

Simulation of transgenic pollen dispersal by use of different grain colour maize

Diss. ETH No. 16508



Michael Bannert

financial support by
BUWAL * Swissem * Fenaco

Dedicated to Dr. Alberto Soldati
*22.3.1945 †17.3. 2004

Alberto Soldati was born in Lugano (Canton Tessin) in 1945 into a farming family. After attaining his diploma and doctorate from the Swiss Federal Institute of Technology (ETH) Zurich, he became the well-known and highly respected manager of the ETH Research Station in Eschikon-Lindau. Alberto was instrumental in planning the development of this station, from its modest beginnings to the modern facility it became and which he managed until his untimely death in 2004.

Due to his expertise and his warm, friendly personality Alberto succeeded in establishing an efficient working environment. He initiated the maize cross-pollination research in the Agronomy and Plant Breeding group in 2002, the result of which is this dissertation.



Alberto Soldati was my supervisor, who supported me not only in scientific but also in personal matters. In 17. March 2004 his sudden death forced me to finish our project without him. But his working part should be never forgotten like also his person.

Diss. ETH No. 16508

Simulation of transgenic pollen dispersal by use of different grain colour maize

A dissertation submitted to the

SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH

For the degree of

Doctor of Sciences

Presented by

Michael Bannert

Dipl. Ing. Agr.

Technical University Munich

born June 13, 1973

Germany

accepted on the recommendation of

Prof. Dr. Peter Stamp, examiner

Prof. Dr. Klaus Amman, co-examiner

2006

Table of contents

SUMMARY	8
ZUSAMMENFASSUNG	11
1 GENERAL INTRODUCTION	
1.1 Development of plant breeding	14
1.2 General acceptance, risk assessment and coexistence	15
1.3 Biology of flowering in maize	16
1.4 Pollination of maize	18
1.5 Pollen viability	19
1.6 Silk viability and time of fertilization	20
1.7 Anthesis silking interval	21
1.8 Outline of the thesis	24
1.9 References	24
2 MEASUREMENT OF POLLEN DISPERSAL IN MAIZE BY GRAIN COLOUR	
2.1 Methods for measuring pollen dispersal	30
2.2 Experimental set-ups with white grain maize	31
2.3 Average grain number per ear and sample number	32
2.4 Sample size and detection probability	33
2.5 Evaluation of the colour marker system	34
2.6 Comparison of colour marker results to PCR analysis results	36
2.7 Analytical error of PCR and grain colour marker assessments	37
2.8 Tissue specific DNA ratio maternal / paternal	37
2.9 References	38
3 CROSS-POLLINATION OF MAIZE IN LONG DISTANCE	
3.1 Abstract	43
3.2 Introduction	43
3.3 Material and methods	44
3.3.1 Site descriptions	44
3.3.2 Maize varieties	46
3.3.3 Cultural procedures and crop management	46
3.3.4 Synchronisation of flowering	46

Table of contents

3.3.5 Meteorological measurements	46
3.3.6 Sampling	47
3.3.7 Data analysis	47
3.4 Results	48
3.4.1 Rate and frequency of cross-pollinations	48
3.4.2 Distribution of cross-pollinations	49
3.4.3 Horizontal pollen dispersal distance and wind velocity	52
3.5 Discussion	52
3.5.1 The white grain marker hybrid for cross-pollination measurements	52
3.5.2 Rate and frequency of cross-pollinations	53
3.5.3 Pollen dispersal and pollen competition	53
3.5.4 Vertical pollen dispersal	54
3.6 Conclusion	55
3.7 References	55

4 VARIATION IN CROSS-POLLINATION OF MAIZE IN SHORT DISTANCE

4.1 Abstract	59
4.2 Introduction	59
4.3 Material and methods	62
4.3.1 Site descriptions	62
4.3.2 Maize varieties	62
4.3.3 Field experiment design	62
4.3.4 Cultural procedures and crop management	64
4.3.5 Synchronisation of flowering	64
4.3.6 Meteorological measurements	64
4.3.7 Sampling	65
4.3.8 Data analysis	65
4.4 Results	65
4.4.1 Impact of wind on cross-pollination	65
4.4.2 Impact of pollen donor / receptor ratios	69
4.4.3 Impact of asynchronous flower	70
4.5 Discussion	72
4.5.1 Rapid decrease with distance	72
4.5.2 Highest variation in initial distance	73
4.5.3 Impact of pollen donor and receptor field sizes	74
4.5.4 Flower synchrony and cross-pollination rate	74

Table of contents

4.6 Conclusion	76
4.7 References	76

5 GENERAL CONCLUSIONS AND OUTLOOK

5.1 The grain colour system	79
5.2 Current knowledge of cross-pollination in maize	79
5.3 Challenges for cross-pollination research	81
5.4 Limits of predictions and modelling	82
5.5 Conclusions for agricultural practice	82
5.6.1 Different types of cultivation	82
5.6.2 Organic farming	83
5.6.3 Mixing harvest and equal mixing	83
5.6.4 Maize seed production	84
5.6.5 Speciality seeds: Topcross high oil maize	84
5.6.6 Pharmaceutical GM maize plants	84
5.7 Cross-pollination of next generation transgenic maize	85
5.8 References	87

ACKNOWLEDGEMENTS

CURRICULUM VITAE

Summary

Cross-pollination of maize was studied by plant breeders in former years in order to guarantee seed purity. Today, the cultivation of transgenic maize varieties has world-wide increased rapidly. As a result, cross-pollination research has made a comeback in the context of managing the coexistence of conventional and transgenic maize. This situation is different to the plant breeding situation, where female plants get detasseled and are highly receptive both to the pollen from the male parent but also to adventitious pollen from neighbouring fields. Maize is a wind-pollinated crop that produces large amounts of pollen and favours cross-pollination by protandry of flowers. Therefore maize has a biological potential to cross-pollinate by pollen dispersal into neighbouring fields.

Studies about cross-pollination in maize have been published in recent years in many countries of the world. However, so far no experiments have been carried out under the Swiss alpine conditions, which differ in constellations of potentially important influence factors like weather conditions and topography. Twenty-two field experiments were conducted in 2003 and 2004, mainly in two different regions that are typical of Switzerland: In an alpine region of canton Uri and in the Swiss midlands of canton Zurich. Cross-pollination was not directly measured by use of transgenic maize varieties. Instead of this an alternative approach was used by simulating transgenic cross-pollination with maize varieties of different grain colours. In this visual marker system, yellow grain maize is assumed to be transgenic and white grain maize as conventional. Because of the xenia effect the dominant yellow grain colour can be transferred by pollen to white grain maize and such cross-pollinations are visible as yellow grains on the white grain maize ears. The experimental white grain hybrid, DSP17007, was tested in pilot experiments and identified as being comparable to modern hybrids. The method of measuring cross-pollination by counting the number of yellow grains on white grain ears was time and cost effective and enabled high sample rates. This made it possible to investigate complex cross-pollination patterns in high detail in order to optimize sampling procedures as well as to analyse cross-pollination events at very low levels of incidences.

Cross-pollination in long distance was investigated at distances of 50 to 4500 m in the alpine region of canton Uri. Thirteen fields of white grain maize were arranged in different wind orientations to the yellow grain pollen donor. The rate of cross-pollination of total fields was always below 0.02 %. Cross-pollination patterns were in most cases like randomly dispersed, with mainly single cross-pollination events and an average cross-pollination frequency of 1.8% of the sampled ears. In four fields, located in distances of 50 to 370 m in the main wind direction to the yellow grain pollen donor field, a low but marked cross-pollination occurred at the field border indicating pollen dispersal by horizontal winds. The potential distance of pollen dispersal was calculated according to the measured wind conditions in relation to settling height and speed. Thereby it was confirmed that most of the shed pollen will not reach distances

Summary

beyond 50 m. A pollen take-off experiment corroborated that only a very small portion of pollen will move vertically above the field, due to special events like thermals or gusts. These results help to explain the low rates of long distance pollen dispersal. A few “hot-spot” areas with higher cross-pollination rates existed. But whenever the surrounding plants were checked in detail, pure yellow grain contamination plants were found. Therefore “hot-spots” may be more often an effect of seed contamination rather than of extreme or special atmospheric events. This should be carefully taken in account for any cross-pollination experiment.

Cross-pollination experiments in short distance were focused in the Swiss midlands of the canton Zurich on the situation that maize fields are adjacent to each other. Such situations may arise when a farmer cultivates different maize types on the same field or when neighbouring farmers plant their maize crops without another separation crop between them. Moreover, such a situation is of scientific interest as an extreme situation. With different field experiment settings different constellations of influence factors were checked in order to investigate the variability of the cross-pollination rate and to define “worst-case” situations of maximum cross-pollination. In a wind exposed location there was a clear effect of the main wind direction; in most of the other cases the wind effect was probably minimized by the variable topography of the Swiss midlands, which are characterized by a small scale mix of hills, scattered woods and settlements. An important effect of the size ratios of pollen-donor and pollen-receptor fields was hypothesized. The investigated size ratios varied from about 4 : 1 to 1 : 8. However there was no visible impact on the cross-pollination gradients. Probably because of the high settling speed of the pollen only a small portion of pollen will be dispersed beyond distances of 10 m and therefore different field size ratios will not modify the cross-pollination rate significantly. A strong effect was shown by flower asynchrony. When the pollen-donor field sheds pollen five days later than the emergence of silks in the receptor field the cross-pollination rate was lower than 0.9%, even in the neighbouring row at a distance of 1 m, which is in accordance of observed wilted (already fertilized) silks around five days after emergence. It was proven that asynchronous flowering can strongly be modified by the synchrony or homogeneity of flowering within a receptor field. One pollen donor field flowered seven days later than the mid silk emergence of the receptor field. Therefore, little or no cross-pollination should have occurred. A surprisingly high cross-pollination rate was due to a high number of yellow grains on small weak ears that were late in flowering when the availability of white grain pollen was probably already quite low in relation to the inflow from the yellow grain donor. In all field experiments variation in cross-pollination was high at close distance to the pollen donor but the rates decreased rapidly with distance and beyond 15 m they were more or less below 0.9 % in all experiments.

The results of this Swiss study supported and complemented the results of international studies. In general cross-pollination rates were lower, which might be a feature of special Swiss conditions for climate, topography and landscape patterns or the special flower biology of the used maize varieties.

Summary

All values for cross-pollination were calculated for the heterozygous case of transgenic varieties at present. Therefore the cross-pollination values were halved as yellow varieties were homozygous for the transferred yellow grain colour marker.

Zusammenfassung

Schon vor langer Zeit untersuchten Züchter die Fremdpollenbefruchtung von Mais um die Reinheit von Saatgut garantieren zu können. Heute, bei einem raschen weltweiten Anstieg der Kultivierung von gentechnisch modifizierten Maissorten, ist die Fremdpollenbefruchtung von Mais erneut aktuell, um die Koexistenz von gentechnisch veränderten und konventionellen Mais zu regeln. Die Koexistenz Thematik unterscheidet sich deutlich von der Saatgutproduktion, bei der von den Mutterpflanzen die männlichen Blütenstände entfernt werden und diese dann sowohl für den Pollen der Vaterpflanzen als auch für Pollen von ausserhalb der Anlage sehr empfänglich sind. Mais ist eine windbestäubte Kulturpflanze und einige biologische Merkmale der Blüte, wie die Produktion von hohen Pollenmengen und die Protandrie (Vormännlichkeit), begünstigen Fremdpollenbefruchtung.

In den letzten Jahren wurden weltweit einige Studien über die Fremdpollenbefruchtung von Mais veröffentlicht. Aber bis jetzt gab es noch keine Untersuchungen unter den alpinen Bedingungen der Schweiz, bei der wichtige mögliche Einflussfaktoren wie Wetterbedingungen und Topographie anders sind. In den Jahren 2003 und 2004 wurden hauptsächlich an zwei unterschiedlichen Standorten, die typisch für Schweizer Verhältnisse sind, 22 Feldversuche durchgeführt: In der alpinen Region des Kantons Uri und dem Schweizer Mittelland von Kanton Zürich. Die Fremdpollenbefruchtung wurde nicht direkt mit transgenen Maissorten untersucht sondern durch die Verwendung von Maissorten mit unterschiedlicher Kornfarbe simuliert. Bei diesem visuellen Marker System simuliert gelbkörniger Mais die transgene Sorte und weisskörniger Mais die konventionelle Sorte. Befruchtet Pollen von gelbkörnigen Sorten in weisskörnigem Mais entsteht dort aufgrund des Xenien Effekts mit jeder erfolgreichen Befruchtung ein gelbes Korn. Als weisskörnige Sorte wurde die Test-Hybride DSP17007, die sich in Pilotversuchen als geeignet und vergleichbar mit heutigen Maissorten gezeigt hatte, verwendet. Diese Methode, die Fremdpollenbefruchtung durch einfaches Zählen von gelben Körnern auf der weisskörnigen Sorte zu ermitteln, ist bezüglich Zeit- und Kostenaufwand effektiv und ermöglicht hohe Stichprobenzahlen. So lassen sich auch detaillierte Verbreitungsmuster untersuchen und seltene Fremdpollenbefruchtungen können noch entdeckt werden.

In den alpinen Regionen des Kantons Uri wurde die Fremdpollenbefruchtung über lange Strecken zwischen 50 m und 4500 m untersucht. In beiden Versuchsjahren zusammen wurden 13 weisskörnige Versuchsfelder in verschiedenen Abständen und Wind Orientierungen zu den gelbkörnigen Feldern angelegt. Die Fremdpollenbefruchtung des gesamten Feldes blieb immer unter 0.02%. Die Verbreitungsmuster der Einkreuzungen waren hauptsächlich durch zufällige über das gesamte Feld verstreute Einkreuzungen, meist einzelne Einkreuzungen auf durchschnittlich 1.8% der untersuchten Kolben, charakterisiert. Bei vier Feldern, die in Abständen von 50 bis 370 m in Hauptwindrichtung eines gelbkörnigen Feldes orientiert waren, konnten an dem zur Pollenquelle zugewandten Feldrand in der Randreihe etwas höhere

Einkreuzraten gefunden werden. Diese Einkreuzungen zeigen vermutlich den Polleneintrag durch horizontale Windverfrachtung. Die durchschnittliche mögliche Pollenverfrachtungs Distanz wurde aufgrund der Windmessungen in Bezug zur Sinkgeschwindigkeit des Pollens errechnet. Damit konnte gezeigt werden, dass der meiste Pollen innerhalb von 50 m Reichweite zu Boden geht. Ein Versuch zur vertikalen Verfrachtung des Pollens zeigte dass nur eine sehr kleine Menge des ausgeschütteten Pollens durch Windböen oder thermische Aufwinde in 4 m Höhe über das Maisfeld gelangt. Diese Ergebnisse bestätigen die gefundenen niedrigen Auskreuzungsraten jenseits 50 m Distanz. Einige „hot-spots“, Bereiche mit abweichend hoher Einkreuzung, wurden gefunden. In den Fällen, in denen die nähere Umgebung dieser „hot-spots“ genauer untersucht wurde, konnten gelbkörnige Verunreinigungspflanzen als Ursache festgestellt werden. Die auch von anderen Experimentatoren beobachteten „hot-spots“ sind daher vermutlich eher auf Saatgutverunreinigungen als auf extreme Wetterereignisse zurückzuführen.

Schwerpunkt am Versuchsstandort im Schweizer Mittelland im Kanton Zürich waren Versuche zur Fremdpollenbefruchtung über kurze Distanz bis 50 m für den Fall eines Anbaus von gelbkörnigem Mais direkt neben weisskörnigem Mais. Solche Situationen treten auf wenn ein Landwirt verschiedene Maissorten kultiviert oder wenn zwei benachbarte Landwirte ihre Maisfelder ohne einer anderen Feldfrucht dazwischen anlegen. Besonders aber ist diese Situation von wissenschaftlichem Interesse weil so die maximal mögliche Fremdpollenbefruchtung nachgewiesen werden kann. Mit verschiedenen Feldversuchsanlagen wurde die Variabilität der Fremdpollenbefruchtung, verursacht durch die verschiedenen Konstellationen der Einflussfaktoren, untersucht, um „worst-case“ Situationen mit maximal hoher Fremdpollenbefruchtung zu ermitteln. Bei einem windexponierten Standort konnte ein klarer Effekt durch die Orientierung zur Hauptwindrichtung beobachtet werden. In den anderen Fällen war aber kein deutlicher Windrichtungseffekt zu erkennen, was auf die besondere klein strukturierte Topographie des Schweizer Mittellandes mit Hügeln, verstreuten Waldstücken und Siedlungen zurückzuführen sein könnte. Ein wichtiger Einfluss des Feldflächen Verhältnisses von Pollendonor und Rezeptor wurde vermutet. In Versuchen wurden Verhältnisse von 4 : 1 bis 1 : 8 untersucht. Jedoch ergab sich dadurch kein erkennbarer Einfluss auf die Fremdpollenbefruchtung, was vermutlich darauf zurückzuführen ist, dass nur sehr wenig Pollen über eine Distanz von 10 m hinaus verfrachtet wird. Einen starken Einfluss zeigte eine ungleiche Blütezeit. Wenn die Pollenausschüttung des Donor Feldes fünf Tage nach dem Erscheinen der Seiden im Rezeptorfeld mit der Pollenausschüttung begann war die Fremdpollenbefruchtung sogar in unmittelbarer Distanz von 1 m weit unter 0.9%. Dieser Befund stimmte auch mit der Beobachtung überein, dass die Seiden bereits fünf Tage nach ihrem Erscheinen verwelkt (bereits befruchtet) sind. Aber es wurde auch festgestellt, dass die Synchronie der Blütezeit von Feldern von der Gleichmässigkeit der Entwicklung der Pflanzen innerhalb des Bestandes abhängt. Ein Pollendonor Feld begann sieben Tage nach dem Erscheinen der Seiden des Rezeptorfeldes

Zusammenfassung

mit der Pollenausschüttung und eine sehr geringe Fremdpollenbefruchtung wäre zu erwarten. Jedoch stieg die Fremdpollenbefruchtung wieder aufgrund von einzelnen kleinen Maiskolben mit hoher Einkreuzung an. Diese kleinen Kolben sind wahrscheinlich auf eine verzögerte Pflanzenentwicklung und Blüte zurückzuführen. Zu diesem Zeitpunkt der Blüte war vermutlich die Pollenausschüttung des eigenen Feldes im Verhältnis zur gerade beginnenden maximalen Pollenausschüttung des Pollendonors Feldes bereits reduziert. Obwohl es auf kurze Distanz, vor allem den ersten Metern, hohe Variabilität in der Fremdpollenbefruchtungsrate gab, nahm bei allen Feldversuchen die Rate mit der Entfernung schnell ab und war ab 15 m bereits unter 0.9%. Nur in seltenen Ausnahmefällen gab es auch jenseits der 15 m Distanz leicht erhöhte Werte („hot-spots“).

Die Ergebnisse der Studie in der Schweiz unterstützen und ergänzen bereits bestehende internationale Studien in detaillierter und verlässlicher Weise. Im Allgemeinen waren die Raten der Fremdpollenbefruchtung aber etwas geringer, was auf die besonderen Bedingungen in der Schweiz bezüglich Klima, Topographie und Landschaftsmuster sowie auf die besondere Blütenbiologie der verwendeten Maissorten zurückzuführen sein könnte.

Alle Fremdpollenbefruchtungs Werte in dieser Studie wurden auf den heterozygoten Zustand derzeitiger transgener Sorten berechnet: Werte von Experimenten mit Kornfarben Marker, wie hier verwendet, wurden halbiert um den homozygoten Zustand der verwendeten gelbkörnigen Sorten zu berücksichtigen.

1 General Introduction

1.1 Development of plant breeding

Since agriculture was incepted more than 8,000 years ago in different parts of the world, farmers chose promising plant species and turned them into crops by adapting them slowly to their needs. Food supply often failed until 150 years ago in the developed world and up to present days in developing countries, causing severe hardships for affected people. Innovative progress in science and technology brought mankind in developed countries on the way to a society of wealth these days. One important step was the development of modern agriculture. As plants did not evolve to meet the needs of humans Plant Breeding based on Mendel's laws was an important factor in this development (*Odenbach, 1997*). Modern maize breeding started more than a hundred years ago in North America but the enormous increase in the yield of modern maize varieties was achieved only after the development of hybrid maize breeding 80 years ago. This was one driving factor to make maize one of the three most important crops world-wide. Genetic improvement led to increased productivity, earlier maturity, reduction of lodging due to stronger root systems, shorter and stronger stalks and resistance to destructive disease pathogens and insect pests (*Poehlman and Sleper, 1995*). Thus, yield could be increased five- to six-fold due to hybrid breeding (*Zscheischler et al., 1990*).

Breeding methods still have to be adapted these days according to new demands and goals for speciality crops in the developed world and for food security in developing countries. Many of these new demands and goals for agriculture at present and in future can not be achieved by classic breeding methods and depend on the introduction of sophisticated breeding technologies like plant biotechnology with a broad variety of different methods. Because of its effective large-scale cultivation and its global economic importance maize became a main target crop implementing plant biotechnology in breeding programs. Although in developed countries food production is high right now, farmers have to maintain or increase productivity and to cope with economic pressure. Problems related to large-scale cultivation must also be solved in the most sustainable way. Important breeding targets are improvements of pest and herbicide resistance.

In 1996 the first transgenic plants were sown commercially in the USA. Today, nine years later, transgenic plants grow on 81 million ha world-wide and are an important part of agricultural practice in many countries (*Transgen, 2005a*). GM-maize (genetic modified maize) is grown mostly in the USA, Argentina, Canada, Spain, South Africa, Uruguay and the Philippines. For the year 2015 its cultivation is predicted for 30 countries and on a total level of 150 million ha (*Biosicherheit, 2005b*). Although there are a considerable number of genetically modified varieties in maize, at present there are only three different transgenic traits with

global significance for agriculture: herbicide resistance, BT-resistance to European corn borer (*Ostrinia nubilalis*) and BT-resistance to corn rootworm (*Diabrotica virgifera virgifera*).

Herbicide resistant maize produces through genetic modification an altered biosynthesis of an enzyme, thereby making it resistant to herbicides like “Roundup Ready” (glyphosate). These maize varieties have a great advantage to cope effectively with weeds in large-scale cultivation. Beside economic benefits, by using these maize varieties, new environment-friendly cultivation systems like conservation tillage systems and extended inter-cropping management that improve soil fertility and reduce soil erosion can be implemented (*GKB, 2001*).

The BT-transgenic maize varieties are built up on an old concept of fighting pests. *Bacillus thuringiensis* (BT) species produce a group of proteins (BT-protein) that kill certain insect species. Since 1964 compounds of BT protein are used in organic farming. In transgenic BT-maize genes for the expression of BT proteins were transferred. Because of a high specificity, Bt-maize resistant against European cornborer and corn rootworm have different kinds of BT-protein (*Biosicherheit, 2005a*).

1.2 General acceptance, risk assessment and coexistence

The current developments in agriculture using methods of biotechnology show some promising benefits on the one hand. However, on the other hand there are a lot of public concerns about potential hazards and the ethics of implementing these new methods. There is a responsibility to use new technologies in such a way that hazards are avoided in a reasonable way. Besides concern about the impact of transgenes on the environment (non target organism, horizontal gene transfer) and human health, there is a clear demand for a coexistence of conventional and GM-plants to guarantee free choice for farmers and consumers in Europe.

In many countries there are labelling requirements for products containing or derived from GMOs (genetically modified organism). In EU countries, the threshold for the positive labelling of food and feed products, containing or derived from GM-crops, is set at 0.9% at the moment (Regulation EC1829/2003 on GM-food and -feed; *EurLex, 2003*). In Switzerland labelling requirements are similar to those in the EU (*Transgen, 2005b*). In other countries labelling of unintended GM content of plant products is quite different depending on the attitude to GM-plants. For example in the USA and Argentina GM labelling is voluntary but not compulsory, in Canada exists a voluntary threshold of 5%, a level that is mandatory in Japan (*Choices, 2003*). The countries that introduced legislation for mandatory-labelling of GM-foods have done so to give their consumers a choice in selecting the foods they feel comfortable with (*Ahmed, 2002*).

Unwanted GM contamination in conventional or organic production can result by events like seed lot contamination, volunteers, mixing seed at sowing, cross-pollination, harvest, and trade

(Sanvido *et al.*, 2005). Under conditions in central Europe contamination by volunteers in the field is unlikely as maize kernels do not survive low winter temperatures north of the Alps in contrast to tropical regions, where seed companies usually continue breeding in winter time (Hütter *et al.*, 2000). As maize is a wind-pollinating crop, the genetic material of GM-maize can be dispersed by pollen in adjacent fields of conventional maize. The thesis focuses on this aspect of cross-pollination, as one of the crucial components of research for handling coexistence of conventional and genetically modified (GM) maize.

1.3 Biology of flowering in maize

Maize is a monoecious plant and develops unisexual male and female flowers in separated parts of the plant. The male florescence (tassel) arises from the shoot apical meristem, while the female inflorescences (ears) originate from the axillary bud apices. In every axillary bud of a leaf a female inflorescence is initiated, but only one, or in some cases two, will develop into a fertile ear (Maiskomitee, 2003). Abortion or growth cessation of a second ear usually occurs during or just after silking of the uppermost ear (Tollenaar *M.*, 1977; Harris *et al.*, 1976). Studies have suggested that abortion of late-pollinated ovaries (e.g. from the tip of the apical ear or subapical ear) is triggered after the fertilisation of the early-pollinated ovaries, what can be defined as a primigenic dominance e.g. the dominance exerted by the organ formed first (Bangerth, 1989). Although many modern hybrids are prolific types, high yielding maize crops usually have only one ear and do not exhibit prolificacy and second ears at the common high plant density required for high yield (Duvick, 1992). As the tassel is on the top of the plant and the silk in the middle part of the stem, the flowers are separated vertically by about 1 to 1.3 m on a plant that can become up to 3 m tall. This plant architecture favours outcrossing. Protandry usually occurs and the male inflorescence (tassel) appears around two to four days before silk emergence, favouring cross-pollination (Maiskomitee, 2003). Although modern breeding has tended to reduce protandry (floral asynchrony) the tassel may begin to shed pollen before silks emerge. The degree of male and female floral synchrony is genotype-specific and sensitive to plant population, soil fertility and environmental stress. The tassels usually emerge before pollen shedding begins (Burris, 2001). Thus, maize is a naturally cross-pollinating crop; about 95% of the ovules on a shoot are usually cross-pollinated and 5% self-pollinated in a normal field stand (Poehlman and Sleper, 1995). Most of the pollen that pollinates an ear of maize comes from plants in the immediate vicinity, although the wind may carry pollen over large distances. In the fields there is often non-uniform plant growth (different plant size) and development (start of phenological stages of flowering) resulting in longer periods until flowering is completed and a probably asynchronous pollination within the population (Ma *et al.*, 2004).

Pollen is produced in the anthers of the tassel at the top of the plant. When mature, the anthers suspend on filaments that emerge from the tassel, and pollen is released from an opening at the

tip of the anther. Even a slight breeze or vibration (e.g., as caused by gently tapping the stem) will release the pollen into the air when it is mature. The low wind speed threshold for maize pollen release has implications for its dispersal. Usually most pollen will be shed under low wind conditions with only low potential travelling distances (*Aylor et al., 2003*).

The male inflorescence (tassel) can produce considerably more pollen grains than required for pollination of a single plant (*Schoper et al., 1987*). A single tassel from a normal plant may produce up to 25 million pollen grains or an average of 25,000 pollen grains for each female flower on an ear of 750 to 1000 kernels (*Poehlman and Sleper, 1995*).

Jones and Newell (1948) and *Raynor et al. (1972)* found production rates between 9,000 to 50,000 pollen grains per kernel set, depending on the genotype. This range is typical of wind-pollinated species (*Burris, 2001*). There is large variation in the estimates of pollen numbers per tassels; *Burris (2001)* reports estimates ranging from 14 to 50 million in older varieties (*Miller, 1985*) to 2 to 5 million in modern hybrids. Because of selection for reduced male dominance, the size of an average dent hybrid tassel has declined over the last three decades; this was due to selection for closer spacing that demanded smaller tassel size and reduced pollen production (*Duvick and Cassman, 1999*).

Changes in the tassel size of maize hybrids have been observed since the 1930s as breeders focused on increasing grain yield. Tassel dry weight decreased by 36% from 1967 to 1991, improving light interception by the canopy as well as reducing the demand for carbohydrate resources (*Duvick and Cassman, 1999*). This trend continues. *Mickelson et al. (2002)* described molecular markers for tassel traits. They suggest that the ideal maize genotype would have a smaller tassel at current plant densities, sufficient for reproduction and seed set in a dense field stand. In conclusion, modern hybrids with reduced pollen shed contribute less to cross-pollination but their pollen competition to adventitious pollen is also lower.

The typical tassel may shed pollen for 2 to 14 days depending on the genotype and environmental factors with the major shed occurring for five to eight days beginning on approximately the third day after the tassel is expanded. During the shedding period the pollen is released for approximately four to five hours commencing approximately one hour after sunrise. The period may be delayed by one to two hours if the weather is cool and cloudy (*Burris, 2001*). Similarly, *Jarosz et al. (2003)* reported maize pollen shed for five to eight days, with the major portion of the daily release usually occurring during midmorning to midday. *Treu and Emberlin (2000)* and *Wolt et al. (2003)* also reported that pollen anthesis continues for five to eight days, but under favourable conditions the vast majority of pollen shedding may occur within a two day period. *Paterniani and Stort (1974)*, reported a flowering time of a tropical maize field of about 10 to 15 days.

Maize pollen is released mainly during dry and drying conditions. Furthermore, maize pollen can be released during unsettled atmospheric conditions, preceding fronts or thunderstorms (Aylor, 2003); In experiments in France (Jarosz *et al.*, 2005), pollen release started once the air vapour pressure deficit increased above 0.2 to 0.5 kPa. The dynamic of release was correlated with the dynamic of air vapour pressure deficit surrounding the tassels. The concentration at night did not fall to zero, suggesting that pollen may have been resuspended (Jarosz *et al.*, 2005).

In summary, there is considerable variation in pollen shedding, mostly depending on weather conditions and the developmental stage of the plant.

1.4 Pollination of maize

Wind is the main agent in pollination of maize. Maize pollen grains are one of the heaviest and largest (about 90 – 100 μm in diameter) among the wind-dispersed pollen grains, thus limiting the distance maize pollen can travel (Raynor *et al.* 1972). In a study of settling velocities of various pollen types, Di-Giovanni *et al.* (1995) reported that maize settled nearly ten times faster than other pollen types. Under natural conditions, most of the pollen grains usually fall on the soil surface between the rows. However, it is assumed that a small amount of pollen can be transported over longer distances given favourable wind speeds and appropriate humidity (Kiesselbach, 1949; Garcia *et al.*, 1998). Washout by rain is disadvantageous for maize pollen dispersal. Fresh pollen grains have an internal osmotic potential of about -1.2 Mpa (Westgate and Boyer, 1986). When placed in pure water, they take up water readily until they burst open and become incapable of germination. It is likely that washout by rain can severely limit maize pollen dispersal both by rapidly removing airborne pollen from the air and by preventing germination and fertilization (Aylor *et al.*, 2003). Frequent rainy weather during flowering time can result in badly fertilized ears (Maiskomitee, 2003).

Honeybees collect pollen for feeding and breeding their larvae. They collect them around a distance of 2 km of their beehive. The female flowers of maize are not attractive to honeybees because they produce no nectar. Male and female flowers are spatially separated. During maize pollen collection at the top of the plants the bees usually do not come in contact with the female silks at the middle height of the stalks (Transgen, 2005c). Kohl (1993) reported that honeybees collected pollen in maize fields, even when there were enough possibilities to collect pollen from other plants. During late flowering of maize in mid-July to the end of July, half of the collected pollen originated from *Zea mays* and *Helianthus annuus*. In Germany (Transgen, 2005d) field experiments were conducted on pollen collection by honeybees. When the bee populations were established before the onset of maize flowering, bees did not collect pollen of maize despite direct proximity. They had already looked for more attractive flowers. In conclusion, honeybees usually do not contribute to fertilization and cross-pollination in maize (Transgen, 2005c).

1.5 Pollen viability

In-vitro tests showed that UV-B treatment interferes with flowering (delay), pollen ultrastructure and anther maturation, but there was no effect on pollen germination, regardless of treatment during plant development and/or pollen germination (*Santos et al., 1998*). During the ontogenic development of reproductive organs, pollen grains are enclosed in anthers and it has been demonstrated that anther walls attenuate UV-B radiation by at least 98% (*Flint and Caldwell, 1983*). Maize pollen was found to be much more resistant to UV-C than the relatively resistant conidia of *Venturia inaequalis* and the urediniospores of *Uromyces appendiculatis* (*Aylor, 2004*). Maize plants specifically, were classified as tolerant with respect to this stress condition (*Krupa and Kickert, 1993*).

Because pollen germination appears to be relatively insensitive to solar radiation *Aylor (2004)* concluded that dehydration is the main factor in maize pollen mortality. Several other studies have shown the sensitivity of pollen to dehydration (*Barnabas, 1984; Buitink et al., 1996; Luna et al., 2001*). Water is lost from a pollen grain at a rate that depends mainly on the conductivity of the pollen wall and to the drying power of the atmosphere, which depends on the energy input from the sun and the vapour pressure deficit of the ambient air (*Aylor, 2004*).

Maize pollen can sustain a water loss of up to 80% (*Barnabas, 1984*). According to *Kerhoas et al. (1987)* pollen is shed in partly dehydrated state at anthesis when the water content of maize pollen is 57 to 58%. Maize pollen can withstand drying until a water content of 28% is reached, at which point 60 to 80% of the individuals show a negative reaction in a fluorochromatic viability test. At this water content, there is no more crystallisable water and metabolism decreases, leading to oxidative damage. It is interesting to note that each grain in the pollen population develops independently according to its tolerance to drying.

The water content of maize pollen directly affects its dispersal in the atmosphere through its effect on settling speed and viability. Depending mainly on the vapour pressure deficit of the air, the water status of maize pollen can change from being fully hydrated to being nearly dehydrated within 1 to 4 h (*Aylor, 2003*). Several physical changes take place during drying. For example, the shape of maize pollen changes from a prolate spheroid to a crinkled, prismatic solid, its specific gravity increases by about 16%, and its settling speed decreases by about 34% (*Aylor, 2002*).

Aylor (2004) determined the survival of maize pollen for various periods of exposure to atmospheric conditions outdoors on different days by in vitro germination tests. Initial germination percentages were on average about 35 %. The germination percentage of freshly collected pollen exposed to direct sunlight tended to increase initially, remained relatively high for 60 to 120 min, and then decreased more or less rapidly over the next 60 to 120 min. *Luna et al. (2001)* measured the longevity of pollen exposed outdoors in Mexico in the wintertime

using a complete fertilization criterion. They found a relative loss in pollen viability of 80% in 1 h and a 100% loss in viability in 2 h, and suggested that pollen death was mainly due to dehydration, controlled primarily by the vapour pressure deficit of the air. However, depending on the environmental factors, mainly temperature (*Goss, 1968; Schoper et al., 1987; Jemison and Vayda, 2001*), humidity (*Goss, 1968; Barnabas, 1984; Garcia et al., 1998; Traore et al., 2000; Jemison et Vayda, 2001*) and atmospheric water potential (*Luna et al., 2001*), pollen can remain viable for up to 24 h after shedding. Cool temperatures and high humidity favour pollen longevity.

Barnabas (1984) found that during the dehydration process adhesion, germination percentage and length of the pollen tube decrease substantially. Severe dehydration clearly reduced pollen fitness and not only quantitative, but also qualitative pollen competition could occur.

Pollen that has travelled over long distances will certainly be in disadvantage compared to freshly shed pollen of a field. Water capacity of soil or additional watering could also vary the pollen vitality and competition. Pollen transported in the atmosphere will usually come in contact with cooler air. Also there is higher humidity on higher air layers for rehydration. Clouds show 100% relative humidity. Thus, if hydration is the only limiting factor of viability, pollen could theoretically survive quite long and transport of viable pollen over long distances could be possible.

1.6 Silk viability and time of fertilization

The silks are the styles of the female part of the flower. Every single silk directs the germ tube of a germinating pollen grain to one ovule, which must be fertilized in order for a kernel to develop. Silks emerge from the top of the ear and continue to grow until fertilized. Some reaching lengths of up to 15 cm or more (*Bassetti and Westgate, 1993a*). The spikelets close to the base of the ear produce the longest silks which become the first to protrude from the husk. Silks originating from the base or the middle of the ear emerge next followed by the apical silks. The number of female spikelets per ear varies by genotype and environment but rarely all the spikelets are fertilized and develop into seed. The silks are receptive at emergence and can remain receptive for more than 10 days. Once fertilized the silk stops elongation and desiccates rapidly. If not fertilized, the silk will continue to elongate until it is fertilized or cellular elongation is complete (*Burris, 2001*). Silks follow a well-defined sequence of emergence, elongation and senescence (*Bassetti and Westgate, 1993a*) with a clearly defined window of time for fertilization. Consequently, the ability of pollen grains to fertilize is also depending on a receptive state of the female silks and eggs (*Aylor et al., 2003*).

Senescence of silks started 7 to 8 d after emergence from the husk, as shown by the collapse of the basal region of the silk (*Bassetti and Westgate, 1993b*). Pollen tubes are unable to grow through this senescing area, thus preventing fertilization and contributing significantly to ear barrenness, especially in open pollinated varieties where pollen supply is less limiting.

Water deficit also inhibit the growth of silks, especially during the day (*Herrero and Johnson, 1981; Westgate and Boyer, 1986*). Water stress delays the senescence of recently exposed silks but accelerates senescence in silks that already exposed for several days to the atmosphere (*Bassetti and Westgate, 1993c*). Pollen survival alone is not sufficient for effective gene dispersal since the hydration of the silk and ovules can have a large impact on seed set (*Westgate and Boyer, 1986*). Water deficit has been implicated in damage to the embryo sac of the developing ovary when imposed at the megaspore mother cell development stage, and has resulted in an 80 to 90% reduction in grain weight per plant (*Moss and Downey, 1971*).

The silks themselves do not readily retain pollen unless it is captured by the trichomes (hairs) on the silks. However, on a crop canopy basis the silk area density is very low. Clearly, many of the airborne pollen grains are intercepted by other plant parts or the ground surface and do not reach the intended target (*Aylor et al., 2003*). As pollen grains drift through the maize canopy the vegetative parts can filter most of the pollen from the air, competing with the silks for available pollen. However, fresh maize pollen has a tendency to roll on plant surfaces and can be easy dislodged from a leaf flapping in a breeze. Thus, if pollen lies on surfaces sloping downward toward the leaf tip it can readily roll off the leaf and get a second chance of reaching silks. However, if pollen was deposited on surfaces sloping downward towards the stem, it might become trapped in the leaf axle. When fresh pollen arrives, very little pollen will stick to the silks themselves, but the hairs on the silks are covered with pollen as they are sticky and retain deposited pollen against even the force of an air jet having speeds of 15-30 m s⁻¹ (*Aylor et al., 2003*). Only very small points of a silk are receptive and for this reason a large amount of pollen is necessary to ensure contact to the right parts. Many pollen grains can be deposited on a given silk, but only one will fertilize the ovule to which it is attached; each silk has multiple deposition sites but only a small fertilization site (*Aylor et al., 2003*).

1.7 Anthesis silking interval

When maize is subjected to stress at flowering because of deficits of water, light, nutrients, and sometimes by long photoperiods and lack of thermal adaptation, ear growth slows in relation to tassel growth and the interval between pollen shedding and silk emergence, the anthesis-silking interval (ASI), increases. This appears to be a general response of the plant to a reduction in photosynthates formed during this growth stage (*Edmeades et al., 2000*). If maize cultivars are grown outside their area of thermal adaptation, for example during breeding phases in tropical countries at wintertime, the ASI may increase, probably because of poor adaptation. When cultivars, which were adapted to the cool highlands of Mexico, were grown in hot lowland tropical environments, the ASI increased from 2 to 7 days; conversely, when a lowland tropical population was transferred to the highlands, its ASI increased from 0.3 to 5 days (*Lafitte et al., 1997*). Thus introgression of new, exotic germplasm could result in higher cross-pollination susceptibility. Nitrogen deficiency results in delayed flowering in maize (*Hanway, 1962*) and in

slow silk emergence from distal spikelets (*Lemcoff and Loomis, 1994*). When drought occurs at flowering, silking is delayed though anthesis and pollen dehiscence may be accelerated slightly by the higher air temperatures and lower relative humidity (*Srinivasan et al., 1997*). *Herrero and Johnson (1981)* observed that drought during silking had a greater effect on female than on male floral development. Silk delay at high plant densities has been known for a long time (*Kohnke and Miles, 1951; Lang et al., 1956*).

The anthesis silking interval (ASI) can be observed on an individual plant basis but at the plot level ASI is usually measured as the date when 50% of plants have visible silks minus the date when 50% of plants first extrude anthers. The ASI is an ubiquitous indicator of the level of stress under which the crop is growing at flowering and a good predictor of grain yield and barrenness under stress (*Edmeades et al., 2000*).

The adverse effects of drought at flowering are well known for many years. *Lonnquist and Jugenheimer (1943)* observed that tassels exposed to heat and drought resulted in poorly pollinated ears. *Kiesselbach (1950)* reported silk delay in response to drought. *Sadras et al. (1985)* determined that the period during which silks are exposed to pollen may condition final kernel set. According to *Schussler and Westgate (1991)*, severe water stress occurring during silking decreased grain number by up to 99%. This was explained by the fact that in maize the number of kernels is mainly related to the current flux of assimilates around silking that is negatively influenced by stress (*Edmeades and Daynard, 1979*). Early attempts to quantify the relationship between ASI and grain yield were made by *DuPlessis and Dijkhuis (1967)*, who reported an 82% decline in grain yield as the ASI increased from 0 to 28 days under drought stress (*DuPlessis and Dijkhuis, 1967*).

Detailed studies by Hall and co-workers in Argentina (*Hall et al., 1980, 1981, 1982*) indicated that lack of pollen for late-emerging silks can be a significant cause of barrenness. For individual plants of different cultivars *Hall et al. (1982)* reported a pollen shed period that varied from 7 to 9 days. In a stand of a modern hybrid, pollen shedding has been shown to occur over a period of 16 days with 90% occurring over a period of around 10 days when pollen shed density exceeds 100 grains cm⁻² d⁻¹, an important pollen density for successful fertilization. At the peak of pollen production, more than 500 pollen grains cm⁻² d⁻¹ are shed (*Bassetti and Westgate, 1994*); grain set declines when pollen shed density falls below 100 grains cm⁻² d⁻¹, in agreement with *Sadras et al. (1985)*.

Bassetti and Westgate (1994) reported that grain set in a maize crop declines because of pollen supply when the ASI was < -5 or > 5 days. Further losses could be expected when silks are exposed for >6 days because of silk senescence (*Bassetti and Westgate, 1993b*). When ASI exceeded 7 days in individual plants, grain set was limited by both pollen availability and silk exposure at the ear tip and a typical pattern emerged where grain set is restricted to the midbase of the ear and tips show barrenness. So under stress with increased ASI, there is a small period of increased susceptibility to cross-pollinations.

Modern breeding efforts have tended to reduce protandry (floral asynchrony) and the tassel size steadily over the last three decades. To compensate for this in the production of pure hybrid seed it is necessary to increase the size of the border rows and the number of male plants (*Burris, 2001*). As mentioned above, a larger tassel size may be particularly important under stress environments when pollen availability for silks is often reduced (*DuPlessis and Dijkhuis, 1967*). By selecting for tassel traits, breeders must balance the shading effect of the tassel with the need for an adequate pollen supply, particularly in stress inducing environments where silking is delayed.

Hall et al. (1982) suggested to use a mix of hybrids, including a late pollinator to reduce the negative effects of a lack of pollen. Although these studies imply that a lack of pollen is the primary cause of reduced kernel set under stress conditions, the addition of viable pollen to late-appearing silks does not guarantee an increase in final kernel number (*Otegui et al., 1995*). Pollinated ovaries of water-stressed plants are fertilized, but kernel abortion occurs after a few cellular divisions (*Westgate and Boyer, 1986*). *Bassetti and Westgate (1993a)* reported a decrease in kernel number caused by the loss of silk receptivity during water deficit, whereas *Otegui et al. (1995)* described this phenomenon with respect to kernel abortion. *Westgate and Boyer (1986)*, addressing effects of water stress on the ASI grain yield relationship, proposed that these causes could be grouped as a combination of several effects. Lack of pollen can be due to asynchrony, nonviability or because anthers do not exert. Damage to the embryo sac during megasporogenesis will usually prevent pollination even though silking may still occur (*Moss and Downey, 1971*). A slow rate of spikelet growth results in a large ASI, silk senescence and abortion following pollination. *Westgate and Boyer (1986)* concluded that the reduction in grain number per plant under drought is almost always due to a combination of pollen supply and spikelet fitness.

Water deficit during silking reduces elongation (*Bassetti and Westgate, 1993d*) and promotes asynchrony between pollen shedding and silk appearance (*Hall et al., 1981, 1982*), which may result in insufficient pollen availability for late-appearing silks from the tip of the ear. Thus, the tip of the ear is most susceptible to cross-pollination by foreign pollen.

These facts have consequences for cross-pollination. Whenever there is a perfect temporal alignment of pollen shed and silk emergence in a field, pollen grains from local sources greatly outnumber (and out-compete) pollen from adventitious sources. On the other hand, if there is imperfect temporal congruence of pollen release and silk receptivity there is an increased opportunity for pollen from outside sources to fertilize the ear. Adverse environmental conditions, such as drought, can alter the timing of silk emergence in relation to pollen shed, resulting in poor fertilization (*Hall et al., 1982; Bruce et al., 2002;*), giving pollen from a distant field a greater competitive advantage than is usually expected (*Aylor et al., 2003*). The importance of synchronous flowering in avoiding outcrossing in seed production is summed up in an old maize breeders adage: „If you have a perfect nick, then spatial isolation is easy,

however, if you have an imperfect nick, then you can never have enough spatial isolation“ (Aylor *et al.*, 2003).

1.8 Outline of the thesis

Although there are different maize cultivation systems with different consequences for cross-pollination, the thesis will focus on the most common system of growing maize for silage or grain harvest. Some results on cross-pollination are already available, but no study has been carried out so far about cross-pollination under conditions of alpine regions. It was assumed that the special climatic conditions, topography and landscape of Switzerland could result in different cross-pollination patterns.

In Switzerland, large scale field experiments with transgenic plants are not desirable today. Successful preliminary studies with different kernel colour markers, to simulate transgenic cross-pollination, were carried out. With a broad series of different field experiments at different locations the fluctuation margin of cross-pollination was to be examined and important factors of influence to be identified in order to develop data sets that allow for a more rational discussion about threshold levels.

1.9 REFERENCES

- Ahmed E. (2002) Detection of genetically modified organisms in foods. *Trends Biotechnol.* Vol. 20, No. 5, 215-223.
- Aylor D.E. (2002) Settling speed of corn (*Zea mays*) pollen. *Journal of Aerosol Science.* 33: 1599-1605.
- Aylor D.E. (2003) Rate of dehydration of corn (*Zea mays* L.) pollen in the air. *J. Exp. Bot.* Vol. 54. No. 391:2307-2312.
- Aylor D.E. (2004) Survival of maize (*Zea mays*) pollen exposed in the atmosphere. *Agric. For. Meteorol.* 123:125-133
- Bangerth F. (1989) Dominance among fruit/sinks and the search for a correlative signal. *Physiol. Plant.* 76:608-614
- Barnabas B. (1984) Effect of water loss on germination ability of maize (*Zea mays* L.) pollen. *Ann. Bot.* 55:201-204.
- Bassetti P. and Westgate M.E. (1993a) Water deficit affects receptivity of maize silks. *Crop Sci.* 33:279-282
- Bassetti P. and M.E. Westgate. (1993b) Senescence and receptivity of maize silks. *Crop Sci.* 33:275-278.

Bassetti P. and Westgate M.E. (1993c) Water deficit affects receptivity of maize silks. *Crop Sci.* 33:279-282

Bassetti P. and Westgate M.E. (1993d) Emergence, elongation, and senescence of maize silks. *Crop Sci.* 33:271-275

Bassetti, P. and M.E. Westgate. (1994) Floral asynchrony and kernel set in maize quantified by image analysis. *Agron. J.* 86:699-703.

Biosicherheit (2005a) Gentechnik Insektenresistenz: Mit den Waffen von Bakterien gegen Fraßinsekten. <http://www.biosicherheit.de/mais/146.doku.html>

Biosicherheit (2005b) 81 Millionen Hektar: Zuwachs für gv-Pflanzen weiter ungebrochen. <http://www.transgen.de/gentechnik/pflanzenbau/531.doku.html>

Bruce W.B., Edmeades G.O., Barker T.C. (2002) Molecular and physiological approaches to maize improvement for drought tolerance. *J. Exp. Bot.* 53:13-25

Buitink J., Walters-Vertucci C., Hoekstra F.A., Leprince O. (1996) Calorimetric properties of dehydrating pollen: analysis of a desiccation-tolerant and intolerant species. *Plant Physiol.* 111:235-242.

Burris. J.S. (2001) Adventitious pollen intrusion into hybrid maize seed production fields. *Proc. 56th Annual Corn and Sorghum Research Conference 2001.* American Seed Trade Association, Inc., Washington, DC.

Choices. (2003) International approaches to labelling genetically modified foods. <http://www.choicesmagazine.org/2003-2/2003-2-01.htm>. The magazine of food, farm, and resource issues.

Di-Giovanni, Kevan F.P.G., Nasr M.E. (1995) The variability in settling velocities of some pollen and spores. *Grana.* 34:39-44

DuPlessis D.P. and Dijkhuis F.J. (1967) The inheritance of lag time between pollen shedding and silking on the yield of maize. *S. Afr. J. Agric. Sci.* 10:667-674

Duvick D.N. (1992) Genetic contributions to advances in yield of U.S. maize. *Maydica* 37: 69-79

Duvick D.N. and Cassman K.G. (1999) Post-green revolution trends in yield potential of temperate maize in the North-Central United States. *Crop Sci.* 39:1622-1630

Edmeades G.O. and Daynard T.B. (1979) The relationship between final yield and photosynthesis at flowering in individual maize plants. *Can. J. Plant Sci.* 59:585-601

Edmeades, G.O., Bolanos J., Elings A., Ribaut J.-M., Bänziger M. (2000) The role and regulation of the anthesis-silking interval in maize. In: M.E. Westgate and K.J. Boote (ed.) Physiology and modeling kernel set in maize. ASA and CSSA, Madison, WI.

EurLex. (2003) Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. <http://europa.eu.int/eur-lex/lex/LexUriServ/LexUriServ.do?uri=CELEX:32003R1829:EN:HTML>

Flint S.D. and Caldwell M.M. (1983) Influence of floral optical properties on the ultraviolet radiation environment of pollen. *Am. J. Bot.* 70:1416-1419

Garcia M.C., Figueroa M.J., Gomez L.R., Townsend R., and Schoper J. (1998) Pollen control during transgenic hybrid maize development in Mexico. *Crop Sci.* 38:1597-1602

GKB. (2001) Konservierende Bodenbearbeitung. <http://www.gkb-ev.de> Gesellschaft für konservierende Bodenbearbeitung

Goss J.A. (1968) Development, physiology and biochemistry of corn and wheat pollen. *Bot. Rev.* 34:333-358

Hall A.J., Ginzo H.D., Lemcoff J.H., Soriano A. (1980) Influence of drought during pollen-shedding on flowering, growth and yield of maize. *J. Agron. Crop Sci.* 149:287-298.

Hall A.J., Lemcoff J.H. and Trapani N. (1981) Water stress before and during flowering in maize and its effects on yield, its components, and their determinants. *Maydica.* 26:19-38

Hall A.J., Vilella F., Trapani N., Chimenti C. (1982) The effect of water stress and genotype on the dynamics of pollen shedding and silking in maize. *Field Crops Res.* 5:349-363

Hanway, J.J. (1962) Corn growth and composition in relation to soil fertility. I. Growth of different plant parts and relation between leaf weight and grain yield. *Agron. J.* 54:145-148.

Harris R.E., Moll R.H., Stuber C.W. (1976) Control and inheritance of prolificacy in maize. *Crop Sci.* 16:843-850

Herrero M.P. and Johnson R.R. (1981) Drought stress and its effects on maize reproductive systems. *Crop Sci.* 21:105-110

Hütter E., Bigler F., Fried P.M. (2000) Verwendung transgener schädlingsresistenter Nutzpflanzen in der Schweiz. Schriftenreihe Umwelt Nr. 317. Bundesamt für Umwelt, Wald und Landschaft, Bern, p.161

Jarosz N., Loubet B., Durand B., Foueillassar X., Huber L. (2005) Variations in maize pollen emission and deposition in relation to microclimate. *Environ. Sci. Technol.* 39:4377-4384

- Jarosz N., Loubet B., Durand B., McCartney H.A., Foueillassar X., Huber L. (2003) Field measurements of airborne concentration and deposition rate of maize pollen (*Zea mays* L.) downwind of an experimental field plot. *Agric. For. Meteorol.* 119:37-51
- Jemison, J.M., Jr., and Vayda M.E. (2001) Cross-pollination from genetically engineered maize: Wind transport and seed source. *AgBioForum.* 4:87-92
- Jones M.D. and Newell L.C. (1948) Longevity of pollen and stigmas of grasses: Buffalograss, *Buchloe dactyloedes* Engelm., and corn, *Zea mays* L. *J. Am. Soc. Agron.* 40(3):195-204
- Kerhoas C., Gay G., Dumas C. (1987) A multidisciplinary approach to the study of the plasma membrane of *Zea mays* pollen L. during controlled dehydration. *Planta* 171:1-10
- Kiesselbach T.A. (1949) The structure and reproduction of corn. *Research Bull.* P161. *Agric. Exp. Stn. Univ. Nebraska, Lincoln, NE.*
- Kiesselbach T.A. (1950) Progressive development and seasonal variations of the corn crop. *Nebraska Agric. Exp. Stn. Bull.* 166.
- Kohl A. (1993) Das Pollensammelverhalten der Honigbiene (*Apis mellifera* L.) im Naturschutzgebiet Taubergießen (Oberrhein, Südwestdeutschland). *Zeitschrift für Ökologie und Naturschutz.* 2:163-169
- Kohnke H. and Miles S.R. (1951) Rates and patterns of seeding corn on high fertility land. *Agron. J.* 43:488-493.
- Krupa S.V. and Kickert R.N. (1993) The greenhouse effect: the impacts of carbon dioxide (CO₂), ultraviolet-B (UV-B) radiation and ozone (O₃) on vegetation (crops). *Vegetatio.* 104/105:223-238
- Lafitte H.R., Edmeades G.O., Johnson E.C. (1997) Temperature responses of tropical maize cultivars selected for broad adaption. *Field Crops Res.* 49:215-229.
- Lang, A.L., Pendleton J.W., Dungan G.H. (1956) Influence of population and nitrogen levels on yield and protein and oil contents of nine corn hybrids. *Agron. J.* 48:284-289.
- Lemcoff J.H. and Loomis R.S. (1994) Nitrogen and density influences on silk emergence, endosperm development, and grain yield in maize (*Zea mays* L.). *Field Crops Res.* 38:63-72.
- Lonnquist J.H. and Jugenheimer R.W. (1943) Factors affecting the success of pollination in corn. *J. Am. Soc. Agron.* 35:923-933.
- Luna V.S., Figueroa M.J., Baltazar M.B., Gomez L.R, Townsend R., Schoper J.B. (2001) Maize pollen longevity and distance isolation requirements for effective pollen control. *Crop Sci.* 41:1551-1557.

Ma B.L., Subedi K.D., Reid L.M. (2004) Extend of Cross-fertilization in maize by pollen from neighboring transgenic hybrids. *Crop Sci.* 44:1273-1282

Maiskomitee. 2003. Fachinfo Morphologie. http://www.maiskomitee.de/fb_fachinfo/02_01_02_04.htm. Deutsches Maiskomitee e.v.

Mickelson S.M., Stuber C.S., Senior L., Kaeppler S.M. (2002) Quantitative trait loci controlling leaf and tassel traits in a B73 x Mo17 population of maize. *Crop Sci.* 42:1902-1909

Miller P.D. (1985) Maize pollen: Collection and enzymology. Chapter 45. pp.279-282. In: Sheridan W.F. (ed.). 1985. Maize for biological research. A special publication of the plant molecular biology association, USA.

Moss G.I. and Downey L.A. (1971) Influence of drought stress on female gametophyte development in corn (*Zea mays* L.) and subsequent grain yield. *Crop Sci.* 11:368-372.

Odenbach, W. (1997) *Biologische Grundlagen der Pflanzenzüchtung*. Parey, Berlin. p. 1-4

Otegui M.E., Andrade F.H., Suero E.E. (1995) Growth, water use, and kernel abortion of maize subjected to drought at silking. *Field Crops Res.* 40:87-94

Paterniani E. and Stort A.C. (1974) Effective maize pollen dispersal in the field. *Euphytica.* 23:129-134

Poehlman J.M. and Sleper D.A. (1995) *Breeding field crops*. Iowa State University Press, Ames. XV, 494 pp.

Raynor G.S., Ogden E.C., Hays J.V. (1972) Dispersion and deposition of corn pollen from experimental sources. *Agron. J.* 64:420-427

Sadras V.O., Hall A.J., Schlichter T.M. (1985) Kernel set of the uppermost ear in maize: I. Quantification of some aspects of floral biology. *Maydica.* 30:37-47

Santos A., Almeida J.M., Santos I., Salema R. (1998) Biochemical and Ultrastructural Changes in Pollen of *Zea mays* L. Grown Under Enhanced UV-B Radiation. *Ann. Bot.* 82: 641-645

Sanvido O., Widmer F., Winzeler M., Streit B., Szerencsits E., Bigler F. (2005) Koexistenz einer Landwirtschaft mit und ohne Gentechnik. *Agrarforschung.* 12(4):140-145

Schooper J.B., Lambert R.J., Vasilas B.L. (1987) Pollen viability, pollen shedding, and combining ability for tassel heat tolerance in maize. *Crop Sci.* 27:27-31

Schussler J.R. and Westgate M.E. (1991) Maize kernel set at low water potential: II. Sensitivity to reduced assimilates at pollination. *Crop Sci.* 31:1196-1203

Srinivasan, G., Bänziger M., Edmeades G.O., Lothrop J.E., Torres J.L. (1997) Identification of drought tolerance in elite tropical highland maize germplasm. p. 308-312. In: G.O. Edmeades et al. (ed). Developing drought and low-N tolerant maize. CIMMYT, El Batan, Mexico.

Tollenaar M. (1977) Sink-source relationships during reproductive development in maize: A review. *Maydica*. 23:49-75.

Transgen. (2005a) Anbau gv-Pflanzen: Globaler Anstieg, Stagnation in Europa. <http://www.transgen.de/gentechnik/pflanzenbau>

Transgen. (2005b) Kennzeichnung und Zulassung in der Schweiz: Deklaration nun wie in der EU. <http://www.transgen.de/recht/kennzeichnung/281.doku.html>

Transgen. (2005c) BT-Mais: Pollen im Honig. <http://www.transgen.de/einkauf/lebensmittel/208.doku.html>

Transgen. (2005d) Begleitforschung Bayern: GV-Maispollen im Honig kaum nachweisbar. <http://www.transgen.de/erprobungsanbau/begleitforschung/554.doku.html>

Traore S.B., Carlson R.E., Pilcher C.D., Rice M. (2000) Bt and non-Bt maize growth and development as affected by temperature and drought. *Agron. J.* 92:1027-1035

Treu R., Emberlin J. (2000) Pollen dispersal in the crops maize (*Zea mays*), oil seed rape (*Brassica napus* spp. *oleifera*), potatoes (*Solanum tuberosum*), sugar beet (*Beta vulgaris* ssp. *vulgaris*) and wheat (*Triticum aestivum*). Report for the soil association, National pollen research unit, University college Worcester, UK

Westgate M.E. and Boyer J.S. (1986) Reproduction at low silk and pollen water potentials in maize. *Crop Sci.* 26:951-956.

Wolt J.D., Peterson R.K.D., Bystrak P., Meade T. (2003) A screening level approach for nontarget risk assessment: transgenic Bt corn pollen and the monarch butterfly (*Lepidoptera: Danaidae*).- *Environ. Entomol.* 32:237-246

Zscheischler J., Estler M. C., Staudacher W., Groß F., Burgstaller G., Streyl H., Rechmann T. (1990) *Handbuch Mais*. p. 16-18. DLG-Verlags-GmbH, Frankfurt am Main.

2 Measurement of pollen dispersal in maize by grain colour markers

2.1 Methods for measuring pollen dispersal

There are several methods established for measuring real or simulated transgenic cross-pollination in maize. Most common are PCR detection, germination assay of herbicide resistant maize, visual markers like waxy and sugary grain texture or grain colour markers.

PCR detection assays are based on the parallel amplification of transgenic specific sequences and an endogenous reference gene, which serves as an internal control for the quality of the extracted DNA. After amplification, the total amount of target DNA present in the sample can be determined (*Hernandez et al., 2003*). The germination assay of herbicide resistance, that is sometimes used in combination with PCR detection, is based on transgenic herbicide resistant plants as pollen donors. If pollen from these fields fertilizes plants in a conventional field the grains will bear this transgenic information. When progenies from the latter are sown, only seedlings with transgenic herbicide resistance will survive after herbicide treatment. Counting the rate of dead and surviving seedlings will show the cross-pollination rate (*Biosicherheit, 2002*). Visual detection of cross-pollination on waxy maize is a possible tool, because conventional pollen cross-pollinating on waxy maize can be recognized by a simple colour test practised directly on the grains of the ear (*Fouellassar and Fabié, 2005*). Direct visual detection would be possible by using sweet corn maize as the pollen receptor if varieties of suitable vigour are available.

Another approach is also based on xenia phenomenon: Transmission of grain colour. In the case of dominance, the grain colour is characteristic of the pollen parent after fertilization. In maize, the seed coat is usually transparent and is determined by the genotype of the mother plant, whereas pigmentation occurs in the aleurone tissue and is thus determined by the genotype of both parents. In the case of dominance the male gene controls the colour over two copies of the female parent (*Coe, 2001*). Grain colours caused by pigmentation in the aleurone tissue of endosperm are easy to observe and can be used as markers in genetic studies or tools for evaluating cross-fertilization. The colours black, blue and red are based on anthocyan pigments, yellow is due to carotenoids, and white is the lack of any pigmentation. The system of dominant blue colour was used rarely (*Byrne and Fromherz 2003*), probably because of the complex genetics, instabilities of blue colour inheritance and lack of suitable hybrids for experiments in temperate conditions. But the system of dominant yellow pigmentation by pollen transfer on white grain maize is well known from a number of experiments on cross-pollination so far (*Salamov, 1940; Jones and Newell, 1948; Jones and Brooks, 1950; Hutchcroft, 1958; Raynor et al., 1972; Paterniani and Stort, 1974; Garcia et al., 1998; Ma et al., 2004; Stevens et al., 2004*). In early years this system was used to investigate cross-pollination in seed production to improve seed purity. Today the system has been rediscovered in determination of cross-

pollination of transgenic maize.

The production of white maize worldwide is estimated to be about 65 to 70 million tons, representing 12 to 13% of the annual world output of all maize. However, in Switzerland commodity maize is uniformly of yellow grain colour. In this study, yellow grain hybrids act as dominant pollen donors to white grain hybrids, which are the recessive receptors. The yellow grain hybrids simulate transgenic hybrids and the white grain hybrid the conventional hybrid. When pollen of yellow grain hybrids fertilizes in a white grain hybrid the simulated transgenic contamination can be measured by simply counting the number of yellow grains on white grain ears. This is a great advantage compared to other methods, especially expensive PCR analysis, and enables a high number of samples. Thus, it is possible to gain knowledge of potential transgenic cross-pollination without using transgenic plants.

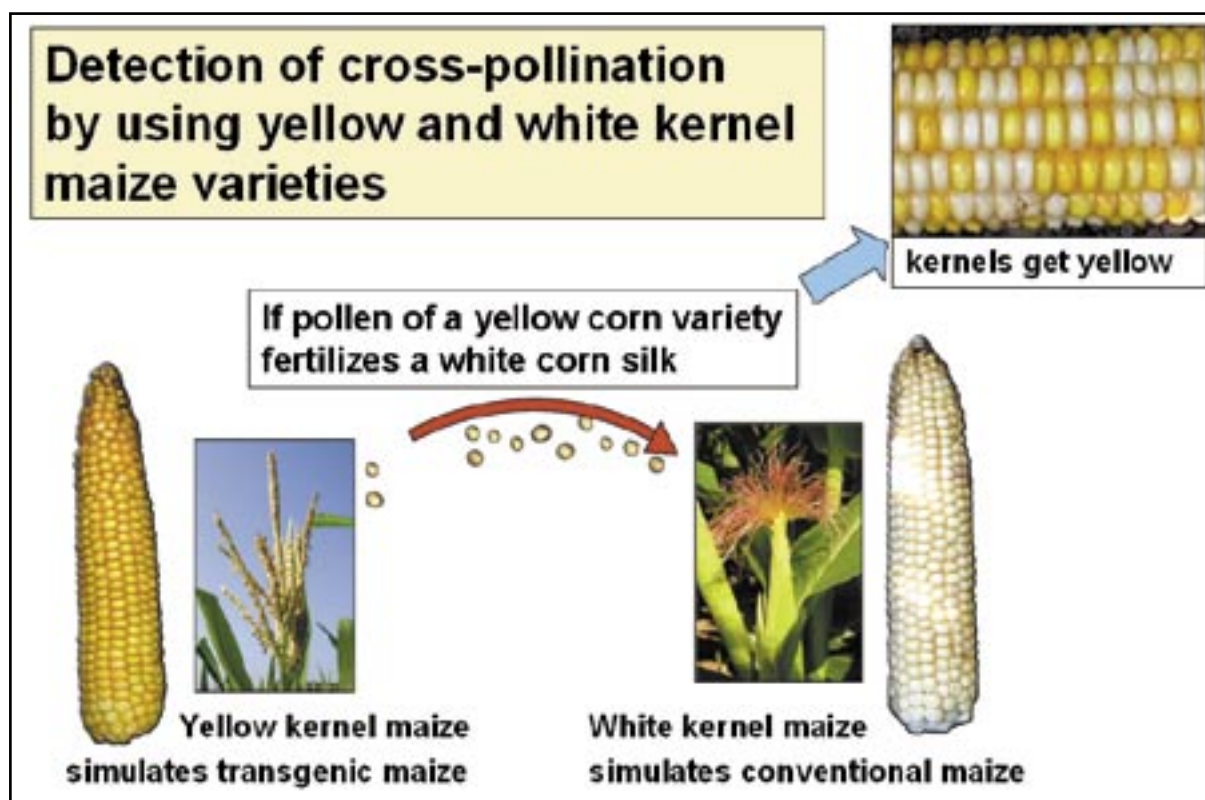


Figure 2.1: System of cross-pollination detection by use of yellow and white grain maize

2.2 Experimental set-ups with white grain maize

A well suitable white grain maize test hybrid, DSP17007, was bred by Delley Seeds and Plants (DSP) in Switzerland and was first tested by *Weingartner et al. (2002)*. It is medium early for central European conditions and the yield level is close to modern maize hybrids in this class. Given the fact that farmers usually cultivate hybrids that are homozygous for yellow grain colour, patterns of pollen dispersal can be studied in a network approach with farmers who agree to grow maize in desirable combinations with white maize test hybrids in selected field situations. Thus, different geographical situations can be chosen and analyzed. Sowing in a

particular region should be done preferably in collaboration with the local contractor in order to ensure a sufficient control on complete cleaning of the machine before a new colour type is sown; this is one of the crucial error factors.

An important decision is how and how much to sample. Sample sizes must be big enough to detect contaminants at a predetermined expected threshold of cross-pollination. Ultimately, the optimum sampling strategy is a balance between sensitivity, cost and confidence (*Ahmed, 2002*). For pioneering scientific test evaluations one can go back to the technically highest possible sample number according to field size, available manpower and harvest time period. The number of samples can be limited by the short period of time available for harvest when cooperating with farmers, especially in the case of silage maize as the grain needs some maturation before yellow grains can be distinguished from white grains but harvest will be earlier than for grain use.

To calculate the possible maximum number of samples it has to be considered how many fields will be sampled, their total size, the quota of fodder maize with only a short period of time for harvest and the number of man hours available during this period. A technical solution to optimizing the logistics may be to sample and store only ears with more than about ten yellow grains per ear; ears with fewer yellow grains can be preferably counted in the field. This enables to check up high numbers of ears per field to determine dispersal patterns when high spatial resolution is required.

2.3 Average grain number per ear and sample number

The calculation of the cross-pollination rate is based on average grain number of the field. There are several factors that influence the final grain number of an ear that can lead to a considerable variability between ears of the same field. In maize, the number of potential grain ovules is established early in plant development and can be affected by carbon and nitrogen supply, while the number of ovules that ultimately develop into mature grains is affected by the degree of pollination and extent of grain abortion (*Below et al., 2000*). Under optimum conditions, grain sink capacity and number of grains are genetically determined (*Jones et al., 1996*). In general, small-grain hybrids (grain weight < 300 mg) have a larger grain number than large-grain hybrids (*Otegui and Andrade, 2000*). The actual grain sink capacity under field conditions is clearly affected by environmental perturbations such as high temperature and water deprivation (*Jones et al., 1996*). Presumably, starch content in maize ovaries supports reproductive growth and ovary starch levels are associated with final grain number (*Zinselmeier et al., 1985*). Furthermore *Schussler and Westgate (1995)* reported that assimilate flux plays a key role during the critical stage of reproductive development. Stress factors that limit assimilate flux will reduce final grain number. Water deficit around anthesis (*Westgate and Boyer, 1985; Schussler and Westgate, 1991; Ober et al., 1991; Quattar et al., 1987*), weed stress (*Tollenaar et al., 1997*) and inadequate nitrogen supply (*Mozafar, 1990; Uhart and Andrade, 1995*) will

result in grain losses.

This response to stress is especially strong during a relatively narrow period of time from early ear-shoot growth prior to pollination through the early period after pollination. In contrast, induction of storage product accumulation and later phases of grain filling are relatively insensitive to stress (*Ober et al., 1991; Quattar et al., 1987*). *Grant et al. (1989)* reported that the interval during which grain number was sensitive to moisture stress began 2 to 7 days after silking and ended 16 to 22 days after silking. *Tollenaar (1977)* reported that grain number is determined during a period ranging from approximately 10 days before until 15 days after anthesis.

The main advantage of the use of white grain colour marker is the fast assessment of yellow grains on an ear; their number will be mostly low when the experimental conditions are targeted to low contamination thresholds like 0.9%. The superiority of the system could not be maintained if the total grain number of each ear had to be counted. Therefore a representative sampling pattern has to be devised for the whole field or field parts, in the case of large fields, to calculate an average grain number per ear. Depending on the homogeneity of a field stand, the number of sampled ears per area unit will vary. On this average grain number per ear the percentage of the outcrossing can be based.

2.4 Sample size and detection probability

Sample sizes must be big enough to detect contaminants at the expected threshold. Too small sample sizes or improper sampling technique can result in “jackpot” distributions of positive and false negative results that will not represent the real situation (*Anklam et al, 2002*).

The USA Department of Agriculture provides a formula to determine sample size for single sample qualitative testing (*USDA, 2000*). Given the desired lot concentration and probability of detection, a sample size is computed with the following formula:

$$N = \log [1-(G/100)/\log(1-(P/100))]$$

N: sample size (number of grains)

G: probability in percent of rejecting a lot concentration

P: Percent concentration in the lot

The relationship between sample size and the probability of detection is exponential, so that the sample size would have to be increased significantly to increase the detection limit and probability. The Ministry of Agriculture and Forestry (MAF) of New Zealand shows this relationship in an example (table 2.1) of detecting GM (genetic modified) seeds (*MAF, 2002*).

2 - Measurement of pollen dispersal

Sample size Grain number (~g)	Chance of detecting 0.1% GM	Chance of detecting 0.05% GM	Chance of detecting 0.01% GM
1400 (~500)	75 %	50 %	13 %
3200 (~1000)	95 %	79 %	27 %
6400 (~2000)	99 %	95 %	47 %
10 000 (~3000)	99 %	98 %	63 %

Table 2.1: Interplay of sample size, detection limit and detection probability

To detect contamination of 0.1% GM a probability of 75% can be achieved by 1,400 grains, but for detection of 0.01 % sampling 10,000 grains would achieve only 63% probability (*MAF, 2002*). If the detection confidence is sought 95% at 0.1% contamination, then seed samples should be bigger than 4,300 seeds and for 99% confidence bigger than 6,600 (*Heinemann et al., 2004*). Very low concentrations of GM contaminations are always difficult to detect. For example to detect a 0.01% percent lot concentration with a 99% probability the required sample size is 46,050 grains. Increasing detection probability on low concentrations of GM contamination will increase sample size in a way it is not able to handle any more. Thus, compromise must be made between detection probability and sample size handling (*USDA, 2000*).

Inhomogeneous variation found in field by checking the ears can decline during harvest, transport and storage due to different mixing processes to more homogeneous variation. If in a PCR check of a maize harvest the raw materials are not systematically mixed during harvest and storage, the resulting strata can seriously invalidate assumptions associated with simple random sampling (*Anklam et al., 2002*). This raises the additional issue of determining the number of samples (of the size recommended in the calculations described above) to quantify confidence limits associated with any heterogeneity because of incomplete mixing (*Heinemann et al., 2004*). However, it can be expected that heterogeneous cross-pollinations in fields get more and more homogenous due to the different procedures starting from harvest to the milling of large numbers of seeds for PCR analysis or product processing at the end, according to Boltzmanns law of physical entropy of all mixed matter.

2.5 Evaluation of the colour marker system

In the present study the formula (*USDA, 2002*) must be transformed to check which detection threshold is obtained on a probability of 95%, the standard discussed for seed control, according to recommendation of a tolerable 5% risk for consumers by the EU expert group of seed experts (*Kruse, 2004*).

2 - Measurement of pollen dispersal

$$P = - (10^{\lceil \lg(1-(G/100)) \rceil / N} - 1) * 100$$

- P: detectable cross-pollination concentration in lot
 N: sample size (number of grains)
 G: probability in percent of rejecting a lot concentration

Distribution of cross-pollination is different in short distance (distances up to 50 m) in comparison to a long distance (distances greater than 50 m). On short distances there are strong gradients (Figure 2.2) of cross-pollination related to distance but on long distance cross-pollinations are more homogeneously distributed (Figure 2.3), probably as a result of a kind of “fall-out” of a “pollen-cloud” above the field.

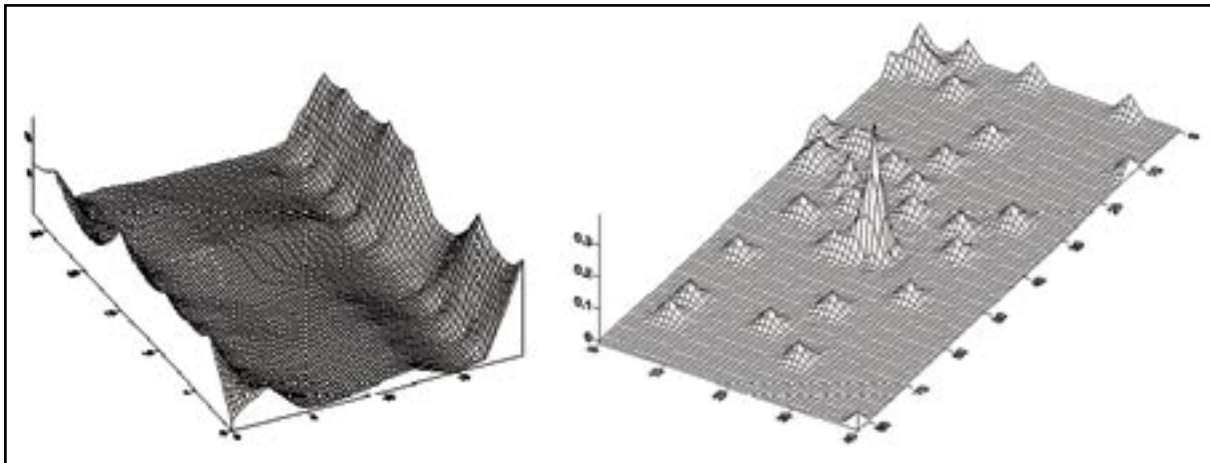


Fig.2.2 (left): Short distance cross-pollination: Strong gradients

Fig.2.3 (right): Long distance cross-pollination: Homogeneous distribution

Such features have to be considered for calculation of detection thresholds in a different way for short distance and long distance. For long distances the calculations can relate to the whole field but for short distances the calculations can only relate to a certain distance within the gradient of similar cross-pollination levels.

For example, calculating the detection threshold for a long-distance experiment (P_{LD}) with distances above 50 m was based with the following assumptions: homogeneous distribution of cross-pollination (Figure 2.3) and number of sampled ears in a one ha field; 1000 ear samples per field with an average of 315 grains per ear resulting in 315,000 grain samples.

$$P_{LD} = - (10^{\lceil \log(1-(95/100)) \rceil / 315000} - 1) * 100 = 0.00095$$

2 - Measurement of pollen dispersal

According to the formula above a homogeneous cross-pollination of about 0.001 % can be detected with a probability of 95%.

Detection thresholds in short-distance experiments (P_{SD}) would be principally the same as in long-distance experiments. However, at short distance the distribution of cross-pollinations is more heterogeneous with strong gradients (Figure 2.2). For calculation of detection threshold only samples within similar distances to a foreign pollen source are representative for the cross-pollination in this area. Based on the assumptions of a row with 100 sampled ears and an average ear grain number of 315, resulting in 31,500 investigated grains, detection threshold can be calculated.

$$P_{LD} = - (10^{[\log(1-(95/100)]/31500} - 1)) * 100 = 0.0095$$

With the described sample system there would be with a probability of 95% a detection threshold for whole field of 0.001 % cross-pollination and for a single row, with a certain distance, a detection threshold of about 0.01 %, which is quite sufficient for a scientific in depth analysis of cross-pollination rates at short distance.

2.6 Comparison of colour marker results to PCR analysis results

For a comparison of cross-pollination values it has to be considered that PCR analysis methods and the GM background itself can both be very heterogeneous. There are different copy numbers of transgenes in different GM hybrids. For comparison often calculation factors are needed. Transgenic events are generated in maize lines selected for high transformation rates and other interesting traits for laboratory use and manipulation. Once a transformed plant is obtained, the transgenic locus is transferred by back-crosses to different existing lines. Thus, one transformation event can be introduced into many cultivars. In seed production the crossing of a homozygous transgenic parent with a non-transgenic parent results in heterozygous hybrids for the gene of interest (*Trifa and Zhang, 2004; Vaughn et al., 2005*). For Bt11 and Mon810 hybrids, the two main types of present BT maize, the cry1Ab gene for BT Toxin is expected in only 50% of the pollen and egg cells from heterozygous parents (*Chilcutt and Tabashnik, 2004*). As yellow hybrids are usually homozygous to yellow colour, 100% of the pollen will deliver yellow colour information on the white grain. For a comparison of transgenic outcrosses with the simulated outcrosses by grain colour, the latter results have to be halved.

Maize hybrids with more than one transgene exist already, e.g. herbicide tolerance or different BT proteins affecting different pest (*Vaughn et al., 2005*). For these transgenic hybrids the probability of transgenes in their pollen has to be calculated on an individual basis, according to the number of transgenic copies in the parental lines.

2.7 Analytical error of PCR and grain colour marker assessments

The analytical uncertainty of quantitative PCR methods is generally assumed to be around 25% for the best PCR methods available. It is difficult to estimate the uncertainty introduced by variation between different cultivars, harvests and grains (*Holst-Jensen et al., 2003*). During the German „Erprobungsanbau“ in 2004 a comparison of four certified laboratories was done. At low levels of GM contents results were similar but in the critical range around 0.9% there was a variation of sometimes more than factor two of the true value (*Weber et al., 2005*).

By using the grain colour marker system, contamination plants can have an impact but if the seed production has been done at sufficient care and the sowing procedures are sufficiently controlled the impact should be small; therefore this method is simple and robust. Errors do not interfere on a large scale. But in comparison with PCR analysed data the possible percentage deviation of the values and thus the range of values have to be kept in mind.

2.8 Tissue specific DNA ratio maternal / paternal

Whereas the grain colour marker just indicates the transgenic event in principle, the quantity of transgenes and the quantity of the transgenic product are subject to complex tissue dependent processes. Grains are composed mainly of three different kinds of tissues: embryo, endosperm and teguments. As the DNA origins are different for these tissues also the quantity of DNA and the number of copies of the genome also vary. Whereas endosperms are triploid, resulting from the fusion of two maternal polar nuclei with one sperm nucleus, embryos are diploid, resulting from the fusion of one haploid maternal nucleus and one haploid male nucleus.

Teguments are diploid and of wholly maternal origin and contribute only negligible amounts of DNA to the grain (maximum 3.5% of total DNA). Microscopic studies have shown that embryos consist of small dense cells, whereas most of the endosperm cells are larger and vacuolated (*Schel et al., 1984*). Results indicate that about half of the total DNA extracted from the maize grains originates from the endosperm tissue and the other half from the embryo tissue (*Trifa and Zhang, 2004*). The endosperm accounts for 80 to 90% of total grain weight (*Kowles and Phillips, 1985*) and the amount of DNA contributed by the endosperm varies over a large range, from 36 to 60% (*Trifa and Zhang, 2004*). The DNA content of the developing endosperm changes during endoreduplication but not in other tissues like the embryo. This result in changes of the DNA ratios of maternal / paternal origin and could change results of PCR analysis depending on the developmental stage of the grain.

2 - Measurement of pollen dispersal

tissue	transgene content	proposed relative DNA amount
teguments	0 %	max. 3.5 %
embryo	50 %	95% (full maturity) – 5 % (endoreduplication)
endosperm	33 %	1 % (full maturity) – 95% (endoreduplication)

Table 2.2: Variation in the transgene content and relative amount of DNA per tissue

In a grain in an earlier phase of development (endoreduplication) the endosperm will contribute considerably to the whole amount of transgenic DNA (33% transgene). However, in fully mature grains with controlled cell-death (*Young et al., 2000*) there will be almost no DNA in the endosperm, except the single layer aleurone tissue, and the total transgenic DNA content will be determined mainly by the embryo (50% transgene). Thus, in mixed samples the sum of all grains and vegetative parts, as defined by a certain developmental stage will finally determine the analytical transgenic content of DNA.

In contrast, the transfer of yellow colour by pollen will always show a clear “yes” or “no” result. This has to be carefully considered when comparing data of fodder maize and fully mature grain maize. Furthermore, grain colour is usually a homozygous trait; thus, 100% of the pollen will contain the yellow colour whereas in most transgenic varieties at present only 50% of pollen will contain the transgene. Thus, the grain colour marker data indicate a maximum of transgenic outcrossing.

2.9 REFERENCES

Ahmed, F.E. (2002) Detection of genetically modified organisms in foods. *Trends Biotechnol.* 20:215-223.

Anklam E., Gadani F., Heinze P., Pijnenburg H., Van den Eede G. (2002) Analytical methods for detection and determination of genetically modified organisms in agricultural crops and plant-derived food products. *Eur. Food Res. Technol.* 214:3-26.

Below F.E., Cazetta J.O., Seebauer J.R. (2000) Carbon/Nitrogen interactions during ear and grain development of maize. In: M.E. Westgate and K.J. Boote (ed.) *Physiology and modelling grain set in maize*. ASA and CSSA, Madison, WI.

Biosicherheit. (2002) Auskreuzung aus transgenem Mais und Quantifizierung der Auskreuzung. <http://www.biosicherheit.de/projekte/106.proj.html>

Byrne P.F. and Fromherz S. (2003) Can GM and non-GM crops coexist? Setting a precedent in Boulder County, Colorado, USA. *Food, Agriculture and Environment* 1(2), 258-261

Chilcutt C.F. and Tabashnik B.E. (2004) Contamination of refuges by *Bacillus thuringiensis* toxin genes from transgenic maize. PNAS. Vol. 101(20):7526-7529

Coe Jr. E.H. (2001) The origins of maize genetics. Nature Genetics. Vol 2:898- 905

Foueillassar X. and A. Fabié. (2005) Conventional waxy production, an experience to evaluate co-existence of GM and conventional maize. Conference Coexistence of GM and non-GM Crops. Poster session. Agroscope FAL Reckenholz

Garcia M., Figueroa J., Gomez R., Townsend R., Schoper J. (1998) Pollen control during transgenic hybrid maize development in Mexico. Crop Sci. 38:1597-1602

Grant R.F., Jackson B.S., Kiniry J.R., Arkin G.K. (1989) Water deficit timing effects on yield components of maize. Agron. J. 81:61-65

Heinemann, J.A., Sparrow A.D., Traavik T. (2004) Is confidence in the monitoring of GE foods justified?. Trends in Biotechnology. Vol. 22. No. 7:331-336.

Hernandez M., Esteve T., Prat S., Pla M. (2003) Development of real-time PCR systems based on SYBR Green I, Amplifluor and TaqMan technologies for specific quantitative detection of the transgenic maize event GA21. Journal of Cereal Science. 39:99-107.

Holst-Jensen A., Ronning S.B., Lovseth A., Berdal K.G. (2003) PCR technology for screening and quantification of genetically modified organisms (GMOs). Anal. Bioanal. Chem. 375:985-993.

Hutchcroft C.D. (1958) Contamination in seed fields of corn resulting from incomplete detasseling. Agron. J. 267-271

Jones M.D. and Brooks J.S. (1950) Effectiveness of distance and border rows in preventing outcrossing in maize. Oklahoma Agricultural Experimental Station Technical bulletin No. 38

Jones M.D. and Newell L.C. (1948) Longevity of pollen and stigmas of grasses: Buffalograss, *Buchloe dactyloedeas* Engelm., and maize, *Zea mays* L. J. Am. Soc. Agron. 40(3):195-204

Jones R.J., Schreiber B.M.N., Roessler J.A. (1996) Kernel sink capacity in maize: Genotypic and maternal regulation. Crop Sci. 36:301-306.

2 - Measurement of pollen dispersal

Kowles, R.V. and Phillips R.L. (1985) DNA amplification patterns in maize endosperm nuclei during kernel development. *Proc. Natl. Acad. Sci. U.S.A.* 82:7010-7014

Kruse M. (2004) Prüfpläne für die Untersuchung von Saatgut auf GVO Verunreinigungen. *Gesellschaft für Pflanzenzüchtung e.v. Vorträge Pflanzenzüchtung.* 67:220-227

Ma B.L., Subedi K.D., Reid L.M. (2004) Extent of cross-fertilization in maize by pollen from neighboring transgenic hybrids. *Crop Sci.* 44:1273-1282

MAF. (2002) Additional questions about genetically modified (GM) maize inadvertently grown by Pacific seeds. Ministry of Agriculture and Forestry New Zealand. <http://www.biosecurity.govt.nz/imports/plants/papers/gm-seeds/gm-seeds-faq3.htm>

Mozafar A. (1990) Kernel abortion and distribution of mineral elements along the maize ear. *Agron J.* 82:511-514.

Ober E.S., Setter T.L., Madison J.T., Thompson J.F., Shapiro P.S. (1991) Influence of water deficit on maize endosperm development. Enzyme activities and RNA transcripts of starch and zein synthesis, abscisic acid, and cell division. *Plant Physiol.* 97:154-164.

Otegui, M.E. and Andrade F.H. (2000) New relationships between light interception, ear growth, and kernel set in maize. In: M.E. Westgate and K.J. Boote (ed.) *Physiology and modeling kernel set in maize.* ASA and CSSA, Madison, WI.

Paterniani E. and Stort A.C. (1974) Effective maize pollen dispersal in the field. *Euphytica.* 23:129-134

Quattar S., Jones R.J., Crookston R.K. (1987) Effects of moisture stress during grain filling on the pattern maize kernel growth and development. *Crop Sci.* 27:726-730.

Raynor G.S., Ogden E.C., Hayes J.V. (1972) Dispersion and deposition of kernel pollen from experimental sources. *Agron. J.* 64:420-427

Salamov A.B. (1940) Über die räumliche Isolierung bei Mais (Translation of the russian original). In: O. Sanvido, F. Widmer, M. Winzeler, B. Streit, E. Szerencsits and F. Bigler. 2005. *Koexistenz verschiedener landwirtschaftlicher Anbausysteme mit und ohne Gentechnik.* Schriftenreihe der FAL 55, Agroscope FAL Reckenholz

Schel J.H.N., Kieft H., Van Lammeren A.A.M. (1984) Interactions between embryo and endosperm during early developmental stages of maize caryopses (*Zea mays*). *Can. J. Bot.* 62: 2842-2853.

Schussler J.R. and Westgate M.E. (1991) Maize kernel set at low water potential: II. Sensitivity to reduced assimilates at pollination. *Crop Sci.* 31:1196-1203.

Schussler J.R. and Westgate M.E. (1995) Assimilate flux determines kernel set at low water potential in maize. *Crop Sci.* 35:1074-1080.

Stevens W.E., Berberich S.A., Sheckell P.A., Wiltse C.C., Halsey M.E., Horak M.J., Dunn D.J. (2004) Optimizing pollen confinement in maize grown for regulated products. *Crop Sci.* 44:2146-2153

Tollenaar M. (1977) Sink-source relationship during reproductive development in maize. A review. *Maydica.* 22:49-75.

Tollenaar M., Aguilera A., Nissanka S.P. (1997) Grain yield is reduced more by weed interference in an old than in a new maize hybrid. *Agron. J.* 89:239-246

Trifa, Y. and Zhang D. (2004) DNA content in embryo and endosperm of maize kernel (*Zea mays* L.): Impact on GMO Quantification. *J. Agric. Food Chem.* 52:1044-1048

Uhart S.A. and Andrade F.H. (1995) Nitrogen deficiency in maize: II. Carbon-nitrogen interaction effects on kernel number and grain yield. *Crop Sci.* 35:1384-1389.

USDA. 2000. Sampling for the detection of biotech grains. US Department of Agriculture. <http://151.121.3.117/biotech/sample2.htm>

Vaughn, T., Cavato T., Brar G., Coombe T., DeGooyer T., Ford S., Groth M., Howe A., Johnson S., Kolacz K., Pilcher C., Purcell J., Romano C., English L., and Pershing J. (2005) A Method of Controlling Maize Rootworm Feeding Using a *Bacillus thuringiensis* Protein Expressed in Transgenic Maize. *Crop Sci.* 45:931-938.

Weber W.E., Bringezu T., Broer I., Holz F., Eder J. (2005) Koexistenz von gentechnisch verändertem und konventionellem Mais: Ergebnisse des Erprobungsanbaus Silomais 2004. *Mais.* 1:14-17

2 - Measurement of pollen dispersal

Weingartner U., Kaeser O., Long M., Stamp P. (2002) Combining Cytoplasmic Male Sterility and Xenia Increases Grain Yield of Maize Hybrids. *Crop Sci.* 42:1848 - 1856

Westgate M.E. and Boyer J.S. (1985) Carbohydrate reserves and reproductive development at low leaf water potentials in maize. *Crop Sci.* 25:762-769

Young T.E. and Gallie D.R. (2000) Regulation of programmed cell death in maize endosperm by abscisic acid. *Plant molecular biology.* 42:397-414

Zinselmeier C., Westgate M.E., Schussler J.R., Jones R.J. (1995) Low water potential disrupts carbohydrate metabolism in maize (*Zea mays* L.) ovaries. *Plant Physiology.* 107:385-391

3 Cross-pollination of maize in long distance

3.1 ABSTRACT

In a two year study in Switzerland, cross-pollination of yellow grain maize in 13 white grain test fields was investigated by using the occurrence of yellow grains on white grain ears as a marker of cross-pollination from neighbouring fields. We investigated distances of 50 to 4500m between pollen-donor and pollen-receptor fields. In no case the cross-pollination of the whole field was above 0.02%. Four fields, located in downwind distances of 50 to 371 m in main wind direction, showed low but marked cross-pollination at the field border orientated to the next yellow grain maize field. In every field there were some low rate cross-pollinations dispersed around, mainly single cross-pollination events, on an average of 1,8 % of sampled ears. Horizontal wind speed measurements during flowering time in relation to settling speed of maize pollen showed a potential horizontal dispersal distance of up to 55 m. Observed cross-pollinations over longer distances could be due to gusts or vertical movements of the wind (e.g. thermal or turbulence effects). In some fields spots of higher cross-pollinations were found that were in checked cases related due to a yellow grain seed contamination (0.004% of seedstock) of the white seeds.

3.2 INTRODUCTION

Maize is a wind-pollinating crop and can cross-pollinate in neighbouring fields. Research on maize pollen dispersal and cross-pollination has been carried out for many years with the goal of improving seed purity (*Salamov A.B., 1940; Jones and Brooks, 1950; Burris, 2001*). Recently, with the introduction of genetic modified (GM) maize and definition of GM tolerance thresholds and labelling requirements in some countries, there is legislation to establish and guarantee coexistence of both ways of agriculture. The interest in long distance pollen transport was fueled by the discovery of transgenic sequences in maize landraces of Mexico, where cultivation of transgenic maize was forbidden to protect biodiversity (*Quist and Chapela, 2001*) and in conclusion pollen dispersal from the USA across the border was suspected. *Bellon and Berthaud (2004)* on the other hand, concluded that these transgenic sequences were due to the custom of Mexican small-scale farmers to incorporate improved commercial varieties into their open pollinated landraces.

Studies of cross-pollination in maize generally showed a rapid decrease over short distances up to 30 m, often accompanied by high variation, and a long tail with low cross-pollinations occurring over several hundred meters (*Jones and Brooks, 1950; Raynor et al., 1972; Luna et al., 2001; Henry et al., 2003; Ma et al., 2004*), which is a general feature of aerobiological dispersal (*Westbrook and Isard, 1999*). Existing studies about the long distance dispersal of maize are heterogeneous and difficult to compare, possibly due to the fact that the genetic material, the size of the donor and receptor field, the topography and the climatic situation are

diverse.

In older studies generally much higher cross-pollination rates were reported. Using different grain colours for detection, *Jones and Brooks (1950)* observed cross-pollination of 5.8% in 75 m and 0.2% in 500 m in Oklahoma (USA) for two open-pollinated varieties; *Salamov A.B. (1940)* found up to 0.79% at distances beyond up to 800 m in the northern Caucasus (Russia), but concluded in this much-cited study that the reason for this was a contamination of white grain with yellow grain maize leading to variation independent of distance.

But cross-pollination rates vary considerably in recent studies as well. In the USA *Jemison and Vayda (2001)* used an herbicide resistant maize field orientated upwind of predominant wind directions to the recipient field. In 1999 there was no cross-pollination at a distance of 350 m, but in year 2000 cross-pollination of 0.7 to 1.4% took place at a distance of 100 m in a field with asynchronous pollen production. In a region characterized by low humidity and frequent high winds (Colorado, USA), using blue grains colour and herbicide marker hybrids as the pollen donor, cross-pollinations of 0.3% at 80 m and of 0.05% at 180 m were detected in organically cultivated maize (*Byrne and Fromherz, 2003*).

In Tsukuba and Tsumagoi (Japan) rates of outcrossing of sweet corn varieties varied by years and locations between 0.1 to 1.2% at 50 m, and 0.04% at 400 m (*Matsuo et al., 2004*).

In recent multi- field trials, throughout England, samples of conventional maize stands in 150 m distance showed evidence of cross-pollination in 19 out of 44 fields; of these, 12 fields had >0.1% GM-DNA and 7 had >0.3% GM-DNA (*Henry et al. , 2003*). In several fields the level of gene flow showed a marked increase at distances of 100 to 150 m from the GM source. This may have been due to the effects of the landscape and air movement over the crop. *Luna et al. (2001)* also found in Mexico more cross-pollination at 200 m from the source than at the shortest distance of 100m.

In our study the definition of long distance dispersal was related to typical small-scale farming patterns in alpine regions. Due to crop rotation maize fields are usually not growing adjacent but are separated by other crops with separation distances greater than 50 m. According to an analysis of aerial photos of an eastern region in Switzerland the distance between maize fields varied from 75 to 149 m, with an average of 112 m (*Flury et al., 2004*). Therefore, our analysis of pollen dispersal focused on distances between 50 and 4500 m.

3.3 MATERIAL AND METHODS

3.3.1 Site Descriptions

Field experiments were carried out in 2003 and 2004 in Urner Reusstal (Canton Uri), Switzerland (latitude 8°37' N, longitude 46°52' W, altitude = 449 m). The climate is temperate

3 - Cross-pollination of maize in long distance

with an annual precipitation of about 1201 mm (maximum of 148 mm in July) and an average temperature of 8.6°C (maximum of 17.1°C in July).



Fig. 3.1: Experiment location Urner Reusstal. Arrows show main wind direction

The valley is characterized by a mixture of grassland and forage maize cultivation with settlements between the fields. It is about 14 km long and by 1.3 to 4 km wide. In this area there are about 20 maize fields with a size of 0.5 to 1.5 ha spread in distances of 50 m up to 4.5 km to the next field. High mountains (up to 3000 m) surrounding the valley act as a barrier to pollen from outside sources, and a lake separates the next maize fields in the north direction by about 13 km. Thus, long-distance cross-pollinations should depend only from the experimental field arrangements within the valley. As the topography forces wind in one main direction (from the north-west to the south-east) yellow and white grain fields could be arranged for different wind situations. The location is characterized by a mountain-valley and land-lake wind system which causes a change in wind direction within almost one hour in most days of the maize flowering period. Over night wind blows from South-East and between 6:00 and 9:00 am wind direction changes to North-West over day. In the evening, between 16:00 and 19:00, wind changes again to South-East over night. This wind systems and changes in wind direction were especially strong in the hot year of 2003 with increased temperature differences due to different warming of different terrain and subsequent compensation by wind circulations. The soil in the valley is quite uniform Eutric Cambisol with more or less a sandy loam texture. Conditions for maize

cultivation were slightly suboptimal for grain set in comparison to main arable land in the Swiss midlands.

3.3.2 Maize varieties

As low cross-pollination rates were expected, the visual marker system with yellow and white grain maize was advantageous as it enables high sampling rates for the detection, also of rare cross-pollination events. Each time pollen from yellow grain hybrids fertilizes a white grain ovary a yellow grain will develop, indicative of cross-pollination. For pollen receptor fields, the white grain hybrid DSP 17007 (Delley Seeds and Plants company, Switzerland) was used, which has comparable agronomic traits to the conventional hybrids cultivated at the test site in both years: LG2185, LG2265, DK287 and Goldville. They are in early maturity group and similar in flowering time to DSP17007. The cross-pollination events were assessed by counting the number of yellow grains on white ears.

3.3.3 Cultural procedures and crop management

Sowing was done by a special instructed local contractor to avoid seed contamination during sowing. The row distance was 80 cm and the density of the final plant stands was around 9 plants per m². Sowing of all fields (yellow and white grain) was done in 2003 between 28 April and 9 May, a short timespan resulting in a good flower synchrony of the fields. In spring 2004 the weather was often cool and rainy and the sowing period was prolonged from 29 April to 28 May, resulting in some field situations with a flower asynchrony of up to seven days (see table 3.1). Agronomic practices were carried out according to the recommended levels of herbicide and fertilizer application (about 120 kg N per ha).

3.3.4 Synchronization of flowering

The flowering period was defined as the time span between the beginning of the first pollen shed and the last wilted silks. These data were recorded for all the white grain fields, whereas for the yellow grain fields, that only act as pollen donors, this was just done for the time span of pollen shed. The main flowering period of the white grain hybrid took about 10 days. However, due to variation of single plant development the complete flowering on the field level lasted about 20 days. Difference in flower synchronization was noted in number of days delay (+) or earliness (-) of mid pollen shedding of the next yellow grain pollen donor field to the mid silking of the white grain receptor field (see table 3.1).

3.3.5 Meteorological measurements

A meteorological station of MeteoSwiss at the experimental site delivered hourly measurements of wind direction, wind speed, air moisture, air temperature and global radiation. In both years a total pollination period of all fields was determined to be around 22 days; during this period the

hours with a vapour pressure deficit lower than 0.2 kPa were eliminated, as it has been observed that this value has to be passed for the start of pollen shedding (*Jarosz et al. 2005*). Furthermore, the evaluated hours were restricted to the time between 6:00 and 18:00, when nearly all pollen shedding occurs (*Jarosz et al. 2003*). This adjusted time span was taken as the actual pollination period with possible cross-pollinations.

3.3.6 Sampling

The experiment fields were in most cases in the shape of a long rectangle with length around 200 m and width around 40 m. The sampling points were distributed as follows: on the short side in every fourth row (every 2.5 m), on the long side at every 10 m. At every sampling point five ears were taken and the average number of yellow grain was calculated. On a hectare basis this amounted to 1200 sampling points and 6000 ear samples. The average number of grains per ear of a field was calculated on 30 ears that were selected randomly from a diagonal of the field. According to *Kruse (2004)*, the detection limit (P_{LD}) can be calculated as $P_{LD} = - (10^{\lceil \log (1 - (95/100)^{315000} - 1) \rceil} * 100 = 0.00095$. With this sampling system cross-pollinations down to 0.001% could be detected with a probability of 95%, which is in the range of the lowest level of cross-pollinations found here.

3.3.7 Data analysis

The cross-pollination rate is calculated by the ratio of yellow grains to all grains of an ear. For the total number of grains per ear the calculated average grain number per ear of the field was used. The cross-pollination rate of a sampling point (CPp%) is the average calculated of the next five adjacent plants to the sample point. It was important to take the average of five ears to compensate variations within a sample point.

$$CP_p \% = [(SPE1+SPE2+SPE3+SPE4+SPE5) / (5 * AVK)]$$

CP_p % Cross-pollination of a sample point

SPE_x Ear number x at sample point (5 sample ears per sample point)

AVK Average grain number of an ear of the field

The cross-pollination rate of the whole field is the ratio of the sum of all the yellow grains found in all samples to the total sum of grains of all ear samples (calculated on the average ear grain number of field).

$$CP_f \% = [\text{sum (yellow grains of all samples)} / \text{sum (grains of all samples)}] * 100$$

CP_f % Cross-pollination rate of whole field

The sum of all the grains of samples is calculated by the average ear grain number of the field multiplied by the number of sample ears. There was a yellow grain seed contamination of the white grain experimental seeds calculated to about 0.004%, averaged over the two experimental years. The resulting few cross-pollination contaminations were not eliminated from the actual field to field cross-pollinations. Visualizations were done by geostatistic Software Surfer 8.0 (www.goldensoftware.com) and gridding was done by the kriging method.

3.4 RESULTS

3.4.1 Rate and frequency of cross-pollinations

Cross-pollination in a total single field was in no case higher than 0.02% (Table 3.1) with a minimum of 0.0002 % (one yellow grain in all the samples of the whole field), although a few sample points showed higher cross-pollination rates in other fields. For example, one yellow grain on an average ear (370 grains) would translate to 0.27% cross-pollination.

Table 3.1: Cross-pollination data for all fields in 2003 and 2004 with respect to distance from the closest yellow grain donor field.

Field: field name; *Distance*: distance to next pollen donor (yellow grain maize); *Wind*: field orientation of receptor field (white grain maize) to main wind directions (+ in direction, - parallel, - - against); *Flower*: flower synchronization in days as timespan between mid-pollen shedding of donor to mid-silking of receptor (e.g. -5 means a mid pollen shedding of donor 5 days earlier than mid silking of receptor; = means synchronous); CP_f = total cross-pollination rate of whole field (calculated to heterozygous pollen traits).

Field	Year	Distance	Wind	Flower	CP_f %
W7	2004	52 m	+	- 7	0,009
W12	2004	85 m	+	=	0,015
W2	2003	105 m	-	- 4	0,003
W3	2003	125 m	-	- 1	0,01
W10	2004	149 m	+	- 1	0,016
W5	2003	150 m	+	- 2	0,007
W1	2003	200 m	-	- 1	0,009
W9	2004	287 m	- -	- 4	0,005
W11	2004	371 m	+	- 5	0,008
W8	2004	402 m	-	- 1	0,005
W13	2004	458 m	+	- 7	0,0002
W6	2003	4125 m	+	- 1	0,006
W14	2004	4440 m	+	- 2	0,0005

3 - Cross-pollination of maize in long distance

Although the years quite differed in meteorological conditions, an exceptional hot and dry summer in 2003 in contrast to moderately cool and wet conditions in 2004, the difference in cross-pollination between the years was not striking. Factors (wind orientation and velocity, flower synchrony, surrounding landscape) varied because the fields could not be arranged in both years identically; thus, only more obvious differences would have been recognizable at the very low rates of cross-pollination that characterized both years. Cross-pollination happened only on 0.06 to 4.1% of the sampled ears per fields with an average of 1.8% of samples in all fields. Of these ears with cross-pollination 89% were single-fertilization events; more than five cross-pollinations per ear were found for only 4.9 % of the ears that showed cross-pollination.

3.4.2 Distribution of cross-pollinations

Cross-pollinations were in most fields like randomly distributed over the entire field (Figure 3.2).

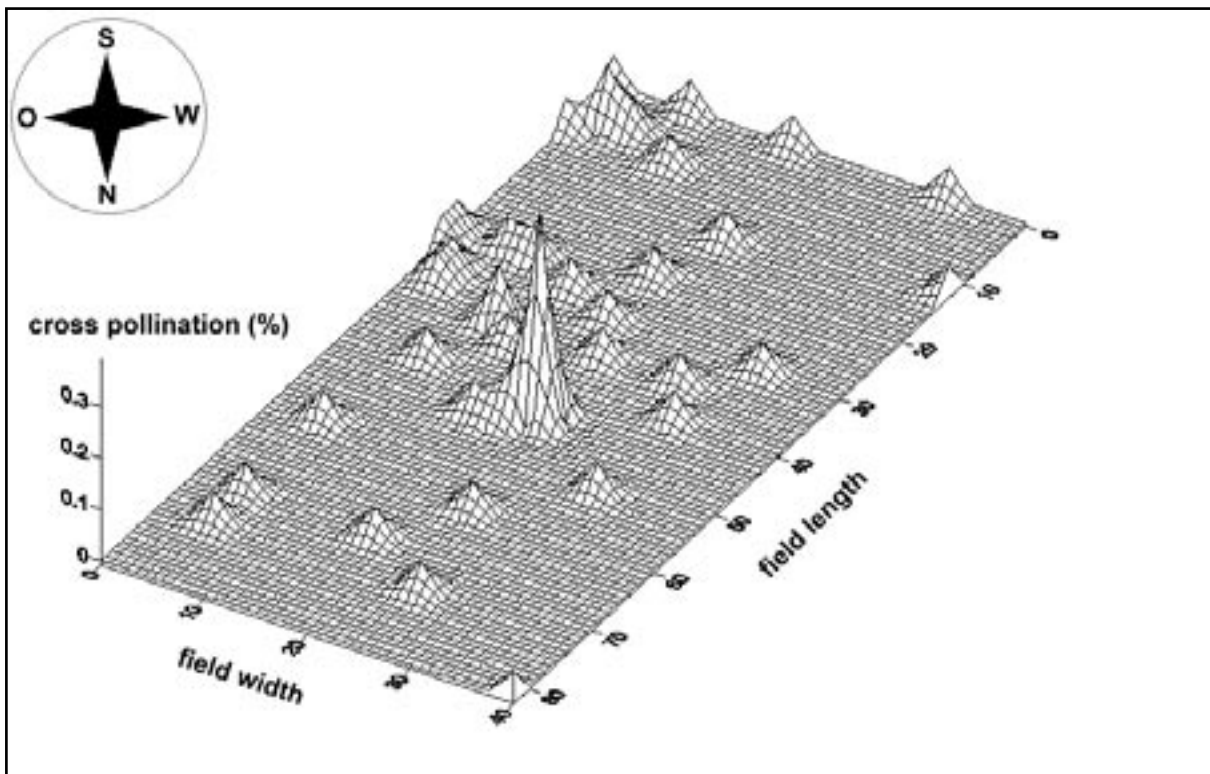


Fig. 3.2: Example of cross-pollination patterns looking like randomly distributed (field W8); maximum value of 0.3%.

Only in four of 13 fields spatial gradients of varying cross-pollination were found. These gradients were characterized by a higher rate of cross-pollination in the border row, orientated to the next yellow grain pollen donor field, with a rapid decrease in direction inside the field (Figure 3.3).

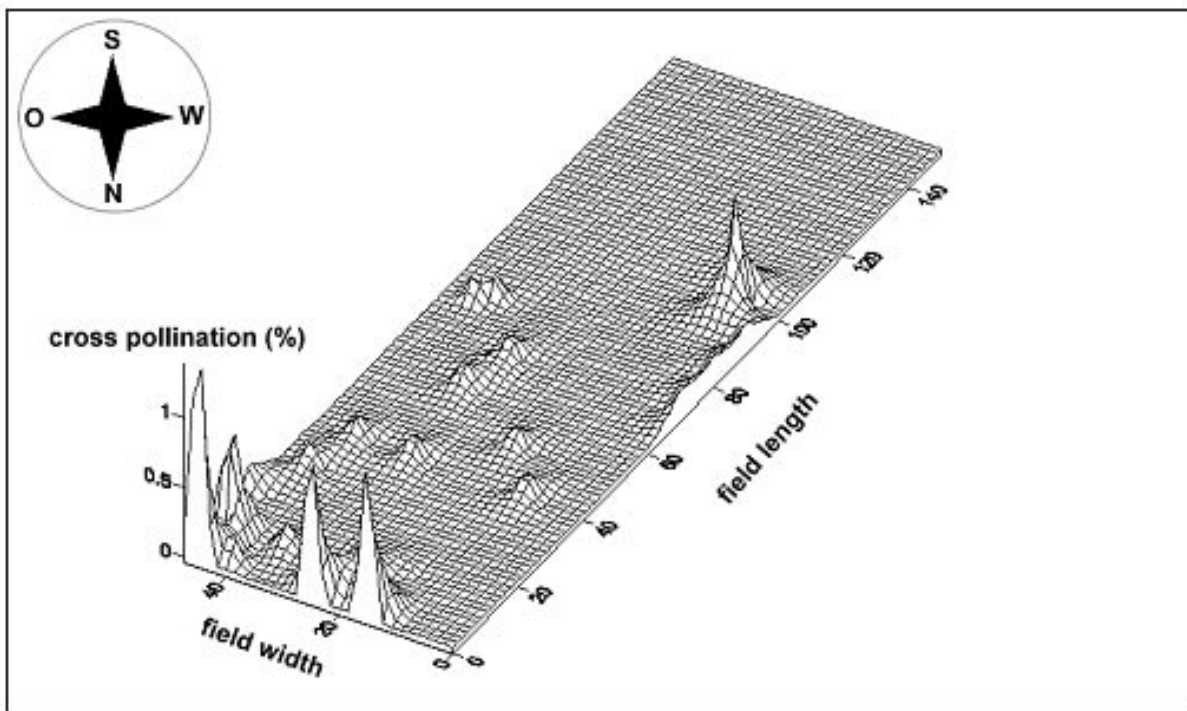


Fig. 3.3: Example of cross-pollination with marked gradients (field W7); maximum values of 1.2%.

A simplified overview of the distribution of different cross-pollination types of the fields is shown in Figure 3.4 for both years in the following way: the centre represents the next yellow grain donor field. Surrounding the yellow grain field, all the white grain fields are shown with regard to direction and distance. Fields with and without a cross-pollination gradient are marked differently.

The four fields with clear gradients had higher rates of cross-pollination in the border row, orientated in the main wind direction with regard to the next yellow grain pollen donor field. It seems that higher rates of cross-pollinations were due to horizontal wind forces only in main wind directions at distances between 52 m and 371 m. In the other cases of random cross-pollination the fields were either not orientated to the direction of the wind or were further away from the pollen donor.

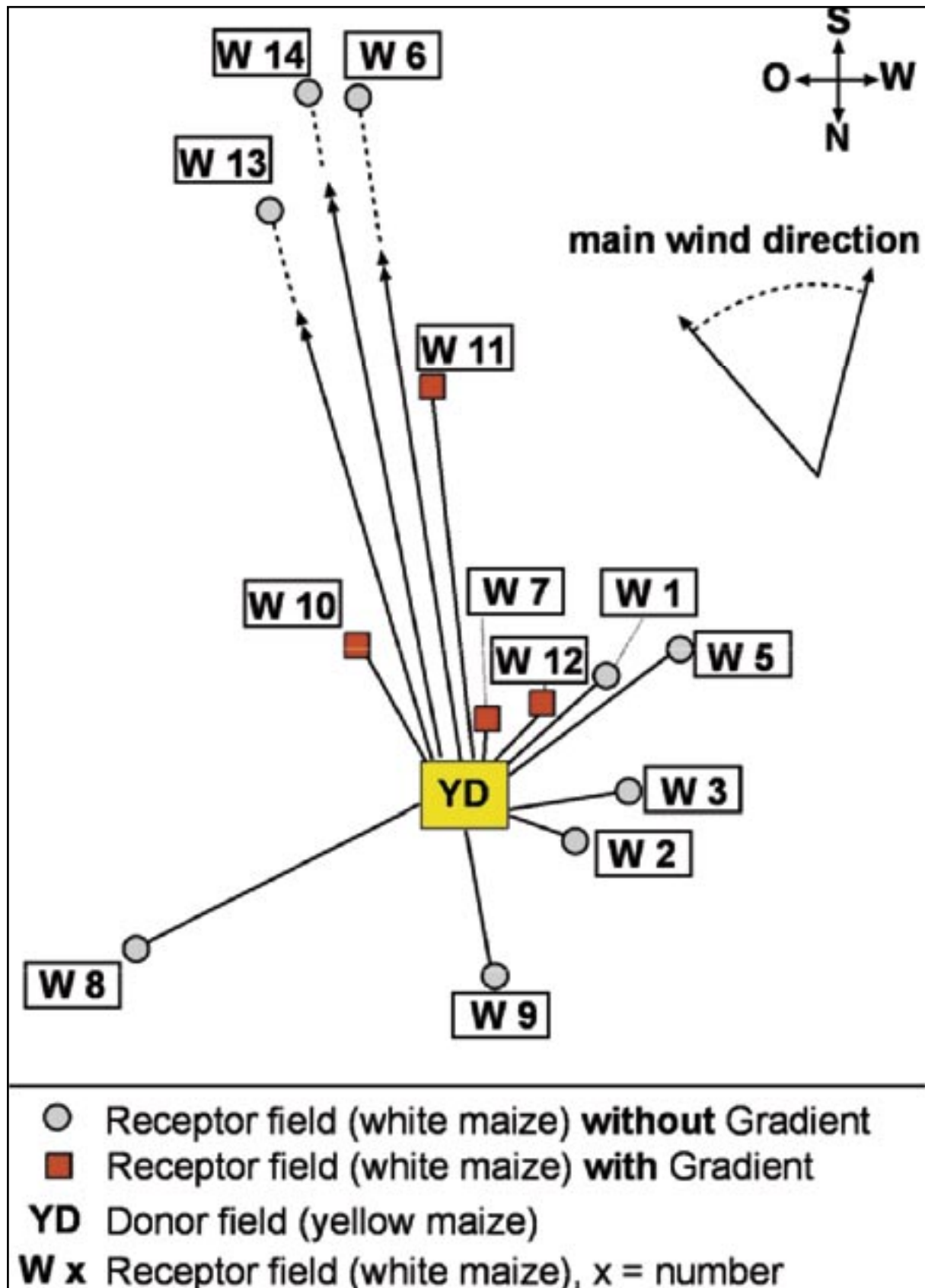


Fig. 3.4: Cross-pollination in fields of white maize with and without marked gradients in relation to distance and direction of the next yellow grain donor field (See Table 3.1 for field details)

3.4.3 Horizontal pollen dispersal distance and wind velocity

Tassels were at a height of about 2.5 m and silks at a height of about 1 m. In consequence, pollen had a settling distance of about 1.5 m to reach the silks. According to *Aylor (2002)* the settling speed of maize pollen is in the range of 21 cm s^{-1} to 32 cm s^{-1} , depending on the hydration status. In the best case for dispersal there is an available time span of about 5 s between settling from the height of the tassels to the silks if no vertical forces counteract gravity and settling speed. Based to these deliberations the possible dispersal distance per actual wind speed was classified for both years, which were similar (Figure 3.5).

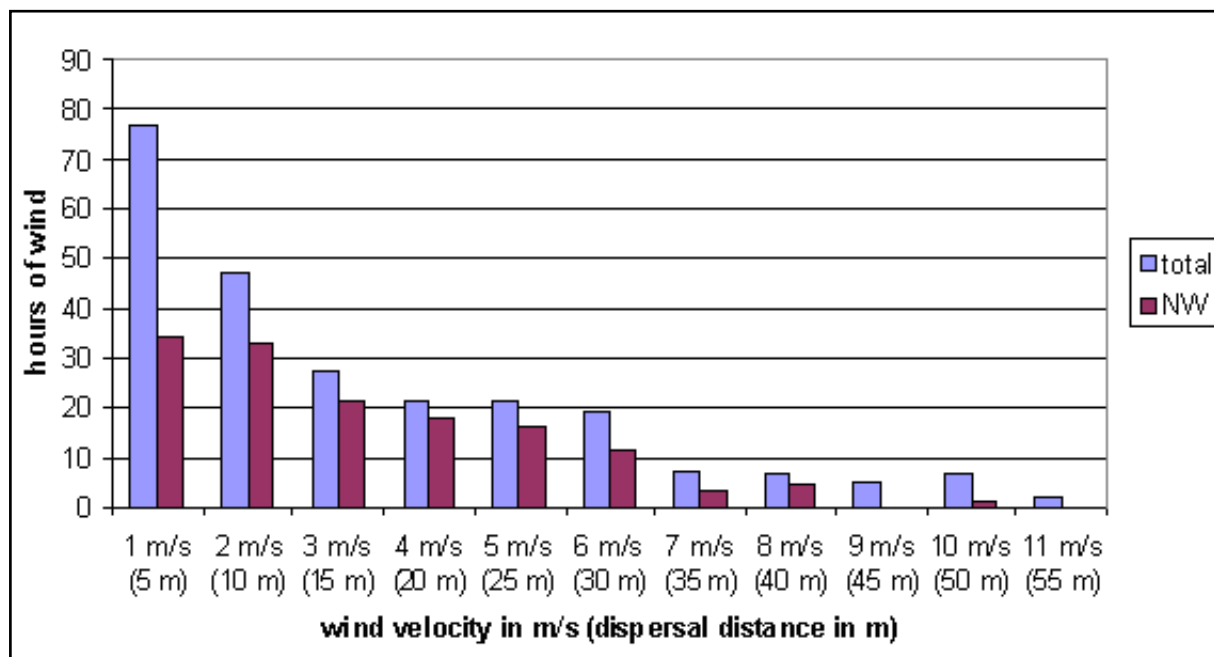


Fig. 3.5: Possible dispersal distance of pollen as influenced by the duration of wind speed classes in total and in the main wind direction North-West (NW) in year 2003;

Most of the wind was of low velocity, especially when it was not in the main direction (North-West) (Fig. 3.5).

3.5 DISCUSSION

3.5.1 The white grain marker hybrid for cross-pollination measurements

In control experiments on short distance cross-pollination rates up to 80% were found for single late developed plants of the white grain hybrid with long anthesis silking intervals and reduced pollen competition. This maximum cross-pollination potential of our white hybrid is in similar range to that of varieties reported in other studies (*Ma et al., 2004; Jones and Brooks, 1950; Stevens et al., 2004*). Although male flowering of modern hybrids can be reduced by reduced tassel branches and pollen shedding (*Duvick and Cassman, 1999*), the white grain hybrid had

normal tassel size and was a good pollinator.

Usually 100% of yellow grain pollen will show cross-pollination because present varieties are homozygous for the yellow colour (except varieties like Goldenso). However, many commercial GM - maize hybrids are at present heterozygous for the transgenic trait with only 50% of pollen containing the transgene in contrast to the yellow colour gene. Therefore, cross-pollination rates were divided by factor two in order to make results comparable to cross-pollination of today's GM-hybrids.

A few cases of yellow grain seed contamination within white grain seeds occurred. Within 22,800 sampled ears there was one ear with completely yellow grain, i.e. a seed contamination of 0.004%. Some background contamination with yellow grain seeds was reported in other studies as well (*Stevens et al., 2004; Salamov A.B., 1940*). *Rieger et al. (2002)* monitored effective pollen dispersal from herbicide resistant canola. A remarkable feature of these data was the high variability in the level of outcrossing at long distances. *Aylor et al. (2003a)* concluded that this variability is a hallmark of long-distance dispersal in the atmosphere due to the long-extending tail that results from the properties of atmospheric turbulence. *Henry et al. (2003)* also found in several of their 55 maize fields that the level of gene flow showed a marked increase at distances of 100 to 150 m from the GM source; they concluded that effects of the landscape and of air movement above the crop could explain this phenomenon. Such spots of increased yellow grains in the white maize fields were found in this study too. In some cases a time-consuming check of plants surrounding these spots was done and an ear with completely yellow grains was detected to be a contamination plant. However, such spots were rare events (maximum 2 spots per field) because the measured contamination rate of white grain seeds (0.004%) was also low.

3.5.2 Rate and frequency of cross-pollinations

In comparison to other studies on long distance pollen dispersal of maize with very varying rates, the results here are close to the lowest rates as found by *Matsuo et al. (2004)* with 0.23% in 100 m and 0.06% in 200 m distance and by *Byrne and Fromherz (2003)* with 0.05% in 183 m distance. However, it is not quite clear from some other reports if these cross-pollination rates are related to a single-sample point or a defined area, where the cross-pollination rate of a single sample gets diluted by the frequency of the total number of cross-pollination events.

3.5.3 Pollen dispersal and pollen competition

As mature pollen is released even by a slight breeze or vibration most pollen will be shed at low wind speeds with very short dispersal range (*Aylor, 2003b*). However, there are also a few hours with strong winds in turbulent weather conditions (e.g. thunderstorms) that may be responsible for the dispersal of a small portion of pollen in different directions over long distances, as indicated by the randomly distributed low rate of cross-pollination events over

all the fields. Without any support by vertical wind movement, e.g. thermals and outstanding gusts, pollen would disperse only to a maximum of 55 m, with most of the pollen dispersed on shorter distances. This may explain the low cross-pollination rates beyond this distance and that cross-pollination gradients were found only in four fields that were orientated in main wind direction.

Even though some very long distance dispersals for pollen of trees with similar size to maize pollen have been reported in range up to 500 km (*Rousseau et al., 2003*), it is improbable to get maize cross-pollinations over this distance. Pollen grains marking long distance transport are generally in very low number (*Campbell et al., 1999*); they will be confronted by intense pollen competition from the pollen of the receptor field. About six million (*Jarosz et al., 2005*) respectively 25 million (*Poehlman and Sleper, 1995*) pollen are produced by a maize plant, depending on the variety. Thus, there will be more than 25,000 pollen for each single grain set. In conclusion, successful cross-pollination is very unlikely at long distances from the pollen-donor field.

The effects of pollen competition, as well as the spatial variation of pollen dispersal and deposition, were tested in a small experiment plot of about 500 detasseled white grain maize plants located between yellow and white grain fields. In the main wind direction and at a distance of around 625 m to next maize fields still 20% of the detasseled plants had at least one grain per ear instead of an average of 1.8% of samples with cross-pollination in male fertile white grain fields. Thus, pollen competition may be a strong protection against cross-pollination. In all the samples of this experimental plot the ratio of yellow to white grains was 1:1 which was in good relation to their planted areas.

3.5.4 Vertical pollen dispersal

Though horizontal winds allow just for a low pollen dispersal range, some pollen grains are able to fertilize plants over long distances. The parameters for successful long distance dispersal must be the effects of vertical forces, such as thermals and atmospheric mixing turbulence which hinders pollen from settling down and thus extends the dispersal distance. For an approximate determination of the pollen take-off by vertical movement, in 2004 a scaffold was installed with horizontal cords of adhesive pollen traps (*Fonseca et al., 2002*) at heights up to 5m from 0 to 12 m into a maize field. On days with good thermal mixing conditions, indicated by low height clouds around midday, 250 pollen cm⁻² were found at the height of the tassels between the rows whereas at 4m height only 2 pollen cm⁻² were deposited; this indicates that only a very small (0.8%) portion of the shed pollen could take-off and reach higher air layers with better conditions for long distance dispersal. Most take-off was found in the border row, where wind meets the plant wall and in consequence turbulences can lift the pollen more easily (*Zeng and Takahashi, 2000*). Such pollen grains can then be caught by sucking airflows. *Irwin and Thresh*

(1988) reported that convective thermals with around 1 m s^{-1} vertical velocity are sufficient to distribute biota within the lowest 1000 m of the atmosphere within 15 min. Jarosz *et al.* (2003) found a somewhat less dramatic decrease in pollen density with distance but the measurements were done differently. At the Swiss experiment site the speeds and duration of wind were generally higher than in the lowlands. This may have favoured horizontal pollen movement but may also have reduced the generation of thermal convective cells that are dependent on conditions of high heat fluxes at low wind speed (Gryning and Batchvarova, 1999). More representative data are still required in order to elucidate the preliminary data about pollen take-off as a potential factor for long distance dispersal.

3.6 CONCLUSION

Although there are many factors that influence pollen dispersal, most pollen will settle down over short distance and probably will not interact with most of these factors. Orientation to prevailing horizontal wind speed is important, with an effect of up to 350 m. Low cross-pollination events found on longer distances are more an effect of a very low seed contamination than an effect of specific meteorological events with vertical movements. However, the risk could be excluded that commodity maize would be contaminated with regard to threshold level of 0.9%, and even to technical detection limit of PCR analysis (0.1%), at distances above 100 m under the present experimental conditions. The diffusely distributed cross-pollination events at longer distances still could require more detailed studies in cases where any cross-pollination has to be strictly avoided.

3.7 REFERENCES

Aylor D. E. (2002) Settling speed of corn (*Zea mays*) pollen. *J. Aerosol Sci.* 33:1601–1607

Aylor D.E., Schultes N.P., Shields E.J. (2003a) An aerobiological framework for assessing cross-pollination in maize. *Agric. For. Meteorol.* 119:111-129

Aylor D.E. (2003b) Rate of dehydration of corn (*Zea mays* L.) pollen in the air. *J. Exp. Bot.* Vol. 54. No. 391:2307-2312

Bellon M.R. and Berthaud J. (2004) Transgenic maize and the evolution of landrace diversity in Mexico. The importance of farmers' behaviour. *Plant Physiol.* Vol. 134. pp. 883-888

Burriss J.S. 2001. Adventitious pollen intrusion into hybrid maize seed production fields. In 56th Annual Corn and Sorghum Research Conference, in Washington DC.

Byrne P.F. and Fromherz S. (2003). Can GM and non-GM crops coexist? Setting a precedent in Boulder County, Colorado, USA. *Food, Agriculture and Environment* 1(2), 258-261

Campbell I.D., McDonald K., Flannigan M.D., Kringayark J. (1999) Long-distance transport of pollen into the Arctic. *Nature*. Vol. 399. No. 6. p. 29-30

Duvick D.N. and Cassman K.G. (1999) Post-green revolution trends in yield potential of temperate maize in the North-Central United States. *Crop Sci.* 39:1622-1630

Flury C., Gotsch N., Rieder P., Szerencsits E., Schüpbach B. (2004) Projekt Greifensee – interdisziplinäre Forschung für die Landwirtschaft. *Agrarforschung* 11(10):428-433

Fonseca A.E, Westgate M.E., Doyle R.T. (2002) Application of fluorescence microscopy and image analysis for quantification dynamics of maize pollen shed. *Crop Sci.* 42:2201-2206

Gryning S.E. and Batchvarova E. (1999). Regional heat flux over the NOPEX area estimated from the evolution of the mixed-layer. *Agric. For. Meteorol.* 98-99:159-167

Henry C., Morgan D., Weeks R., Daniels R.E., Boffey C. (2003) Farm scale evaluations of GM crops: Monitoring gene flow from GM crops to non-GM equivalent crops in the vicinity – Part I: Forage maize. DEFRA report. Central Science Laboratory Sand Hutton, Centre for Ecology and Hydrology Dorchester, p. 25

Irwin M.E. and J.M. Thresh. (1988) Long-range aerial dispersal of cereal aphids as virus vectors in North America. *Phil. Trans. Roy. Soc. Lond. B* 321

Jarosz N., Loubet B., Durand B., Foueillassar X., Huber L. (2005) Variations in Maize Pollen Emission and Deposition in Relation to Microclimate. *Environmental Science & Technology*. Vol. 39, No. 12 : 4377-4384

Jarosz N., Loubet B., Durand B., McCartney A., Foueillassar X., Huber L. (2003) Field measurements of airborne concentration and deposition rate of maize pollen. *Agric. For. Meteorol.* 119: 37 - 51

Jemison J.M. and Vayda M.E. (2001). Cross pollination from genetically engineered corn: wind transport and seed source. *AgBioForum* 4 (2), 87-92

Jones M.D. and Brooks J.S. (1950) Effectiveness of distance and border rows in preventing outcrossing in corn. Oklahoma Agricultural Experiment Station Technical Bulletin No. T-38, Stillwater, OK, 18 pp.

Kruse M. (2004) Prüfpläne für die Untersuchung von Saatgut auf GVO Verunreinigungen. Gesellschaft für Pflanzenzüchtung e. v. Vorträge Pflanzenzüchtung. 67:220-227

Luna S., Figueroa V.J., Baltazar M.B., Gomez M.R., Townsend L.R., Schoper J.B. (2001) Maize pollen longevity and distance isolation requirements for effective pollen control. *Crop Sci.* 41:1551-1557

Ma B.L., Subedi K.D., Reid L.M. (2004) Extent of cross-fertilization in maize by pollen from neighboring transgenic hybrids. *Crop Sci.* 44:1273-1282

Matsuo K., Amano K., Shibaïke H., Yoshimura Y., Kawashima S., Uesugi S., Misawa T., Miura Y., Ban Y., Oka M. (2004) Pollen dispersal and outcrossing in *Zea mays* populations: A simple identification of hybrids detected by xenia using conventional corn in simulation of transgene dispersion of GM corn. Poster at 8th international symposium on the biosafety of genetically modified organisms, September 26 – 30, 2004, in Montpellier

Poehlman J.M. and Sleper D.A. (1995) *Breeding field crops*. Iowa State University Press, Ames. XV, 494 pp.

Quist D. and Chapela I.H. (2001) Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 414:541-543

Raynor G.S., Odgen E.C., Hayes J.V. (1972) Dispersion and deposition of corn pollen from experimental sources. *Agron. J.* 64:420-427

Rieger M.A., Lamond M., Preston C., Powles S.B., Rousch R.T. (2002) Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science*. 296:2386-2388

Rousseau D.D., Duzer D., Cambon G., Jolly D., Poulsen U., Ferrier J., Schevin P., Gros R. (2003) Long distance transport of pollen to Greenland. *Geophysical research letters*. Vol. 30. No. 14. p. 1765

Salamov A.B. (1940) About isolation of maize. German translation in: Sanvido O., Widmer F., Winzeler M., Streit B., Szerencsits E. and F. Bigler. 2005. Koexistenz verschiedener landwirtschaftlicher Anbausysteme mit und ohne Gentechnik. Schriftenreihe der FAL 55. FAL Reckenholz

3 - Cross-pollination of maize in long distance

Stevens W.E., Berberich S.A., Sheckell P.A., Wiltse C.C., Halsey M.E., Horak M.J., Dunn D.J. (2004) Optimizing pollen confinement in maize grown for regulated products. *Crop Sci.* 44:2146-2153

Westbrook J.K. and Isard S.A. (1999) Atmospheric scales of biotic dispersal. *Agric. For. Meteorol.* 97(4):263-274

Zeng P. and Takahashi H. (2000) A first-order closure model for the wind flow within and above vegetation canopies. *Agric. For. Meteorol.* 103:301-313

4 Variation in cross-pollination of maize in short distance

4.1 ABSTRACT

Numerous field experiments on cross-pollination have been carried out, but so far the results differ widely, probably to some extent due to the different experimental designs and settings. This makes comparisons and generalizations complicated. In 2003 and 2004, six field experiments were carried out in the hilly regions of Switzerland. Cross-pollination of colour dominant yellow grain maize in direct neighbourhood of colour recessive white grain maize was checked at the field level. The main factors of influence tested were wind direction, flower synchrony and the size of the donor and receptor field parts. The quick visual assessment of single yellow grains on white grain ears enables high sampling densities, so that the patterns of distribution could be followed. Field size ratios varying from 3.6 : 1 to 1 : 8 had nearly no influence on the cross-pollination rate. The synchrony between donor pollen shedding and receptor silking was an important component for cross-pollinations. The time slot for good synchrony and high rates of cross-pollinations is quite narrow. In relation to silk emergence of the receptor field, high cross-pollination was possible if the pollen donor did not start pollen shed earlier than five days and not later than three days. Furthermore, in receptor fields with inhomogeneous plant development weak plants were silking later when internal pollen dispersal was exhausted. This resulted in increased cross-pollination susceptibility. Only in one well wind exposed location a clear effect of the main wind direction was found, otherwise the wind effect was probably minimized by the varied topography. Clearly recognizable variations were generally found at shorter distances up to 15 m, where pollen concentration is usually high enough to interact with factors to a measurable extent. In distances beyond 15 m all experiments had a similar low cross-pollination rate.

4.2 INTRODUCTION

Today GM-maize (genetically modified maize) is widely distributed over the world and the areas of cultivation increases every year. Nevertheless, cultivation is still limited to a few countries. Quite a number of countries, especially countries in the EU, prepare to introduce GM-maize varieties at present or in the near future. As maize is a wind-pollinating crop and can cross-pollinate in neighbouring fields, for a coexistence of both types of cultivation, conventional and GM-crops, countries had to define certain thresholds of intolerable GM content in a conventional crop product and to elaborate guidelines how to handle the situation. An important part of these guidelines is the definition of appropriate isolation distances to limit outcrossing of transgenic maize to the defined thresholds. Although there are several studies on cross-pollination so far (see Figure 4.1) a clear decision about appropriate separation distances is still difficult because experimental set-ups are very heterogeneous and accordingly the results vary within an appreciable range. Data exist from experiments with visual grain colour markers, simulating

4 - Variation in cross-pollination of maize in short distance

transgenic maize, and from true GM material. As the latter is heterozygous for the transgene, contrary to homozygous yellow grain colour, all data derived from studies with visual grain markers are divided by a factor two to enable a correct comparison. Existing studies worldwide with exact data tables of cross-pollination at sample points of certain distance were used to sum up in a diagram and to show maximal and commonly found cross-pollination rates.

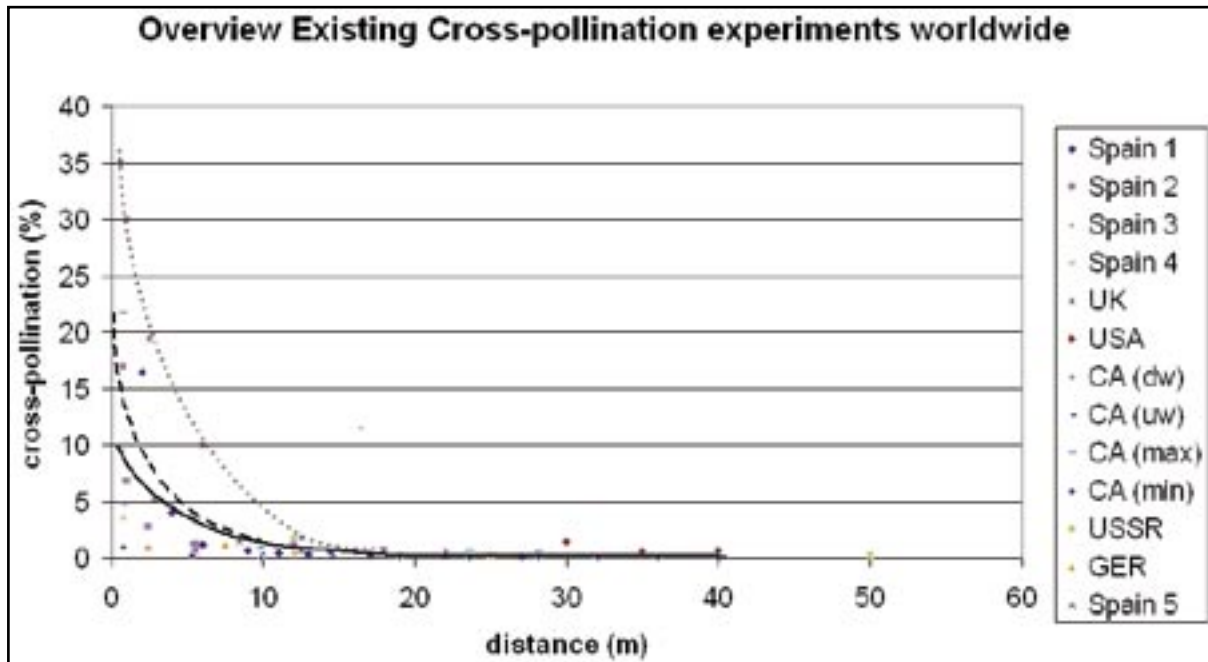


Figure 4.1: Maximum cross-pollination rate found by several studies

Dotted line: old study of 1947; *Dashed line:* the maximum cross-pollination rates of new studies; *Bold line:* maximum range of most new studies.

Data sources: Spain 1 (Molina, 2004); Spain 2 (Brookes et al., 2004); Spain 3 (Brookes et al., 2004); Spain 4 (Brookes et al., 2004); UK (Bateman, 1947); USA (Jones and Brookes, 1950); CA (Ma et al., 2004): dw = downwind, uw = upwind, max = maximum, min = minimum; USSR (Salamov, 1940); GER (Wilhelm, personal communication); Spain 5 (Molina, 2004).

Figure 4.1 shows that most cross-pollination data are in a similar range, but there also exist some extraordinary higher cross-pollination rates shown as dotted line (highest rate of old studies) and dashed line (highest rate of new studies). So far the data of all relevant experiments have in common that the cross-pollination rate rapidly decreases within the first 30 m distance and sometimes a long tail with low cross-pollination rates exists over a hundred meters or further. The initial cross-pollination rate in the first adjacent rows (distance 0.75 – 1 m) varies between different experiments from highest rates of about 35 % in old studies (*Bateman, 1947*) to values of about 22 % in recent studies with modern hybrids (*Byrne and Fromherz, 2003; Ma et al., 2004*) to lowest initial cross-pollination rates of 2 % (*Ma et al., 2004*). Variation in the first row adjacent to the pollen donor, the maximum cross-pollination rate, may be a general indicator, how good conditions (e.g. weather, flower synchrony) were for cross-pollination. Beyond the

initial 30 m, cross-pollination values are in most cases in a similar way below 0.9% (*Henry et al., 2003; Bénétrix, 2004; Bénétrix and Bloc, 2003; Melé, 2004; Weber et al. 2005a; Weber et al. 2005b*). There are also examples of higher rates, which may be the result of special climatic and topographic conditions or maize varieties that are not comparable to pollination biology and seed purity of modern hybrids today. A certain constant „background noise“, extending over longer distances, and the occurrence of „hot spots“ with single extraordinary high cross-pollinations may often be a result of seed contaminations. 100% seed purity is usually not guaranteed as assumed or observed by several authors (*Stevens et al., 2004; Salamov A.B., 1940*). *Jemison and Vayda (2001)* found a seed contamination by 0.16% of GM variety in the control of their maize variety.

Quite a number of other studies do not provide detailed data for comparisons but the results apparently fit within the variation range of cross-pollination rates shown in Figure 4.1. *Henry et al. (2003)* conducted 55 field experiments with a source field next to a receptor field. Using a regression equation they fitted the cross-pollination rates: 0.9% at 24.4 m and 0.3% at 80 m. In France *Bénétrix (2004)* found in a source field surrounded by a recipient field at the initial 10 m distance cross-pollinations of 1-2%, in distances greater than 10 m values were below 0.9%. *Bénétrix and Bloc (2003)* found a clear effect of wind in a recipient field next to a source, with values below 1% in distances of 10-12 m downwind and 5-7 m upwind and 25 m downwind of a site with very strong winds. In field designs in Spain with a source field surrounded by recipients fields values were below 0.9% at 10 m downwind and 2 m upwind (*Melé, 2004*). Data from 27 field experiments in France corroborated the decrease in cross-pollinations below 0.9% at distances greater than 25 m from the source field (*Fabié, 2004*). In some experiments (*Weber et al., 2005a; Weber et al., 2005b*) the researchers did not take sample points at specified distances but took a mixed sample directly from the harvest machine. These mixed samples are representative of a certain distance area where dilution effects can be expected. This may be a realistic approach with respect to analytical control of coexistence in agricultural practice but the data from mixed samples do not reveal distinct distribution patterns as do approaches with sample points; therefore they were not considered for further deliberations, although the data seem to fit in the same range of cross-pollination.

In conclusion, the data of already existing experiments show variation of cross-pollination rates within a certain range. Some assumptions about the reasons of this variation have been drawn but in most cases there are no clear explanations available yet. Therefore, in this study experimental settings were chosen in representative farming regions with different constellations of factors that influence the rate of cross-pollination rate. These factors are wind, size ratios of pollen donor and receptor fields as well as the synchrony of donor and receptor plant stands. The focus was on short distances for the case of adjacent transgenic maize and conventional maize fields.

4.3 MATERIAL AND METHODS

4.3.1 Site descriptions

Six field experiments were conducted in Switzerland in the canton Zurich (latitude 8° 40' 60" N, longitude 47° 25' 60", altitude 516 m) and in the canton Zug (latitude 8° 25' 0" N, longitude 47° 10' 0", altitude 445 m) in 2003 and 2004. The climate is temperate with an annual precipitation of about 1,100 mm (with a maximum of 140 mm in June and July) and an average temperature of 7.9°C with highest values in July (16.7°C). Both locations are typical of the Swiss midlands with a mixture of woods, settlements and intensive agricultural areas with wheat, maize and grassland in a hilly topography. All soils were Eutric Cambisols with a sandy loam texture.

4.3.2 Maize varieties

Xenia effect were used to detect cross-pollination by choosing maize varieties with different grain colour inheritance. Yellow grain colour is dominant over white grain colour. If pollen of a yellow grain variety fertilizes in a white grain variety field there will result a yellow grain on the white grain ear for every successful cross-pollination event. The cross-pollination rate is then measured by counting the number of yellow kernels on ears of the white grain variety. In pollen receptor fields the white grain experimental hybrid DSP17007 (Delley Seeds and Plants Company, Switzerland) was used, which has comparable agronomic traits to modern hybrids. Yellow grain varieties were Monopol, Romario and PR39G12. All four hybrids are in early maturity group, pretested in 2001 and 2002. Seed contamination of the white grain variety by a yellow grain variety was very low; none were directly detected in this study. In other extensive experiments with the same seed stock the contamination rate was calculated to be around 0.004%.

4.3.3 Field experiment design

The field experiments were designed to focus on certain factors that influence on cross-pollination.

Table 4.1: Overview of field experiment designs

Asynchronous flowering was calculated as the difference in days between the date of mid-yellow grain maize pollen shedding to the date of mid-white grain maize silking, e.g. + 2 days means that the yellow maize pollen shedding was two days later than the white maize mid silking.

Name	Design See Figure:	Year	Size white grain (ha)	Size yellow grain (ha)	Field size ratio yellow : white	Sowing date white grain	Flower asynchrony (d)
A03	4.2-A	2003	0.96	1.00	1: 1	25.04.03	+ 1
B03-A	4.2-B	2003	0.91	0.38	1: 2.5	05.05.03	+ 3
B03-B	4.2-B	2003	0.28	1.02	3.6: 1	05.05.03	+ 3
C04	4.2-C	2004	1.04	0.13	1: 8	18.05.04	0
D04	4.2-D	2004	2.12	1.46	1: 1.5	07.05.04	+ 5
E04	4.2-E	2004	1.08	0.95	1: 1	27.04.04	+ 7

The white grain test hybrid had an anthesis silking interval of around two to four days. The sowing dates of the yellow grain varieties varied, therefore in Table 4.1 the more relevant flower asynchrony is recorded.

Fig. 4.2.: Field layouts of test fields

Letters “Y” (yellow grain) and “W” (white grain) mark the positions of the yellow grain donor and the white grain receptor field parts in relation to the geographic direction; for further information see Table 4.1

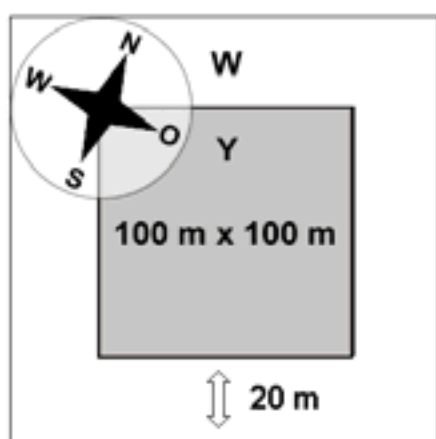


Fig. 4.2 - A (field A03)

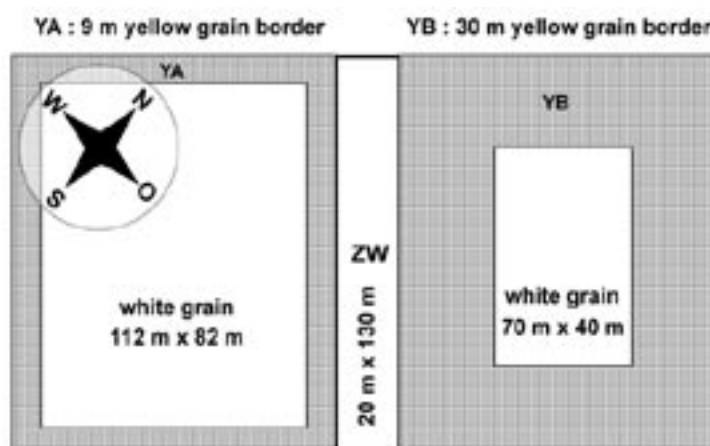


Fig. 4.2 – B (field B03-A and B03-B)

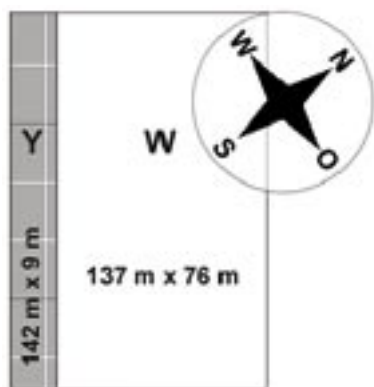


Fig. 4.2 – C (field C04)

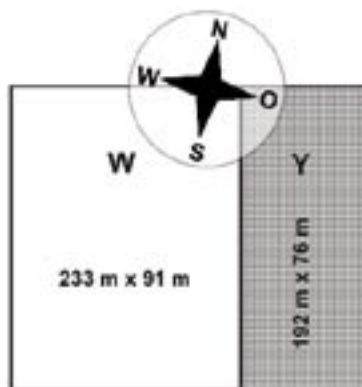


Fig. 4.2 – D (field D04)

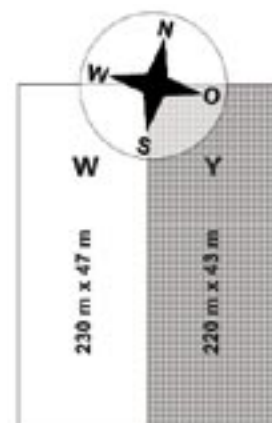


Fig. 4.2 – E (field E04)

4.3.4 Cultural procedures and crop management

Sowing of the white grain maize was done in close collaboration with one local contractor in order to guarantee that, before sowing white grain colour maize, the machine was carefully cleaned of remaining yellow grain seeds by using a pneumatic jet of water. The distance between rows was 80 cm and the final plant density about 9 plants per m². Cultivation (e.g. herbicide treatment and fertilizer application) was done according to recommended agricultural practice for a grain yield of 9 t per ha.

4.3.5 Synchronisation of flowering

The flower synchronisation of all fields was checked by regular counting at four positions every third day, taking into account the start of pollen shed in white and yellow grain maize and the time of mid-silking only of the white grain maize fields. The main fertilization took place in all the fields within about ten days, but in some fields there was a variation in the plant development of single plants, thus extending the complete fertilization time of some fields to around 20 days. Difference in the synchronisation of male yellow grain pollen shedding and female white grain silking was calculated as the number of days later than (+) or earlier than (-) the white grain pollen receptor field.

4.3.6 Meteorological Measurements

Hourly measurements of wind direction, wind speed, air moisture, air temperature and global radiation were delivered by a nearby meteorological station of MeteoSwiss. In both years it took around 22 days from the first fertilization to the last fertilisation of all test fields. For this pollination period those hours were eliminated as not valid for pollination that had a vapour pressure deficit lower than 0.2 kPa (Jarosz *et al.*, 2005). The daily hours taken into account for pollen shedding were from 6:00 to 18:00, the period during which nearly all pollen shedding occurs (Jarosz *et al.*, 2003).

4.3.7 Sampling

The experiment fields were in most cases in the shape of a long rectangle. Every fourth row was a sample row (2.4 m distance) and within these sample rows every 10 m a sampling point was marked where five ear samples were checked (about 1200 sampling points and 6000 ear samples per ha). To determine the average number of grains per ear of a field, 30 ears of a field were selected randomly and all grains counted. This was the basis for calculating the percentage of cross-pollination, because the five ears from the sampling point were checked only for the number of yellow grains.

4.3.8 Data analysis

The cross-pollination rate (CP%) of a sampling point is calculated as the number of the yellow grains of five ears divided by the average total grain number of five ears.

$$\text{CP\%} = [(\text{SPE1} + \text{SPE2} + \text{SPE3} + \text{SPE4} + \text{SPE5}) / (5 * \text{AVK})]$$

CP% Cross-pollination of a sampling point

SPE_x Ear number x at sample point (five sample ears per sample point)

AVK Average grain number of ears of a field

As the yellow grain colour is homozygous (100% of pollen will contain the yellow grain colour gene) but transgenic varieties at present are heterozygous, with only 50% of pollen containing the transgene, our data were divided by two to make them comparable to results with transgenic maize. To compare the cross-pollination rates with other data with visual markers, the values here must be multiplied by factor two.

4.4 RESULTS

4.4.1 Impact of wind on cross-pollination

Two fields (Fig. 4.2-A and 4.2-B) were chosen to assess the impact of wind on pollen dispersal, one with an inner pollinator core and one with a pollinator mantle. Wind measurements showed a similar wind situation during the main flowering period of maize for both years; the wind orientation was mainly from the directions West-Northwest and East-Northeast, with much lower impacts from other directions (Fig. 4.3).

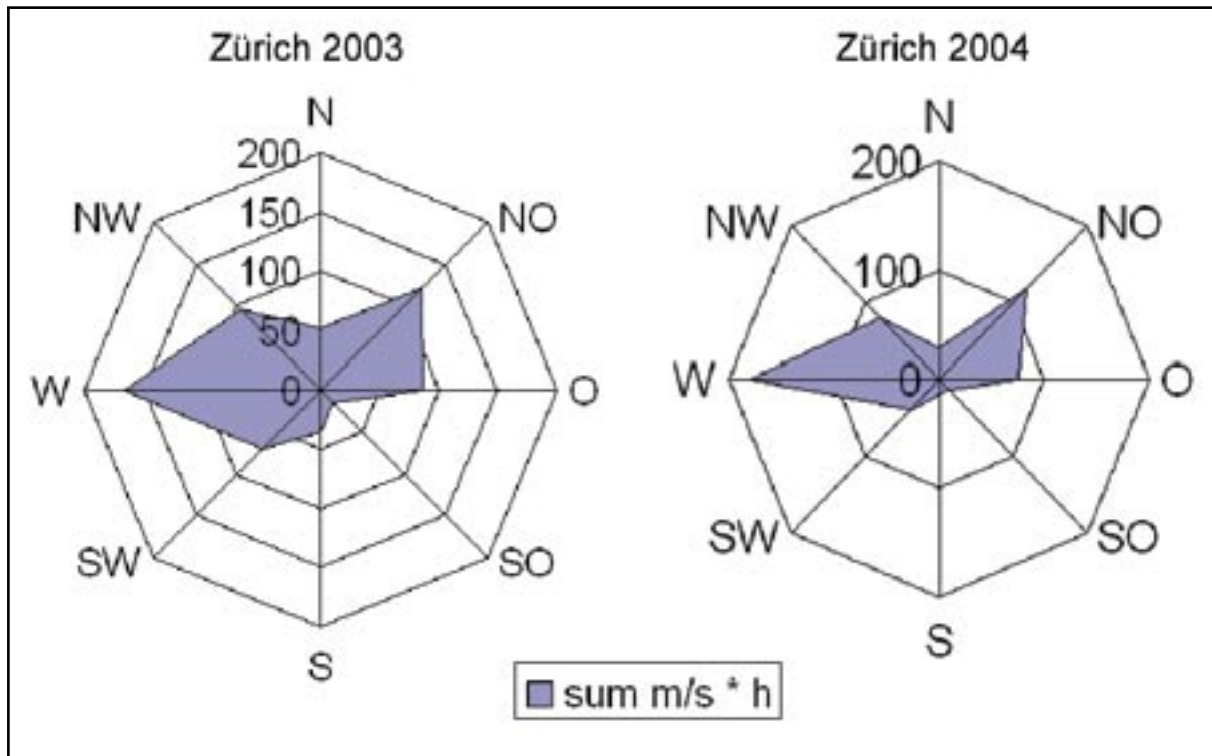


Fig. 4.3.: Hours of wind summed up as $m/s \cdot h$ per direction in 2003 (left) and 2004 (right)

Due to a very diverse small structured topography with hills and woods, typical of the Swiss midlands, the actual impact of wind at a certain time on a certain spot in the field may have deviated from the average in some cases. For the early maturing hybrids used in the experiments, pollen shedding of the tassels occurred at a height of about 2.5 m and silking at about 1 m height. As a consequence, pollen had a settling distance of about 1.5 m. Aylor (2002) reported a settling speed of maize pollen between 21 and 32 cm s^{-1} depending on the hydration status. In the worst-case of light pollen there would result a timespan of around 5 s for pollen to settle down from the tassel to the silk if other vertical forces were not acting against gravity. According to these deliberations the wind speed could be assigned to corresponding dispersal distances (Fig. 4.4).

Figure 4.4 shows a similar potential dispersal distance by horizontal wind of up to 30 m of both years, with most of the pollen probably settling over shorter distances. As mature pollen is shed, even due to a slight breeze or vibrations (Aylor 2003), the prevailing low wind speeds will release the highest quantity of pollen under conditions with only a short horizontal dispersal. The wind is a complex phenomenon with gusts, turbulence and thermal processes sometimes enabling a certain amount of pollen to reach much greater distances.

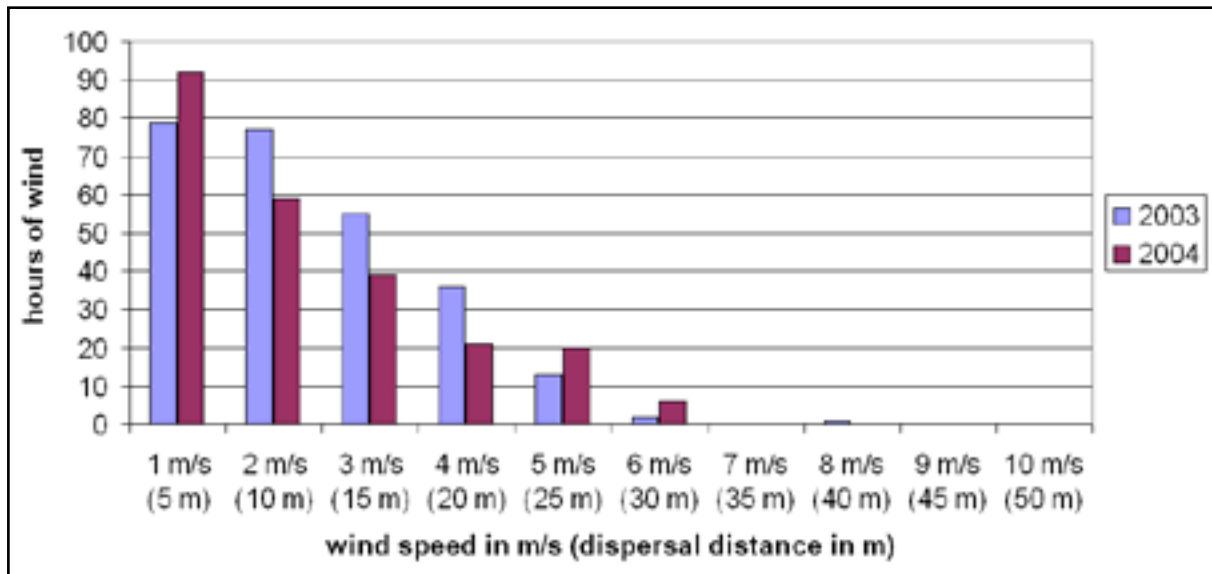


Fig. 4.4.: Wind speed and corresponding dispersal distances, assuming that it took about 5 s for pollen to settle from the tassel to the silk in 2003 and 2004

The field site “A03” (Figure 4.2-A) was chosen because it was exposed to the prevailing wind direction and embedded in a landscape with wheat fields and grassland, which have only low friction and distraction effect. As seen in the metedata the prevailing wind direction was from the north; as a consequence the highest cross-pollination rates were found in a southerly direction (Fig. 4.5), in the initial distance (0.8 m) nearly twice higher comparing to the other directions. Due to the fact that the velocity of the wind was generally low, the cross-pollination rate decreased rapidly with distance. Averaging all the directions, cross-pollination rates started with a rate of around 5% at the border to the field of yellow grain maize and decreased to below 0.9% at a distance of 13 m. Observations of single plants showed maximum rates of up to 16% and minimum rates of 1.5% yellow grains at the border row, indicating considerable plant to plant variability.

The Field design of experiment B03-A (Figure 4.2-B) was arranged to study the influence of wind direction and of field size ratios on the rate of cross-pollination. A core of white grain maize was surrounded by a nine-meter yellow grain mantle to check how far and strong cross-pollination will reach in the core field. The landscape of the Swiss midlands is variable. This site (B03-A) was shielded against the wind by settlements, hills and woods in contrast to the field A03. Although in some directions deviation was observed at 0.8m distance from the pollen source, these differences were not significant (Fig. 4.6). Cross-pollination rates in all directions varied close to the yellow grain pollen source by about 3 - 4 %, similar to the variation of about 3 – 5 % of the non wind affected directions in experiment A03 (Fig. 4.5), which showed in main wind direction a clear wind effect by an initial cross-pollination of around 9%.

4 - Variation in cross-pollination of maize in short distance

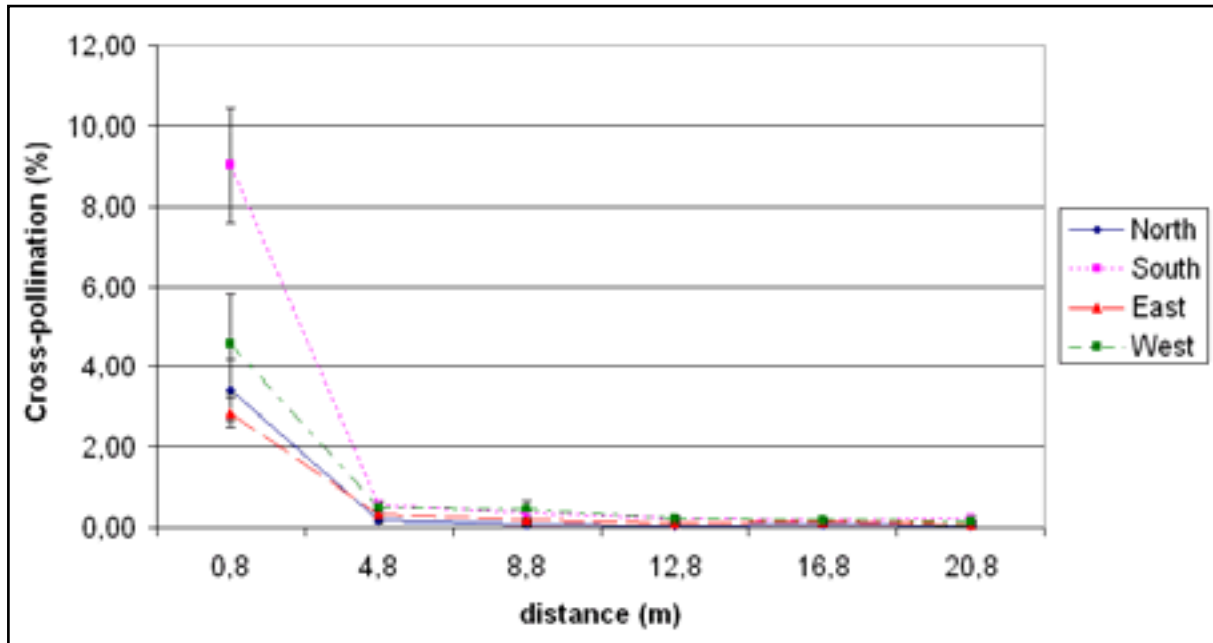


Figure 4.5.: Field A03: cross-pollination rates in different wind directions from the central pollen source core.

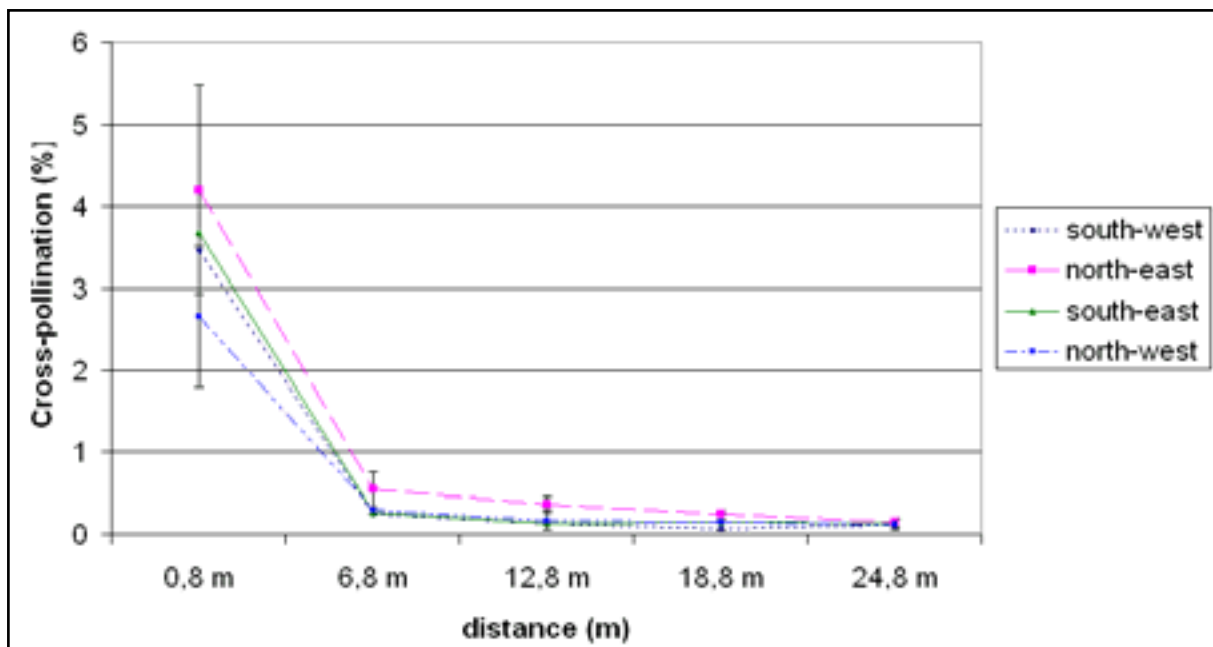


Figure 4.6.: Field B03-A: different lines show the difference in cross-pollination rates in different directions from the pollen source core.

4.4.2 Impact of pollen donor / receptor ratios

The second main purpose of experiments B03-A and B03-B (Fig. 4.2-B) was to study the impact of different field ratios of the pollen donor and receptor. White grain maize was surrounded in one part of the field (WA, Fig. 4.2-B) by a 9 m wide yellow grain maize (YA) and in the second part of the field (WB, Fig. 4.2-B) by a 30 m wide yellow grain maize (YB). The white grain stripe ZW, 20 m wide, served as a buffer space. In the case of WB a much stronger effect of yellow grain „pollen pressure“ could have resulted in higher cross-pollination rates; however, the values in both cases were nearly the same, independent of the distance to the pollen donor (Fig. 4.7).

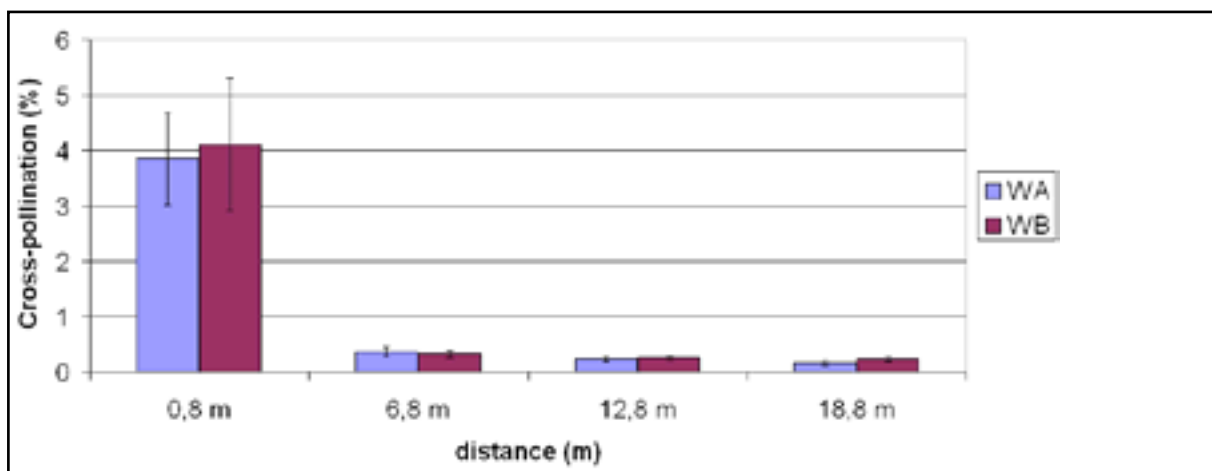


Figure 4.7.: Cross-pollination rates of the two white grain parts WA and WB of field B03 with different pollen donor / receptor ratios of 1: 2.5 and 3.6: 1, respectively. Shown data are the average of all four wind directions; vertical bars indicate the standard deviation.

In the case of field C04 (Fig. 4.2-C) a very low pollen donor - receptor ratio of 1:8 was tested, which may have decreased the initial rate of outcrossing at close and longer distances, but this was not the case (Fig. 4.8).

This experiment was carried out in the following year under different meteorological conditions. Sampling was carried out at longer distances from the source, up to twice the distance than had been tested in the other experiments. There was no deviation from the results described before (Fig. 4.7).

4 - Variation in cross-pollination of maize in short distance

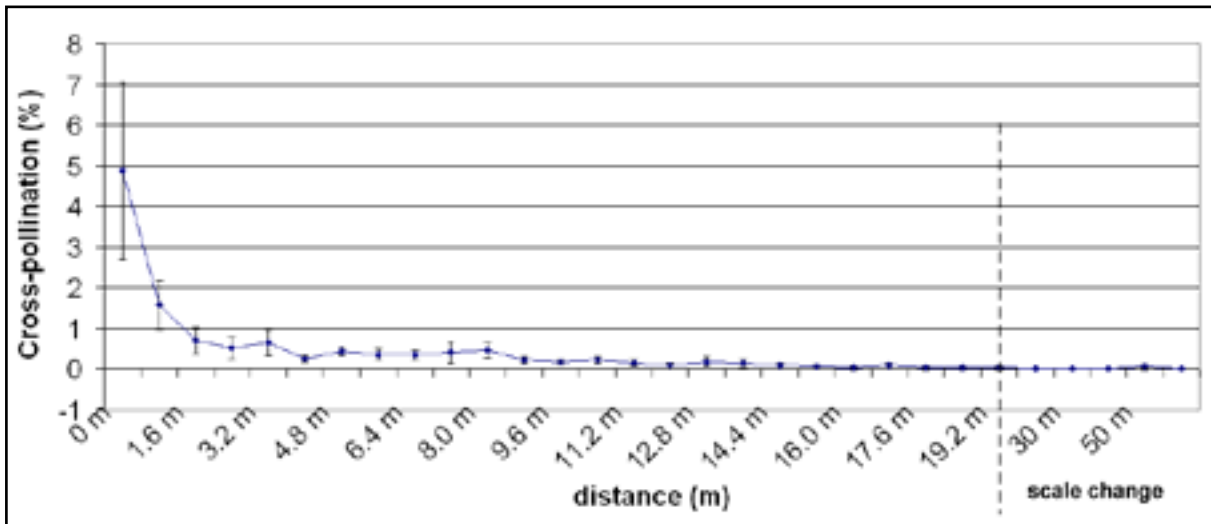


Figure 4.8.: Field size ratio and rate of cross-pollination (experiment C04). Pollen donor - receptor ratio of 1:8; vertical bars indicate standard deviation.

4.4.3 Impact of asynchronous flower

All experiments presented above had a close flower synchrony from mid pollen shedding of yellow grain maize to mid silking of white grain maize. In a wider sense, this could be regarded as the international expression of the Anthesis Silking Interval (ASI), although this is usually used with respect to isogenic internal field situations. Furthermore, for the purpose of studying cross-pollination, the average field ASI is just one factor; as described below, different developmental patterns of the individual plant level can have a much larger impact on the ASI. In experiments D04 (Fig. 4.2-D) and E04 (Fig. 4.2-E), a greater asynchrony of flowering was induced by delayed sowing of the pollen donor.

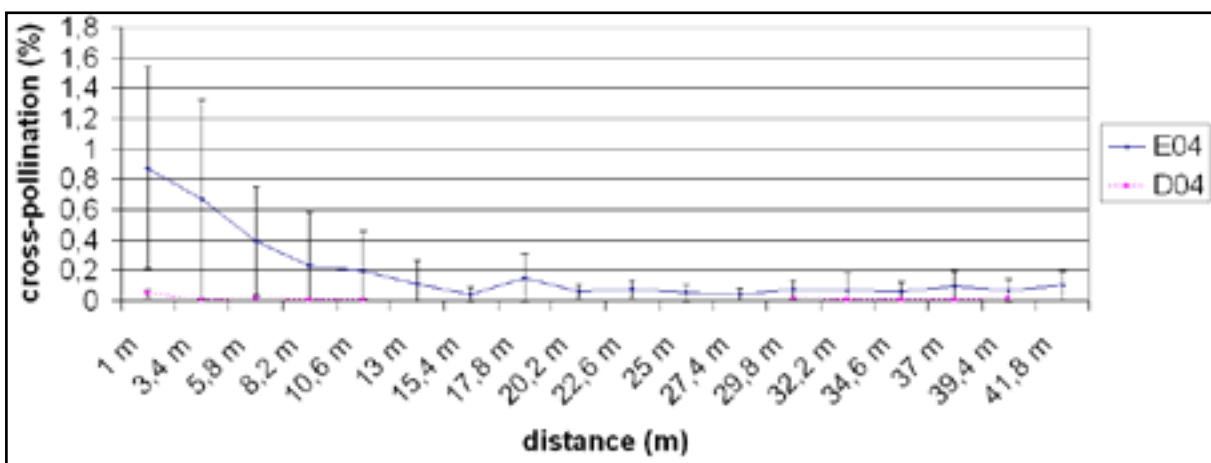


Fig. 4.9: Impact of asynchronous flowering on rates of cross-pollination with a retardation of mid-pollen shedding of the donor to mid silking of the receptor by + 7d (experiment E04) and +5 d (Experiment D04); vertical bars indicate the standard deviation.

4 - Variation in cross-pollination of maize in short distance

In experiment D04 (Fig. 4.2-D), shedding of yellow grain pollen was delayed by five days to the mid-silking of the white grain maize. The resulting rate of cross-pollination was very low compared to the three previously described experiments which had good synchrony and started with average rates of cross-pollination of about 4% at 0.8 m distance, whereas here the average value for D04 was 0.05% in the first adjacent row (Fig. 4.9). The low rate of cross-pollination was due to the fact that the silks were mostly fertilized within a few days after emergence (observable by wilted silks five to six days after their appearance) depending on weather conditions.

In experiment E04, inspite of the greater asynchrony of flowering by seven days, cross-pollinations commenced with an average of 1.15% in the first adjacent row and showed large variation (Fig. 4.9).

A detailed analysis at the single ears level showed that single plants had numerous cross-pollinations, mainly on those plants with small ears (Fig. 4.10. and Fig. 4.11.). This was particular to this field, indicating heterogeneous early development of white grain plants and, accordingly, probably a considerable number of weak plants that were in the silking stage after most of the neighbouring plants had already finished pollen shedding.

Fig. 4.10



Fig. 4.11

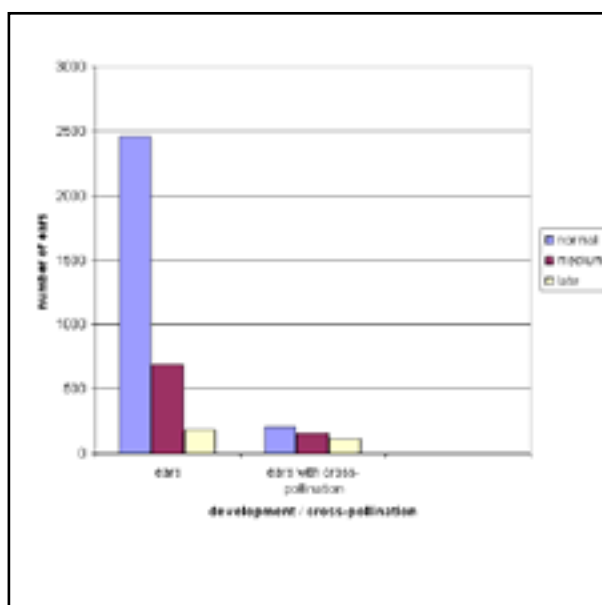


Fig. 4.10.: Normal (> 16 cm), medium (12 - 16 cm) and small ears (< 12 cm), probably corresponding to normal, medium and late plants in this experiment field.

Fig. 4.11.: Ear size and cross-pollination: numbers of sampled normal, medium and small ears (left-hand bars); number of ears per class with cross-pollination (right-hand bars).

4 - Variation in cross-pollination of maize in short distance

Ears were classified according to length by first checking the range of ear size and, second, by defining the maximum and minimum size classes. Due to different yields and ear size levels of fields every field has to be classified by an own field specific ear length scale. Plants that developed normally had probably already finished fertilization when pollen shedding of the yellow variety started, resulting in a small rate and low frequency of cross-pollination. However, small ears, probably from late-developed plants, started silking during pollen shedding of the donor field and exhibited high rates of cross-pollination (values up to 80%). As these ears were small with a low number of grains, a high rate of cross-pollination had a much lower impact on the average of the whole field. Nevertheless, they contributed to 47% of the total cross-pollination of the field, although they made up only 5% of all the ears. This heterogeneity of the white-grain field development would explain the discrepancy that, despite a seven days instead of a five days difference the former still showed a considerably higher rate of cross-pollination.

4.5 DISCUSSION

4.5.1 Rapid decrease with distance

All the experiments showed a rapid decrease in cross-pollination with distance from the pollen source, except for two experiments with asynchronous flower of five and seven days later pollen shedding of the donor, that already started with quite small values in the closest rows. The rates of cross-pollination of all experiments are summed up in a diagram (Fig. 4.12).

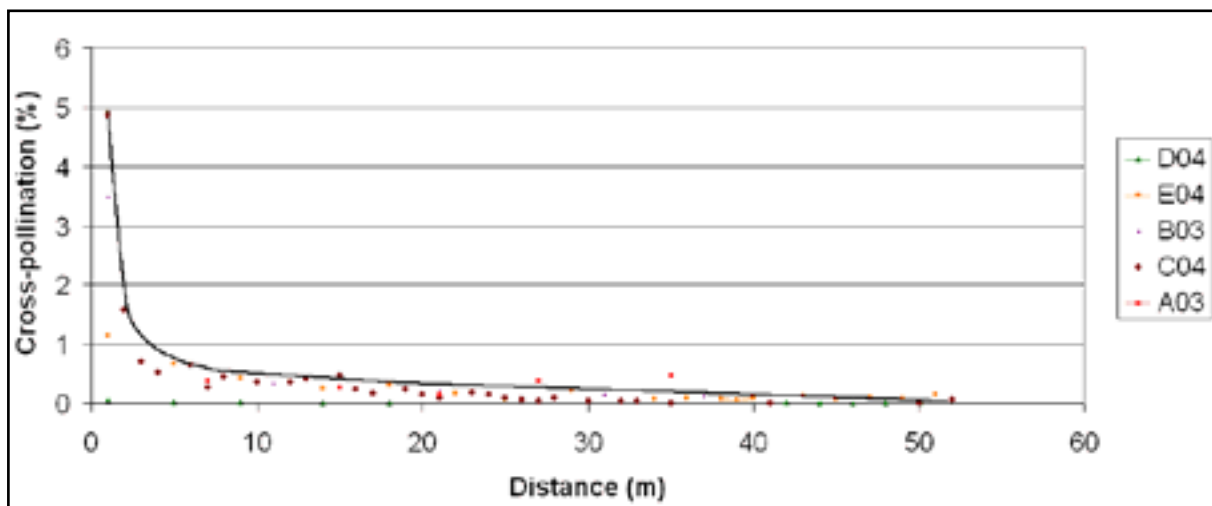


Figure 4.12.: Variation in cross-pollination across all experiments (Swiss experiments 2003 / 2004); the bold line follows the maximum cross-pollination rates (“worst-case”)

At greater distances (beyond 15 m) cross-pollination was in most experiments similarly lower than 0.9% except for a few peaks that could have resulted from contamination of the seed stock or from some wind turbulence events. The variation in the rates of cross-pollination was in a

similar range as in most of the other experiments conducted in various parts of the world (Fig. 4.1). A rather large standard deviation at close distance to the pollen source within and between the experiments in this study cannot be explained without a large investment in monitoring. The generally lower values here might be due to the special climatic conditions and small structured landscape of Switzerland as well as due to the pollination biology of the white grain test hybrid. Its ASI of about three days ensures a good supply of pollen at start of silking, which is also a feature of modern hybrids with a short flowering time.

The rapid decrease in the rate of cross-pollination can be explained by the interaction of the speed of pollen settling and the horizontal winds (Fig. 4.4). As the wind was mostly of low speed, most of the pollen was released with a low potential range of dispersal. Due to complex atmospheric processes like gusts and thermal upwinds some pollen may reach greater distances. However this pollen will be confronted to strong pollen competition from pollen shedding in the receptor maize field with high pollen concentrations just around the plant; therefore, the chance of successful cross-fertilization is low.

4.5.2 Highest variation in initial distance

The highest variation in cross-pollination occurred in the initial 10 m distance. This phenomenon can be explained by the fact that pollen concentrations are highest at this distance and factors affecting cross-pollination will have the greatest effect there. Comparison of the data of the first adjacent row shows most clearly how conditions have been for cross-pollination.

In the first adjacent row (0.8 m distance) cross-pollination was usually around 3-6 % (experiment A03, B03, C04). Similar rates were found in experiments of other researchers (*Ma et al., 2004; Brookes et al., 2004; Molina, 2004; Fig. 4.1*). The experimental fields of this study were located in a typical landscape of the Swiss midlands with a mixture of settlements, hills and woods. So in many cases the fields were only accessible to slowed down or distracted winds, which could explain the similar cross-pollination rates without a clear wind direction effect on some locations.

In a wind exposed location the cross-pollination rate did rise up to 9% in the first row (0.8 m) downwind of the donor field (compare Fig. 4.5 and Fig. 4.6). In eight field experiments, *Ma et al. (2004)* found variations in the rates of cross-pollination in the row adjacent to the donor between 1 - 22% in the downwind direction. Cross-pollination rates decreased in the case of asynchronous flowering of five days (experiment D04) to 0.05 % and in case of flower asynchrony of seven days (experiment E04) to 1.14 % in the first adjacent row (0.8 m).

Though many other factors can modify the effect of wind on cross-pollination, synchronous flowering is probably one of the most effective modification factor. Single plants in our experiments here had cross-pollination rates of up to 16%. These plants may have been in best synchrony for external pollen sources. There may be considerable variety in the impact on cross-pollination with respect to the amount of total pollen released and the dynamics of pollen

release during the daylight hours and throughout the days of pollen shedding.

4.5.3 Impact of pollen donor and receptor field sizes

It is generally assumed that the effect of the ratio of the size of pollen donor and pollen receptor fields must be of high importance on short- and long-distance outcrossing but no clear studies have been reported so far. However, there was no difference in rates of cross-pollination at source receptor ratios as different as 1: 8, 1: 2.5 and 3.6: 1 (Figures 4.7 and 4.8; 9 m and 30 m wide pollen sources). This was probably due to the fact that most cross-pollination occurred within the initial 10 m from the donor, as found in other experiments. If pollen donors are further than 10 m from the receptor field most of the pollen will not reach inside the receptor field because of the short dispersal distance. It is assumed that a field-size ratio effect may be higher at a wind exposed location. But it was obvious (Fig. 4.5) that the wind mainly affected cross-pollination rates at close distances. Therefore, it is assumed that a field size-ratio effect will be similarly low at more wind exposed locations. Whether these findings apply to large field sizes and to long distance transports as well remains still to be determined.

4.5.4 Flowering synchrony and cross-pollination rate

To investigate the impact of synchronous flowering on cross-pollination a sum-up of all experiments has been done (Fig. 4.13). Synchronous flowering is represented in relation to mid male flowering of the pollen-donor field to the mid silk emergence of the silks in the pollen receptor field. For example, - 4 days means that the start of pollen shedding of the donor was four days earlier than the emergence of the silks of the receptor and + 2 days means that the pollen shedding of the donor was two days later than the receptor silks. It is observable that other factors such as wind direction and exposition modifies the effect of synchronous flowering, as the cross-pollination rate at the end is always the result of all factors of influence. But synchronous flowering is a prerequisite of cross-pollination and that factors of influence have the chance to affect the rate.

In general, apart from some variation, highest cross-pollination rates occurred at synchrony levels of - 4 to + 3 days, with a clear decrease beyond this range. The decrease in cross-pollination by a seven days earlier start of flowering of the donor field, accompanied by an anthesis silking interval of two to four days of the receptor field, may be the effect of beginning decrease of pollen shedding of the donor field after nine days. On the other hand, the very low rate of cross-pollination of a five days delayed pollen shedding of the donor field may indicate that most silks in the receptor field were already completely fertilized by this time, as it was observable that at the field level silks were wilted (fertilized) after five to six days.

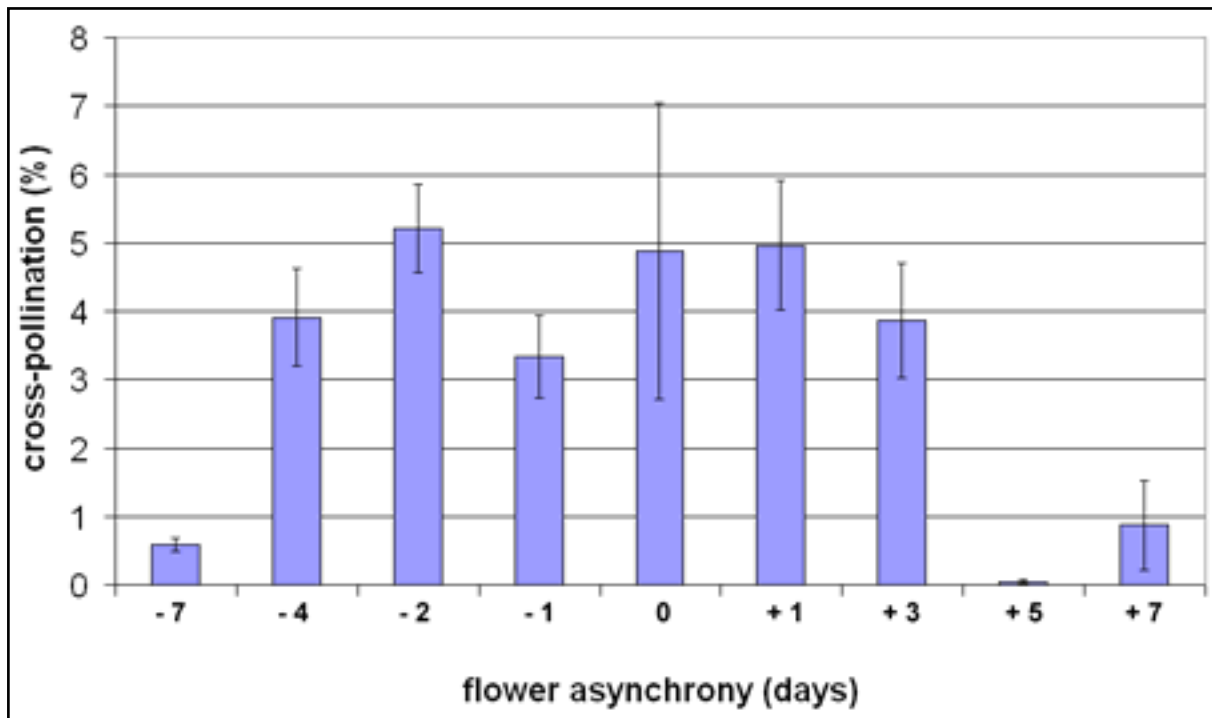


Fig. 4.13: Summary of rates of cross-pollination across all experiments in relation to flower synchrony. Flower synchrony is presented as the difference in days between time of mid-yellow grain maize pollen shedding to the date of mid-white grain maize silking (Table 4.1.). The rate of cross-pollination is related to the first adjacent row (0.8 m distance).

In conclusion, the time slot for good synchrony and high rates of cross-pollination may be very narrow and could be modified by factors like weather conditions. Very wet weather will prevent pollen shedding and in contrast warm and dry conditions could accelerate pollen shedding and fertilization processes. Furthermore, this time slot will be affected by the state of the field, how equally the field is developed. A heterogeneously developed field could prolong flowering and the possibility of cross-pollination (Figures 4.11 and Fig. 4.13). Although pollen shedding of yellow-grain maize started seven days later, the cross-pollination rate rose due to frequent cross-pollination on small ears, which probably correspond to late developed plants. In such cases even an extremely late pollen donor can be successful because the receptor field would already have passed the main phase of pollen shedding. This is a clear indication that homogenous development of the receptor field is another important factor in keeping cross-pollination rates low. This may be problematic in cases of low-input agriculture without intensive preparation of the seed bed or reduced input of fertilizers and in fields with areas of natural heterogeneous soil.

4.6 CONCLUSION

Some assumed major factors of influence on cross-pollination were studied for their effect. None was effective beyond the initial 15 m of a receptor field; this was due to a generally limited distance of pollen dispersal. With respect to the legal threshold values for GMO-free products the initial variation of cross-pollination will be diluted with field size. As some important factors related to cross-pollination are not predictable (e.g. meteorological conditions) discussions about cross-pollination should be orientated to a „worst-case“ scenario with maximum cross-pollination rates. Such „worst-case“ scenarios will be more likely when the receptor field is downwind of a GM field in a wind-exposed landscape and when flower synchrony is favourable. All the studies worldwide, including those presented here, prove that variation will always be quite high and will never be fully explained at the practical field level. Nevertheless, some factors like the specificity of variety flowering biology and of topographic patterns should be evaluated further to close some knowledge gaps. However, it is difficult to separate the effect of certain influence factors as cross-pollination rate at the end is always the sum of several effects. Some of them, like meteorological conditions, are not possible to plan ahead.

4.7 REFERENCES

- Aylor D. (2002) Settling speed of corn (*Zea mays*) pollen. *J. Aerosol Sci.* 33:1601-1607
- Aylor D. (2003) Rate of dehydration of corn (*Zea mays* L.) pollen in the air. *J. Exp. Bot.* 54: 2307-2312
- Bateman A. (1947) Contamination of seed crops. II. Wind pollination. *Heredity* 1:235-246
- Bénétrix F. (2004) Programme opérationnel d'évaluation des cultures issues des biotechnologies: bilan des programmes 2002/2003. Arvalis, Institut du végétal.
- Bénétrix F. and Bloc D. (2003) Mais OGM et non-OGM: possible coexistence. *Perspectives agricoles* 294:14-17
- Brookes G., Barfoot P., Melé E., Messeguer J., Bénétrix F., Bloc D., Foueillassar X., Fabié A., Poeydomenge C. (2004) Genetically modified maize: pollen movement and crop co-existence. PG Economics Ltd. <http://www.pgenomics.co.uk/pdf/Maizepollennov2004final.pdf>
- Byrne P.F. and Fromherz S. (2003) Can GM and non-GM crops coexist? Setting a precedent in Boulder County, Colorado, USA. *Food, Agriculture and Environment* 1(2), 258-261

Fabié A. (2004) Research on coexistence in the field – French experiments for maize. COPA-COGECA colloquy on the co-existence and thresholds of adventitious presence on GMOs in conventional seeds. <http://www.copa-cogeca.be/pdf/8bis.pdf>

Henry C., Morgan D., Weeks R., Daniels R.E., Boffey C. (2003) Farm scale evaluations of GM crops: Monitoring gene flow from GM crops to non-GM equivalent crops in the vicinity – Part I: Forage maize. DEFRA report. Central Science Laboratory Sand Hutton, Centre for Ecology and Hydrology Dorchester, p.25

Jarosz N., Loubet B., Durand B., Foueillassar X., Huber L. (2005) Variations in maize pollen emission and deposition in relation to microclimate. *Environ. Sci. Technol.* 39:4377-4384

Jarosz N., Loubet B., Durand B., McCartney A., Foueillassar X., Huber L. (2003) Field measurements of airborne concentration and deposition rate of maize pollen. *Agric. For. Meteorol.* 119:37-51

Jemison J. and Vayda M. (2001) Cross pollination from genetically engineered corn: Wind transport and seed source. *AgBioForum* 4:87-92

Jones M. and Brooks J. (1950) Effectiveness of distance and border rows in preventing outcrossing in corn. *Oklahoma Agricultural Experiment Station Technical Bulletin*, T-45

Ma B. L., Subedi K. D. and Reid L.M. (2004) Extent of cross-fertilization in maize by pollen from neighboring transgenic hybrids. *Crop Sci.* 44:1273-1282

Melé E. (2004) Spanish study shows that coexistence is possible. *ABIC* 3: 2, http://www.abic2004.org/download/ABIC2004_newsletter_no3.pdf

Molina O. J. (2004) Results of the studies into the coexistence of genetically modified and conventional maize. COPA-COGECA colloquy on the co-existence and thresholds of adventitious presence on GMOs in conventional seeds. <http://www.copa-cogeca.be/pdf/9.pdf>.

Salamov A.B. (1940) About isolation of maize. German translation in: Sanvido O., Widmer F., Winzeler M., Streit B., Szerencsits E. and F. Bigler. 2005. Koexistenz verschiedener landwirtschaftlicher Anbausysteme mit und ohne Gentechnik. Schriftenreihe der FAL 55. FAL Reckenholz

Stevens W., Berberich S., Sheckell P., Wiltse C., Halsey M., Horak M., Dunn D. (2004) Optimizing pollen confinement in maize grown for regulated products. *Crop Sci.* 44:2146-2153

Weber W.E., Bringezu T., Broer I., Holz F., Eder J. (2005a) Koexistenz von gentechnisch verändertem und konventionellem Mais: Ergebnisse des Erprobungsanbaus Silomais 2004. *Mais* 1:14-17

Weber W.E., Bringezu T., Broer I., Holz F., Eder J. (2005b) Koexistenz von gentechnisch verändertem und konventionellem Mais: Ergebnisse des Erprobungsanbaus Körnermais 2004. *Mais* 2:62-65

5 General Conclusions and Outlook

5.1 The grain colour system

The system based on grain colour has been widely approved for the detection of cross-pollination for many decades all over the world, mostly by using dominant yellow- and recessive white-grain maize varieties (*Salamov, 1940; Jones and Brooks, 1950; Hutchcroft, 1958; Raynor et al., 1972; Paterniani and Stort, 1973; Garcia et al., 1998; Ma et al., 2004; Stevens et al., 2004*). Our results surely represent one of the largest data sets generated by this system and they corroborate the results from literature mentioned before. Therefore, we conclude that the system of detecting cross-pollination by yellow- and white-grain maize varieties is a very reliable and efficient system for studying other issues of cross-pollination in the context of coexistence. Advantages of this system are low cost and therefore the potential of intensive sampling with numerous repetitions. Together with the simplicity of the method, which reduces experimental error, this method enables to generate very detailed and robust data about cross-pollination.

5.2 Current knowledge of cross-pollination in maize

When all available data of cross-pollination in maize are considered together some regularities become apparent, although there remains always some variations within a certain range. The rate of cross-pollination decreases rapidly within the initial distance of 15 m from a pollen source, a phenomenon that has been found to a similar extent by many other research groups all over the world (*Ma et al., 2004; Molina, 2004; Brookes et al., 2004; Henry et al., 2003; Bateman, 1947*). The rapid decrease in our experiments related to pollen settling speed with a potential dispersal distance in this range, modified by the prevailing wind speeds (see Chapter 4 and Fig. 4.4).

Different factors affect cross-pollination especially at initial distance from the donor field where concentration of pollen is high, but only to low effect on greater distances beyond 30 m. In wind-exposed locations the rate of cross-pollination in the first adjacent row to the pollen source can double in prevailing wind direction, but this wind effect had only a great impact on the initial 5 m; beyond 15 m the cross-pollination rate was similar in all directions (See Chapter 4 and Fig. 4.5). Thus, it is concluded that, even in alpine regions at a small field dimension, this additional wind effect gets diluted to a low effect on the cross-pollination rate of the whole field. Flower synchrony has a great impact on the rate of cross-pollination. As silks get fertilized and start senescence quickly on the single plant level there may be only a small time slot of a few days for potential cross-pollination. Thus, a well-synchronized development of plants would surely minimize the possible time slot for potential cross-pollination at the field level. From various experiments here the main time slot for possible cross-pollinations had a range between seven days earlier and 4 days later start of pollen shedding by the donor field stand, in relation to the main silking date of the pollen receptor stand. The decrease in cross-pollination due to

earlier flowering depends on how long and how massive pollen shedding of the pollen donor field is. Maximum amounts of pollen are shed when the tassel branches start to shed pollen, a few days after the start of anthesis. A reduction in cross-pollination was observed, when pollen shedding of the donor starts seven days earlier than that of the receptor; this indicates that pollen shedding decreased under these weather conditions for at least nine days, because the anthesis silking interval of the receptor plants was around three days. Highest rates of cross-pollination are expected when pollen donor and receptor field start flowering at same time. In this case most silks usually emerge around two days after start of male flower (depending on weather and anthesis silking interval of the variety) and the pollen donor field reaches maximum pollen shedding by flowering of the tassel branches. Such a tight synchrony did not occur in this study. Even when pollen shedding of the donor was seven days later than the receptor field unexpected higher cross-pollination rates occurred on some ears. But this could be explained mainly by considerable numbers of late-developed plants in the receptor field; this favoured cross-pollination due to low pollen competition.

Widely differing field size ratios of the pollen donor and receptor plants did not have an effect on the rate of cross-pollination at the investigated distances probably due to the short dispersal distance of maize pollen. Beyond 10 m only a small portion of pollen reaches the receptor field, but due to high quantitative pollen competition in the receptor field the donor pollen has only a low chance of fertilizing receptor plants.

At long distances (greater than 100 m) the rate of cross-pollination was very low and did in no case exceed 0.02 % in total of the field (field sizes 0.5 to 1.5 ha). Only in fields that were orientated in the prevailing wind direction clear but low cross-pollination rates on the field borders were observable at distances of 50 m to 371 m. These findings are in agreement with the measurement of horizontal wind speeds that showed a potential dispersal range of up to 55 m, with most pollen settling within much shorter distances. In addition, an experiment on pollen take-off showed that only a very small portion of pollen moves into higher air layers with potential longer dispersal distances. The very small amount of pollen dispersed over long distances will meet strong pollen competition in the receptor field and the chance of cross-pollination is probably very low. Rare “hot-spots” of unusually high cross-pollinations in some fields were related to contamination plants whenever checked for in detail. In conclusion, such “hot-spots”, also observed by other authors (*Henry et al., 2003; Ma et al., 2004*) may be more often the results of seed contamination than of extreme weather events.

Obviously, cross-pollination at short distances follows different mechanisms than at long distance. At short distances the most important effects will be those of flower synchrony, wind exposition and wind velocity. Furthermore, canopy architecture, canopy wind turbulence, and pollen retention on plant surfaces may be important factors as described by *Aylor (2005)*.

On long distance dispersal additional factors of influence get important. Certain meteorological mechanisms like wind gusts or thermals are necessary to lift pollen into higher air layers and initiate dispersal over long distance. The properties of the surrounding landscape may interact in a complex way with atmospheric dispersal mechanisms. Physiological parameters of the pollen, such as viability, may change during the dispersal and may also impact on cross-pollination probability (Aylor, 2004).

5.3 Challenges for cross-pollination research

Although the knowledge of cross-pollination in maize has increased, there are still some important gaps; it is not clear to which extent understanding of cross-pollination can be improved by filling them.

The type of landscape and the corresponding meteorological conditions like development of thermals or secondary wind systems are difficult to understand and measure. Atmospheric motion systems are generally positively correlated in space and time scales. Motions which occur in small volumes of air have short life spans and motions involving extensive volumes have substantially longer time periods. For example, wind blowing across a rough surface generates tiny eddies that persist for a few seconds. Isolation on a dry desert surface can cause thermal convective circulations (dust devils), which may be several meters in horizontal diameter, 100 m high, and persist for several minutes (Russel and Wilson, 1996). In the experimental locations investigated in this study the landscape was small structured and mixed, which is typical of many parts of Switzerland. Under these conditions strong atmospheric circulations may evolve less than in much more homogenous landscapes where stronger effects may be found. Also the question remains, if there could be special weather events that might result in “hot-spots” of higher cross-pollination beyond the expected distances. However, the extensive results of the long distance experiments indicate that “hot spots” are more probably due to seed contamination than due to special meteorological events.

So far, experiments were conducted with mainly one variety of receptor maize. It is not fully understood to which extent varieties may differ in the susceptibility to cross-pollination; this could be due to their flowering biology like anthesis silking interval or physiological parameters like pollen competition during germination and tube elongation on the silks. The search for pollen incompatibility factors (Frei, 2005) have shown that there exist differences in competition during pollen tube growing on the silks. Some researchers have observed ratios of grain colours that deviated from the expected values after selfing maize that was heterozygous for grain colour; this may indicate different pollen competition ability depending on pollen genotype (personal communication X. Foueillassar and U. Grossniklaus). Different cross-pollination susceptibility and ability may modify the found rates of cross-pollination, depending on the combinations of maize genotype (receptor and donor). The physiological differences may be affected in a different way by environmental conditions. This could lead to

different cross-pollination under similar conditions but with different genotypes.

A number of important environmental impacts cannot be controlled in field experiments; thus, only long-term monitoring would enable the detection of extreme events that may occur at large temporal scales with unexpected consequences. As experimental factors can be varied only to a limited extent, due to the related costs, it will scarcely be possible to understand in all details, which factor was responsible for a certain effect, as the extent of cross-pollination is always the sum up effect of many different factors. Furthermore, it would require a multidisciplinary approach including the participation of scientists with agricultural, meteorological and geographical expertise to analyze the combination of different impact factors. However, the results of all the experiments here are nevertheless a powerful proof that the most decisive patterns of cross-pollination can be reasonably well explained by the existing knowledge about pollen dispersal. The missing pollen dispersal issues may only contribute to a slight improvement of cross-pollination knowledge in relation to the high input of labour and money to get additional knowledge. It may have to be accepted that there will never be a 100% percent prediction, similar to other complex systems e.g. weather forecast.

5.4 Limits of predictions and modelling

Rates of cross-pollination are dependent upon local conditions like topography and weather. Such site-specific parameters are difficult to measure and limit our ability to generalise results and to predict pollen dispersal. To improve predictions and investigations of cross-pollination, modelling can deliver additional support. Rapid technological developments in computing power have markedly improved the utility and availability of atmospheric transport and dispersion models. Many models were developed for general applications in atmospheric science, and have been adapted for specific applications (*Westbrook and Isard, 1999*).

An already existing model of cross-pollination in maize is MAPOD (*Angevin et al., 2003*). By assuming “worst-case” scenarios it seems to be possible to draw up general guidelines for handling coexistence that can be adapted to more precision by further experiments in future. But there are numerous parameters with influence in cross-pollination rate and by the introduction of every parameter the variability sums up as well as the errors. If the model demands input of too many parameters its use will be limited to intensive scientific conditions and will be less suitable for agricultural practice.

5.5 Conclusions for agricultural practice

Several particular features must be regarded when considering cross-pollination and its management in agriculture.

5.6.1 Different types of cultivation

Different types of maize products exist, ranging from silage maize (complete plant) to exclusively grains; this has consequences for the resulting transgene content in relation to

cross-pollination rate, depending on the harvest product. Only transgenically cross-pollinated grains of conventional maize can deliver transgenic DNA content to the harvest product. If the harvest product also contains conventional material, like silage maize, then transgenic DNA will be diluted by “conventional” DNA. The results of 28 experimental silage maize fields were compared with eight fields of grain maize harvest in the German “Erprobungsanbau”; the calculated rate of cross-pollination was similar, although it was expected that grain maize would have much higher values than silage maize due to the dilution effects mentioned before (Weber *et al.*, 2005a; Weber *et al.*, 2005b). This feature is probably due to the instability of DNA that gets digested rapidly in harvested leaf material by internal and external nucleases. In conclusion, at the end it is only the DNA protected within the grain that will be accounted by PCR analysis and the variation in cross-pollination rate by harvest product type may be low.

5.6.2 Organic farming

Organic farmers usually market their products under protected labels that require GM-freedom; therefore they want coexistence handling orientated to a threshold close to the possibilities of technical analysis (around 0.1%). This is very difficult to achieve, despite cross-pollination occurs mainly over short distance. Over long distances however, there can also occur some cross-pollination at very low rates.

According to the results of experiments in the alpine region in Urner Reusstal (see Chapter 3), organic farming would not be affected by GM-maize in distances above 50 m to 100 m, not taking in account other additional sources of contamination during seed, harvest and transport. No herbicides are allowed in organic farming and the nutrient input is usually low; this may result in a higher weed pressure, which might lead to a longer anthesis-silking interval (as observed in some parts of our fields there). Thus, organic cultivated maize fields are probably more susceptible to cross-pollination. This should mainly affect field borders because of the short dispersal range of pollen.

5.6.3 Mixing of the transgene content at harvest

In most experiments on cross-pollination the samples were harvested from well defined and well distributed sampling points. But in agricultural as well as in future control practices with defined thresholds, samples usually are taken of a large mixed harvest portion. In comparison to the experimental single-spot observations in science there will be usually dilution by harvest due to mixing of areas with high and low cross-pollination in agricultural practice. This effect was checked in the German “Erprobungsanbau” (Weber *et al.*, 2005a; Weber *et al.*, 2005b); mixed samples were taken from larger harvest areas, with distances to the pollen source of 0-10 m, 20-30m up to 50-60 m. At distances up to 20-30 m and 50-60 m the transgene contents were always below 0.9%, although values of up to 1.8 and 3,7% occurred at 0-10 m in grain and silage maize, respectively (Weber *et al.*, 2005a; 2005b).

The mixing effect on the transgene content depends not only on the harvest process but also on the direction of harvest procedure. If it takes place across the gradient of cross-pollination mixing is much more effective than along the cross-pollination gradient.

5.6.4 Maize seed production

The maintenance of genetic purity in cross-pollinated plants is the most important issue for breeders and seed production companies (*Jones and Brooks, 1950; Burris, 2001*). Fields for the production of hybrid seed usually contain rows of pollen producing (male) plants alternated with rows of sterile or detasseled (female) plants acting as pollen receptors. Depending on the planting pattern, as much as 80% of the plants in a field are detasseled female parents and do not produce pollen. As a consequence, they are highly receptive to both the pollen from the male parent but also to adventitious pollen carried in from neighbouring fields by the wind. Due to inbred depression and a general trend to reduced pollen production, the male parent plants usually produce less pollen than commercial maize cultivars. In order to ensure a high degree of purity of the hybrid seed (usually 99.5%), strict cultivation conditions must be employed (*Brookes et al., 2004*). Thus, susceptibility to cross-pollination is much higher during the breeding process and during seed multiplication; special guidelines are necessary and are already implemented independently of the GM issue. The minimum separation distance in the EU is 200 m for all categories of seed production and is considered to be sufficient to maintain inbred lines with 99.9% purity (*MAFF, 2000*).

5.6.5 Specialty seeds: Topcross high oil maize

Some more special types of cultivation, i.e. the production of Topcross high-oil maize by mixing a cytoplasmatic male sterile hybrid with a high oil maize pollinator exist in rare cases. This type of cultivation is similar to the hybrid seed production discussed above. Depending on the ratio of maize plants, with and without pollen production, susceptibility to foreign pollen could increase, because of lower pollen production and competition within the field (*Lambert et al., 1998*). This would be also the case in the Plus-Hybrid system where up to 80% of the plants should be male-sterile in order to ensure xenia effects by cross-pollination with an unrelated hybrid (*Weingartner et al. 2002*).

5.6.6 Pharmaceutical GM maize plants

In the production of pharmaceuticals by GM-plants, sometimes molecules are produced that are not allowed to get in the food chain. Both the US Department of Agriculture (USDA) and the Food and Drug Administration (FDA) have started to make deliberations how to handle the production of pharmaceuticals in crops (*Mascia and Flavell, 2004*). Plants that are engineered for the use as “green factories” have the potential to become important sources of plant-based industrial compounds and plant-based pharmaceuticals (e.g. blood plasma proteins, human hepatitis B vaccine) because of several advantages compared to production in modified

mammalian cell cultures or in microbial bioreactors. The spread of these transgenes could be associated with great actual risks and intensive care is needed to avoid cross-pollination. The USDA in USA regulates this production with some guidelines. In 2003, regulations were drawn up for growing open-pollinating maize of this type require at least 1.6 km spatial isolation from other maize fields (*Stevens et al., 2004*). In future, new methods of gene containment could solve this problem.

5.7 Cross-pollination of next generation transgenic maize

The present first-generation transgenic crops carry only a few simple transgenic traits, such as herbicide resistance or the BT-Protein for certain insect resistance, based on the possibilities of the early tools of plant genetic engineering (*Maliga and Graham, 2004*). In the future there could be many more diverse applications and varieties available. As in classical breeding, it is assumed that different transgenic lines with special characteristics will be combined. In this case, if every parental line of a hybrid contains a transgene, the GM plant will be transgenic homozygous in comparison to the present transgenic heterozygous varieties. As a consequence, these transgenic homozygous plants will shed pollen that contains up to 100% transgenes and not 50% transgenes as at present. To establish guidelines that also fit in the future this has to be considered. Results of experiments with transgenic heterozygous material must be doubled and the results of experiments on cross-pollination with homozygous grain colour may show the maximum cross-pollination by transgenic varieties of the future.

Beyond pharmaceuticals, modern plant biotechnology has opened up further options for producing large quantities of commercially important industrial products in plants. These technologies have made it possible to use well-developed agricultural systems to produce large quantities efficiently and cheaply in comparison to expensive small-scale production in labs by bioreactors. The literature is full of examples of products such as speciality oils, enzymes, plastics, vaccines and antibodies (*Raskin et al. 2002; Ma et al. 2003*). As can be deduced from numerous cross-pollination experiments now, it is possible to handle coexistence to a certain threshold but zero tolerance seems to be impossible. Therefore, commercial interest exists in developing gene-containment technologies to handle coexistence with no tolerance for cross-pollination for a second generation of transgenic plants that are intended for molecular farming and production of biopharmaceuticals. Several different types of gene containments are in development and it is not yet clear which technology will be applicable in which case and at which point of time.

One containment technology focuses on Apomixis, which is the process of asexual reproduction through seed in the absence of meiosis and fertilization, generating clonal progeny of maternal origin. Apomixis might be achieved by de novo engineering of crops in combination with

male sterility. The mechanism is still very complex and poorly understood so far. Therefore, apomixis technology will not be available in the near future (*Spillane et al., 2004; Koltunow and Grossniklaus, 2003*).

The RBF (Recoverable Block of Function) technology blocks gene flow from transgenic plants at the molecular level. It prevents hybridization of transgenic plants with sexually compatible plants. Besides the known barnase and similar “suicidal” genes, other blocking constructs can be used, which can act at the levels of RNA, proteins, metabolism or hormones level (Kuvshinov et al., 2001).

Another approach is based on gametophytic cross-incompatibility genes of maize to establish a fertilization barrier against GM contamination (*Frei, 2005*). In a similar way, the manipulation of DNA methylation may also offer effective approaches for creating hybridization barriers and for controlling gene flow between species (*Bushell et al., 2003*).

The transformation of the foreign genes into the chloroplast genome is also a useful route because, in many plant species, chloroplast DNA is usually not transferred from pollen to egg. A chloroplast-based expression system that is suitable for the commercial production of recombinant proteins in tobacco leaves has been developed (*Maliga 2002*).

A solution, ready to use for the near future, is the Plus hybrid system that combines male-sterile hybrids grown in mixtures with male-fertile non-isogenic pollinator hybrids (*Feil et al., 2003*). The transgenic variety could be made male-sterile by introducing CMS (cytoplasmic male sterility), as usual in hybrid breeding. In addition to the exclusion of transgenic pollen dispersal this system also promises grain yield advantages brought about by CMS and xenia. This system is suitable when it is unnecessary to have a 100% transgenic field, e.g. BT-resistance; In this case an admixture of “conventional” plants is desirable for resistance conservation management. If a 100% transgene field is necessary, as in cases of herbicide resistance, this system is not suitable. However this system would be of great advantage as a further safety measurement in the production of pharmaceuticals as the pollen flow from the transgenic plants could be blocked or reduced.

In conclusion, there are some possibilities for gene containment. The introduction of such containment systems may change the debate and handling of coexistence as there should be no more cross-pollination. However, transgenic breeding is time- and cost-intensive, and after successful development of these containment technologies it will also take time to apply them to new transgenic varieties and finally to introduce them on the market. The old transgenic varieties will be used for as long as possible for economic reasons. New developments might be unattractive for companies because of patent rights and licensing fees. Time will show whether these new technologies will find their way into agriculture, or if coexistence will be handled and accepted with regulations and other methods like separation distances as discussed so far. These developments should be carefully considered carefully with regard to further research requirements to understand in detail all possible impact factors on this process. The time needed

to answer the last cross-pollination riddles may be too long, that in the meantime new gene containment technologies may be ready for applications, making it futile to invest more into research on cross-pollination and pollen dispersal.

So there arises the final question: Is the cross-pollination of maize of interest only today?

5.8 REFERENCES

Angevin F., Roturier C., Meynard J.M. and Klein E.K. (2003) Co-existence of GM, non-GM and organic maize crops in European agricultural landscapes: using MAPOD model to design necessary adjustments of farming practices. GMCC03 – 1st European Conference on the Co-existence of Genetically Modified Crops with Conventional and Organic Crops. http://www.agrisci.dk/GMCC-03/abs_5.htm#m5

Aylor D. E. (2005) *Quantifying maize pollen movement in a maize canopy*. Agric. For. Meteorol. 131:247-256.

Aylor, D.E. (2004) Survival of maize (*Zea mays*) pollen exposed in the atmosphere. Agric. For. Meteorol. 123:125-133.

Bateman A. (1947) Contamination of seed crops. II. Wind pollination. Heredity 1:235-246
Brookes G., Barfoot P., Melé E., Messeguer J., Bénétrix F., Bloc D., Foueillassar X., Fabié A., Poeydomenge C. (2004) Genetically modified maize : pollen movement and crop co-existence. PG Economics Ltd. <http://www.pgenomics.co.uk/pdf/Maizepollennov2004final.pdf>

Brookes G., Barfoot P., Melé E., Messeguer J., Bénétrix F., Bloc D., Foueillassar X., Fabié A., Poeydomenge C. (2004) Genetically modified maize: pollen movement and crop co-existence. PG Economics Ltd. <http://www.pgenomics.co.uk/pdf/Maizepollennov2004final.pdf>

Burris J.S. (2001) Adventitious pollen intrusion into hybrid maize seed production fields. In 56th Annual corn and sorghum research conference, in Washington DC
Bushell C., Spielman M., Scott R.J. 2003. The basis of natural and artificial postzygotic hybridization barriers in Arabidopsis species. Plant Cell. 15:1430-1442

Feil, B., Weingartner U., Stamp P. (2003) Controlling the release of pollen from genetically modified maize and increasing its grain yield by growing mixtures of male-sterile and male-fertile plants. Euphytica 130:163-165.

Frei M. (2005) A gametophytic cross incompatibility gene in maize and its use as a pollen barrier against GM contamination. Abstract of presentation on GM coexistence conference Zürich. <http://www.coexistence.ethz.ch/PDF/coex-Frei.pdf>

Garcia M., Figueroa J., Gomez R., Townsend R., Schoper J. (1998) Pollen control during transgenic hybrid maize development in Mexico. *Crop Sci.* 38:1597-1602

Henry C., Morgan D., Weeks R., Daniels R.E., Boffey C. (2003). Farm scale evaluations of GM crops: monitoring gene flow from GM crops to non-GM equivalent crops in the vicinity --Part I. Forage maize. DEFRA report. Central Science Laboratory Sand Hutton, Centre for Ecology and Hydrology Dorchester, 25 S.

Hutchcroft C.D. (1958) Contamination in seed fields of corn resulting from incomplete detasseling. *Agron. J.* 267 - 271

Jones M.D., Brooks J.S. (1950) Effectiveness of distance and border rows in preventing outcrossing in corn. Oklahoma Agricultural Experiment Station Technical Bulletin No. T-38, Stillwater, OK, 18 pp.

Koltunow A. M. and Grossniklaus U. (2003) Apomixis: A developmental perspective. *Annu. Rev. Plant Biol.* 54:547-574

Kuvshinov V., Koivu K., Kanerva A., Pehu E. (2001) Molecular control of transgene escape from genetically modified plants. *Plant Science.* 160:517-522

Lambert R.J., Alexander D.E., Han Z.J. (1998) A high oil pollinator enhancement of kernel oil and effects on grain yields of maize hybrids. *Agron. J.* 90:211-215.

Ma B.L., Subedi K.D., Reid L.M. (2004) Extent of cross-fertilization in maize by pollen from neighboring transgenic hybrids. *Crop Sci.* 44:1273-1282

Ma J.K., Drake P.M.W., Christou P. (2003) The production of recombinant pharmaceutical proteins in plants. *Nature Reviews*, 4:795-805

Maliga P. (2002) Engineering the plastid genome of higher plants. *Current Opinion in Plant Biology.* 5:164-172

Maliga P. and Graham I. (2004) Molecular farming and metabolic engineering promise a new generation of high-tech crops. *Current opinion in Plant biology.* 7:149-151

Mascia P.N. and Flavell R.B. (2004) Safe and acceptable strategies for producing foreign molecules in plants. *Current Opinion in Plant Biology.* 7:189-195

MAFF. (2000) Ministry of Agriculture, Fisheries and Food. <http://www.agindustries.org.uk/scimac/other-doc/NIABSepDistReview.pdf>

Molina O.J. (2004) Results of the studies into the coexistence of genetically modified and conventional maize. COPA-COGECA colloquy on the co-existence and thresholds of adventitious presence on GMOs in conventional seeds. <http://www.copa-cogeca.be/pdf/9.pdf>

Paterniani E. and Stort A.C. (1973) Effective maize pollen dispersal in the field. *Euphytica*. 23:129-134

Raskin I., Ribnicky D.M., Komarnytsky S., Ilis N., Poulev A., Borisuk N., Brinker A., Moreno D.A., Ripoll C., Yakoby N. (2002) Plants and human health in the twenty-first century. *Trends Biotechnol.* 20:522-531

Raynor G.S., Ogden E.C., Hayes J.V. (1972) Dispersion and deposition of pollen from experimental sources. *Agron. J.* 64:420-427

Russel R.W., and Wilson J.W. (1996) Aerial plankton detected by radar. *Nature*. 381:200-201

Salamov A.B. (1940) Über die räumliche Isolierung bei Mais (Translation of the Russian original). In: O. Sanvido, F. Widmer, M. Winzeler, B. Streit, E. Szerencsits and F. Bigler. 2005. Koexistenz verschiedener landwirtschaftlicher Anbausysteme mit und ohne Gentechnik. Schriftenreihe der FAL 55, Agroscope FAL Reckenholz

Spillane C., Curtis M.D., Grossniklaus U. (2004) Apomixis technology development – virgin births in farmers fields? *Nature Biotechnol.* Vol. 22, No. 6:687- 691

Stevens W.E., Berberich S.A., Sheckell P.A., Wiltse C.C., Halsey M.E., Horak M.J., Dunn D.J. (2004) Optimizing pollen confinement in maize grown for regulated products. *Crop Sci.* 44:2146-2153

Weber W.E., Bringezu T., Broer I., Holz F., Eder J. (2005a) Koexistenz von gentechnisch verändertem und konventionellem Mais: Ergebnisse des Erprobungsanbaus Silomais 2004. *Mais*. 1:14-17

Weber W.E., Bringezu E., Broer I., Holz F., Eder.J. (2005b) Koexistenz von gentechnisch verändertem und konventionellem Mais: Ergebnisse des Erprobungsanbaus Körnermais 2004. *Mais*. 2:62-64

5 - General Conclusions and Outlook

Weingartner U., Kaeser O., Long M., Stamp P. (2002) Combining Cytoplasmic Male Sterility and Xenia Increases Grain Yield of Maize Hybrids. *Crop Sci.* 42:1848-1856

Westbrook, J.L. and Isard S.A. (1999) Atmospheric scales of motion for dispersal of biota. *Agric. For. Meteorol.* 97: 263-274

ACKNOWLEDGEMENTS

I want to thank BUWAL, represented by Dr. phil Hans Hosbach, Swissem and Fenaco for financial support to enable the field experiments in this order. Without them the project could not have been carried out.

Thanks to Meteoschweiz for delivering meteorological data.

Thanks to:

Prof. Dr. Peter Stamp for inviting me to join his research group and start a Phd-thesis.

Dr. Alberto Soldati, who introduced me to the topic of this dissertation.

Kalle Camp and DSP Company for delivering high purity seed of the white test hybrid DSP17007 and support in every question.

Thanks go to the staff of the experimental station in Eschikon: Ernst Merz, Karin Zimmermann, Marianne Wettstein and Paul Jager. Their practical experience, advice and skilled handle was important support.

Thanks for help to the temporal staff of the group: Salome Roner, Anna and Lilly Stamp, Christoph Mörgeli, Daniel, Javi. And thanks to all who suffered for me by counting numerous yellow grains on white ears.

Thanks to the members of the administrative office of the group of Agronomy and Plant Breeding: Inge Demetriou and Madeleine Neuhauser, who saved my scarce research time by support in administrative belongs.

Thanks to Adrian Arnold and Edith Ehrensperger for helping me to find contact to the right farmers for the experiment fields. Thanks to all farmers of Tagelswangen and Urner Reusstal who disposed their fields for growing the white grain maize.

Thanks to Richard Widmer and Jakob Bissig for clean sowing of the test hybrid and for dealing with my special wishes for experiment settings.

Thanks to Marcia Schoenberg for correcting the English of the manuscript and thanks to Susanne Stamp and Boy Feil for finding typing errors.

Thanks and apology to everyone I have forgotten!

CURRICULUM VITAE

Name	Michael Bannert
Date of birth	13 June 1973
Place of birth	Berchtesgaden
Citizenship	Germany
August 1993 - June 1994	Trainee in horticulture: Cramer Orchids and Sommer alpine plants
October 1994 - December 1998	Student at the Faculty of agronomy and horticulture Technical University Munich - Weihenstephan Graduation as Dipl. Ing. Agr
January 1999 - February 2000	Research assistant at Institute of fruit science SFB 607: "Wachstum und Parasitenabwehr" TU Munich - Weihenstephan, Germany
December 2000 - November 2001	Student at Mediadesign Academy Munich Graduation as IT Manager new media
March 2000 - November 2002	Project manager sustainable agriculture Agenda 21 work group, Berchtesgaden, Germany Introduction of hypericum perforatum cultivation in mountain regions
March 2003 - March 2006	Ph.D. student in Agronomy and Plant Breeding Institute of Plant Sciences Swiss Federal Institute of Technology Zurich, Switzerland

For more information or contact visit my Webpage: www.agrisite.de