude of existing references (European Commission, 2003; European Union, 2003). However, Spain, EU top grower of GM maize MON810 (GMO Compass, 2014), still lacks of a national co-existence strategy and regional attempts to regulate this issue have been unfruitful.

Numerous field trials have been performed on maize pollen mediated gene flow in a variety of situations (see *e.g.* Messeguer et al., 2006), enough to treat them statistically (as it has been usefully made for example by Riesgo, Areal, Sanvido, & Rodríguez-Cerezo, 2010). But none of them have been performed in Mediterranean islands. A lot of factors may affect cross-fertilization in maize: "*e.g.* isolation distance between the pollen source and recipient, size, shape and orientation of the pollen source and recipient, wind characteristics, rain, local environment, pollen viability, water status of pollen, male fertility, flowering synchrony, commercial destination of maize, sampling protocol, approaches used to study pollen dispersal, quantification methods, and analyzed plant material" (Devos, Reheul, & Schrijver, 2005). As local climatic conditions affect cross-fertilization and as co-existence seems to be mandatory, scientific studies should evaluate this phenomenon in different cropping areas and situations (Devos et al., 2009, 2005; Messeguer et al., 2006). However, co-existence studies under Mediterranean insular conditions have not been done before.

During the warm season (from May to October, when in Mediterranean area pollination of maize occurs) sea breezes (Estoque, 1961; Fisher, 1960; Neumann & Mahrer, 1971; Rotunno, 1983; Simpson, 1994) are the major low-level mesoscale circulation in coastal Mediterranean areas (Azorin-Molina, Chen, Tijm, & Baldi, 2011). Their influence seems to be even more pronounced in the islands (Melas, Lavagnini, & Sempreviva, 2000) where see breezes have specific particularities such as convergence, which also appear to have effects on climate and meteorology (Azorin-Molina, Connell, & Baena-Calatrava, 2009).

Mountains effects on meteorology and climate are also well-known (Barry & Chorley, 2009; Manabe & Terpstra, 1974) and their over sea and land breezes have been deeply studied (*e.g.* Mahrer & Pielke, 1977; Miao, Kroon, Vilà-Guerau de Arellano, & Holtslag, 2003). Topography highly affects the sea breeze circulation (Miao et al., 2003). In mountainous islands, interactions between sea/land breezes and slope winds may induce temporal and spatial variations of the wind field and the boundary layer structure (Melas et al., 2000). Sea breezes in summer –when maize

pollination occurs– are specially frequent in Palma and Alcúdia bays (Garau, 2013; Ramis & Romero, 1995), reaching in Palma bay around 80% of the days (Ramis & Romero, 1995). Lands surrounding these bays are also the main maize cropping areas of the island.

For all the above mentioned reasons we decided to carry out the first field trial on cross-fertilization between MON810 GM and non-GM maize in Mediterranean insular conditions. The aim of this study was to determine the minimum distance from which it ceases to be cross-pollination. The results of this field trial serve, along with other maize gene flow studies, as an initial scientific basis for creating future regional regulations on co-existence between GM and non-GM maize.

To that end, the experiment was designed to force the maximum cross-fertilization between GM and non-GM maize fields. Adjacent fields (affecting the slope and wind direction) of GM and non-GM fields as well as the synchrony of flowering, were precisely designed with this purpose.

2. Material and methods

2.1. Plant material

Varieties chosen for this experiment were PR34A27 (Pioneer Hi-Bred) for the GM field and P1114 (Pioneer Hi-Bred) for the conventional one. Both varieties were of cycle 500 to ensure developmental and flowering synchronicity.

2.2. Field trial

The main constraints for choosing the field of our experiment were 1) isolation of the fields from other maize plantations; and 2) favorable conditions for cross-fertilization (adjacent fields, slope, wind direction and synchrony of flowering). We finally chose a countryside in Alcúdia Bay (Mallorca), the main maize cropping area in the island. The countryside finally selected is far enough (15 km approximately) from other maize crop fields for avoiding cross-fertilization from alien GM pollen. It allowed to maximize recipient field size without compromising donor field dimensions and its closeness to the sea granted high probabilities of dominant wind favorable to cross-fertilization.

Pollen source was designed sizing 86 m long on average (92 m on NW side to 80 m on SE side) by 60 m wide, with a slight negative slope toward the recipient, that with the same wide than de donor sized 168 m long (the maximum allowed by countryside's geometry and dimensions once discounted the donor). Separation between the source and the donor of 4 m was elected to be the

minimum for boosting cross-fertilization without compromising seed contamination while sowing and safety while farming, monitoring and sampling. Theoretical dominant wind direction was also accurately evaluated and the fields orientated to facilitate pollen flow from the donor to the recipient. For the same reason, seeds for both fields were selected not only with the same growing cycle but also of the same variety (the non-GM variety for the recipient and its GM form for the donor) and sown on the same day. Soil preparation, fertilizing, irrigation and other farming practices were homogeneous in order not to distort the effect of trial main variables (wind direction, donor size and slope, field's separation and flowering synchrony) over cross-fertilization. See Figure 1 for more details.

Sowing rate was 88,000 seeds/hectare with a plantation framework of approximately 0.15 m between plants and 0.75 m between rows, and a seeding depth among 4 and 6 cm. Rows were disposed parallel to the field length in SW-NE direction, separated by sprinkler rows in 4 stripes of 15 m.

Both, the donor field and the recipient were subjected to the same farming practices (chisel plowing, plowing, fertilizing, sowing, rolling, spraying and harvesting) and irrigations at the same days.

2.3. Meteorological data

An automatic weather station at the field daily recorded wind strength (average wind speed, maximum wind speed and minimum wind speed) and wind direction during the whole growing cycle, from the plowing to the day before the harvest. In addition to wind strength and wind direction, temperatures (average, maximum and minimum) and precipitation were also daily recorded.

2.4. Sampling

We followed the stratified method for sampling (Messeguer et al., 2006). The field was divided into four perimeters: 0 m; 3 m; 10 m; 32 m. Then virtual dividing transects were stablished (see Figure 2) so the field was divided into 32 areas. The samples were collected in each intersection. A total of 36 sampling points were taken.

We took 3 cobs from each sampling point and they were collected in duplicate to minimize risks of data losing during transportation, storage and sample processing at the laboratory. A phew samples

were also recollected in the donor field and 1 kg of the original seeds preserved, for purity checking.

2.5. DNA isolation and GMO quantification

Kernels of the tree cobs were mixed thoroughly and milled together to a fine powder (Grindomix GM-200 model, Retch). Genomic DNA extraction was carried out from 5 g powder material using the Nucleospin Food kit (Macherey-Negal, Easton, PA) according to manufacturer's instructions. Two DNA extraction replicates were made for each sample.

MON810 detection and quantification was performed by TaqMan event-specific real-time PCR reaction (qPCR) according to EURL-JRC method (Mazzara, M., Grazioli, E., Savini, C., & Van den Eede, G., 2009).

2.6. Estimation of GM content in the recipient field

The percentages of MON810 obtained in the qPCR are used for the estimation of GM contamination in the recipient field. The MON810 content of each area of the field was calculated by the average of the four sampling points that limited the surface.

Weighted percentage of MON810 was calculated by the formula: (median MON810 in 4 sampling points) * (area of the zone) / (total area of the field).

3. Results and discussion

According to the trial, laboratory and data analysis conducted it seems that in favorable conditions for cross-fertilization between GM and non-GM maize (that includes full synchrony of flowering, adjacent fields, large donor size and favorable wind direction), a maize barrier of 10-30 m is enough as for maintain the remaining maize harvest below the threshold of 0.9% (Figure 3).

These results are in line with previous studies carried out on similar wind conditions (see *e.g.* (Ma, Subedi, & Reid, 2004; Messeguer et al., 2006). This fact, in conjunction with predictability of the wind factor in islands and coastal areas due to the hegemonic frequency of sea breeze phenomenon, draws a very promising scenario not only for the future regional regulation on co-existence, but also for other islands and coastal areas.

However, new studies are required to validate these results as well as to test the effect of flowering asynchrony over cross-fertilization on Mediterranean insular conditions.

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Figure 1 Schematic representation of field design, consisting of a donor field of MON810 GM maize separated by a 4 meters wide clearance of its correspondent non-GM maize recipient field in which samples were taken. Wind direction is favorable to cross-fertilization from the donor field to the recipient.

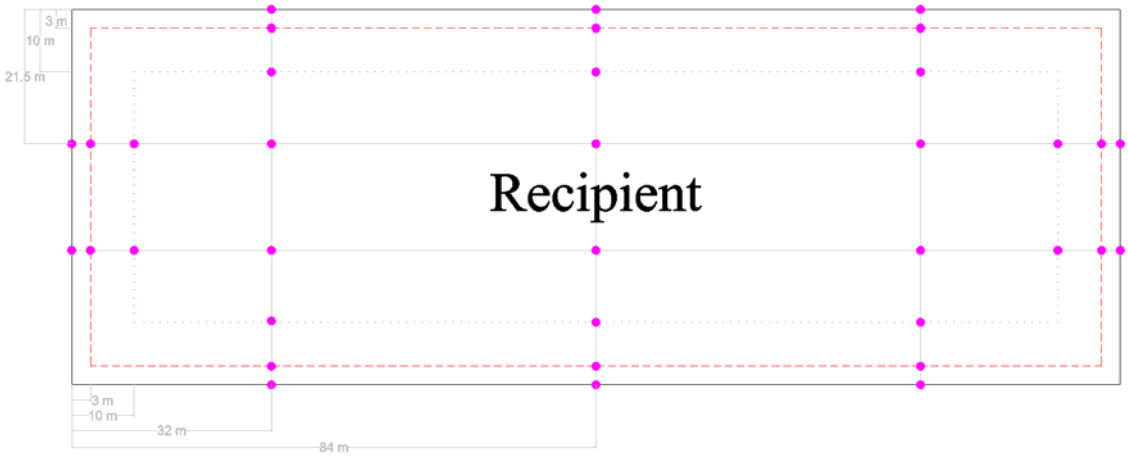

Figure 2 Schematic representation of sampling design. 36 samples were taken in each one of the locations represented on the figure. Each sample consisted of three maize cobs randomly collected in a radius of 1 m from the spot.

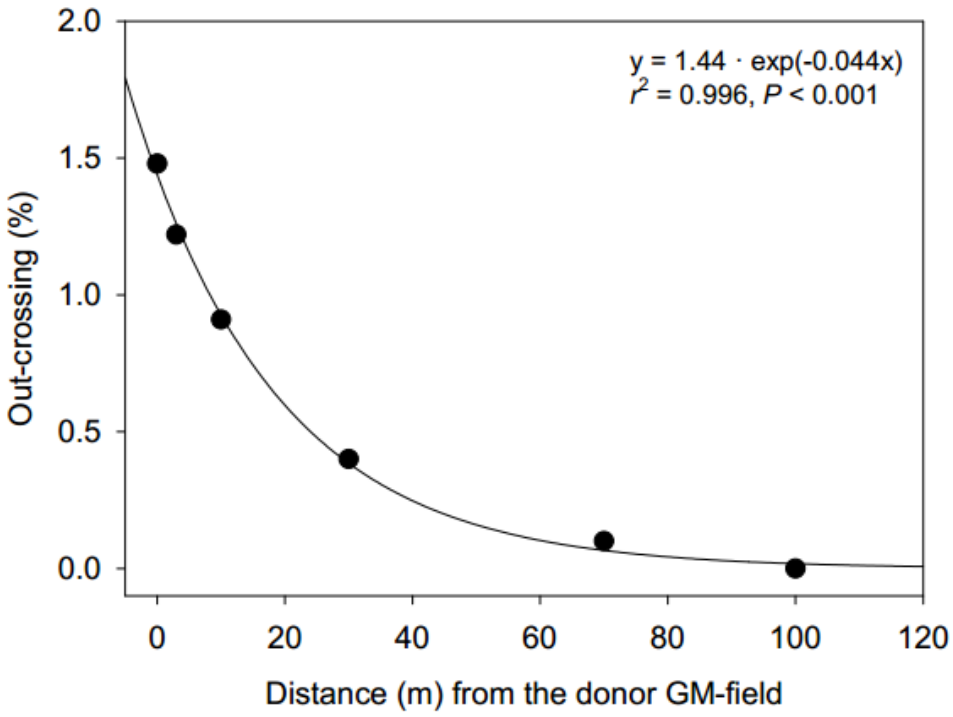

Figure 3 Gene flow frequencies as a function of distance between GM maize donor plants and non-GM maize recipient plants.