Annex 1

ANNOUNCEMENT

Pursuant to Article 21 (1)-(2) of Government Decree No. 132/2004. (IV.29.) Korm. on the procedural regime for authorisation of gene technology operations and on communication with the European Commission in the course of the procedures, **I am banning** the importation of inbred lines and hybrids originating from the MON 810 maize line into the territory of the Republic of Hungary **from 20 January 2005 until the day of the publication of the order revoking the ban.**

The ban extends to plants originating from cross-breeding with any traditionally improved maize varieties and lines**.**

The prohibition does not apply to the use of maize containing the MON 810 genetic construction in the food industry and in animal feed production, nor does it apply to transportation across Hungary without repackaging and any further treatment if it is guaranteed that such maize is not released into the environment.

Attention should be paid to the fact that under the ban on the use of maize specified above, no maize varieties carrying the MON 810 genetic construction may be sown.

Budapest, 20 January 2005

Dr. Imre Németh
Minister of Agriculture and
Rural Development

Annex 2

DATA FOR THE RISK ANALYSIS IN HUNGARY OF BT MAIZE POLLEN AND LARVAE OF PROTECTED BUTTERFLY SPECIES

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*The pollination of the DK 440 BTY (*MON 810 *genetic event) maize variety falls between the 74th and 88th day after sowing. The pollination of Hungarian maize varieties takes place during the course of July and August. In the case of the variety surveyed in our research – generating some 35 kg/hectares of pollen – the pollen coverage drops to 100 pollen grains / cm² at a distance of 5 metres from the edge of the maize field. Caterpillars that hatch during the pollination period and feed on herbs in and near the maize field are most heavily affected by the toxin content of the pollen. The leaf surface/leaf mass ratio of stinging nettle (*Urtica Dioica *L., Urticaceae) is 2.85 times greater than that of common milkweed (*Asclepias syriaca *L. Asclepiadaceae). In the case of equal pollen coverage, this means an approximately three times higher Bt-toxin dose. There are 187 protected butterfly species in Hungary, 16% of which may develop on herbaceous weeds along maize fields as well. In the nettle growths in the drainage ditches of maize fields, the hatching of the larvae of two common butterfly species of the Nymphalidae family – peacock,* Inachis io *(L.) and red admiral (*Vanessa atalanta*) (L.) – coincides with pollination, as a consequence of which they are exposed to the Bt-contents of the maize pollen.*

In 1995 the US Environmental Protection Agency (*EPA*) granted authorisation for the production of transgenic maize varieties producing *Bacillus Thuringiensis* toxin (Bt maize varieties) as a way of protection against the European corn-borer, *Ostrinia Nubilalis* Hübner Pyralidae). Losey et al. (1999) draw attention to the side-effects of toxin-containing pollen settling on the weed species growing in Bt-maize fields. In greenhouse conditions, they treated one milkweed species (Asclepias curassavicea L.; Asclepiadaceae) with large doses of Bt-maize pollen. Thereafter they reported reduced feeding intensity and hindered development as well as increased mortality rates of young but not newly hatched larvae of the monarch butterfly (*Danaus plexippus* (L.) Danaididae). Critics of the report (Beringer 1999, Pimentel and Raven 2000) rightly claimed that the dose triggering the reported effect had not been precisely identified, which applies particularly to areas at greater distances from the edges of maize fields where a reduced pollen density should be found. The first attempt to make up for this shortcoming was made by Jesse and Obrycki (2000). They found that lethal pollen doses may be found on milkweed (*Asclepias syriaca* L., Asclepiadaceae) growing within three metres of the edge of the maize field. *D. plexippus* is a species that is typical of the maize zone in the USA, wintering in Mexico. Some 56% of the *D. plexippus* caterpillars in the state of Iowa are estimated to grow on milkweed growing in maize fields. In the case of other species such as *Papilio polyxenes* Fabricius (Papilionidae), for instance, in the course of laboratory experiments using pollen of – questionably – low Bt-toxin content (2 ng Bt-toxin/g pollen) (Wraight et al. 2000), no increased mortality was found even in the case of an extreme level of pollen density (10,000 pollen grains/cm²). The reasons for the different levels of effectiveness should include: *a)* pollen distribution – different quantities of pollen will settle on and stick to the leaves of different plant species, higher densities of pollen will develop on leaves higher on the stalk of a plant, very few pollen grains will stick to the abaxial surface of a leaf; *b)* the characteristics of the insect species concerned – those feeding on the abaxial surface of the leaves after hatching will be protected to some extent; some species are known to be less sensitive to Bt-toxin; *c)* the response of the population – resistance to Bt-toxin may develop after a longer period of exposure; *d)* and sensitiveness varying by age – newly hatched caterpillars will have a lower detoxification capacity since the allelochemicals contained in the food of caterpillars induce a growing variety of the iso-enzymes involved in detoxification (Darvas 1988).

All of the parts of Bt maize varieties generate shortened *Cry1Ab* toxin, and this toxin is Lepidoptera-specific, i.e. – unless otherwise proven – the caterpillars of all butterfly species are sensitive to the toxin, regardless of the toxin dose and the insect's stage of development. The experiments carried out on *D. plexippus* serve as a model for the national risk analyses proposed by the UN on the basis of the Convention on Biological Diversity (Darvas 2001). As the protected and endangered species that are treated as especially important from the aspect of biodiversity vary substantially between biogeographic regions, it is not possible to form a single uniform judgment on such species.

The risk analysis of the butterfly larvae that are sensitive to Bt-maize pollen comprises four steps (Sears et al. 2001): *a)* identifying the danger; *b)* clarifying dose-effect relationships; *c)* assessing the probability of the occurrence of the effective dose; *d)* describing, on the basis of the above, the risk to which the sensitive butterfly populations are exposed. In the following study, the authors attempt to find out which are the protected butterfly species living in the territory of Hungary whose caterpillars may consume lethal quantities of Bt-toxin containing maize pollen.

Materials and methodology

Our maize field was located, in 2001 and 2002, in a virgin grassland broken up for this purpose in 2000 in an area called *Julianna-major* in a valley between Nagykovácsi and Budapest. No agricultural chemicals were applied in the territory. In view of the large number of samples to be taken, the number of plants was set at 75,000 per hectare. In order to monitor the growth of the maize, the weight of four times 10 plants of the variety of DK 440 BTY (Monsanto-DeKalb variety; referred to as YieldGard, MaisGard in the world market) was measured once a week during the period between sowing and harvesting. To establish the pollen output, the flowers of 10 maize plants were calculated, and then the weight of the pollen falling out of 10 pollen sacks was measured in 10 repetitions. To assess the vertical distribution of maize pollen, the number of pollen grains was counted on four plants, under a binocular microscope, on each of the leaf levels, in 20 fields of vision on different areas of each leaf. No plant releasing pollen that could be mistaken for the large yellow maize pollen globes was found in the area concerned. The measurements were repeated using ten measuring plates of the size of the normal object plate (75 x 25 mm) that had black, lacquered surfaces treated with silica oil and had been fixed on the surface of the maize leaves using paper clippers. The Bt-pollen source (a plot of 9 x 18 metres) was bordered by three lines with the tassels removed, in accordance with the research release permit for *Julianna-major*. To establish the horizontal distribution of maize pollen, the measurements were taken between 27 July and 6 August 2001. Measuring sheets were placed to establish the wind direction, on the last but one row with tassels, along with the fifth leaf level of the first and the third rows, in a radial direction, at eight spots near the pollen source. In the circle segment corresponding to the annual dominant wind direction (in the direction of the valley from Nagykovácsi towards Budapest), measuring sheets were set up at appropriate distances on 8 stakes of 120 cm height, at 1, 5, 10, 20, 50, 100 and 200 metres. No maize was produced in the valley, which is protected by surrounding valleys.

In order to establish the most typical weed species thriving in maize fields in Hungary, surveys were carried out in a dozen maize fields, selected at random, in the most important maize growing regions of Hungary (Fejér, Komárom, Veszprém, Bács-Kiskun, Szolnok and Hajdú-Bihar counties) at the time of maize pollination in the first week of August 2003. In three-metre strips along the edges of the fields and within the fields (at 1, 7 and 50 metres from the outside row), a list of weed species was put together and the coverage by each species (as a percentage of the total surface) was also estimated.

In assessing the leaf mass to leaf surface ratios of maize and some weed species in 2002, we aimed to establish the ratios of the Bt-toxin doses that may be calculated on the basis of the pollen numbers settling on the plants present in the vicinity of Bt-maize to one another in the field in Julianna-major. To establish such a ratio, we measured the mass and surface of 10-20 leaves per species (Model Li-3000; Li-Cor Inc, USA). All suitable data were analysed using the STATISZTIKA program package.

For the analysis of the modes of life of the various protected butterfly species in Hungary, we were looking for corresponding patterns using data collected from collection data held by the Hungarian Museum of Natural Sciences and from the assessment of reports in technical literature (Lampert, 1907, Ronkay 1997) in space (looking for butterfly species feeding on weed species occurring typically in maize fields) and in time (looking for caterpillars hatching during the pollination of maize).

Results

The phenology of DK 440 BTY maize

In 2001 pollination started on the 74th day after sowing, and even on the 88th day pollen was being carried around by wind on the top leaves. There was no rain on these days, which would otherwise almost immediately wash pollen into the funnel that the leaves form around the stalks. On the 81st day after sowing, the pollen sacks were about 60% open. Pollination is a protracted process in wet weather, but it is quickly completed when the weather is dry. The maize we studied shed its pollen – as an average of the two years of the survey – in the second half of July. After the generative phase the plants – simultaneously with the ripening of the produce – lose some of their weight (Figure 1). According to our calculations, pollination of the maize varieties produced in Hungary takes place between early July and mid-August.

Figure 1. The development of the weight of DK 440 BTY maize (g)

Distribution
Standard error
Average

The pollen output of DK 440 BTY maize

The DK 440 BTY variety produces 34-37 kg/ha dry pollen (4.07 ± 0.66 mg dry pollen/10 pollen sacks; 403 ± 108 flowers/tassel; approx. 1209 pollen sacks/tassel; approx. 492 mg dry pollen per plant; 70-75,000 plants per hectare), most of which remain on the hairy maize leaves. Accordingly, the Bt-maize covered by the survey is one of the maize varieties of relatively modest – 35 kg/ha – pollen production.

Vertical distribution of maize pollen

Owing to the substantial degree to which the leaves have closed, only a small quantity of pollen (29 ± 21 pollen/cm^2) can, in the case of silage-maize density, be found on the bottom four leaf levels, which are still green at the end of July (with 1-2 yellow and 3-4 brown, dead leaves). A substantial accumulation of pollen was observed (e.g., 641 ± 79 pollen/cm^2) on the leaf levels containing female flowers (5th-7th leaf from bottom). The maximum pollen density is originally found on the top two leaves (9th and 10th leaves), exceeding 1200 pollen/cm^2, but this is swept down to lower-level leaves by the wind (Figure 2). The difference between the results measured on the maize leaves (whose minute leaf hairs offer an ideal surface for pollen to settle on and stick to) and the sheets treated with silica oil resulted from the fact that no pollen is moved by the wind from the latter surface.

Figure 2. Distribution of maize pollen on maize leaves from bottom to top (pollen/cm^2)

On maize leaves
On sheets with silica oil

Horizontal distribution of maize pollen

The average density of maize pollen on the level of the lowest female flower dropped from 300 to 50 pollen grains/cm^2 in the row on the edge of the field that is the third one from the first external row whose tassels had not been removed, showing adequate effectiveness of such a border of rows of maize with tassels removed (see Figure 3). The quantity of pollen settled in the first 25 metres dropped substantially. The relatively large globules of maize pollen do not easily float in the air. The maximum pollen density was calculated from the results of the radially positioned measurements where the coverage was six times the average in the dominant wind direction. Even on the catching sheets placed at 200 metres, a 1 pollen/cm^2 density was found. According to our calculation, on the foliage of weeds along the field edge in the case of Bt-maize producing 35 kg pollen per hectare the density of pollen dropped to below the 100 pollen grains/cm^2 – the level that is critical for the young caterpillars according to the surveys carried out in the USA (Bt176 event maize pollen – milkweed – *Danaus* larvae) – at a distance of 5 metres from the first line of the field on an average. This can be prevented by a border of three rows of maize with tassels removed (Figure 4) in the dominant wind direction; however, critical border effect should be expected within a distance of 5-10 metres of the edge of the field.

Figure 3. Average change in the distribution of maize pollen on the 5th leaf level from the ground

Pollen/cm^2
Bt-maize (five outside rows)
Border with tassels removed (three rows)

The most typical weed species in Hungarian maize fields during maize pollination

Only a single species in the otherwise very rich weed communities along maize fields was found to nurture caterpillars of protected butterfly species. Stinging nettle (*Urtica dioica* L. (Urticaceae)) was found in four of the ten fields in the survey, only along the edges of the fields, never inside the field. The habitat of stinging nettle extends on adobe soils from the edge of the wet drainage ditches to the edge of the field, with patches extending into wet hayfields (Figure 5). According to our surveys, this species displays the third largest coverage on field edges, which is explained by the fact that the ditch network is a characteristic feature of the domestic arable field structure.

Mass/surface ratios of maize and some weed species

According to the mass/surface ratios of the weed species covered by the survey, three groups of weeds were established: In the case of *A. syriaca, Senecio jacobaea* L. (Asteraceae), *Cirsium arvense* (L.) Scop. (Asteraceae), *Taraxacum officinale* Webber ex. Wiggers (Asteraceae), the same leaf surface was accompanied by a larger mass than in the case of maize. On the other hand, the second group includes – in addition to maize – *Chondrilla juncea* L. (Asteraceae), *Picris hieracioides* L. (Asteraceae), *Daucus carota* subsp. *sativus* (Hoffm.) (Apiaceae), *Rubus* sp. (Rosaceae), *Setaria viridis* (L.) P.B. (Poaceae), and *Consolida regalis* S. F. Gray (Ranunculaceae). In contrast to both groups, the smallest leaf mass is linked to the leaf surface of stinging nettle (Figure 6). This means that in the case of equal maize pollen density the caterpillars feeding on stinging nettle consume about three (2.85) times as much Bt toxin as do others feeding on milkweed.

Figure 4. Horizontal distribution of maize pollen in the case of the three rows of maize stripped of tassels along the edge of the field at a height of 120 cm (values measured with and calculated without the three row border)

Border of three rows without tassels
Bt border

Hatching of larvae of caterpillar species protected in Hungary and the pollen shedding of maize

There are 187 protected caterpillar species in Hungary. Populations of thirty of them may be found along the edges of maize fields (Figure 7). Eight of them survive the winter in the caterpillar stage, nine of the remaining 22 species feed during maize pollination (Figure 8): *Chazara briseis* L. (Nymphalidae), *Colias chrysotheme* Esper (Pieridae), *Inachis io* L. (Nymphalidae), *Papilio machaon* L. (Papilionidae, *Periphanes delphinii* L. (Noctuidae), *Schinia cardui* Hübner (Noctuidae), *Schinia cognata* Freyer (Noctuidae), *Vanessa atalanta* L. (Nymphalidae), *Zygaena laeta* Hübner (Zygaenidae). Smooth foliage with a small surface area is not favourable for the deposition and adhesion of maize pollen, which is why we do not consider it likely that *P. machaon, P. delphinii, Z. laeta* and *C. chrysotheme* feeding on Apiaceae species such as *D. carota, C. regalis, Eryngium campestre* or on *Fabaceae* species (in this order) could consume a hazardous quantity of maize pollen. Other species not affected include *S. cardui* and *S. cognata*, which lived in the flowers of the host species *P. hieracioides, C. juncea* instead of their leaves. *V. atalanta* and *I. Io* caterpillars, however, feed on the leaves of *U. dioica*, which is characterised by a large and hairy surface that is highly suitable for the adhesion of pollen.

Figure 5. Average coverage of the major weed species in maize at pollination (%)

At 50 metres
At 7 metres
At 1 metre
At the edge of the field

Figure 6. Mass/surface ratios of weed species in the *Julianna major* maize field

Distribution
Standard error
Average

Figure 7. Locations of development of the 187 protected butterfly species in Hungary

Caterpillars may feed on herbaceous weeds along field edges
16% 30 species

Butterflies of unknown habitat and host plant
3%

Caterpillars feeding on woody plant species
34%

Caterpillars feeding on herbaceous plants but not along commercial crop on arable land
47%

Discussion

Most maize varieties produce 20-120 kg/ha pollen. The pollen of Bt-maize varieties contains Cry1Ab toxin (40-1600-12360 ng/g dry pollen) depending on the genetic event and the preparation of the sample (Jesse and Obrycki 2000, *U.S. EPA – OPP* 2001, Darvas et al. 2003). We analysed the criteria of the interaction between this substantial quantity of toxin (0.8-192-1483 mg Cry1Ab toxin/ha) and butterfly caterpillars. With the permitted Dipel (*Bacillus thuringiensis* preparation) treatment, Bt toxin quantities smaller by several orders of magnitude than the quantities delivered by the Bt176 genetic event varieties were applied to the area. Bt-pollen appearing in the environment may result in a variety of conflicts.
As a genetic material carrying a new combination of traits, it endangers the genetic purity of non-genetically modified species in the environment. This may be particularly critical in the case of organic farming, producing local varieties. Traditional maize pollinated with Bt pollen will result in crops with Bt-toxin one third as often as transgenic varieties even in the first year (Darvas et al. 2002). In the case of maize, there is a great chance of producing variety hybrids within a few hundred metres. The manuals set the isolation distance at 500 metres, but maize pollen is known to be able to float to distances of several kilometres under very favourable environmental conditions (cloudy weather, high humidity, strong winds) during its 24 hour lifetime. As a matter of course, the chance of variety hybrid production drops exponentially with distance, and this is further reduced by competition for fertilisation with the pollen of other plants. The viability of the pollen is determined by UV radiation and dehydration. According to our assessments, the border rows of Bt maize with tassels removed provide a substantial protection against pollen spreading.

Figure 8. Length of larval stage of protected butterflies developing on weed species that also occur on field edges (the bold frame shows the period during which maize pollen may be shed)

Months

The pollen of the varieties originating from the Bt176 event (e.g., Maximizer, owned by Novartis) contains 40-300 times as much Bt-toxin as does the DK 440 BTY variety originating from the MON 810 event. The pollen output of some varieties may be up to four times as much as the one we surveyed. This means that since the critical 5-10 metre border established for the DK 440 BTY variety may be many times larger, an extended risk analysis, including caterpillars of butterflies living in forest patches between the fields, may be required of variety owners in the case of the varieties of the Bt176 genetic event (Csóti et al. 2003). Rain plays an important role in the elimination of the effects of Bt-pollen. Even a light rain will wash off 50-90% of the pollen on the leaves (Pleasants et al. 2001).

Our experiment did not extend to the fact that loading the environment with sub-lethal doses of Bt-toxin facilitates the development of Bt-toxin resistance. This is helped by the fact that Bt-maize pollen contains only one type of Cry toxin that is not present in the bacterium preparations, which contain 4-8 types of toxin. The likelihood of developing resistance to four toxins is 300 times less than the likelihood of developing resistance to one toxin (Georghiou and Wirth 1997). This may entail the premature depletion of a very highly rated plant protecting product that plays a key role in crop protection in organic farming (Darvas et al. 1979, Darvas and Polgár 1998. Darvas 1999).

Our survey will be continued by an examination of the butterfly communities living on stinging nettle (Darvas et al. 2003). It should be noted in advance that the stage L1 butterfly larvae hatching from the eggs are 12-23 times more susceptible than those of older stages (Hellmich et al. 2001).

Acknowledgements

Our experiments were subsidised by grants from the Ministry of Education (BIO-00042/2000) and of the Ministry of Environment Protection and Water Management (K-36-01-00017/2002). We owe a particular debt of gratitude to *Ede Petró* for the idea of pollen measurement using silica oil that is used in assessing spray distributions.

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