CO-EXTRA

GM and non-GM supply chains: their CO-EXistence and TRAceability

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PP Restricted to other programme participants (including the Commission Services)	**PP**
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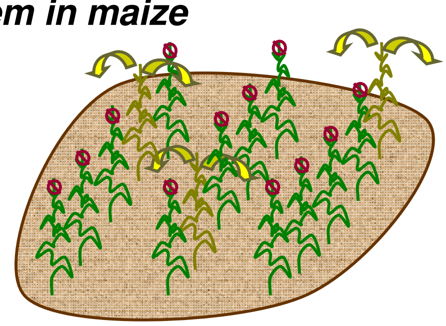
1 Plus-Hybrids in Maize, a new approach of gene flow mitigation increasing the yield

In the current context where the cultivation of genetically modified crops also in Europe seems to be imminent, Plus-Hybrids can be suggested as a method of containment of the transgene. The spread of transgenic pollen and the 'contamination' of conventional or organic fields in the neighborhood appear to be the main preoccupation. One of the biological approaches for gene flow mitigation could be the containment of the transgene in male sterile plants (no release of transgenic pollen) which are cultivated in mixtures with conventional male fertile bred plants, assuming the fertilization (Feil et al., 2003).

1.1 Hypothesis: The Plus-Hybrid system in maize

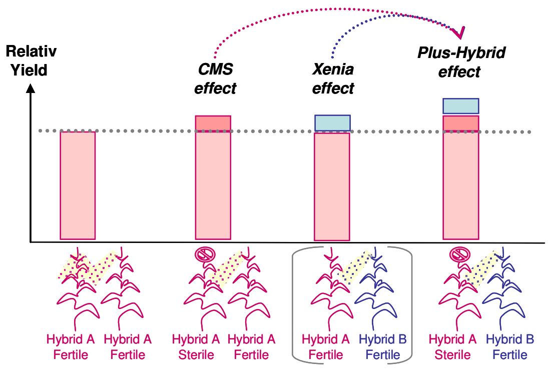
No release of transgenic pollen when a mixture of a **transgenic Cytoplasmic Male Sterile** maize hybrid is grown in combination with a lower proportion of a **non transgenic unrelated fertile** hybrid as pollen donor for the entire field.



1.2 Prerequisites: a high and stable yield for Plus-Hybrids

The Plus-Hybrid system is a promising approach for increasing maize grain yield, not only by heterosis, but also by Plus-Hybrid effect. This effect is a combination of:

- CMS effect: difference in grain yield between an isogenic-pollination {A fertile x A male sterile} and its counterpart {A fertile x A fertile}. This effect has a direct influence on kernel number.
- Xenia effect: is the direct impact from pollen and therefore paternal genetics (A fertile x B fertile}) on grain aspect and on the yield parameter kernel weight.



Yield improvement through Plus-Hybrid effect

The insurance of a high and stable yield is a prerequisite for farmers' acceptance of this type of seed mixtures (with or without GM crops). Breeders must be able to find efficiently "Plus-Hybrids" allowing a real advantage for the farmers in terms of yield. To reach this goal, it is highly essential to determine the optimal combination of already existing hybrids for their suitability within the Plus-Hybrid system.

2 Agronomic and international context

2.1 Current and future global importance of maize

Maize (Zea mays L.) is grown for both human and animal consumption. In the industrialized countries, maize is used primarily as animal feed and secondarily for production of food and industrial products such as starch, oil, sweeteners, and alcohol. In developing countries, maize often is grown as a food-crop for home consumption as well as for the market; increasingly it also is used for animal feed.

The maize production ranks at the very top among field crops. In 2001, global production totals for the three major cereals were: 604 million metric tons for maize, 585 for rice, and 576 for wheat (FAO, FAOSTAT Data, 2002). In the period from 1997 to 1999, the industrialized nations (Western Europe, North America and other high-income countries) produced 48 % of the world's maize crop, utilizing 25 % of the annual 140 million hectares planted to maize globally (Aquino et al., 2001). The disparity between production and planting percentages is because maize yields on average are much higher in industrialized than in developing nations. Yields in 1997 to 1999 averaged 8.3 tons per hectare in high-income countries compared to 2.9 tons per hectare in developing countries (Aquino et al., 2001). Almost the entire maize production in industrialized countries is grown from hybrid seed, whereas in most developing countries open pollinated varieties predominate (Byerlee and López-Pereira, 1994).

For several decades, a considerable shift towards urbanization happened in developing countries and this trend is expected to continue (McCalla, 1994). Shifting people from a rural to urban setting typically increases the demand for meat and eggs and, hence, also the demand for maize as one of the most favored feed grains for livestock and poultry. The world demand in 2020 is predicted to rise to about 138 percent of the 1995 demand and will then surpass the demand for rice and wheat in developing countries (Aquino et al., 2001).

Technically, there are two strategies to satisfy this rising demand: (i) an extension of the maize acreage or (ii) an increase in yield per unit field area. The extension of agriculture to land that has not been under cultivation before is problematic; soil degradation, erosion, and disappearance of forests with shifting cultivation are only some of the keywords. Nevertheless, the area planted to maize is expected to increase during the next 25 years, as it was the case in the past quarter century. The extension of maize acreage will probably be at the expense of other crops, what may have a negative impact on crop rotation. Even with a larger area that is planted with maize, the projected increase in area will not be large enough to satisfy the projected demand for maize. Therefore rising demand can only be satisfied when grain yields can be increased. By emphasizing higher grain yields, two scales have to be considered: an increased and more specialized production by commercial growers, and an increased and more reliable production by smallholders and subsistence farmers.

2.2 Heterosis

"There is abundant evidence which goes to prove that crossing varieties or strains of corn frequently increases the yield, especially in the first hybrid generation. Corn differences from most other plants in that the effect of crossing can be seen the current year. This is due to xenia, or the hybridization of the endosperm as well as of the embryo."(Wolfe, 1915)

Maize yields began to rise rapidly and continually in the industrialized countries when culture and breeding of maize underwent major change, starting in the 1930s in the USA, and in the 1950s in Europe. It was the period when it became known to plant breeders that the progeny from a specific cross can outperform either of the two parents used in the cross. The phenotypic appearance of the progeny is more vigorous and the preferred organs, which constitute yield, are larger than those of either of the two parents. This phenomenon is called "heterosis" and was first described 1908 by Shull (1908). The detection of the usefulness of heterosis for plant breeding has lead to the development of inbred lines that reveal a heterotic yield advantage when they are crossed.
Maize was the first plant species in which heterosis was exploited by introducing commercial hybrids. At the beginning, hybrid maize seed was produced by manual detasseling of the female parent to prevent self-pollination. The major disadvantage of this method is its labor intensity and consequently the high production costs.

2.3 Cytoplasmic male sterility (cms)

"... the experiments indicate that there is more pollen produced by the corn plant than is necessary to produce a maximum crop and that this over production is an exhaustive process." (Watson, 1893)

In 1931, (Rhoades, 1933) was the first to describe cytoplasmic male sterility (cms) in Peruvian maize. In 1944, Rogers detected cms in a Mexican OPV (Golden June) and this was called later the cms-T (Texas) cytoplasm(Rogers and Edwardson, 1952). Jenkins, in the 1950s, detected a new cms type in Teopod maize and called it cms-S (USDA) cytoplasm, which was then developed for commercial use by (Jones et al., 1957). Finally Beckett (1971)found in 1971 a third cms-type in Brazilian maize, which was then named the cms-C (Charrua) cytoplasm. Still nowadays, these three types represent the main classification groups for male-sterile cytoplasm, although many new sources were found and described.

Cms represents a unique example of a maternally inherited trait that is of major economic importance. It was realized that manual detasseling of maize plants would no longer be required. This new male-sterile system was utilized in the commercial production of hybrid seeds after methods were developed for the restoration of fertility with nuclear genes. In the late 1960s, at least 84 sources of cms have been described (Duvick, 1965), but the Texas source was the most extensively utilized because of its ease of fertility restoration and the complete and stable absence of pollen. It is estimated that 85 % of the US hybrid corn was produced with cms-T in 1970 (Ullstrup, 1972). However, in the season 1970, a new race of the pathogen *Helminthosporium maydis* occurred and caused an epidemic of the disease called "southern corn leaf blight". This new race developed its virulence only on maize carrying the T-cytoplasm. Most regions in the US Corn Belt were affected. Whole areas were wiped out and there was an overall 50 % loss in yield, estimated to an economic damage of 1 to 1.5 billion dollars. Immediately most seed suppliers increased their production of lines carrying normal cytoplasm, however seed prices increased rapidly. This is often considered to be the greatest biomass loss of any biological catastrophe. Within 4 years, hybrid maize companies had switched back completely to normal cytoplasm.

It soon became obvious, that any cytoplasm restored by cms-T restorer genes was also susceptible to race T of *Helminthosporium maydis*, no exceptions were found (Duvick and Noble, 1978). In the following years, both S- and C-cytoplasms became prospective for the use in hybrid seed production. Cms-S was imperfectly sterile and genotypes carrying this source were affected to unpredictable restoration to fertility by varying environmental conditions. The C-cytoplasm was the only one to have a certain potential to be used in hybrid seed production, however, the concern that in a few years all maize might be on a very narrow cytoplasmic gene base again prevented its use for many years. It is difficult to obtain actual data regarding the use of cms in hybrid seed production. In 1987, the American Seed Trade Association (ASTA) conducted a survey of the type of cytoplasm used in the production of maize seeds in the USA: 66 % of the seed maize in the USA was produced using normal fertile (N) cytoplasm, while 22 % and 12 % of seed maize was produced using cms-C and cms-S cytoplasm, respectively (Wych, 1988). However, it can be assumed that the rate of commercial hybrids produced with cms-C or cms-S inbred lines is constantly increasing (B. Fabre, Contrôleur national maïs du Service Officiel de Contrôle et de Certification, France, personal communication, 2001; M. Maitz, KWS Saat AG, Germany, personal communication, 2002).

Before 1970, numerous studies were published concerning the effect of male sterility on grain yield. Nowadays, the opposite is true for the actual situation in maize production; except the former thesis (Kaeser, 2002; Weingartner, 2002) no information is available for contemporary germplasm about the effect of male sterility on grain yield and yield components. The only studies which investigated effect of cms on maize grain development were in the context of the special use of cms in the high-oil system (Lambert et al., 1998; Thomison et al., 2002).

2.4 Xenia as the effect of non-isogenic pollination

"Agronomists have been unreasonably slow in accepting that the pollen which fertilizes the silk may influence the size and the weight of the grain produced as well as its color."
(Carrier, 1919)

Pollen from different sources affects characteristics of seeds in the period immediately following fertilization. This has been known for more than a century; it was first described by Focke (1881) and the phenomenon was named "xenia". However, in most cross-pollinating plant species, particularly in agronomic relevant crops, the genetic constitution of the female, fruit-bearing organs is considered to be the main factor determining final grain yield and yield components. Although some traits of the mature grains (e.g. aleurone color) are known to be influenced also by the genetic constitution of the male, pollen-bearing organs, there is only poor knowledge about the importance of the pollen genotype on final grain yield. In maize, the pollen genotype does indeed not play an important role in contemporary cropping systems, since modern maize cultivars have to fulfill certain rules to be accepted on variety lists. According to the UPOV[1] convention, protection may be granted if (i) a variety is clearly distinguishable from other varieties, (ii) it is sufficiently uniform in its relevant characteristics and (iii) it is stable, that means its relevant characteristics remain unchanged after repeated propagation. A consequence of this regulation, as it has evolved over nearly forty years of application, is that in most crops there are no different genotypes planted in the same field, acting as fruit baring female and pollen shedding male parent.

In the maize kernel, the genome of the embryo is derived half and that of the endosperm is derived by one third from the pollen. This means that all the traits related to the embryo and endosperm could potentially be modified by the pollen parent. At maturity, these two kernel compartments together count for 94 % of the kernel weight (Kiesselbach, 1960).

Recently, more attention has been paid to the genotype of the pollen in so called "value-added" production systems (e.g. TopCross[2]). Value-added traits are those qualities of maize that are additional to the traditional harvest yield, harvest moisture, and so on. They include percent starch, percent oil, percent protein, and fatty acid composition, among other traits. Value-added traits have gained interest in the last few years as their potential to improve the nutritional quality and manufacturing and processing characteristics of maize has been proven.

2.5 Combination of cms and xenia

Up to now, no study has been published in which the combined effects of male sterility and xenia were focused on maize grain yield. For our work, it was hypothesized that the combination of this two biological

[1] International Union for the Protection of new Varieties of Plants, Geneva.

[2] TopCross is a registered trademark of DuPont Specialty Grains, Des Moines, Iowa.

factors represents an option to increase maize grain yields, at least in a range of additive positive impacts.

In order to keep a possible practical application in mind, three prerequisites were set up already at the very beginning of the project:

- to avoid outdated information, only currently commercialized hybrids should be investigated,
- to avoid restriction to only one of the three cytoplasm types, it was aimed to have experiments done with all three cytoplasms types (T, C and S).

With this thesis it will be tried to answer the question whether the combination of male sterility and xenia has a potential to increase grain yields for a wide range of European environments and germplasm.

2.6 Effective control of GM pollen through cms-plants

It is proposed that the dispersal of transgenes be controlled by growing 80:20% or similar mixtures of unrestored cms GM maize and male-fertile non-GM maize, whereby the latter component acts as pollen donor for the entire stand (Feil and Schmid, 2002; Feil, 2002; Stamp and Feil, 2001). Since the fertility of the GM hybrid plants is not restored, they release no pollen or no functional pollen and, thus, the transgenes cannot escape from the GM maize field. One major advantage of our system is evident: it can be put into practice immediately, because it is unnecessary to genetically engineer maize for male sterility and inexpensive seed of unrestored cms hybrids can be produced in large quantities using existing standard technology.

From the farmers' point of view, the grain yield is the crucial factor. Weingartner (2002a) reviewed the literature and concluded that the effects of cms on grain yield are variable, suggesting that they are modified by natural and agronomic stresses, heterotic groups, and genotypes. In their experiments, the cms hybrids outyielded their male-fertile counterparts by more than 5%, an outcome which is in line with numerous earlier reports (Stamp et al., 2000)

The Plus-Hybrid system established by Weingartner *et al (2002b)* contains 15 and 20% male-fertile plants, respectively. Since maize produces a large surplus of pollen, a portion of only about 10% male-fertile non-GM maize may suffice to ensure maximum grain set in the whole stand (Poehlman and Sleper, 1995). However, a higher portion of pollen-producing plants and the blending of two or more pollinator hybrids may be advisable to reduce the risk of crop failure.

In this context, it should be noted that mixtures of cms hybrids and male-fertile pollinator plants are already being grown on a large area (> 400,000 ha in 1999) in the USA for the production of high-oil maize.

A major restriction of the system we propose is that it is not fully applicable to herbicide-resistant GM maize, because the herbicide would kill the non-GM pollinator. In this case, the non-GM pollinator can be replaced by a GM pollen donor, which would reduce the flow of GM pollen by about 80% (Feil, 2002). Another important area for the application of gene technology is the development of insect-resistant crops. *Bt* maize was primarily engineered to control the European corn borer (*Ostrinia nubilalis* Hübner). There is concern that the widespread use of *Bt* maize will lead to *Bt* toxin resistance in the insect. Present resistance management strategies rely on a separate refuge composed of non-*Bt* plants to conserve the alleles that encode the susceptibility to the *Bt* toxin. However, maize growers may be unwilling to sacrifice a significant cropping area to delay the onset of resistance. Blends consisting of 80% male-sterile *Bt* maize and 20% male-fertile insect-susceptible maize may help prevent the development of *Bt* toxin-resistant insect populations (Feil et al., 2003) and, thus, reduce the need for separate refuges. However, when both plant types are mixed in a random spatial arrangement, larvae may move between *Bt* and non-*Bt* plants, which may result in a decline in the number of alleles causing the susceptibility to *Bt* toxin (Shelton et al., 2002). Additional research is required to elucidate the consequences of planting mixtures of insect-resistant and insect-susceptible maize for resistance management.

3 Field experiments and Results

3.1 Aims of the research

In order to assess the reliability of the Plus Hybrid system and especially the yield potential lying under the Plus-Hybrid effect, several field trials are carried out in the frame of the WP1, *Biological Approaches For Gene Flow Mitigation.* This study aims to:

1. apply the Plus-Hybrid system to a large number of modern European Hybrids with a broad genetic background:
    - Grouping of modern hybrids according to flowering synchronizations
    - Evaluation of several hybrids abilities within the Plus-Hybrid system (pollinating ability of fertile hybrids and combining ability of cms-hybrids)
    - Identification of high-yielding Plus-Hybrids, putative good pollinators and high-responsive cms-hybrids
    - Phylogenetic classification of the tested hybrids using microsatellites (SSR-markers) & correlation with field results
2. deliver of a high-yielding Plus-Hybrid prototype:
    - Determination of general trends leading to an optimal Plus-Hybrid effect
    - Development of predicting tools for breeders

The experiments have been organized in two phases. During the field season 2005, a large number of modern hybrids have been evaluated for their abilities within the Plus-Hybrid system by adopting a test-cms-hybrid and a test-pollinator. Thus, we could select high responsive hybrids out of them to combine them in orthogonal crosses and in more locations for deeper investigations. This second step is still going on thanks our collaborative partners participating to this European ring experiment.

3.2 Plant material

A large set of modern Hybrids, available in their (cytoplasmic) male sterile and male fertile version, have been collected from several European breeders for the first field trials. The three types of male sterile cytoplasms (T, C and S) and a broad amount of genetic backgrounds are represented.

In the main field trial 2005, 16 hybrids have been evaluated in combination with Silpro-MS and Banguy fertile. These two Hybrids have been cultivated in previous trials done by ETH between 1999 and 2001 and showed respectively good general combining and pollinating abilities.

Breeder	Hybrid	Cytoplasm form
DSP	Silpro	cms-T & fertile
	DSP 25946A31	cms-T & fertile
	DSP 27223B11	cms-S & fertile
	DSP 25673	cms-S & fertile
	DSP 73749A31	cms-T & fertile
	GL2205	cms-C & fertile
Limagrain	Banguy	fertile
	LG22.75	cms-C & fertile
	Anjou265	fertile
	Anjou249	fertile
Syngenta	Ardiles	cms-S & fertile
	Delitop	cms-S & fertile
	Horatop	fertile
KWS	Prinz	cms-S & fertile
	Romario	cms-S & fertile
	Gavott	fertile
Dekalb	DKc3420	fertile
Euralis	EG'Z5824	cms-C & fertile

For the ring experiment 2006, 5 hybrids (in both forms: CMS and fertile) have been selected for deeper investigations. We also included 3 additional pollinators. Some hybrids belonging to a later maturity range have also been integrated in a second small trial, led by Arvalis, in Bulgaria and France.

3.3 Season 2005, Evaluation of a large number of hybrids with common testers

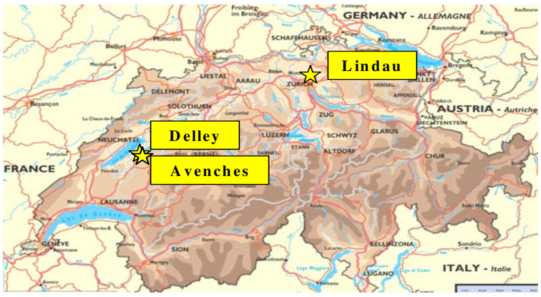
3.3.1 Aims

- Evaluation of mother abilities of cms-hybrids using Banguy as test pollinator
- Evaluation of pollinator abilities of fertile hybrids using Silpro ms as test mother

3.3.2 Locations

These field trials have been carried out by DSP and ETH in 2005 in 3 Swiss locations:

- Delley, Canton Freiburg, DSP
- Avenches, Canton Freiburg, DSP
- Lindau-Eschikon, Canton Zurich, ETH



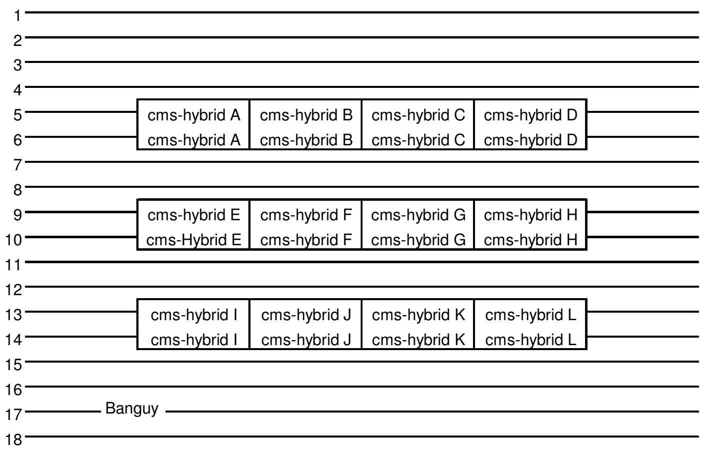
3.3.3 Experimental layout

3.3.3.1 Combining ability of 12 CMS-Hybrids using Banguy fertile as pollinator

Tested hybrids:

- Silpro ms
- Delitop ms
- Ardiles ms
- DSP 25946A31 ms
- DSP 27223B11 ms
- DSP 25673 ms
- LG22.75 ms
- GL2205 ms
- Prinz ms
- EG'Z5824 ms
- Romario ms
- DSP 73749A31 ms

Experimental layout:



CMS plants plots randomly distributed within a pollinator block.

CMS plots: 2 rows
4.5 m. length

Plant density: 10 plants/m^2

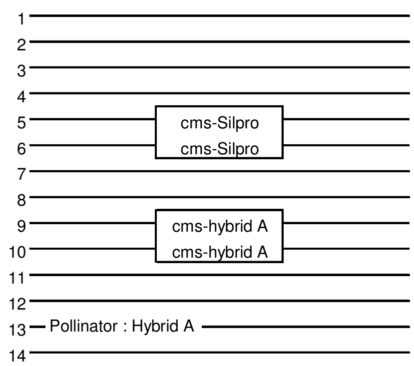
Delley: 3 replications
Avenches: 3 replications
Lindau-Eschikon: 4 replications

3.3.3.2 Pollinating ability of 16 fertile hybrids using Silpro-MS as test-plant

Tested hybrids:

- Silpro
- Delitop
- Ardiles
- DSP 25946A31
- DSP 27223B11
- DSP 25673
- LG22.75
- GL2205
- Prinz
- EG'Z5824
- Romario
- DSP 73749A31
- Horatop
- Anjou265
- DKc3420
- Gavott

Experimental layout:



Sub-plots of Silpro-MS and isogen-CMS within a pollinator plot in order to obtain the cross {CMS-silpro x Hybrid A} but also the reference value {CMS-Hybrid A x Hybrid A} for each hybrid.

CMS plots: 2 rows

4.5 m. length

Plant density: 10 plants/m^2

Delley: 2 replications

Avenches: 2 replications

Lindau-Eschikon: 4 replications

3.3.4 Results

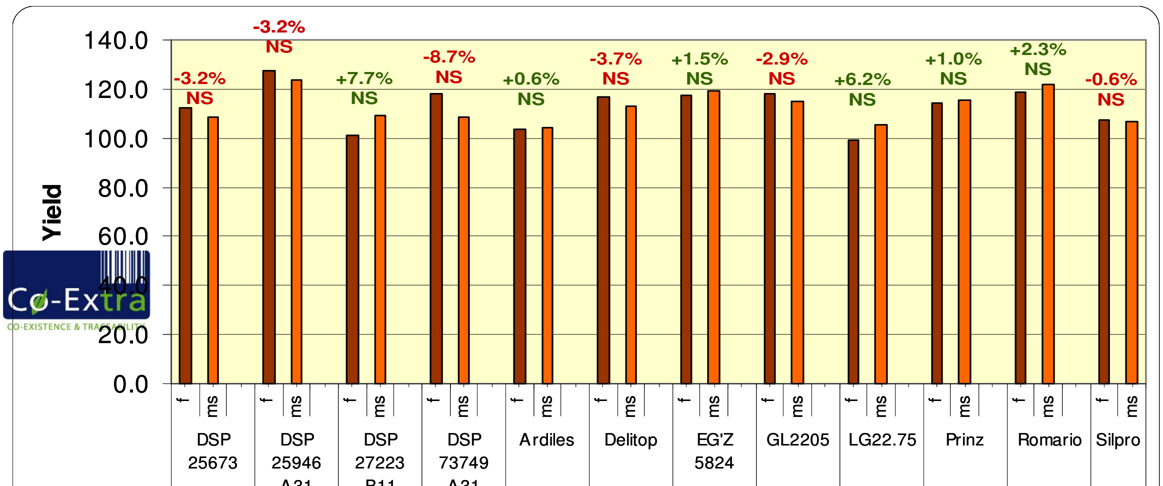
3.3.4.1 Flowering synchrony

As expected, male and female flowering have been relatively synchronous for all tested hybrids. In some cases silks appeared on female plants before the corresponding pollinators fully flowered. Nevertheless pollen was present during the main silking period. The desired combinations have been obtained even if it wasn't always under the best conditions.

3.3.4.2 High variations in yield

We observed high variations in yield between locations, probably due to geologic and climatic differences but also between replications within a location. The consistent size of the field trials (1 to 2 ha) seems to be at the origin of this high variability complicating the statistical assessment of the results.

3.3.4.3 CMS-Effect



1. No statistical significant CMS effect
2. Only 2 CMS-hybrids out of the 12 tested show a consistent positive CMS-effect on yield: DSP27223B11-MS (+7.7%) and LG22.75-MS (+6.2%). This increase in yield was obtained by increasing the number of grain produced (respectively +13.0% and +13.5%) and not the 1000-kernel weight (respectively +1.2% and -3.2%)
3. Most of the CMS-hybrids show a CMS-effect on yield between -3% and +3%.
4. The CMS effect on yield was negative only for 1 CMS-hybrid: DSP73749A31-MS (-8.7%)

3.3.4.4 Combining ability of CMS-hybrids

1. The results obtained in Lindau-Eschikon and Avenches are quite similar. In Delley, a hot and dry period before and directly after flowering led to lower yields.

		Lindau			Avenches		Delley	
Pollinator	Female	Yield q/ha	variation (%) / Hf. x Hf.	variation (%) / Hcms x Hf.	Yield q/ha	var.(%) / Hf. x Hf.	Yield q/ha	var.(%) / Hf. x Hf.
DSP25673 f.	DSP25673 f.	112.3			116.1		107.4	
DSP25673 f.	DSP25673 ms	108.9	-3.2 NS					
Banguy f.	DSP25673 ms	127.7	12.0 ‡	14.7 ‡	127.6	9.0 ‡	108.4	0.9 NS
DSP25946A31f.	DSP25946A31f.	127.3			115.5		104.5	
DSP25946A31 f.	DSP25946A31ms	123.4	-3.2 NS					
Banguy f.	DSP25946A31ms	128.4	0.8 NS	3.9 NS	126.0	8.4 NS	114.6	9.2 NS
DSP27223B11 f.	DSP27223B11 f.	100.8			106.4		107.7	
DSP27223B11 f.	DSP27223B11ms	109.2	7.7 NS					
Banguy f.	DSP27223B11ms	124.8	19.3 *	12.54 ‡	110.4	3.6 NS	109.8	1.8 NS
DSP73749A31 f.	DSP73749A31 f.	117.8			112.7		113.0	
DSP73749A31 f.	DSP73749A31 ms	108.4	-8.7 NS					
Banguy f.	DSP73749A31 ms	133.8	12.0 *	19.0 *	124.6	9.5 ‡	110.7	-2.1 NS
Ardiles f.	Ardiles f.	103.4			107.4		106.5	
Ardiles f.	Ardiles ms	104.0	0.6 NS					
Banguy f.	Ardiles ms	115.0	10.1 NS	9.5 NS	111.2	3.4 NS	97.3	-9.5 NS
Delitop f.	Delitop f.	116.9			109.3		107.0	
Delitop f.	Delitop ms	112.8	-3.7 NS					
Banguy f.	Delitop ms	129.7	9.9 *	13.1 *	117.8	7.2 NS	103.6	-4.0 ‡
EG'Z5824 f.	EG'Z5824 f.	117.4			116.9		111.7	
EG'Z5824 f.	EG'Z5824 ms	119.1	1.5 NS					
Banguy f.	EG'Z5824 ms	137.4	14.6 *	13.3 ‡	146.2	20.0 **	114.3	5.8 NS
GL2205 f.	GL2205 f.	118.1			123.2		110.8	
GL2205 f.	GL2205 ms	114.8	-2.9 NS					
Banguy f.	GL2205 ms	112.1	-5.3 NS	-2.4 NS	124.9	1.3 NS	113.3	2.2 NS
LG22.75 f.	LG22.75 f.	99.0			100.1		105.8	
LG22.75 f.	LG22.75 ms	105.5	6.2 NS					
Banguy f.	LG22.75 ms	110.9	10.7 ‡	4.8 NS	110.3	9.3 ‡	110.6	2.6 *
Prinz f.	Prinz f.	114.4			107.3		103.5	
Prinz f.	Prinz ms	115.6	1.0 NS					
Banguy f.	Prinz ms	118.3	3.3 NS	2.3 NS	107.1	-0.1 NS	92.7	-16.3 ‡
Romario f.	Romario f.	118.8			105.1		107.5	
Romario f.	Romario ms	121.6	2.3 NS					
Banguy f.	Romario ms	129.0	7.9 NS	5.7 NS	118.1	11.1 ‡	114.4	6.0 NS
Silpro f.	Silpro f.	107.3			96.3		96.4	
Silpro f.	Silpro ms	106.6	-0.6 NS		97.8	1.6 NS	102.5	6.0 NS
Banguy f.	Silpro ms	117.8	8.9 NS	9.48 ‡	116.0	17.0 *	95.2	-1.3 NS
Banguy f.	Banguy f.	109.9			95.8		104.8	

2. The average Plus-Hybrid effect observed for the 12 CMS-hybrids pollinated by Banguy fertile was +8.7% in Lindau-Eschikon, +8.3% in Avenches and -0.4% in Delley.
3. The CMS-hybrids showing the best Plus-Hybrid effects are:
   - EG'Z5824-MS: +13.5% over 3 locations and +20.0% in Avenches
   - Romario-MS: +8.3% over 3 locations
   - Silpro-MS: +8.2% over 3 locations and +17.0% in Avenches
   - LG22.75-MS: +7.5% over 3 locations
   - DSP25673-MS: +7.3% over 3 locations
   - DSP73749A31-MS: +6.4% over 3 locations
   - DSP25946A31-MS: +6.1% over 3 locations

4. The other combinations led to a lower or even negative Plus-Hybrid effect.
5. The worse case was Prinz-MS pollinated by Banguy in Delley with a yield decrease of 16.3%.
6. Contrarily to the CMS-effect, the gain in yield due to xenia effect is the result of an increase in kernel size (higher 1000-kernels weight) and not in number of kernels.
7. Pollinating ability of fertile hybrids

3.3.4.5 Pollinating ability of fertile hybrids

		Lindau			Avenches			Delley		
Pollinator	Female	Yield q/ha	variation (%) / S f. x S f.	/ Scms x Sf	Yield q/ha	variation (%) / S f. x S f.	/ Scms x Sf	Yield q/ha	variation (%) / S f. x S f.	/ Scms x Sf
Silpro f. Silpro f.	Silpro f. Silpro ms	113.8 106.6	-6.7 NS		96.3 97.8	1.6 NS		96.4 102.5	6.0 NS	
DSP25673 f.	Silpro ms	108.1	-5.2 NS	1.4 NS	105.9	9.0 ‡	7.6 ‡	96.2	-0.2 NS	-6.6 NS
DSP25946A31 f.	Silpro ms	103.5	-9.9 NS	-3.1 NS	102.5	6.1 ‡	4.6 NS	88.6	-8.7 NS	-15.7 NS
DSP27223B11 f.	Silpro ms	101.4	-12.2 NS	-5.2 NS	95.6	-0.8 NS	-2.4 NS	87.9	-9.6 NS	-16.6 NS
DSP73749A31 f.	Silpro ms	103.6	-9.8 NS	-2.9 NS	90.5	-6.4 NS	-8.1 NS	87.7	-9.8 NS	16.9 NS
Anjou 249 f.	Silpro ms	102.5	-11.0 NS	-4.0 NS				91.2	-5.7 NS	-12.4 ‡
Anjou 265 f.	Silpro ms	93.6	-21.5 NS	-13.9 NS						
Ardiles f.	Silpro ms	103.3	-10.1 NS	-3.2 NS	110.5	12.8 NS	11.5 NS	89.2	-8.0 NS	-14.9 *
Banguy f.	Silpro ms	120.1	5.3 NS	11.2 ‡	116.0	17.0 *	15.7 **	95.2	-1.3 NS	-7.7 ‡
Delitop f.	Silpro ms	106.2	-7.1 NS	-0.4 NS	99.3	3.0 NS	1.5 NS	95.4	-1.0 NS	-7.4 NS
DKc3420 f.	Silpro ms	97.3	-17.0 NS	-9.6 NS						
EG'Z5824 f.	Silpro ms	105.0	-8.3 NS	-1.5 NS	91.4	-5.4 NS	-7.1 **	86.8	-11.0 NS	-18.1 ‡
Gavott f.	Silpro ms	88.8	-28.2 ‡	-20.2 ‡						
GL2205 f.	Silpro ms	112.6	-1.0 NS	5.3 NS	102.0	5.6 NS	4.1 NS	88.9	-8.4 NS	-15.4 ‡
Horatop f.	Silpro ms	90.3	-25.9 ‡	-18.1 ‡						
LG22.75 f.	Silpro ms	105.4	-7.9 NS	-1.1 NS	106.5	9.6 ‡	8.1 **	87.7	-9.8 NS	-16.9 *
Prinz f.	Silpro ms	111.2	-2.3 NS	4.1 NS	110.9	13.2 ‡	11.8 NS	101.7	5.3 NS	-0.8 NS
Romario f.	Silpro ms	100.8	-12.9 NS	-5.8 NS	104.3	7.7 ‡	6.2 ***	96.8	0.4 NS	-6.0 NS

1. The Plus-hybrid effects observed on Silpro-MS by allo-pollination were lower than expected. The high variations between locations complicated the assessment of the results.
2. Only a few combinations led to a higher yield than the reference values of the *Status quo* {Hybrid fertile x Hybrid fertile} – *data not shown*-:
   - Silpro-MS x DSP27223B11 in Lindau-Eschikon
   - Silpro-MS x Ardiles in Avenches
   - Silpro-MS x Banguy in Lindau-Eschikon
   - Silpro-MS x LG22.75 in Avenches
   - Silpro-MS x Prinz in Avenches
   - Silpro-MS x Romario in Avenches

3. Only Banguy fertile and Prinz fertile showed high Plus-hybrid effects up to respectively +17.0% and +13.2% in Avenches in comparison to Silpro fertile x Silpro fertile.

3.3.5 Summary / conclusions

High variations in yield observed between and within location complicated the statistical assessment of the results. However, the potential in term of gain in yield through modern Plus-Hybrids appears promising.

Cms-effects up to +8.0% in the best case have been shown. For 7 out of the 12 CMS-hybrids, the pollination by Banguy fertile led to a gain of yield higher than +6.0% over 3 locations. These results also confirmed that CMS-effect rather acts on the number of kernels produced and that the xenia effect directly acts on the kernel size (significant variations in 1000-kernels weight).

Working with two tester hybrids, Silpro-MS and Banguy fertile allowed the screening of a large set of modern and high-yielding hybrids describing the genetic of several European breeders during this first season. According to these preliminary results, 5 hybrids (CMS- and fertile form) and 3 additionnal pollinators have been selected for deeper investigations in the frame of a European Ring experiment:

- Romario fertile and CMS = KWS1
- EG'Z5824 fertile and CMS = EUR1
- LG22.75 fertile and CMS = LIM1
- DSP73749A31 fertile and CMS =DSP1
- Silpro fertile and CMS = DSP2
- Prinz fertile = KWS2
- Banguy fertile = LIM2
- Delprim fertile =DSP3

3.4 Season 2006, European Maize Plus-Hybrid Ring Experiment

Thanks to a partnership with several European Research Institutes within the Framework of the CO-EXTRA Project, coordinated field trials have been carried out in Switzerland, Bulgaria, France and Germany. This large ring experiment should enable to evaluate the reliability of the Plus-Hybrid system under very different geographical and climatic conditions.

3.4.1 Locations & Institutes involved

- Switzerland: Avenches & Delley, DSP (Magali Munsch, Karl-Heinz Camp)
  Lindau-Eschikon, ETH (Christophe Weider, Peter Stamp)
- Bulgaria: Kostinbrod, ABI (Nikolai Christov, Atanas Atanassov)
- France: Montardon, Arvalis, Institut du Végétal (Xavier Foueillassar)
- Germany: Braunschweig, BBA (Alexandra Hüsken, Joachim Schiemann)

3.4.2 Plant Material

Five hybrids in their CMS and fertile forms, but also 3 additional pollinators were selected for their combining and pollinating abilities according to the results obtained in 2005. The hybrids involved in the Maize Ring experiment belong to 4 different European breeding companies: Delley Seed and Plants, KWS, Euralis and Limagrain.

Plus-Hybrids Table:
- 5 hybrids are tested in an orthogonal design.
- 3 additional good pollinators are tested on the five CMS-hybrids

♀ ♂		Fertile							
		KWS1	EUR1	LIM1	DSP1	DSP2	KWS2	LIM2	DSP3
sterile	KWS1								
	EUR1								
	LIM1								
	DSP1								
	DSP2								

Plus-Hybrid effect
cms effect

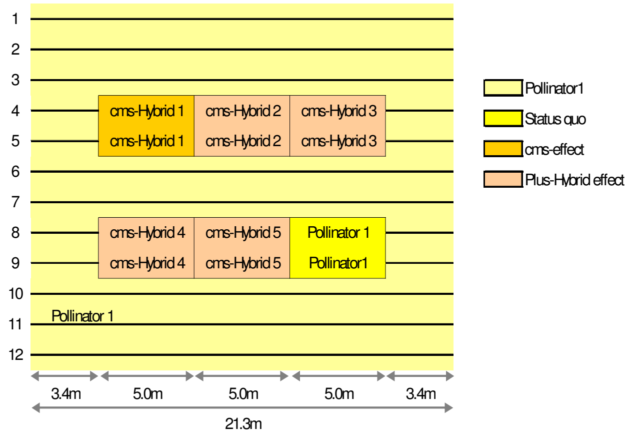
Genotypes used at the six environments in 2006:

CH-Avenches, CH-Delley, CH-Eschikon, Bulgaria, Germany		France	
CMS-hybrids	Pollinators	CMS-hybrids	Pollinators
DSP1-MS	DSP1	DSP1-MS	DSP1
DSP2-MS	DSP2	DSP2-MS	DSP2
EUR1-MS	DSP3	EUR1-MS	EUR1
KWS1-MS	EUR1	LIM1-MS	LIM1
LIM1-MS	KWS1		LIM2
	KWS2		
	LIM1		
	LIM2		

3.4.3 Experimental Layout

The Maize Ring Experiment consists in small plot trials with 3 replications at each location, where 6 subplots are randomly distributed within each pollinator block. The sub-plots are 2 rows wide and 5m long. Two rows of pollinators are sown between the CMS-hybrids to ensure an optimal supply of pollen. Three border rows and rows on the lateral sides (3.4m long) are additional sources of pollen and act as a buffer zone to minimize contamination by pollen from neighboring blocks. The sowing density is 10 plants per scare meter, according to the classical grain maize cultivation methods. The agricultural practices are the same as those implemented locally and the stands are maintained free of weeds and diseases.

Yield parameters like total grain yield, thousand kernel weight (TKW) and number of kernels (KN) produced are investigated. Analysis of the variance and means comparison for total grain yield, TKW and KN were conducted using the mixed procedure (SAS Institute Inc. 1998). Locations and years have been treated as random factors.



Schematic experimental layout of one pollinator block. The pollinator in this example is the Pollinator **1.** This genotype surrounds the sub-plots with the five CMS-hybrids, the first one being the CMS version of pollinator 1. The male fertile version is tested in the sixth sub-plot.

3.4.4 Results

3.4.4.1 Climatic conditions & yield levels

The weather during the field season 2006 was not ideal for maize cultivation.

The drought and hot period observed in all locations was very drastic in Bulgaria where it lasted until 2 weeks after flowering, affecting the vegetative development but also the kernel set.

Switzerland and Germany had a quite mild and wet August leading generally to lower maize grain yields (table1).

Table 1: Average grain yield and yield variations in 2006 at each location

Location	average q.ha-1	maximum q.ha-1	minimum q.ha-1
CH-Lindau	101	118	75
CH-Avenches	113	138	91
CH-Delley	98	117	81
Bulgaria	64	75	46
France	105	128	82
Germany	101	120	87

3.4.4.2 CMS-effects

1. Across 6 locations, there was no significant yield variation through CMS-effect.

Table 2: CMS effect on grain yield and TKW across 6 environments

Female	Pollinator	Grain Yield average (q.ha-1)	Grain Yield CMS effect (%)		TKW average (g.)	TKW CMS effect (%)	
DSP1	-	95.9			257.2		
DSP1-MS	DSP1	93.2	*-2.77*	*NS*	244.7	*-4.83*	*†*
DSP2	-	85.9			284.5		
DSP2-MS	DSP2	88.0	*2.47*	*NS*	284.5	*0*	*NS*
EUR1	-	97.8			264.2		
EUR1-MS	EUR1	100.5	*2.75*	*NS*	269.5	*2.03*	*NS*
KWS1	-	96.5			282.1		
KWS1-MS	KWS1	100.6	*4.26*	*NS*	288.7	*2.35*	*NS*
LIM1	-	92.9			251.8		
LIM1-MS	LIM1	93.2	*0.26*	*NS*	248.5	*-1.34*	*NS*

NS, not significant; †, Significant at the 0.2 level

2. Like in 2005, most of the CMS-effects observed on the five hybrids tested were between +/-3% yield variations.
3. KWS1 showed the highest yield increase over 6 locations with 4.3% CMS effect (NS).
4. As expected, the variations in yield are mainly due to variations in KN (data not shown) and not in TKW.
5. The CMS-effect seems to be quite variable and genotype as well as genotype-environment dependent (See annex1).
6. Across the 3 Swiss locations, four hybrids out of five showed more than 6% yield increase through CMS-effect, going up to 14% for DSP2.
7. CMS effect seems to be mostly expressed in cases where the yield potential is not achieved like in Delley or Lindau. In France and Avenches, most of the fertile hybrids showed a high yield level and frequently the CMS-counterpart could not outperform the status quos' yield (see annex 1).

3.4.4.3 Combining abilities of 5 CMS-hybrids

Most of the allo-pollinations had a positive but not always significant effect on all CMS-hybrids. Grain yield and TKW as well as their respective Plus-Hybrid and Xenia effects across the 6 locations are shown in annex 2.

1. The average Plus-Hybrid effect obtained on these 5 CMS-hybrids is always positive and between 3.8% for EUR1-MS and 5.2% for KWS1-MS across the 6 locations.
2. For some specific combinations, and especially after having been pollinated by KWS2, the Plus-hybrid effect led to more than 10% yield increase over 6 locations.
3. By considering the different locations separately (See annex 4), we can see that the responses from a specific CMS-hybrid to a pollinator are quite similar from a location to the other but the level of the response can be very different. The level of the Plus-Hybrid effect as well as that of the Xenia effect seem to be environment and genotype-environment dependant. Some combinations at a specific location led to yield variations through Xenia effect of up to 20% (annex 4)

Table 3 presents a selection of the results obtained on DSP1-MS and LIM1-MS after pollination by the different fertile hybrids.

Table 3: Combining abilities of DSP1-MS and LIM1-MS: Grain yield and thousand kernel weight (TKW) tested in 6 environments and relative differences (%) to the corresponding isogenic pollination of the CMS-hybrid (Xenia effect).

		Grain yield			TKW		
		average	Xenia		average	Xenia	
female	pollinator	q.ha-1	%		g.	%	
DSP1-MS	DSP1	93.23			244.74		
-	DSP2	104.14	*11.70*	**	282.86	*15.58*	***
-	DSP3	99.87	*7.12*	†	279.20	*14.08*	***
-	EUR1	102.76	*10.22*	*	270.38	*10.48*	***
-	KWS1	103.16	*10.65*	*	282.53	*15.44*	***
-	KWS2	101.25	*8.60*	†	273.63	*11.80*	***
-	LIM1	100.64	*7.95*	†	267.73	*9.39*	**
-	LIM2	101.42	*8.78*	†	287.13	*17.32*	***
	average		***9.29***			***13.44***	
LIM1-MS	LIM1	93.16			248.45		
-	DSP1	88.86	*-4.62*	†	248.80	*0.14*	*NS*
	DSP2	101.30	*8.74*	**	277.51	*11.70*	***
	DSP3	96.20	*3.26*	*NS*	264.08	*6.29*	**
	EUR1	94.58	*1.52*	*NS*	264.40	*6.42*	**

KWS1	95.67	*2.69*	*NS*	270.46	*8.86*	***
KWS2	102.73	*10.27*	**	261.68	*5.33*	*
LIM2	100.14	*7.49*	*	269.37	*8.42*	***
average		***4.20***			***6.74***	

NS, not significant; †, *. **, *** Significant at the 0.2, 0.05, 0.01, 0.001 level respectively

1. DSP1 and LIM1 presented an average yield increase through Xenia effect of respectively 9.29% and 4.20%.
2. All the allo-pollinations tested on DSP1-MS led to a yield increase over 7% and even over 10% for three of them across the 6 locations. Eleven combinations (given pollinator on a given location) showed a Xenia effect over 15% (See annex 4).
3. Most of the pollinators led to a positive Xenia effect on LIM1-MSs' yield: over 7% for 3 of them and up to 10.3% for the best one.
4. In both cases, the Xenia effect induced by different pollinators on the TKW is significantly positive. In the case of DSP1-MS, allo-pollinations induced an average TKW increase of 13.4% with a maximum of 17%. For LIM1-MS, 6 pollinators out of 7 led to a TKW increase of over 5% and up to 11% across 6 locations.
5. These data confirm that yield variations through Xenia effect are mainly due to variations in terms of kernel size rather than in number of kernels produced.

3.4.4.4 Pollinating abilities of 8 fertile hybrids

Grain yield and TKW as well as their respective Plus-Hybrid and Xenia effects induced by the eight pollinators on the five CMS-hybrids across 6 locations are shown in annex3.

1. Seven out of 8 pollinators tested acted mostly positively on the grain yield of the five CMS-hybrids.
2. Five pollinators led to an average yield increase through Plus-Hybrid effect on the 5 CMS-hybrids of over 5% and even over 8% for LIM2 and KWS2.
3. For 3 combinations, the Plus-Hybrid effect on yield over 6 locations was over 10% and up to 15.53%. For this last case, the high Plus-Hybrid effect clearly results from a combination of CMS-effect on DSP2-MS and Xenia effect induced by LIM2's fertilization.
4. Only one pollinator, DSP1, induced a yield decrease on all the 5 CMS-hybrid tested.

The table 4 presents a selection of the results obtained after pollination by LIM2 and KWS2.

Table 4: Pollinating abilities of LIM2 and KWS2: Grain yield and thousand kernel weight (TKW) tested in 6 environments and relative differences (%) to the corresponding isogenic pollination of the CMS-hybrid (Xenia effect).

female	pollinator	Grain Yield		TKW	
		average	Xenia	average	Xenia
		q.ha-1	%	g.	%

DSP1-MS	LIM2	101.4	*8.78*	*†*	287.1	*17.32*	*****
DSP2-MS	LIM2	99.2	*12.75*	*****	290.5	*2.11*	*NS*
EUR1-MS	LIM2	103.5	*2.99*	*NS*	282.2	*4.72*	*†*
KWS1-MS	LIM2	103.2	*2.56*	*NS*	289.4	*0.25*	*NS*
LIM1-MS	LIM2	100.1	*7.49*	***	269.4	*8.42*	*****
	average		***6.92***			***6.56***	
DSP1-MS	KWS2	101.3	*8.60*	*†*	273.6	*11.80*	*****
DSP2-MS	KWS2	92.9	*5.53*	*NS*	269.4	*-5.31*	***
EUR1-MS	KWS2	106.9	*6.38*	*†*	267.8	*-0.65*	*NS*
KWS1-MS	KWS2	106.5	*5.84*	*†*	287.9	*-0.28*	*NS*
LIM1-MS	KWS2	102.7	*10.27*	****	261.7	*5.33*	***
	average		***7.33***			***2.18***	

NS, not significant;†, *. **, *** Significant at the 0.2, 0.05, 0.01, 0.001 level respectively

1. The average yield increase induced by LIM2 on these 5 CMS-hybrids is ~7% across 6 locations.
2. LIM2 led to a Xenia effect on yield over 7% on 3 CMS-hybrids and going up to 12.7% in the best case.
3. KWS2 induced a positive Xenia effect (over 5% and up to 10% across 6 locations) on yield on all the 5 CMS-hybrids tested with an average of 7.3%.
4. The response to allo-pollination by these two pollinators in term of TKW is quite variable and seems to be female dependant. As mentioned before, DSP1-MS and LIM1-MS are significantly affected by allo-pollination with a TKW increase of up to 17% and 11%, respectively. The response in terms of TKW of the other CMS-hybrid is more contrasting and seems influenced by the pollinator but also the environment (data not shown).

3.4.5 Summary / conclusions

The European Maize Ring Experiment conducted under several environments confirmed the expectations we had after the large screening conducted in Switzerland in 2005.

High CMS-effects have been assessed but the impact of the environment and the climatic conditions seems to be the origin of the high variability in CMS-effects observed for a given hybrid. The part of CMS-effect in the Plus-Hybrid effect appears very significant under conditions where the full yield-potential can not be expressed. It suggests that CMS-hybrids are able to compensate and yield better than their fertile counterpart under limiting conditions, but express a lower yield potential under optimal cultivation conditions. This could be related to the fact that CMS-hybrids are often smaller (less biomass) than the fertile forms.

Most of the pollinators used in the Ring Experiment showed interesting general pollinating abilities, often inducing a consistent yield increase compared to the isogenic pollination of the CMS-hybrid. The Xenia effect obtained for a given combination is quite similar and reliable from one location to another, but the level of this response seems affected by environmental conditions. The next step would be to evaluate the stability of the Plus-Hybrid effect under contrasting environmental and climatic conditions.

DSP1-MS presents a high general combining ability (highly responsive to allo-pollination) and the fertile form of DSP1 mostly induced no or negative Xenia effects as pollinator on other CMS-hybrids. This is also observed for specific reciprocal combinations and suggests that reciprocal Plus-Hybrids always show contrary results.

This first year of Maize Ring Experiment showed also that the Plus-Hybrid effect acts on both main yield parameters (i) the number of kernels produced (KN), influenced by CMS-effect, and (ii) the TKW, mostly influenced by Xenia effect.

Two high-responsive CMS-hybrids and two general good pollinators have been identified, and further investigations are ongoing on harvested samples aiming at elucidating the mechanisms leading to high Xenia effects. The genetic distances between the hybrids have also been investigated in our experiments, but the existence of a positive relationship between genetic distance and a high Xenia effect could not be proven yet.

In order to confirm these first results and trends, the European Maize Ring Experiment will be repeated in 2007 at 6 locations over 4 countries.

4 Aknowledgments

We would like to thank our European partners, Swiss Federal Institute of Technology Zürich (Switzerland), Biologische Bundesanstalt für Land und Forstwirtschaft (Germany), AgroBioInstitute (Bulgaria) and Arvalis, Institut du Végétal (France) for their participation and the good work they have done during the maize ring experiment. Our best thanks are also going to the breeding companies which generously provided the seeds for the 3 years of field trials, Delley Seed and Plants, Limagrain, KWS, NK and Euralis.

5 Annexes

5.1 Annex 1: CMS effect on grain yield at different locations

Female	Pollinator	CH-Avenches G. Yield	CH-Avenches CMS effect		CH-Delley G. Yield	CH-Delley CMS effect		CH-Lindau G. Yield	CH-Lindau CMS effect	
		q.ha-1	%		q.ha-1	%		q.ha-1	%	
SP1	-	106.4			85.6			86.6		
SP1-MS	DSP1	105.2	*-1.17*	*NS*	95.3	*11.26*	*NS*	99.7	*15.08*	*†*
SP2	-	93.3			84.4			74.7		
SP2-MS	DSP2	97.9	*5.01*	*NS*	89.2	*5.72*	*NS*	98.0	*31.27*	**
UR1	-	119.1			90.3			104.4		
UR1-MS	EUR1	116.7	*-1.99*	*NS*	102.9	*13.87*	*NS*	113.1	*8.42*	*NS*
WS1	-	118.3			94.0			93.9		
WS1-MS	KWS1	116.7	*-1.37*	*NS*	103.3	*9.91*	*†*	97.3	*3.57*	*NS*
IM1	-	106.7			93.4			105.8		
IM1-MS	LIM1	96.6	*-9.4*	*†*	98.3	*5.24*	*NS*	102.3	*-3.27*	*NS*

Female	Pollinator	Bulgaria G. Yield	Bulgaria CMS effect		France G. Yield	France CMS effect		Germany G. Yield	Germany CMS effect	
		q.ha-1	%		q.ha-1	%		q.ha-1	%	
SP1	-	64.4			125.9			103.8		
SP1-MS	DSP1	59.0	*-8.39*	*NS*	106.4	*-15.52*	**	91.8	*-11.58*	*NS*
SP2	-	62.1			104.1			95.2		
SP2-MS	DSP2	63.8	*2.74*	*NS*	81.9	*-21.29*	*	96.4	*1.22*	*NS*
UR1	-	65.4			97.1			111.0		
UR1-MS	EUR1	59.8	*-8.59*	*NS*	89.6	*-7.64*	*NS*	119.6	*7.68*	*NS*
WS1	-	69.6			./.			99.6		
WS1-MS	KWS1	71.5	*2.76*	*NS*	./.			105.3	*5.69*	*NS*
IM1	-	69.1			91.7			94.4		
IM1-MS	LIM1	61.6	*-10.8*	*NS*	107.1	*16.84*	***	93.1	*-1.43*	*NS*

NS, not significant; †, *. **, *** Significant at the 0.2, 0.05, 0.01, 0.001 level respectively

5.2 Annex 2: Combining abilities of the CMS-hybrids: grain yield and TKW over 6 locations

ıle	pollinator	Grain yield q.ha-1	Plus-Hybrid %		Xenia %		TKW g.	Plus-Hybrid %		Xenia %	
'1	-	95.89					257.17				
'1-MS	DSP1	93.23	*-2.77*				244.74	*-4.83*	†		
-	DSP2	104.14	*8.60*	†	*11.70*	**	282.86	*9.99*	***	*15.58*	***
-	DSP3	99.87	*4.15*		*7.12*	†	279.2	*8.57*	**	*14.08*	***
-	EUR1	102.76	*7.16*	†	*10.22*	*	270.38	*5.14*	†	*10.48*	***
-	KWS1	103.16	*7.58*	†	*10.65*	*	282.53	*9.86*	***	*15.44*	***
-	KWS2	101.25	*5.59*		*8.60*	†	273.63	*6.40*	*	*11.80*	***
-	LIM1	100.64	*4.95*		*7.95*	†	267.73	*4.11*	†	*9.39*	**
-	LIM2	101.42	*5.77*	†	*8.78*	†	287.13	*11.65*	***	*17.32*	***
	average		***5.13***		***9.29***			***6.36***		***13.44***	
DSP2	-	85.89					284.52				
'2-MS	DSP2	88.01	*2.47*				284.51	*0.00*			
-	DSP1	82.05	*-4.47*		*-6.77*	†	269.92	*-5.13*	*	*-5.13*	*
-	DSP3	90.14	*4.95*		*2.42*		275.88	*-3.04*	†	*-3.03*	†
-	EUR1	87.19	*1.51*		*-0.93*		272.59	*-4.19*	†	*-4.19*	†
-	KWS1	89.99	*4.77*		*2.25*		284.08	*-0.15*		*-0.15*	
-	KWS2	92.88	*8.14*	†	*5.53*		269.39	*-5.32*	*	*-5.31*	*
-	LIM1	85.36	*-0.62*		*-3.01*		267.23	*-6.08*	**	*-6.07*	**
-	LIM2	99.23	*15.53*	***	*12.75*	***	290.52	*2.11*		*2.11*	
	average		***4.04***		***1.75***			***-2.73***		***-3.11***	
EUR1	-	97.78					264.16				
ꝛ1-MS	EUR1	100.47	*2.75*				269.51	*2.03*			
-	DSP1	94.7	*-3.15*		*-5.74*		249.18	*-5.67*	†	*-7.54*	*
-	DSP2	100.66	*2.95*		*0.19*		283.05	*7.15*	*	*5.02*	†
-	DSP3	103.25	*5.59*		*2.77*		279.31	*5.74*	†	*3.64*	
-	KWS1	103.88	*6.24*		*3.39*		279.52	*5.81*	*	*3.71*	
-	KWS2	106.88	*9.31*	†	*6.38*	†	267.77	*1.37*		*-0.65*	
-	LIM1	98.83	*1.07*		*-1.63*		269.46	*2.01*		*-0.02*	
-	LIM2	103.47	*5.82*		*2.99*		282.23	*6.84*	*	*4.72*	†
	average		***3.82***		***1.19***			***3.16***		***1.27***	
KWS1	-	96.53					282.06				
S1-MS	KWS1	100.64	*4.26*				288.68	*2.35*			
-	DSP1	95.6	*-0.96*		*-5.01*	†	272.77	*-3.29*		*-5.51*	
-	DSP2	99.76	*3.35*		*-0.87*		284.41	*0.83*		*-1.48*	
-	DSP3	105.88	*9.69*	*	*5.21*	†	284.42	*0.84*		*-1.48*	
-	EUR1	102.51	*6.19*	†	*1.86*		282.53	*0.17*		*-2.13*	
-	KWS2	106.52	*10.35*	*	*5.84*	†	287.88	*2.06*		*-0.28*	
-	LIM1	98.24	*1.77*		*-2.38*		272.69	*-3.32*		*-5.54*	*
-	LIM2	103.22	*6.93*	†	*2.56*		289.4	*2.60*		*0.25*	
	average		***5.20***		***1.03***			***0.28***		***-2.31***	
LIM1	-	92.92					251.82				
ı1-MS	LIM1	93.16	*0.26*				248.45	*-1.34*			
-	DSP1	88.86	*-4.37*		*-4.62*	†	248.8	*-1.20*		*0.14*	
	DSP2	101.3	*9.02*	**	*8.74*	**	277.51	*10.20*	***	*11.70*	***
	DSP3	96.2	*3.53*	*	*3.26*		264.08	*4.87*	*	*6.29*	**
	EUR1	94.58	*1.79*		*1.52*		264.4	*5.00*	*	*6.42*	**
	KWS1	95.67	*2.96*		*2.69*		270.46	*7.40*	**	*8.86*	***
	KWS2	102.73	*10.56*	**	*10.27*	**	261.68	*3.92*	†	*5.33*	*
	LIM2	100.14	*7.77*	*	*7.49*	*	269.37	*6.97*	**	*8.42*	***
	average		***3.94***		***4.20***			***4.48***		***6.74***	

NS, not significant; †, *. **, *** Significant at the 0.2, 0.05, 0.01, 0.001 level respectively

5.3 Annex 3: Pollinating abilities of the fertile hybrids: grain yield and TKW over 6 locations

male	pollinator	Grain yield q.ha-1	Plus-Hybrid %		Xenia %		TKW g.	Plus-Hybrid %		Xenia %	
SP1-MS	DSP1	93.23	-2.77				244.74	-5.03	†		
SP2-MS	DSP1	82.05	-4.47		-6.77	†	269.62	-5.24	*	-5.23	*
UR1-MS	DSP1	94.7	-3.12		-5.74		249.18	-5.67	†	-7.54	*
WS1-MS	DSP1	95.6	-0.96		-5.01	†	272.77	-3.29		-5.51	*
IM1-MS	DSP1	88.86	-4.37		-4.62	†	248.80	-1.20		0.14	
	average		**-3.14**		**-5.53**			**-4.09**		**-4.54**	
SP1-MS	DSP2	104.14	8.60	†	11.70	**	282.86	9.76	***	15.58	***
SP2-MS	DSP2	88.01	2.47				284.51	0.00			
UR1-MS	DSP2	100.66	2.92		0.19		283.05	7.15	*	5.02	†
WS1-MS	DSP2	99.76	3.35		-0.87		284.41	0.83		-1.48	
IM1-MS	DSP2	101.3	9.02	**	8.74	**	277.51	10.20	***	11.70	***
	average		**5.27**		**4.94**			**5.59**		**7.70**	
SP1-MS	DSP3	99.87	4.15		7.12	†	279.20	8.34	**	14.08	***
SP2-MS	DSP3	90.14	4.95		2.42		275.88	-3.04	†	-3.03	†
UR1-MS	DSP3	103.25	5.54		2.77		279.31	5.74	†	3.64	
WS1-MS	DSP3	105.88	9.69		5.21	†	284.42	0.84		-1.48	
IM1-MS	DSP3	96.2	3.53		3.26		264.08	4.87	*	6.29	**
	average		**5.57**		**4.16**			**3.35**		**3.90**	
SP1-MS	EUR1	102.76	7.16	†	10.22	*	270.38	4.92	†	10.48	***
SP2-MS	EUR1	87.19	1.51		-0.93		272.59	-4.19	†	-4.19	†
UR1-MS	EUR1	100.47	2.72				269.51	2.03			
WS1-MS	EUR1	102.51	6.19	†	1.86		282.53	0.17		-2.13	
IM1-MS	EUR1	94.58	1.79		1.52		264.40	5.00	*	6.42	**
	average		**3.88**		**3.17**			**1.58**		**2.64**	
SP1-MS	KWS1	103.16	7.58	†	10.65	*	282.53	9.63	***	15.44	***
SP2-MS	KWS1	89.99	4.77		2.25		284.08	-0.15		-0.15	
UR1-MS	KWS1	103.88	6.18		3.39		279.52	5.81	*	3.71	
WS1-MS	KWS1	100.64	4.26				288.68	2.35			
IM1-MS	KWS1	95.67	2.96		2.69		270.46	7.40	**	8.86	***
	average		**5.15**		**4.75**			**5.01**		**6.97**	
SP1-MS	KWS2	101.25	5.59		8.60	†	273.63	6.18	*	11.80	***
SP2-MS	KWS2	92.88	8.14	†	5.53		269.39	-5.32	*	-5.31	*
UR1-MS	KWS2	106.88	9.21	†	6.38	†	267.77	1.37		-0.65	
WS1-MS	KWS2	106.52	10.35	*	5.84	†	287.88	2.06		-0.28	
IM1-MS	KWS2	102.73	10.56	**	10.27	**	261.68	3.92	†	5.33	*
	average		**8.77**		**7.33**			**1.64**		**2.18**	
SP1-MS	LIM1	100.64	4.95		7.95	†	267.73	3.89	†	9.39	**
SP2-MS	LIM1	85.36	-0.62		-3.01		267.23	-6.08	**	-6.07	**
UR1-MS	LIM1	98.83	1.06		-1.63		269.46	2.01		-0.02	
WS1-MS	LIM1	98.24	1.77		-2.38		272.69	-3.32		-5.54	*
IM1-MS	LIM1	93.16	0.26				248.45	-1.34			
	average		**1.49**		**0.23**			**-0.97**		**-0.56**	
SP1-MS	LIM2	101.42	5.77	†	8.78	†	287.13	11.42	***	17.32	***
SP2-MS	LIM2	99.23	15.53	***	12.75	***	290.52	2.11		2.11	
UR1-MS	LIM2	103.47	5.76		2.99		282.23	6.84	*	4.72	†
WS1-MS	LIM2	103.22	6.93	†	2.56		289.40	2.60		0.25	
IM1-MS	LIM2	100.14	7.77	*	7.49	*	269.37	6.97	**	8.42	***
	average		**8.35**		**6.92**			**5.99**		**6.56**	

NS, not significant; †, *, **, *** Significant at the 0.2, 0.05, 0.01, 0.001 level respectively

5.4 Annex 4: Yield and Yield variations through Plus-Hybrid and Xenia effects at six locations

		CH-Lindau			CH-Avenches			CH-Delley			Bulgaria			France			Germany		
		yield	P-H eff	Xen eff	yield	P-H eff	Xen eff	yield	P-H eff	Xen eff	yield	P-H eff	Xen eff	yield	P-H eff	Xen eff	yield	P-H eff	Xen eff
DSP1-F	DSP1	86.60			106.44			85.64			64.38			125.90			103.77		
DSP1-MS	DSP1	99.66	15.08%		105.19	-1.17%		95.28	11.26%		58.98	-8.39%		106.37	-15.52%		91.76	-11.58%	
	DSP2	104.56	20.74%	4.92%	123.08	15.63%	17.01%	109.47	27.83%	14.90%	66.90	3.91%	13.43%	124.69	-0.96%	17.23%	96.34	-7.16%	4.99%
	DSP3	105.15	21.42%	5.51%	122.45	15.04%	16.41%	82.30	-3.89%	-13.62%	68.21	5.95%	15.65%				104.82	1.01%	14.23%
	EUR1	116.18	34.15%	16.58%	116.02	9.00%	10.29%	101.38	18.38%	6.40%	73.19	13.69%	24.10%	104.52	-16.99%	-1.74%	103.55	-0.22%	12.85%
	KWS1	99.51	14.90%	-0.15%	119.08	11.87%	13.20%	102.88	20.13%	7.98%	66.65	3.52%	13.00%				109.06	5.10%	18.86%
	KWS2	104.00	20.09%	4.35%	130.64	22.74%	24.20%	104.76	22.33%	9.95%	56.65	-12.01%	-3.95%				95.82	-7.67%	4.42%
	LIM1	101.29	16.96%	1.64%	112.56	5.75%	7.00%	98.36	14.86%	3.24%	71.16	10.53%	20.65%	117.85	-6.39%	10.80%	100.46	-3.19%	9.48%
	LIM2	105.14	21.41%	5.50%	135.37	27.18%	28.69%	91.45	6.78%	-4.02%	59.81	-7.10%	1.40%	128.06	1.71%	20.40%	93.87	-9.54%	2.30%
DSP2-F	DSP2	74.65			93.24			84.36			62.11			104.08			95.19		
DSP2-MS	DSP1	86.31	15.61%	-11.93%	91.06	-2.34%	-7.00%	89.75	6.38%	0.63%	46.47	-25.19%	-27.18%	85.16	-18.18%	3.95%	92.39	-2.94%	-4.11%
	DSP2	98.00	31.27%		97.91	5.01%		89.19	5.72%		63.82	2.74%		81.92	-21.29%		96.35	1.22%	
	DSP3	97.87	31.10%	-0.13%	99.12	6.30%	1.23%	80.51	-4.56%	-9.73%	52.78	-15.03%	-17.30%				117.40	23.32%	21.84%
	EUR1	90.49	21.21%	-7.66%	96.78	3.79%	-1.16%	85.92	1.84%	-3.67%	57.51	-7.41%	-9.88%	81.64	-21.56%	-0.34%	109.85	15.40%	14.01%
	KWS1	95.57	28.02%	-2.48%	98.34	5.47%	0.43%	93.78	11.17%	5.15%	52.58	-15.35%	-17.61%				106.44	11.81%	10.47%
	KWS2	106.66	42.87%	8.84%	113.12	21.32%	15.53%	95.16	12.80%	6.69%	53.79	-13.40%	-15.71%				96.42	1.29%	0.07%
	LIM1	92.02	23.26%	-6.10%	100.30	7.57%	2.44%	84.44	0.09%	-5.33%	49.84	-19.76%	-21.90%	89.15	-14.34%	8.82%	97.25	2.16%	0.93%
	LIM2	111.62	49.52%	13.90%	113.25	21.47%	15.67%	98.43	16.68%	10.37%	70.29	13.17%	10.15%	97.14	-6.66%	18.58%	105.17	10.48%	9.15%
EUR1-F	EUR1	104.35			119.07			90.34			65.44			97.05			111.03		
EUR1-MS	DSP1	98.33	-5.78%	-13.09%	112.32	-5.67%	-3.75%	94.37	4.47%	-8.25%	57.72	-11.80%	-3.51%	117.37	20.94%	30.93%	87.47	-21.22%	-26.84%
	DSP2	92.37	-11.49%	-18.36%	124.60	4.64%	6.77%	105.69	16.99%	2.74%	65.45	0.01%	9.40%	119.38	23.00%	33.17%	97.94	-11.79%	-18.08%
	DSP3	101.48	-2.75%	-10.30%	116.55	-2.12%	-0.13%	104.30	15.46%	1.40%	66.48	1.58%	11.12%				114.93	3.51%	-3.87%
	EUR1	113.14	8.42%		116.70	-1.99%		102.86	13.87%		59.82	-8.59%		89.64	-7.64%		119.56	7.68%	
	KWS1	104.11	-0.23%	-7.98%	115.86	-2.70%	-0.72%	111.75	23.70%	8.64%	60.56	-7.46%	1.23%				114.16	2.82%	-4.52%
	KWS2	112.62	7.93%	-0.45%	132.78	11.52%	13.78%	117.01	29.53%	13.76%	64.36	-1.65%	7.59%				99.28	-10.58%	-16.96%
	LIM1	87.51	-16.14%	-22.65%	114.56	-3.79%	-1.83%	108.72	20.35%	5.70%	68.05	3.98%	13.74%	115.80	19.32%	29.18%	97.11	-12.54%	-18.77%
	LIM2	104.61	0.25%	-7.54%	138.08	15.97%	18.32%	100.52	11.27%	-2.28%	66.62	1.81%	11.37%	121.21	24.90%	35.22%	94.78	-14.63%	-20.72%
KWS1-F	KWS1	93.93			118.29			94.01			69.62						99.60		
KWS1-MS	DSP1	102.82	9.46%	5.69%	98.90	-16.39%	-15.23%	105.22	11.93%	1.83%	67.97	-2.37%	-5.00%				89.74	-9.90%	-14.75%
	DSP2	99.76	6.20%	2.55%	114.80	-2.94%	-1.60%	103.58	10.18%	0.24%	73.07	4.97%	2.15%				98.17	-1.44%	-6.75%
	DSP3	114.36	21.75%	17.56%	123.45	4.36%	5.81%	100.03	6.40%	-3.19%	74.70	7.30%	4.41%				108.29	8.72%	2.87%
	EUR1	100.11	6.58%	2.91%	115.68	-2.20%	-0.85%	100.20	6.59%	-3.02%	75.06	7.82%	4.92%				111.45	11.90%	5.87%
	KWS1	97.28	3.57%		116.67	-1.37%		103.33	9.91%		71.54	2.76%					105.27	5.69%	
	KWS2	118.22	25.85%	21.52%	129.81	9.74%	11.26%	110.50	17.54%	6.94%	72.04	3.49%	0.70%				95.38	-4.25%	-9.40%
	LIM1	107.94	14.91%	10.96%	111.27	-5.93%	-4.62%	96.73	2.89%	-6.39%	71.34	2.48%	-0.28%				93.81	-5.82%	-10.89%
	LIM2	95.58	1.75%	-1.75%	129.72	9.67%	11.19%	111.38	18.47%	7.79%	70.30	0.99%	-1.73%				103.53	3.94%	-1.66%
LIM1-F	LIM1	105.80			106.66			93.37			69.10			91.67			94.42		
LIM1-MS	DSP1	96.60	-8.70%	-5.61%	92.44	-13.33%	-4.35%	97.48	4.41%	-0.78%	55.82	-19.22%	-9.45%	102.40	11.71%	-4.39%	88.51	-6.26%	-4.90%
	DSP2	107.03	1.16%	4.58%	113.28	6.20%	17.22%	113.33	21.38%	15.34%	71.77	3.86%	16.43%	107.80	17.59%	0.65%	97.49	3.25%	4.75%
	DSP3	111.01	4.93%	8.47%	109.02	2.22%	12.81%	93.53	0.17%	-4.81%	56.93	-17.61%	-7.63%				108.71	15.14%	16.81%
	EUR1	102.40	-3.22%	0.05%	111.12	4.18%	14.98%	96.50	3.36%	-1.78%	65.51	-5.20%	6.27%	97.08	5.90%	-9.36%	99.00	4.85%	6.37%
	KWS1	95.76	-9.49%	-6.43%	108.23	1.47%	11.99%	98.74	5.76%	0.50%	65.63	-5.02%	6.48%				104.74	10.94%	12.54%
	KWS2	117.70	11.25%	15.01%	122.45	14.81%	26.71%	109.08	16.83%	11.02%	63.39	-8.26%	2.84%				98.22	4.03%	5.54%
	LIM1	102.34	-3.27%		96.64	-9.40%		98.25	5.24%		61.64	-10.80%		107.10	16.84%		93.07	-1.43%	
	LIM2	106.40	0.57%	3.97%	119.66	12.19%	23.82%	99.70	6.78%	1.47%	61.57	-10.89%	-0.11%	115.59	26.09%	7.92%	103.34	9.46%	11.04%
DSP3-F	DSP3	89.56			95.16			85.89			54.68						100.25	6.18%	7.72%
KWS2-F	KWS2	103.89			103.47			94.24			61.72						95.51		
LIM2-F	LIM2	88.71			103.93			96.41			68.89			105.75			97.44		

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