Aus der Landessaatzuchtanstalt der Universität Hohenheim

Apl. Prof. Dr. T. Miedaner

Genetics of resistance to ear diseases and mycotoxin accumulation in the pathosystems maize/*Fusarium* and wheat/*Fusarium*

Dissertation

zur Erlangung des Grades eines Doktors

der Agrarwissenschaften

vorgelegt

der Fakultät Agrarwissenschaften

von

Master of Science

Martin Messerschmidt

aus

Göppingen

Stuttgart – Hohenheim

2010

Die vorliegende Arbeit wurde am 30.06.2010 von der Fakultät Agrarwissenschaften der Universität Hohenheim als „Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften (Dr. sc. agr.)“ angenommen.

Tag der mündlichen Prüfung: 09.07.2010

1. Prodekan: Prof. Dr. Andreas Fangmaier

Berichterstatter 1. Prüfer: Apl. Prof. Dr. Thomas Miedaner

Mitberichterstatterin, 2. Prüferin: Prof. Dr. Chris-Carolin Schön

3. Prüfer: Prof. Dr. Ralf T. Vögele

Contents

[1] Löffler M., Kessel B., Ouzunova M., Miedaner T. (2010a) Population parameters for resistance to *Fusarium graminearum* and *F. verticillioides* ear rot among large sets of early, mid-late and late maturing European maize (*Zea mays* L.) inbred lines. Theor Appl Genet 120:1053-1062 DOI 10.1007/s00122-009-1233-9

[2] Löffler M., Miedaner T., Kessel B., Ouzunova M. (2010b) Mycotoxin accumulation and corresponding ear rot rating in three maturity groups of European maize inoculated by two *Fusarium* species. Euphytica 174:153-164 DOI 10.1007/s10681-009-0080-8

[3] Löffler M., Kessel B., Ouzunova M., Miedaner T. (2010c) Covariation between line and testcross performance for reduced mycotoxin concentrations in European maize after silk channel inoculation of two *Fusarium* species. Theor Appl Genet (accepted)

[4] Löffler M., Schön C.-C., Miedaner T. (2009) Revealing the genetic architecture of FHB resistance in hexaploid wheat (*Triticum aestivum* L.) by QTL meta-analysis. Mol Breed 23:473-488 DOI: 10.1007/s11032-008-9250-y

1. General introduction

Maize (*Zea mays* L.) is grown in whole Europe except the Scandinavian countries with a total acreage of 14 million hectares in 2008 (FAOstat 2009), whereof 37 % are grown as silage maize. In Central and Eastern Europe maize is mainly used for feeding, predominantly as silage maize. In Southern Europe it is used for feeding and human food (i.e. corn flakes, polenta). According to the agroclimatical conditions maize breeding material is divided into different maturity groups: “Early” for Denmark, Germany, Northern France and The Netherlands, “Mid-late” for Southern France and Hungary and “Late” for Spain, Italy and the Balkan states. In all maturity groups dents are used and in the early maturity group additionally flints.

Wheat (*Triticum aestivum* L.) is the most grown cereal in the European Union (EU27) with 24.8 million hectares and an average yield of 4.9 tons per hectare in 2007 (FAOstat 2009). It is grown from the Mediterranean Sea to Southern Scandinavia. Wheat with high baking quality is mainly used for baking of pastries, biscuits and waffles whereas wheat with low quality is used for animal feeding.

***Fusarium* in maize and wheat**

The genus *Fusarium* comprises a diverse array of phytopathogenic fungi causing ear rot in maize and *Fusarium* head blight (FHB) in wheat. FHB and red ear rot are mainly caused by *Fusarium graminearum* (teleomorph *Gibberella zeae*) and to a lesser extent by *F. culmorum* and other *Fusarium* spp. (Bottalico 1998; Görtz et al. 2008; Miedaner 1997; Munkvold 2003). In addition, maize can also be infected by other *Fusarium* spp. like *F. verticillioides* (formerly *F. moniliforme*; teleomorph *Gibberella moniliformis*), *F. proliferatum* and *F. subglutinans* (Bottalico 1998; Logrieco et al. 2002). The most important species in Europe are *F. graminearum* in Central or Eastern Europe with cool and wet climates and *F. verticillioides* in Southern Europe with warm to hot and dry climates (Bottalico 1998; Logrieco et al. 2002). But in Germany *F. verticillioides* was the most frequent isolated species from maize kernels in the warm year 2006, whereas *F. graminearum* dominated in the cool following year showing high influences of weather on the occurrence of species (Görtz et al. 2008).

Fusarioses of maize and wheat are floral infection diseases and maize ears and wheat heads are most susceptible at flowering (Kang and Buchenauer 2000; Reid et al. 1996a). In wheat initial infection of kernels occurs on the inner surfaces of lemma, glume and palea. From infected spikelets the infection spreads over the whole head taking the color of a ripe head (Kang and Buchenauer 2000). The two main infection pathways of *Fusarium* spp. into the maize kernels are either by silk infection with growth of mycelium along the silk into the ear and kernels or secondary infection after wounding of kernels, i.e. by hail, birds or insects like the European corn borer (*Ostrinia nubialis*) (Munkvold 2003). *F. verticillioides* can also infect kernels by systemic transmission from seeds or roots to kernels. Infection of *F. graminearum* starts at the tip of the ear and develops a red or pink mold covering a large contiguous proportion of the ear surface. Symptoms of infection with *F. verticillioides* are a white or light pink mold occurring on random kernels or groups of kernels. A complex relationship between maize and *F. verticillioides* exists as indicated by the dual nature of *F. verticillioides* as both pathogen and a symptomless endophyte indicates (Bacon et al. 2008). Abiotic factors may change the balanced endophytic relationship into a disease.

Fusarium infection in maize and wheat causes yield and quality losses and contaminate the grains with mycotoxins causing severe diseases in animals and humans and are, therefore, of economic importance (Martin and Johnston 1982; Presello et al. 2008; Vigier et al. 2001). In wheat FHB can cause yield losses up to 70 % (Martin and Johnston 1982), whereas in maize losses up to 48 % were reported (Vigier et al. 2001). Economic impacts of the fumonisins (FUM), mycotoxins produced by *F. verticillioides,* in the USA were estimated to be US$ 1 – 20 million in a normal year and US$ 31 – 46 million in years with a significant *Fusarium* ear rot outbreak (Wu 2007). Mycotoxins of major concern produced by *F. graminearum* in maize and wheat are deoxynivalenol (DON) and zearalenone (ZEA)(Bottalico 1998; Kang and Buchenauer 2000). Acute symptoms of DON intake are vomiting (“vomitoxin”) (Pestka 2007). Long-time intake causes immunosuppression and reproductive failure in swine. ZEA is a causal agent of hyperestrogenism in pigs and may cause premature thelarche in humans (Zöllner et al. 2002). FUM may be the causal agent of esophageal or liver cancer and neural tube defects in humans and equine leukoencephalomalacia and porcine pulmonary edema in animals (Voss et al. 2007). Since food processing does not necessarily reduce the bioavailability of these toxins (Humpf and Voss 2004; Lauren and Smith 2001) the European Union released legal limits (EC No. 1126/2007). In unprocessed wheat maximum DON and

ZEA concentrations are 1.25 and 0.1 mg kg^{-1} and in maize 1.75 and 0.35 mg kg^{-1}, respectively. For FUM maximum concentrations in unprocessed maize are 4.0 mg kg^{-1}. Maize for direct human consumption has limits of 0.75, 0.1 and 1.0 mg $kg^{-1}$of DON, ZEA and FUM, respectively. Guidelines of maximum concentrations in maize of DON and FUM for animal feeding vary between 2 – 8 mg kg^{-1} depending on species and age and of ZEA between 0.25 – 0.5 mg kg^{-1}.

Application of fungicides has very limited effects on FHB (Miedaner, personal communication) and in maize no effective fungicides has been admitted (D. Seyfang, personal communication). In tight maize-wheat crop rotations maize residuals can be a source of inoculum in the following wheat increasing the FHB severity and also DON contamination (Maiorano et al. 2008). Maize genotypes carrying a *Bt* (*Bacillus thuringienses*) gene expressing *Cry* proteins had reduced ear rot severity and mycotoxin contaminations after wounding by European corn borer (Bakan et al. 2002; Magg et al. 2002; Munkvold et al. 1997; Munkvold et al. 1999). But the effectiveness depended on factors like promoters and the genetic background and only infection after wounding is addressed. Furthermore, transgenic varieties are not allowed in most European countries at present. Agronomic methods like plowing and optimization of plant density, sowing date, nitrogen fertilization and insecticide application affected ear rot severity and fumonisin concentrations in Italy (Blandino et al. 2008a; Blandino et al. 2008b; Blandino et al. 2008c; Maiorano et al. 2008). An integrated field program regarding most of these factors could reduce fumonisin concentrations from approximately 12.4 to 1.7 mg kg^{-1} by maintaining yield levels under natural infection in Northern Italy (Blandino et al. 2009). Nevertheless, in years with adverse growing conditions, the FUM concentrations were still above the legal limits of 1.0 mg kg^{-1} for direct human consumption. Therefore, resistance breeding could be a valuable tool for a resource efficient and sustainable reduction of FHB, maize ear rot and contamination with mycotoxins.

Resistance breeding

A successful resistance breeding program requires reliable inoculation techniques. In maize, two inoculation techniques have been found to address the two main infection pathways best (Chungu et al. 1996b; Reid et al. 1996a): (1) Injection of inoculum into the silk channel simulating silk infection and (2) wounding of three to four kernels by punching four nails previously dipped into inoculum into the kernels simulating kernel or wound infection. Both resistance mechanisms were reported to be correlated moderately (Presello et al. 2004; Schaafsma et al. 2006). Resistances to maize ear rot caused by *F. graminearum* or *F. verticillioides* after silk channel or kernel inoculation are quantitatively inherited with a continuous distribution of ratings among F_1 progenies (Ali et al. 2005; Ding et al. 2008; Pérez-Brito et al. 2001; Robertson-Hoyt et al. 2006). Generation mean and diallel analyses after inoculation with *F. graminearum* or *F. verticillioides* and corresponding toxins indicated a mainly additive inheritance, but also dominant and digenic dominant × dominant effects could be found (Butrón et al. 2006; Chungu et al. 1996a; Clements et al. 2004; Gendloff et al. 1986; Nankam and Pataky 1996; Williams and Windham 2009).

In wheat a large number of QTL (quantitative trait loci) studies revealed that QTL having large and environmentally-stable effects exist (reviewed Buerstmayr et al. 2009; Paper 4), particularly the QTL *Fhb1* on chromosome 3BS (Pumphrey et al. 2007). Markers tightly linked to resistance QTL or even diagnostic markers (Liu et al. 2008) were found accelerating breeding progress by marker assisted selection (MAS). In contrast, only few QTL studies have been conducted in maize for ear rot resistance in which QTL with low effects or high dependency on environments were found (Ali et al. 2005; Ding et al. 2008; Pérez-Brito et al. 2001; Robertson-Hoyt et al. 2006). Therefore, marker-assisted selection is still not promising and resistance to both infection pathways can only be improved by phenotypic selection. Strategies could be (1) selection in variety development, (2) recurrent selection in combination with (1) or (3) introgression of resistance alleles from resistant germplasms into adapted material by backcrossing. Breeding for any trait requests genotypic variation within the breeding material. Significant genotypic differences in Canadian and US materials were reported for resistance to *F. graminearum* (Reid et al. 1996b; Schaafsma et al. 1997) and *F. verticillioides* (Clements et al. 2004; Robertson et al. 2006) and also to related mycotoxin accumulation.

Reduction of mycotoxins in the harvest is the major concern in *Fusarium* resistance breeding. But quantification of mycotoxin concentrations is expensive (~ 5 – 7 € per sample without labor for immunotests), laborious and time consuming. In contrast, ear rot rating is less laborious, cheaper and faster. Therefore, indirect selection on reduced toxin concentrations by ear rot rating could increase responses to selection assuming a fixed budget. But strong genetic associations between both traits are necessary for successful indirect selection. Strong associations between ear rot rating and mycotoxin concentrations has been reported for FUM and DON in US or Canadian inbred lines (Kleinschmidt et al. 2005; Reid et al. 1996b; Robertson et al. 2006; Vigier et al. 2001). No clear association between symptoms and ZEA concentration has been reported (Bakan et al. 2002; Cullen et al. 1983; Hart et al. 1984).

The aim of breeders is to provide maize hybrids resistant to ear rot and mycotoxin accumulation. For that, two approaches can be assessed: (1) Selection in inbred lines for ear rot resistance or (2) selection in testcrosses or a combination of both. A prerequisite for successful selection in inbred lines for hybrid resistance is a strong association between line and testcross performance. Only little information about quantitative-genetic parameters like genetic variation, genotype × environment interaction, heritabilities and correlations between ear rot severity and mycotoxin concentrations or line and testcross performance has been reported in early European elite maize (Bolduan, personal communication), but for mid-late and late European maize it is totally lacking.

Meta-analysis of *Fusarium* resistance QTL in maize or wheat

Since the advent of QTL studies a large number of species have been studied for numerous markers and traits. Having QTL data of different populations it would be interesting whether a QTL found in one population corresponds with QTL in other populations concerning the same or even other traits. Compilation of genetic information from multi-experiment data can be followed by an empirical comparison of genomic regions in form of a bibliographical review supported by statistical and graphical representation as suggested by Chardon et al. (2004) and performed for FHB resistance recently (Buerstmayr et al. 2009; Holzapfel et al. 2008). A more advanced approach is to combine results from independent published QTL studies by a statistical meta-analyses approach (Goffinet and Gerber 2000) which was implemented in the software package ‘MetaQTL’ (Veyrieras et al. 2007). This analysis reduces the main disadvantages over the empirical review. i.e. the unavailability of error parameters, like confidence intervals. QTL meta-analyses have been conducted for flowering time in maize (Chardon et al. 2004) and earliness in wheat (Hanocq et al. 2007). In the former study 62 so-called meta-QTL (MQTL) were found and resulted in 19 associations between maize and QTL and genes in rice and Arabidopsis. In the latter study the function of known major genes was confirmed and four additional MQTL were identified as candidates for use in MAS.

In maize QTL studies on ear rot resistance have been conducted in only six populations using either *F. graminearum* or *F. verticillioides* and different inoculation techniques addressing either silk or kernel resistance (Ali et al. 2005; Ding et al. 2008; Pérez-Brito et al. 2001; Robertson-Hoyt et al. 2006). In contrast, many QTL studies have been performed on FHB resistance in wheat (reviewed by Buerstmayr et al. 2009). But up to now, no QTL meta-analysis has been conducted on resistances to *Fusarium* in maize and wheat compiling the information of the different published QTL analyses.

Objectives

The objectives of this study were to

1) Analyze methods of ear rot testing by
    a. comparing two artificial inoculation techniques and natural infection for evaluation of resistance to *Fusarium* in maize in the late maturity group
    b. comparing different isolates of both *F. graminearum* and *F. verticillioides* for their aggressiveness
    c. determining the relationship of resistance to *F. graminearum* and *F. verticillioides* in the early maturity group
2) Estimate population parameters by
    a. assessing the genetic variation and heritabilities of ear rot resistance caused by *F. graminearum* or *F. verticillioides* of inbred lines in large sets of three European maturity groups (early, mid-late, late)
    b. examining the association between mycotoxin concentrations and ear rot rating in subsets of all inbred lines
    c. determining the relationship of line per se and testcross performance for ear rot severity and mycotoxin concentrations
3) Summarize different published QTL studies by a statistical meta-analysis approach for resistance to ear rot in maize and to FHB in wheat

References

Ali ML, Taylor JH, Jie L, Sun G, William M, Kasha KJ, Reid LM, Pauls KP (2005) Molecular mapping of QTLs for resistance to *Gibberella* ear rot, in corn, caused by *Fusarium graminearum*. Genome 48:521-533

Bacon CW, Glenn AE, Yates IE (2008) *Fusarium verticillioides*: Managing the endophytic association with maize for reduced fumonisins accumulation. Toxin Rev 27:411-446

Bakan B, Melcion D, Richard-Molard D, Cahagnier B (2002) Fungal growth and *Fusarium* mycotoxin content in isogenic traditional maize and genetically modified maize grown in France and Spain. J Agric Food Chem 50:728-731

Blandino M, Reyneri A, Colombari G, Pietri A (2009) Comparison of integrated field programmes for the reduction of fumonisin contamination in maize kernels. Field Crop Res 111:284-289

Blandino M, Reyneri A, Vanara F (2008a) Effect of plant density on toxigenic fungal infection and mycotoxin contamination of maize kernels. Field Crop Res 106:234-241

Blandino M, Reyneri A, Vanara F (2008b) Influence of nitrogen fertilization on mycotoxin contamination of maize kernels. Crop Prot 27:222-230

Blandino M, Reyneri A, Vanara F, Pascale M, Haidukowski M, Saporiti M (2008c) Effect of sowing date and insecticide application against European corn borer (Lepidoptera: Crambidae) on fumonisin contamination in maize kernels. Crop Prot 27:1432-1436

Bottalico A (1998) *Fusarium* diseases of cereals: Species complex and related mycotoxin profiles, in Europe. J Plant Pathol 80:85-103

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for *Fusarium* head blight resistance in wheat: A review. Plant Breed 128:1-26

Butrón A, Santiago R, Mansilla P, Pintos-Varela C, Ordás A, Malvar RA (2006) Maize (*Zea mays* L.) genetic factors for preventing fumonisin contamination. J Agric Food Chem 54:6113-6117

Chardon F, Virlon B, Moreau L, Falque M, Joets J, Decousset L, Murigneux A, Charcosset A (2004) Genetic architecture of flowering time in maize as inferred from quantitative trait loci meta-analysis and synteny conservation with the rice genome. Genetics 168:2169-2185

Chungu C, Mather DE, Reid LM, Hamilton RI (1996a) Inheritance of kernel resistance to *Fusarium graminearum* in maize. J Hered 87:382-385

Chungu C, Mather DE, Reid LM, Hamilton RI (1996b) Comparison of techniques for inoculating maize silk, kernel, and cob tissues with *Fusarium graminearum*. Plant Dis 80:81-84

Clements MJ, Maragos CM, Pataky JK, White DG (2004) Sources of resistance to fumonisin accumulation in grain and *Fusarium* ear and kernel rot of corn. Phytopathology 94:251-260

Cullen D, Caldwell MW, Smalley EB (1983) Susceptibility of maize to *Gibberella* ear rot: Relatoinship to host genotype, pathogen virulence, and zearalenone contamination. Plant Dis 67:89-91

Ding J-Q, Wang X-M, Chander S, Yan J-B, Li J-S (2008) QTL mapping of resistance to *Fusarium* ear rot using a RIL population in maize. Mol Breed 22:395-403

FAOstat (2009) http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor verified 15/7/2009

Gendloff EH, Rossman EC, Casale WL, Isleib TG, Hart LP (1986) Components of resistance to *Fusarium* ear rot in field corn. Phytopathology 76:684-688

Goffinet B, Gerber S (2000) Quantitative trait loci: A meta-analysis. Genetics 155:463-473

Görtz A, Oerke E, Steiner U, Waalwijk C, Vries I, Dehne H (2008) Biodiversity of *Fusarium* species causing ear rot of maize in Germany. Cereal Res Commun 36:617-622

Hanocq E, Laperche A, Jaminon O, Lainé A, Le Gouis J (2007) Most significant genome regions involved in the control of earliness traits in bread wheat, as revealed by QTL meta-analysis. Theor Appl Genet 114:569-584

Hart LP, Gendloff E, Rossman EC (1984) Effect of genotypes on ear rot infection by *Gibberella zeae*. Plant Dis 68:296-298

Holzapfel J, Voss H-H, Miedaner T, Korzun V, Häberle J, Schweizer G, Mohler V, Zimmermann G, Hartl L (2008) Inheritance of resistance to *Fusarium* head blight in three European winter wheat populations. Theor Appl Genet 117:1119-1128

Humpf H-, Voss KA (2004) Effects of thermal food processing on the chemical structure and toxicity of fumonisin mycotoxins. Mol Nutr Food Res 48:255-269

Kang Z, Buchenauer H (2000) Cytology and ultrastructure of the infection of wheat spikes by *Fusarium culmorum*. Mycol Res 104:1083-1093

Kleinschmidt CE, Clements MJ, Maragos CM, Pataky JK, White DG (2005) Evaluation of food-grade dent corn hybrids for severity of *Fusarium* ear rot and fumonisin accumulation in grain. Plant Dis 89:291-297

Lauren DR, Smith WA (2001) Stability of the *Fusarium* mycotoxins nivalenol, deoxynivalenol and zearalenone in ground maize under typical cooking environments. Food Addit Contam 18:1011-1016

Liu S, Pumphrey MO, Gill BS, Trick HN, Zhang JX, Dolezel J, Chalhoub B, Anderson JA (2008) Toward positional cloning of FHB1, a major QTL for *Fusarium* head blight resistance in wheat. Cereal Res Commun 36:195-202

Logrieco A, Mulé G, Moretti A, Bottalico A (2002) Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. Eur J Plant Pathol 108:597-609

Magg T, Melchinger AE, Klein D, Bohn M (2002) Relationship between European corn borer resistance and concentration of mycotoxins produced by *Fusarium* spp. in grains of transgenic Bt maize hybrids, their isogenic counterparts, and commercial varieties. Plant Breed 121:146-154

Maiorano A, Blandino M, Reyneri A, Vanara F (2008) Effects of maize residues on the *Fusarium* spp. infection and deoxynivalenol (DON) contamination of wheat grain. Crop Prot 27:182-188

Martin RA, Johnston HW (1982) Effects and control of *Fusarium* diseases of cereal grains in the Atlantic Province. Can J Plant Pathol 4:210-216

Miedaner T (1997) Breeding wheat and rye for resistance to *Fusarium* diseases. Plant Breed 116:201-220

Munkvold GP (2003) Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. Eur J Plant Pathol 109:705-713

Munkvold GP, Hellmich RL, Rice LG (1999) Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and nontransgenic hybrids. Plant Dis 83:130-138

Munkvold GP, Hellmich RL, Showers WB (1997) Reduced *Fusarium* ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. Phytopathology 87:1071-1077

Nankam C, Pataky JK (1996) Resistance to kernel infection by *Fusarium moniliforme* in the sweet corn inbred IL125b. Plant Dis 80:593-598

Pérez-Brito D, Jeffers D, González-de-León D, Khairallah M, Cortés-Cruz M, Velázquez-Cardelas G, Azpíroz-Rivero S, Srinivasan G (2001) QTL mapping of *Fusarium moniliforme* ear rot resistance in highland maize, mexico: Cartografía de qtl de la resistencia a la pudrición de la mazorca (*Fusarium moniliforme*) en maíz de valles altos, méxico. Agrocencia 35:181-196

Pestka JJ (2007) Deoxynivalenol: Toxicity, mechanisms and animal health risks. Anim Feed Sci Technol 137:283-298

Presello DA, Botta G, Iglesias J, Eyhérabide GH (2008) Effect of disease severity on yield and grain fumonisin concentration of maize hybrids inoculated with *Fusarium verticillioides*. Crop Prot 27:572-576

Presello DA, Reid LM, Mather DE (2004) Resistance of Argentine maize germplasm to *Gibberella* and *Fusarium* ear rots. Maydica 49:73-81

Pumphrey MO, Bernardo R, Anderson JA (2007) Validating the Fhb1 QTL for *Fusarium* head blight resistance in near-isogenic wheat lines developed from breeding populations. Crop Sci 47:200-206

Reid LM, Hamilton RI, Mather DE (1996a) Screening maize for resistance to *Gibberella* ear rot. Agriculture and Agri-Food Canada Ottawa, ON.Tech Bull Publ 1996-5E

Reid LM, Stewart DW, Hamilton RI (1996b) A 4-year study of the association between *Gibberella* ear rot severity and deoxynivalenol concentration. J Phytopathol 144:431-436

Robertson LA, Kleinschmidt CE, White DG, Payne GA, Maragos CM, Holland JB (2006) Heritabilities and correlations of *Fusarium* ear rot resistance and fumonisin contamination resistance in two maize populations. Crop Sci 46:353-361

Robertson-Hoyt LA, Jines MP, Balint-Kurti PJ, Kleinschmidt CE, White DG, Payne GA, Maragos CM, Molnár TL, Holland JB (2006) QTL mapping for *Fusarium* ear rot and fumonisin contamination resistance in two maize populations. Crop Sci 46:1734-1743

Schaafsma AW, Tamburic-Ilincic L, Reid LM (2006) Fumonisin B1 accumulation and severity of *Fusarium* ear rot and *Gibberella* ear rot in food-grade corn hybrids in Ontario after inoculation according to two methods. Can J Plant Pathol 28:548-557

Schaafsma AW, Nicol RW, Reid LM (1997) Evaluating commercial maize hybrids for resistance to *Gibberella* ear rot. Eur J Plant Pathol 103:737-746

Veyrieras J, Goffinet B, Charcosset A (2007) MetaQTL: A package of new computational methods for the meta-analysis of QTL mapping experiments. BMC Bioinformatics 8:49

Vigier B, Reid LM, Dwyer LM, Stewart DW, Sinha RC, Arnason JT, Butler G (2001) Maize resistance to *Gibberella* ear rot: Symptoms, deoxynivalenol, and yield. Can J Plant Pathol 23:99-105

Voss KA, Smith GW, Haschek WM (2007) Fumonisins: Toxicokinetics, mechanism of action and toxicity. Anim Feed Sci Technol 137:299-325

Williams WP, Windham GL (2009) Diallel analysis of fumonisin accumulation in maize. Field Crops Res 114:324-326

Wu F (2007) Measuring the economic impacts of *Fusarium* toxins in animal feeds. Anim Feed Sci Technol 137:363-374

Zöllner P, Jodlbauer J, Kleinova M, Kahlbacher H, Kuhn T, Hochsteiner W, Lindner W (2002) Concentration levels of zearalenone and its metabolites in urine, muscle tissue, and liver samples of pigs fed with mycotoxin-contaminated oats. J Agr Food Chem 50:2494-2501

2. Paper 1: Population parameters for resistance to *Fusarium graminearum* and *Fusarium verticillioides* ear rot among large sets of early, mid-late and late maturing European maize (*Zea mays* L.) inbred lines

M. Löffler, B. Kessel, M. Ouzunova, T. Miedaner

M. Löffler, T. Miedaner, State Plant Breeding Institute, Universität Hohenheim (720),
Fruwirthstraße 21, 70593 Stuttgart-Hohenheim, Germany

B. Kessel, M. Ouzunova, KWS SAAT AG, Grimsehlstraße 31, 37555 Einbeck, Germany

Theor Appl Genet 120:1053-1062

DOI 10.1007/s00122-009-1233-9

The original publication is available at http://springerlink.metapress.com/content/100386/

Abstract

Infection of maize ears with *Fusarium graminearum* (FG) and *F. verticillioides* (FV) reduces yield and contaminate the grain with mycotoxins. Breeding varieties resistant to both *Fusarium* spp. is one alternative to minimize these problems. The objectives of our study were to draw conclusions on breeding for ear rot resistance by estimating variance components, heritabilities and correlations between resistances to FV and FG severity and to investigate different inoculation methods. In the years 2007 and 2008 three maturity groups (early, midlate, late) each comprising about 150 inbreds were tested in Germany, France, Italy, and Hungary according to their maturity group. Silk channel inoculation was applied for FG (early) and FV (all groups). In the late maturity group, additionally kernel inoculation was applied in a separate trial. The percentage of mycelium coverage on the ear was rated at harvest (0-100 %). In all maturity groups significant ($P<0.01$) genotypic variances of ear rot severity were found. Inoculation was superior to natural infection because of higher disease

severities and heritabilities. In the early maturity group FG caused significantly ($P<0.01$) higher ear rot severity than FV (Flint vs. dent: 61.7 and 55.1 % FG *vs.* 18.2 and 11.1 % FV ear rot severity, respectively). FV inoculation in Southern Europe (midlate, late) resulted in similar means between 10.3 and 14.0 %. Selection is complicated due to significant ($P<0.01$) genotype × environment interactions. Correlation between FG and FV severity was moderate in flints and dents ($r=0.59$ and 0.49, respectively) but lines resistant to both fungi exist. In conclusion, chances for selecting improved European elite maize material within the existing germplasms is promising by multi-environmental inoculation trials.

3. Paper 2: Mycotoxin accumulation and corresponding ear rot rating in three maturity groups of European maize inoculated by two *Fusarium* species

M. Löffler, T. Miedaner, B. Kessel, M. Ouzunova

M. Löffler, T. Miedaner, State Plant Breeding Institute, Universität Hohenheim (720), Fruwirthstraße 21, 70593 Stuttgart-Hohenheim, Germany

B. Kessel, M. Ouzunova, KWS SAAT AG, Grimsehlstraße 31, 37555 Einbeck, Germany

Euphytica 174:153-164

DOI 10.1007/s10681-009-0080-8

The original publication is available at http://springerlink.metapress.com/content/102881/

Abstract

In maize, *Fusarium graminearum* (FG) and *F. verticillioides* (FV) produce the mycotoxins deoxynivalenol (DON), zearalenone (ZEA) and fumonisins (FUM), respectively. The EU released limits for these toxins in food. Breeding resistant varieties is one alternative to fulfill these limits. Measurement of mycotoxin concentrations is expensive and time consuming. If indirect selection based on cheap and fast ear rot rating is feasible, efficiency of selection could be increased. The objective of this study was to analyze correlations between mycotoxin concentrations and ear rot rating by inoculating three maturity groups (early, mid-late, late) each comprising about 50 inbred lines tested in Central and Southern Europe. In the early maturity group flint lines were more susceptible in all instances except ZEA than dent lines. Broad ranges and significant ($P<0.01$) genotypic variances were detected, but also genotypic × environment interaction variances were significant ($P<0.01$). Similar or even higher heritabilities of ear rot rating than those of mycotoxin concentrations were found (0.61

- 0.93 and 0.56 – 0.89, respectively). Although high genotypic correlations between FUM and DON or ZEA were found (0.77; 0.76, respectively), separate testing of FV and FG and corresponding mycotoxins is necessary since genotypes resistant to FV were not necessarily resistant FG and *vice versa*. Identification of lines with reduced mycotoxin concentrations by ear rot rating is promising due to moderate to high heritabilities and high genotypic correlations between ear rot and corresponding mycotoxin concentrations (0.87 – 0.99). Assuming fixed budgets indirect selection based on cost efficient ear rot rating increases selection intensity and therefore is more effective than direct selection for reduced mycotoxin concentrations.

4. Paper 3: Covariation between line and testcross performance for reduced mycotoxin concentrations in European maize after silk channel inoculation of two *Fusarium* species

M. Löffler, B. Kessel, M. Ouzunova, T. Miedaner

M. Löffler, T. Miedaner, State Plant Breeding Institute, Universität Hohenheim (720), Fruwirthstraße 21, 70593 Stuttgart-Hohenheim, Germany

B. Kessel, M. Ouzunova, KWS SAAT AG, Grimsehlstraße 31, 37555 Einbeck, Germany

Theor Appl Genet (accepted)

The original publication will be available at

http://springerlink.metapress.com/content/100386/

Abstract

Fusarium spp. in maize can contaminate the harvest with mycotoxins harmful to humans and animals. Breeding resistant varieties is one alternative to reduce contamination with mycotoxins. Little is known about population parameters for resistance to ear rot and mycotoxins deoxynivalenol, zearalenone and fumonisins after infection with *F. graminearum* or *F. verticillioides*, respectively. The objectives of this study were to draw conclusions for resistance breeding to one of the *Fusarium* spp. in three maize maturity groups after silk channel inoculation. For that, variation and covariation of line and testcross performance and correlations between both species and between mycotoxins and ear rot resistance were calculated. Means of ear rot after infection with *F. graminearum* were considerably higher than with *F. verticillioides*. Medium phenotypic correlations (r=0.46 – 0.65) between resistances to both *Fusarium* spp. implicate separate testing. Analyses of variance revealed significant (P<0.01) differences among inbreds in line and testcross performance for 30 to 60 entries per maturity group. Multi-environmental trials for accurate selection are necessary due

to significant ($P<0.1$) genotype × environment interactions. High genotypic correlations between ear rots and mycotoxins ($r \geq 0.80$) and similar heritabilities of both traits revealed the effectiveness of indirect selection for mycotoxin concentrations based on ear rot rating. Medium genotypic correlations between line and testcross performance were found ($r=0.64$ – 0.83). One moderately to highly susceptible tester is sufficient due to the high genotypic correlations between testcrosses of different testers ($r=0.80$ – 0.94). Indirect selection for testcross performance based on per se line performance is less effective by regarding relative efficiencies. In conclusion, selection for resistance to ear rot and mycotoxin accumulation should be started among testcrosses tested first for general combining ability based on ear rot data in parallel with a negative selection for line per se performance.

5. Paper 4: Revealing the genetic architecture of FHB resistance in hexaploid wheat (*Triticum aestivum* L.) by QTL meta-analysis

M. Löffler, C.-C. Schön, T. Miedaner

M. Löffler, T. Miedaner, State Plant Breeding Institute, Universität Hohenheim (720), Fruwirthstraße 21, 70593 Stuttgart-Hohenheim, Germany

C.-C. Schön, Plant Breeding, Technische Universität München, Am Hochanger 4, 85350 Freising, Germany

Mol Breed (2009) 23:749-488

DOI 10.1007/s11032-008-9250-y

The original publication is available at http://springerlink.metapress.com/content/100317/

Abstract

In wheat *Fusarium* head blight (FHB) results in reduced yield and quality by accumulation of mycotoxins. The objective of this study were identifying genomic regions in wheat involved in the control of FHB resistance applying a QTL meta-analysis approach by combining QTL of 30 mapping populations to propose independent meta-QTL (MQTL). At first, a consensus map was created on which initial QTL were projected. On twelve chromosomes nineteen MQTL comprising two to thirteen initial QTL with widely varying confidence intervals were found. Some of them colocated with genomic regions previously identified (e.g. chromosomes 3BS, 6B), however, some MQTL were newly detected by this study. Separate analysis of populations with the same resistant parent showed a high consistency for the Chinese spring wheat donor ‘Sumai 3’, but only few consistency for the Chinese donor ‘Wangshuibai’ and the Swiss donor ‘Arina’. According to our results breeders can in future (1) choose parents for crossing not comprising the same resistance loci or QTL intervals, (2)

exploit new MQTL, and (3) select markers of some of these MQTL to be used in marker-assisted selection.

6. General discussion

Methodical aspects

Is artificial inoculation necessary?

In maize breeding labor and time are limited resources particularly during the work peaks at flowering. Thus, it is important to know whether natural infection is sufficient or laborious and time consuming inoculation should be applied to cause reliably sufficient ear rot severity. In all maturity groups and locations of our studies artificial inoculation and natural infection resulted in significant ($P<0.01$) ear rot differentiation among the inbred lines and testcrosses illustrating that both natural infection and inoculation were capable to statistically distinguish resistant from susceptible germplasm. Nevertheless, natural infection caused only low ear rot severity at all locations of the early maturity group and at MUR of the mid-late. Visual differentiation and consequently selection on such low levels of ear rot severity is difficult and, therefore, inoculation should be applied to achieve a reliably sufficient infection increasing visual differences among genotypes. At ALZ of the mid-late and at MCE of the late maturity group ear rot severities of natural infection and inoculation were similar. Thus, a breeder could save extra trials meaning less monetary input and less work while the work peak at flowering. But several reasons support the application of inoculation: (1) Ear rot data were collected only for the three experimental years and, therefore, it cannot be assumed that sufficient high natural infection pressure occurs each year, (2) statistical power is increased because the ratio σ_e^2 / σ_g^2 decreased considerably in inoculation trials, (3) genotypes resistant to artificial inoculation were resistant to non-inoculation in all maturity groups but not vice versa, (4) occurrence of disease escape, i.e. visually non-infected plants will be rated as resistant and (5) by ear rot rating the *Fusarium* species cannot be distinguished and, thus, a breeder does not know which *Fusarium* resistance is addressed. The last point is of interest because 13 *Fusarium* species were isolated from naturally infected maize in Germany with the prevalent species changing between years according to weather (Görtz et al. 2008) and because resistances to different *Fusarium* spp. were correlated only moderately (Paper 1; Presello et al. 2004; Schaafsma et al. 2006). In conclusion, we suggest to apply artificial inoculations for the evaluation of ear rot resistance in all maturity groups to get sufficient, repeatable and more accurate ear rot severity for sufficient visual differentiation and to ensure testing for the desired *Fusarium* sp. resistance.

In Southern Europe secondary *Fusarium* infection of kernels after wounding mainly caused by European corn borer plays also a major role beside silk infection (Munkvold 2003). Both methods caused similar ear rot severities and heritabilities and, therefore, are capable to differentiate resistant from susceptible genotypes as suggested by Chungu et al. (1996b). But FUM concentrations after silk channel inoculation were higher than after kernel inoculation which is in contrast to a previous study (Schaafsma et al. 2006). The moderate correlations between both inoculation methods for ear rot resistance and FUM concentrations indicate that at least some QTL involved in resistance might be acting against both ways of entry. The found correlations were similar to correlations found in Argentinean and Canadian maize (Presello et al. 2004; Schaafsma et al. 2006). Consequently, breeding for ear rot resistance requires application of both inoculation methods, particularly if varieties should be developed for areas in which damage by European corn borer occurs frequently like in Southern Europe.

Were the used isolates appropriate for testing resistance to ear rot and toxin accumulation?

The selection of *Fusarium* isolates for evaluation of resistance to ear rot and mycotoxin accumulation was based on one-year data of 2006 (Bolduan, personal communication). But each year aggressiveness tests for *F. verticillioides* and *F. graminearum* were conducted in parallel with seven to eight isolates, respectively (Figure 1). Inoculation and disease assessment were done as recently described (Miedaner et al. 2010). The data reveal that the *F. graminearum* isolates were capable to cause high ear rot severity although most of them were isolated from wheat indicating the low pathogenic specialization of *F. graminearum* (Miedaner et al. 2008). No significant differences among inbred lines could be found since all three were classified as susceptible within the trials for resistance evaluation. The selected isolate for testing ear rot resistance (IFA66; Figure 1A encircled) was found to be among the most aggressive isolates causing sufficient ear rot for visual differentiation and high DON and ZEA concentrations how resistance tests (PAPER 2) and also high performance liquid chromatography (HPLC) analyses by project partners have shown (Data not shown).

The means of the different isolates of the *F. verticillioides* and *F. proliferatum* aggressiveness test were lower than those of the *F. graminearum* test (Figure 1) which is in agreement with studies from Canadian or US maize for *F. graminearum* and *F. verticillioides*, respectively (Clements et al. 2004; Reid et al. 2002; Robertson et al. 2006). The lower aggressiveness might be caused by the dual nature of *F. verticillioides* either as an endophyte with symptomless infection or a pathogen causing symptoms more randomly (Bacon et al. 2008). The isolates FV216/1 and FV234/1, isolated from Italian maize, were the most stable isolates across the years. The other *F. verticillioides* and *F. proliferatum* isolates isolated from various regions of the world varied considerably between years indicating higher influence of weather on ear rot severity than of the two “adapted” isolates. Although the chosen isolate (FV234/1; Figure 2B encircled) was not among the most aggressive ones in every year, we considered it as sufficient aggressive causing ear rot severity and also high FUM concentrations how resistance tests (Paper 1, 2, 3) and HPLC analyses have shown.

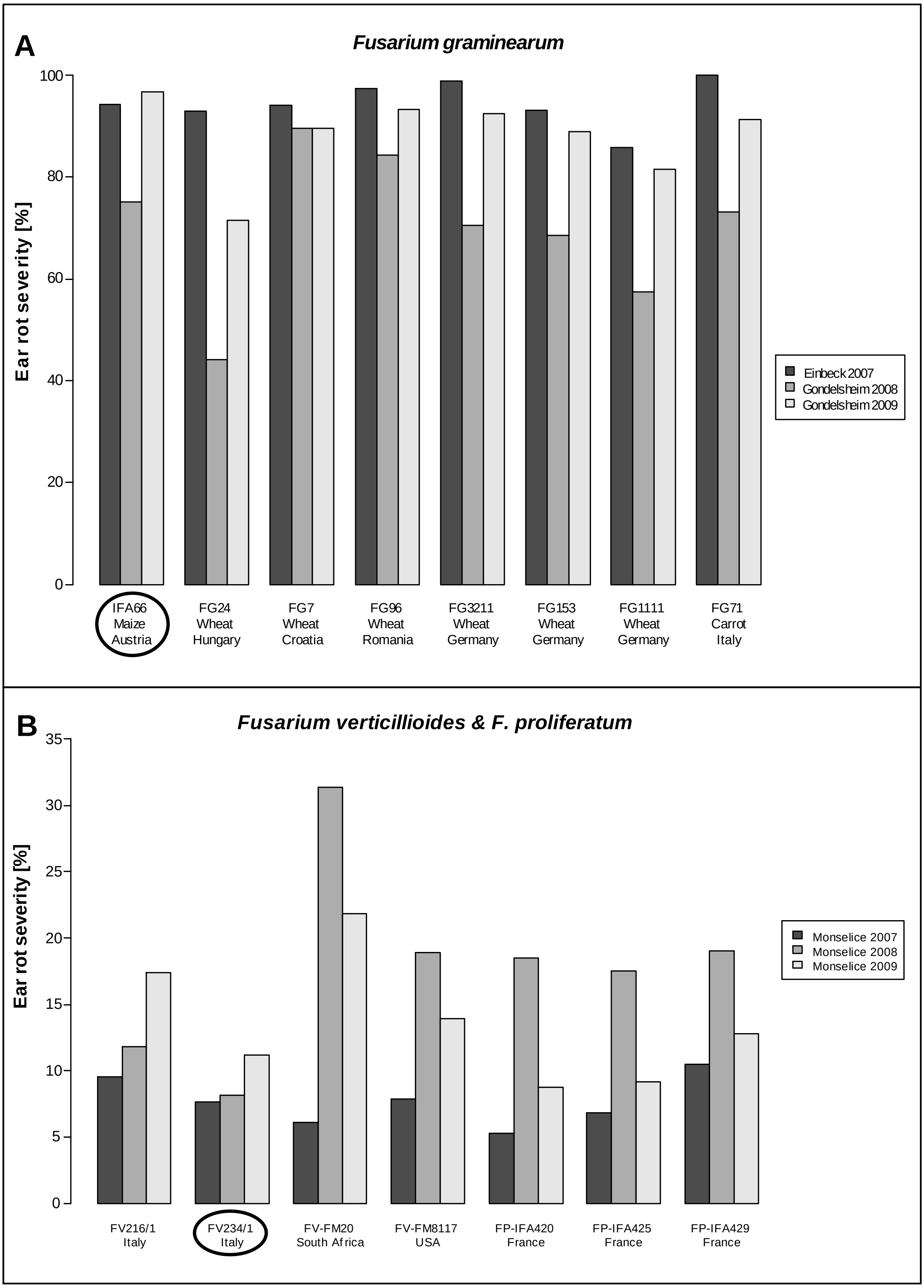

Figure 1: Means of ear rot severity of isolates of *F. graminearum* (FG) differing in geographic origin and host from where they were isolated (A) and *F. verticillioides* (FV) or *F. proliferatum* (FP)(B) isolated from maize across three genotypes tested in three subsequent years. Encircled isolates were used in resistance evaluation

Is separate testing of resistance to *F. graminearum* and *F. verticillioides* and corresponding mycotoxins necessary?

The natural occurrence of *Fusarium* spp. highly depends on weather with a varying species composition from season to season in Germany (Görtz et al. 2008). Thus, a breeder should know whether testing resistance to one *Fusarium* spp. is sufficient or testing resistance to different *Fusarium* spp. needs to be conducted separately. The significant (P<0.01) but moderate associations between resistances to *F. graminearum* and *F. verticillioides* in flints and dents are in agreement with other studies with other germplasms (Presello et al. 2004; Schaafsma et al. 2006). Although genetic correlations between FUM and DON or ZEA were high (0.77; 0.76, respectively) genotypes resistant to DON or ZEA accumulation were not necessarily resistant to FUM accumulation. The moderate to high genotypic correlations might be explained by species-specific resistance mechanisms attributed to the different ways of spreading within the ear. Other resistance mechanisms could be common to both species, i.e. unspecific resistance factors like a thick wax layer (Sampietro et al. 2009). Interestingly, genotypes resistant to *F. graminearum* (<30 % ear rot severity) also had a low *F. verticillioides* severity (<10 %). Similarly, Reid et al. 2009 reported that selection on resistance to *F. graminearum* resulted indirectly in increased resistance to *F. verticillioides* in Canadian maize. In conclusion, both *Fusarium* spp. should be tested separately to gain a resistance as broad as possible, but if resources are limited (i.e. seeds in early inbred line testing large populations), a pre-testing for resistance to *F. graminearum* should be preferred, followed by a *F. verticillioides* resistance test.

Conclusions drawn by estimation of population parameters

Is there genetic variation in the existing breeding material and is it heritable?

One prerequisite for selection of a certain trait is the existence of genetic variation. Additionally, desirable and usable levels should be reached which are in our case low ear rot severity or mycotoxin concentrations. Broad ranges and significant (P<0.01) genetic variation of both ear rot severity and mycotoxin concentrations were found in dents of all maturity groups even in the chosen subsets. Despite heavy infections due to artificial inoculation inbred lines and testcrosses were found having low levels of *F. verticillioides* severity and FUM concentrations close to or even lower than legal limits (Paper 2, 3). Genotypes fairly resistant to ear rot could be found in the early dents although the aggressive *F. graminearum* isolate was inoculated. In about 18 % of naturally infected samples DON concentrations were higher than the legal limit with up to 150 mg kg $^{-1}$ in our study (Paper 2). Nevertheless, all these samples were genotypes susceptible to very susceptible after inoculation indicating that selection for ear rot resistance has the potential to reduce mycotoxin concentrations after natural infection. In conclusion, breeding in dents seems to be promising since breeders can select for genotypes resistant to ear rot caused either by *F. verticillioides* or *F. graminearum* and corresponding mycotoxins gaining quickly in resistant genotypes.

Flint lines were generally more susceptible to ear rots and the mycotoxins DON and FUM than dent lines (Paper 1, 2, 3). This higher susceptibility could be caused historically since flints were created out of few Central European landraces by selfing and had have few influx of new alleles from other germplasms (Reif et al. 2005) and/or missing co-occurrence of the flints and *F. verticillioides* during a substantial amount of time since flints had been selected for cooler climates whereas *F. verticillioides* occurs in hot and dry climates. Additionally, the higher resistance of dents might be due to the fact that dents are used as seed parents and, therefore, have been selected indirectly for seed quality and, hence, ear rot resistance. Both parents of a hybrid need to express resistance on a certain level to gain resistant hybrids since results of testcrosses and also other studies indicated that inheritance of ear rot resistance is mainly additive (Butrón et al. 2006; Chungu et al. 1996a; Clements et al. 2004; Gendloff et al. 1986; Nankam and Pataky 1996; Williams and Windham 2009). Consequently, the resistance level in the flints should be improved.

The varying means among years and locations indicate high influences of weather on trait expression resulting in significant ($P<0.01$) genotype × environment interactions. Mainly, the significant triple genotype × location × year interactions found in the early and mid-late maturity group in line performance stress the high influences of weather. Nevertheless, entry-mean heritabilities were moderate to high which is likely caused by multi-environmental tests and the high genotypic variances in the maturity groups, but also in sub-groups. Due to these significant genotype × environment interactions resistance evaluation should be conducted in multi-environmental trials to increase entry-mean heritabilities gaining in more accurate and reliable selection of resistant genotypes. This is illustrated by the fact, that two environments did not suffice in the late maturity group to significantly differentiate the lines (Paper 3).

Altogether, there is genetic variation in all germplasms, which also reaches desirable levels despite inoculation, but multi-environmental trials are necessary for an accurate selection.

Is indirect selection for low mycotoxin concentrations by symptom rating effective?

Practical breeders aim to maximize response to selection with the restriction of a limited budget. Since mycotoxin analyses are expensive, laborious and time-consuming, but ear rot rating is cost efficient and fast, a breeder wants to know if indirect selection based on ear rot rating gains similar or even higher responses to selection than by direct mycotoxin analyses. High phenotypic and genotypic correlations between ear rot severity and mycotoxin concentrations are very promising (Paper 2, 3). However, the merit of indirect selection also depends on the precision of field trials (i.e. heritability) which is combined in the formula of relative efficiency (Falconer and Mackay 1996) without regarding selection intensities. Heritabilities of ear rot severity and mycotoxins concentrations were mostly similar in lines and testcrosses and, thus, relative efficiencies varied closely around one (Paper 2, 3). This indicates that indirect selection is similarly effective as direct selection. In lines of the mid-late maturity the relative efficiency for FUM and *F. verticillioides* ear rot severity was 0.82 (Paper 2) showing that indirect selection would be less effective without regarding economic aspects. But assuming a fixed budget the application of cost efficient and fast ear rot rating could increase heritabilities and/or selection intensities by testing in more environments and/or more genotypes making indirect selection more effective than direct selection for reduced mycotoxin concentrations. Another reason supporting ear rot rating is that selection can be conducted prior to sowing the winter nursery additionally saving resources and time. But indirectly selected genotypes should be tested for mycotoxin concentrations directly since visually symptomless kernels or resistant genotypes may still contain high mycotoxin concentrations, particularly FUM after *F. verticillioides* infections (Paper 2, 3; Desjardins et al. 1998). In conclusion, indirect selection based on ear rot rating is more effective than direct selection for low mycotoxin concentrations, but indirectly selected genotypes should be screened for FUM concentrations directly.

How is resistance inherited?

Direct comparisons of means and variance components between line and testcross performance were not suitable since different inoculum volumes were used (Paper 3). Therefore, direct comparisons between lines and testcrosses only could be done by dimensionless values like heritabilities and correlations. Despite a relatively high ratio $\sigma_{gt}^{2}/\sigma_{g}^{2}$ for *F. verticillioides* ear rot severity and FUM concentrations in the early maturity group the genotypic correlations between testcrosses of different testers were high (≥0.80). The high genotypic correlations indicate that general combining ability effects are more important than specific combining ability effects for all regarded traits. Additionally, the moderate to high genotypic correlations between lines and testcrosses also indicate a mainly additive gene action (Smith 1986) but also non-additive gene actions might be possible. In generation mean and diallel analyses also mainly additive gene actions were found (Butrón et al. 2006; Chungu et al. 1996a; Clements et al. 2004; Gendloff et al. 1986; Nankam and Pataky 1996; Williams and Windham 2009). In conclusion, additive inheritance is predominant but also non-additive gene-action can occur.

Is testcross performance predictable by line per se performance?

Essential for hybrid breeding is the relationship between line and hybrid performance because the number of possible testcrosses can be reduced tremendously by discarding lines. Genotypic correlations between line and testcross performance were moderate for both ear rot severity and mycotoxin concentrations (Paper 3). Beside genotypic correlations, comparisons of selection in lines with selection in testcrosses require also the heritabilities both comprised in the equation of relative efficiency (Falconer and Mackay 1996). Relative efficiencies of indirect selection for testcross performance based on line performance never reached a level close to 100 % due to moderate correlations between lines and testcrosses and similar heritabilities. Furthermore, selection based on line performance is not appropriate due to practical reasons: (i) Directly after creation and propagation of doubled haploid (DH) lines sufficient seeds might not be available of all genotypes to conduct separate tests for ear rot resistance and (ii) testing 7,000 – 10,000 DH lines or even more in each heterotic group per year (Schmidt 2004) and, of course, the numerous testcrosses created and selected the years before would require high amounts of manpower. Altogether, prediction based on line performance for testcross performance is not effective due to moderate to high genetic correlations and the mentioned practical reasons.

Which type and number of testers should be used for evaluation of testcross performance?

Genetic variation among lines in testcrosses is important for selection. Significant variances among lines in testcross performance revealed genetic variation in all maturity groups and are likely attributed to the use of moderately to highly susceptible testers (Paper 3). Resistant testers can diminish genotypic differences due to presence of masking dominant genes. Furthermore, susceptible testers having a low gene frequency of favorable alleles are expected to be most effective testers from a theoretical but also an empirical view (Allison and Curnow 1966; Rawlings and Thompson 1962). Particularly for evaluation of resistance to *F. verticillioides* ear rot susceptible testers should be applied to obtain sufficient visual differentiation. Therefore, moderately to highly susceptible testers of the opposite gene pool are suitable for selection of resistant lines by topcross testing especially for *F. verticillioides* ear rot resistance.

Application of only one tester would be more cost efficient than of two testers. A prerequisite is a high genotypic correlation between testcrosses created with different testers. These correlations were high although significant genotype × tester interactions were found in the early maturity group (Paper 3). Consequently, one tester is sufficient for the evaluation of general combining ability of lines in topcross tests due to the high correlations and since resistance is inherited mainly by additive effects like indicated above.

Conclusions for resistance breeding to ear rot and mycotoxin accumulation

In this study different situations have been identified each requesting a different proceeding for resistance breeding. (1) In Central Europe only resistance in lines is important to ensure a high seed quality whereas resistance in testcrosses is not important due to low natural infection, but (1a) a highly critical problem of the heterotic group of flint is the absence of usable genetic variation. (2) In Southern Europe resistance in lines, but also in hybrids is important due to the high natural infection pressure to ensure low toxin concentrations in the harvest of the farmers.

Regarding scenario 1a a prerequisite for successful breeding of resistant varieties is the creation of genetic variation with usable levels since both parents need to be resistant to obtain resistant varieties due to the mainly additive inheritance. One approach could be the introgression of resistance alleles from other germplasms. However, heterotic patterns and genetic similarity to the germplasm in which they should be introgressed need to be regarded. Non-Stiff-Stalk lines are most suitable for introgression into the flint germplasms “exploiting the specific adaptation of European flint germplasm and the excellent combining ability of US germplasm in European maize breeding programs” (Reif et al. 2009). Unfortunately, no resistance donors like Sumai 3 in wheat carrying *Fhb1* have been identified in maize up to now. But sources for alleles of *F. graminearum* resistance might be the CO lines from the breeding program of Eastern Cereal and Oilseed Research Center, Ontario, Canada, if lines belong to the heterotic groups of flint or non-Stiff-Stalk. Three of the four CO lines used as checks in the early maturity group (Paper 1) were more resistant than most flint lines indicating their potential. US cornbelt non-Stiff-Stalk lines like the moderately resistant GE440 (Robertson et al. 2006; J. Holland, personal communication) might be a source for *F. verticillioides* resistance alleles. However, recurrent selection for other traits like chilling tolerance or agronomic traits should be conducted after introgression to achieve similar levels like before introgression. After successful creation of usable genetic variation in flints this heterotic group can be treated like dents of the early maturity group.

Our further conclusions for breeding of resistance to ear rot and mycotoxin accumulation are implemented in a breeding scheme applying only DH lines (Schmidt 2004). Briefly, 7,000 – 10,000 or even more DH lines are created in each heterotic group in each cycle of variety development. Out of these 1,000 – 2,000 lines are selected based on observation trials and then topcrossed to one single-cross tester. With results of the topcrosses 50 – 200 lines are selected for topcross evaluation in a second year. Finally, a few combinations are selected for extensive tests in factorial crosses.

Common to scenario 1 and 2 is that selection for resistance to mycotoxin accumulation is always based on ear rot rating since this is more effective. Nevertheless, selection in lines for testcross performance is ineffective due to low efficiencies of indirect selection and the practical reasons mentioned above especially the high number of lines in every year. Therefore, selection in lines should be based on other traits like tolerances and resistances to abiotic and biotic stresses, respectively, and seed yield and quality. In all scenarios the selected lines are tested in topcrosses for general combining ability of agronomic traits like yield. In parallel to these topcrosses all parental lines should be grown and inoculated. After topcross selection only parental lines of the selected topcrosses would be rated saving resources. This is feasible because testcrosses can be harvested some weeks before rating of lines must be conducted. Here a “negative” selection is conducted meaning that only susceptible to very susceptible lines are discarded to ensure high seed quality. In scenario 2, additionally, resistance in hybrids is important. Therefore, first evaluation for ear rot resistance of the 1,000 – 2,000 lines in topcrosses should be conducted with one susceptible tester of the opposite gene pool. The topcross used for resistance evaluation is one of those used to assess the general combining ability for agronomic traits to avoid creation of additional topcrosses. The same procedure is repeated with the 50 – 200 selected lines in the subsequent year. In the following factorial crosses resistance to ear rot and mycotoxin accumulation also should be conducted. Of course, all collected ear rot data can be used for selection of lines for recurrent selection increasing resistance in heterotic groups successively. All tests for resistance should be conducted multi-environmentally to account the genotype × environment interactions but separately to tests for agronomic traits. In conclusion, phenotypic selection should be effective to develop varieties resistant to ear rot and, even more important, mycotoxin accumulation by applying our suggestions.

QTL meta-analysis

In maize, QTL analyses have been conducted on six populations by kernel wounding and/or silk channel inoculation by *F. verticillioides* or *F. graminearum* (Table 1). The QTL meta-analysis of ear rot resistance in maize was conducted like described in Paper 4 except regarding quality aspects. The IBM2 2004 Neighbors map (Anonymous 2009) was used as the reference map. Results of the QTL meta-analysis in maize are presented in Table 2.

In contrast to the QTL meta-analysis in wheat (Paper 4) none of the 14 MQTL comprised more than three initial QTL which is likely caused by the low number of available populations. Nevertheless, it is noteworthy that MQTL comprising initial QTL of different studies were found although populations of each study originated from different geographical regions. The AICc (modified Akaike-information-criterion) values of clustered QTL on chromosomes 1, 2, 6 and 7 were not significantly different to the model in which no MQTL was found (difference of AICcs was smaller than two; Jansen and Stam (1994)). This and the low number of initial QTL comprised in a MQTL indicate that results of a QTL meta-analysis in maize for ear rot resistance are too preliminary and should be repeated with more studies. Contrary, in wheat a large number of studies was available and, therefore, a QTL meta-analysis on FHB resistance was conducted to circumvent the limitations of low population number (Paper 4). The results of Paper 4 were confirmed by another meta-analysis study (Liu et al. 2009).

Nevertheless, the results of the meta-analysis in maize showed that there were few MQTL comprising only initial QTL found after kernel inoculation (MQTL 5, 6, 11, 12). Similarly to spray inoculation in wheat, silk channel inoculation in maize covers resistances to initial infection (type I) and spread within the ear (type II). Kernel inoculation corresponds to single floret inoculation in wheat which addresses only type II resistance. The differentiation of MQTL according to inoculation techniques supports the moderate correlations between silk channel and kernel inoculation (Paper 1, 2; Presello et al. 2004; Schaafsma et al. 2006) indicating that some QTL involved in resistance might be acting against only one way of entry and others against both ways. MQTL 2, 6, 9 and 10 comprised only initial QTL detected after inoculation with *F. verticillioides*. No pure *F. graminearum* MQTL could be found since only one QTL study using *F. graminearum* was included in the QTL meta-analysis. The pure *F. verticillioides* MQTL indicate that at least some QTL are *Fusarium* species specific while others are responsible for common resistance mechanisms like a thick wax layer (Sampietro et al. 2009). This observation is supported by moderate correlations between *F. verticillioides* and *F. graminearum* resistances (Paper 1, 2; Presello et al. 2004; Schaafsma et al. 2006).

In general, both QTL meta-analyses revealed that precision of initial QTL affects precision of MQTL. Regarding studies in which initial QTL were calculated only on single environment basis (i.e. Ali et al. 2005) the mean explained phenotypic variances of a MQTL are likely overestimated due to overestimated effects of initial QTL. Applying the formula of Darvasi and Soller (1997) the overestimated effects might affect results of the meta-analysis mainly by narrowing the CI of a MQTL. Therefore, precision should be increased already while conducting QTL analyses by regarding (a) population size, (b) the precision of phenotyping e.g. measured by heritability (Beavis 1998), (c) method used for QTL detection (i.e. ANOVA, SIM, CIM, MIM)(see Lynch 1998), (d) marker coverage or density and (e) conducting a combined analysis across environments.

Breeders aim to aggregate different resistance QTL in a variety to gain a high resistance level since crossing genotypes having the same resistance QTL would not increase resistance level. Thus, information about the origin of resistance QTL in parents is valuable for aggregating QTL of different origins in progenies, particularly if inheritance is mainly additive like

Fusarium resistance in maize and wheat (Chungu et al. 1996a; Snijders 1990). The results of the QTL meta-analyses in wheat showed that there were QTL specific to an origin (e.g. *Fhb1* on 3B). But also MQTL could be found across populations from different origins (e.g. MQTL 14 on 5A) indicating that the comprised QTL can be found in different materials but also might be stable in different genetic backgrounds and across environments. If such QTL additionally have high effects, they will be desirable for use in resistance breeding. In conclusion, a QTL meta-analysis can be a valuable tool for selection of parents not comprising the same resistance QTL and exploit appropriate MQTL together with their respective markers in MAS.

Table 1: Overview of characteristics of studies used for meta-analysis in maize

Parents (first is resistant, second is susceptible)	Map density (cM)	*Fusarium* spp[a]	Total number of environments	Type of population	Population size	Inoculation technique[b]	Method used[c]	References
CO387 x CG62	13.8	FG	4	F_2	144	K	CIM	Ali et al. 2005
CO387 x CG62	13.8	FG	4	F_2	144	S	CIM	Ali et al. 2005
87-1 x Zong3	7.3	FV	4	$F_{8:9}$	187	K	CIM	Ding et al. 2008
3 x 18	11.4	FV	4	F_2	238	K	CIM	Perez-Brito et al. 2000
5 x 18	15.4	FV	4	F_2	206	K	CIM	Perez-Brito et al. 2000
GE440 x FR1064	18.8	FV & FP	4	$BC_1F_{1:2}$	213	S & K	CIM	Robertson-Hoyt et al. 2006
NC300 x B104	17.6	FV & FP	3	$F_{2:3}$	143	S & K	CIM	Robertson-Hoyt et al. 2006

[a] FG, *F. graminearum* ; FP, *F. proliferatum* ; FV, *F.verticillioides*

[b] K, kernel inoculation; S, silk channel inoculation

[c] Composite interval mapping

Table 2: Characteristics of detected meta-QTL (MQTL) of *Fusarium* resistance in maize

Chrom	Meta-QTL	Position on consensus map (cM)	Number of initial QTL comprised in MQTL	Mean explained variance of QTL (R^2)	Most precise CI of initial QTL (cM)	Mean initial CI (cM)	MQTL CI (95%)	Coefficient of reduction in length from **most precise** initial to meta-QTL CI	Coefficient of reduction in length from **mean** initial to meta-QTL CI	Inoculation methods combined in MQTL[a]	Inoculated *Fusarium* sp.[b]
1	1	257.2	2	20.7	3.7	30.6	3.7	1.0	8.3	S/K	FG/FV
	2	830.6	3	9.3	110.0	152.5	80.5	1.4	1.9	S/K	FG/FV
2	3	76.8	2	4.7	98.3	100.9	98.3	1.0	1.0	S/K	FV
	4	610.7	3	16.2	36.8	132.8	80.1	0.5	1.7	S/K	FG/FV
3	5	93.8	2	11.5	43.5	56.6	36.9	1.2	1.5	K	FG/FV
	6	215.4	2	10.5	39.4	100.6	37.8	1.0	2.7	K	FV
	7	269.9	2	19.5	89.5	95.7	71.4	1.3	1.3	S/K	FG/FV
	8	450.4	2	15.6	89.5	114.5	369.1	0.2	0.3	S/K	FG/FV
4	9	298.9	3	5.0	135.7	189.2	99.3	1.4	1.9	S/K	FV
5	10	33.7	2	6.9	25.3	132.4	25.2	1.0	5.3	S/K	FV
	11	469.2	2	10.4	38.9	156.3	38.5	1.0	4.1	K	FG/FV
6	12	206.8	2	12.0	64.1	160.8	72.0	0.9	2.2	K	FG/FV
	13	402.9	3	9.2	84.2	115.5	319.5	0.3	0.4	S/K	FG/FV
7	14	276.4	3	11.0	22.6	69.0	16.7	1.4	4.1	S/K	FG/FV

[a] S, Silk channel inoculation; K, Kernel inoculation

[b] FG, *F. graminearum* ; FV, *F. verticillioides*

Outlook

Generally, we have shown that breeding of varieties resistant to ear rot and mycotoxin accumulation in maize is feasible and promising by phenotypic selection. Nevertheless, evaluation of ear rot resistance remains laborious and time consuming. Therefore, cost efficient MAS for resistance to ear rot and mycotoxin accumulation might enhance selection progress assuming a fixed budget. For that QTL with high effects in different genetic backgrounds would be most suitable like *Fhb1* in wheat. But up to now, such QTL have not been identified in maize and the probability to find such QTL is very low. Nevertheless, conducting QTL analyses and, afterwards, meta-analyses in maize may also enhance selection since more would be understood about the genetic architecture of different important traits meaning coincident positions of QTL like shown in wheat for FHB resistance and plant height (Mao et al. 2010). Additionally, parents having different resistance QTL could be selected and crossed like it was shown for FHB resistance in wheat likely accelerating selection progress.

References

Anonymous (2009) http://www.maizegdb.org/cgi-bin/displaycompletemaprecord.cgi?id=934700 verified 5/14/2009

Ali ML, Taylor JH, Jie L, Sun G, William M, Kasha KJ, Reid LM, Pauls KP (2005) Molecular mapping of QTLs for resistance to *Gibberella* ear rot, in corn, caused by *Fusarium graminearum*. Genome 48:521-533

Allison JCS, Curnow RW (1966) On the choice of tester parent for the breeding of synthetic varieties of maize (*Zea mays* L.). Crop Sci 6:541-544

Bacon CW, Glenn AE, Yates IE (2008) *Fusarium verticillioides*: Managing the endophytic association with maize for reduced fumonisins accumulation. Toxin Rev 27:411-446

Beavis W (1998) Paterson AH (eds) QTL Analyses: Power, precision and accuracy; Molecular dissection of complex traits. Boca Raton, USA, CRC Press, pp 145-162

Butrón A, Santiago R, Mansilla P, Pintos-Varela C, Ordás A, Malvar RA (2006) Maize (*Zea mays* L.) genetic factors for preventing fumonisin contamination. J Agric Food Chem 54:6113-6117

Chungu C, Mather DE, Reid LM, Hamilton RI (1996a) Inheritance of kernel resistance to *Fusarium graminearum* in maize. J Hered 87:382-385

Chungu C, Mather DE, Reid LM, Hamilton RI (1996b) Comparison of techniques for inoculating maize silk, kernel, and cob tissues with *Fusarium graminearum*. Plant Dis 80:81-84

Clements MJ, Maragos CM, Pataky JK, White DG (2004) Sources of resistance to fumonisin accumulation in grain and *Fusarium* ear and kernel rot of corn. Phytopathology 94:251-260

Darvasi A, Soller M (1997) A simple method to calculate resolving power and confidence interval of QTL map location. Behav Genet 27:125-132

Desjardins AE, Plattner RD, Lu M, Claflin LE (1998) Distribution of fumonisins in maize ears infected with strains of *Fusarium moniliforme* that differ in fumonisin production. Plant Dis 82:953-958

Ding J-Q, Wang X-M, Chander S, Yan J-B, Li J-S (2008) QTL mapping of resistance to *Fusarium* ear rot using a RIL population in maize. Mol Breed 22:395-403

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics. Longman group Limited, Harlow

Gendloff EH, Rossman EC, Casale WL, Isleib TG, Hart LP (1986) Components of resistance to *Fusarium* ear rot in field corn. Phytopathology 76:684-688

Görtz A, Oerke E, Steiner U, Waalwijk C, Vries I, Dehne H (2008) Biodiversity of *Fusarium* species causing ear rot of maize in Germany. Cereal Res Commun 36:617-622

Jansen R, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447-1455

Liu S, Hall MD, Griffey CA, McKendry AL (2009) Meta-Analysis of QTL associated with *Fusarium* head blight resistance in wheat. Crop Sci 49:1955-1968

Lynch M,B. (1998) Genetics and analysis of quantitative traits. 23 Plumtree Road, Sunderland, MA 01375 USA, Sinauer Associates, Inc

Mao S-, Wei Y-, Cao W, Lan X-, Yu M, Chen Z-, Chen G-, Zheng Y- (2010) Confirmation of the relationship between plant height and *Fusarium* head blight resistance in wheat (*Triticum aestivum* L.) by QTL meta-analysis. Euphytica (In press)

Miedaner T, Bolduan C, Melchinger AE (2010) Aggressiveness and mycotoxin production of eight isolates each of *Fusarium graminearum* and *Fusarium verticillioides* for ear rot on susceptible and resistant early maize inbred lines. Eur J Plant Pathol (In press)

Miedaner T, Cumagun CJR, Chakraborty S (2008) Population genetics of three important head blight pathogens *Fusarium graminearum, F. pseudograminearum* and *F. culmorum*. J Phytopathol 156:129-139

Munkvold GP (2003) Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. Eur J Plant Pathol 109:705-713

Nankam C, Pataky JK (1996) Resistance to kernel infection by *Fusarium moniliforme* in the sweet corn inbred IL125b. Plant Dis 80:593-598

Pérez-Brito D, Jeffers D, González-de-León D, Khairallah M, Cortés-Cruz M, Velázquez-Cardelas G, Azpíroz-Rivero S, Srinivasan G (2001) QTL mapping of *Fusarium moniliforme* ear rot resistance in highland maize, mexico: Cartografía de qtl de la resistencia a la pudrición de la mazorca (*Fusarium moniliforme*) en maíz de valles altos, méxico. Agrocencia 35:181-196

Presello DA, Reid LM, Mather DE (2004) Resistance of Argentine maize germplasm to *Gibberella* and *Fusarium* ear rots. Maydica 49:73-81

Rawlings JO, Thompson DL (1962) Performance level as criterion for the choice of maize tester. Crop Sci 2:217-220

Reid LM, Zhu X, Parker A, Yan W (2009) Increased resistance to *Ustilago zeae* and *Fusarium verticilliodes* in maize inbred lines bred for *Fusarium graminearum* resistance. Euphytica 165:567-578

Reid LM, Woldemariam T, Zhu X, Stewart DW, Schaafsma AW (2002) Effect of inoculation time and point of entry on disease severity in *Fusarium graminearum*, *Fusarium* *verticillioides*, or *Fusarium subglutinans* inoculated maize ears. Can J Plant Pathol 24:162-167

Reif JC, Fischer S, Schrag TA, Lamkey KR, Klein D, Dhillon BS, Utz HF, Melchinger AE (2009) Broadening the genetic base of European maize heterotic pools with US Cornbelt germplasm using field and molecular marker data. Theor Appl Genet 120:301-310

Reif JC, Hamrit S, Heckenberger M, Schipprack W, Maurer HP, Bohn M, Melchinger AE (2005) Trends in genetic diversity among European maize cultivars and their parental components during the past 50 years. Theor Appl Genet 111:838-845

Robertson LA, Kleinschmidt CE, White DG, Payne GA, Maragos CM, Holland JB (2006) Heritabilities and correlations of *Fusarium* ear rot resistance and fumonisin contamination resistance in two maize populations. Crop Sci 46:353-361

Robertson-Hoyt LA, Jines MP, Balint-Kurti PJ, Kleinschmidt CE, White DG, Payne GA, Maragos CM, Molnár TL, Holland JB (2006) QTL mapping for *Fusarium* ear rot and fumonisin contamination resistance in two maize populations. Crop Sci 46:1734-1743

Sampietro DA, Vattuone MA, Presello DA, Fauguel CM, Catalán CAN (2009) The pericarp and its surface wax layer in maize kernels as resistance factors to fumonisin accumulation by *Fusarium verticillioides*. Crop Prot 28:196-200

Schaafsma AW, Tamburic-Ilincic L, Reid LM (2006) Fumonisin B1 accumulation and severity of *Fusarium* ear rot and *Gibberella* ear rot in food-grade corn hybrids in Ontario after inoculation according to two methods. Can J Plant Pathol 28:548-557

Schmidt W (2004) Hybridmaiszüchtung bei der KWS SAAT AG. Bericht über die 54. Arbeitstagung 2003 der Vereinigung österreichischer Pflanzenzüchter, BAL Gumpenstein, 25.-27.Nov.2003 1-6

Smith OS (1986) Covariance between line per se and testcross performance. Crop Sci 26:540-543

Snijders CHA (1990) The inheritance of resistance to head blight caused by *Fusarium culmorum* in winter wheat. Euphytica 50:11-18

Williams WP, Windham GL (2009) Diallel analysis of fumonisin accumulation in maize. Field Crops Res 114:324-326

7. Summary

Infection of ears and spikes of maize and wheat, respectively, with *Fusarium graminearum* reduces yield and, more important, contaminate the harvest with mycotoxins. In maize, *F. verticillioides* is an economically important cause of ear rot. Among other mycotoxins, *F. verticillioides* produces the fumonisins (FUM) and *F. graminearum* produces deoxynivalenol (DON) and zearalenone (ZEA). All three mycotoxins are harmful to humans and animals. Therefore, the European Union released legally enforceable limits. In Europe, no effective fungicide for control of *Fusarium* infection has been released. One alternative to reduce ear rot severity and mycotoxin concentrations is breeding and growing varieties resistant to *Fusarium* infections. However, few is known about breeding parameters for resistance to *Fusarium* infections and mycotoxin accumulation in European maize breeding material. In maize, only a low number of QTL (quantitative trait loci) studies have been published on *Fusarium* resistance, whereas a large number of QTL studies on *Fusarium* resistance exists in wheat. Nevertheless, the information of these QTL studies has not been compiled yet by a QTL meta-analysis approach.

The main objective of this thesis was to draw conclusions for breeding of resistance to head blight or ear rot and mycotoxin accumulation with special attention on three European maize maturity groups. In detail, we investigated methodical aspects like (1) the comparison of natural and artificial inoculation to evaluate ear rot resistance, (2) the aggressiveness of several isolates of *F. verticillioides* and *F. graminearum*, and (3) the necessity of separate testing of *F. verticillioides* and *F. graminearum*. Furthermore, quantitative-genetic parameters like heritabilities and correlations were estimated to draw conclusions about (4a) genetic variation in line and testcross performance and the relationships (4b) between ear rot severity and mycotoxin concentrations in lines and testcrosses and (4c) between line and testcross performance. Additionally, (5) the positions of so-called meta-QTL (MQTL) of ear rot and *Fusarium* head blight resistance in maize and wheat, respectively, were analyzed by a QTL meta-analysis approach.

Three maturity groups (early, mid-late, late) each comprising about 150 maize inbred lines were evaluated for ear rot resistance to *F. verticillioides.* The same genotypes of the early maturity group were additionally evaluated for resistance to *F. graminearum* in separate, but adjacent trials. Field evaluation was conducted in two to six environments with silk channel inoculation and natural infection, respectively. In the late maturity group kernel inoculation was conducted additionally. Out of the 150 lines, 50 to 60 lines per maturity group were crossed with two unrelated testers of the opposite heterotic group. The concentrations of toxins FUM, DON and ZEA of the chosen lines and their testcrosses were analyzed by immunotests. Eight *F. graminearum* isolates and each of four and three *F. verticillioides* and *F. proliferatum* isolates, respectively, were tested for their aggressiveness at one location each year. QTL meta-analyses were conducted by (1) collecting published QTL studies, (2) creating a consensus map and (3) comparing the positions of QTL with the software package “MetaQTL” in wheat and maize.

Despite significant genotypic differences among the inbred lines after inoculation or natural infections, inoculation was found to be superior due to easier visual differentiation and increased accuracy. Therefore, inoculation should be conducted. In the late maturity group silk channel inoculation (simulating infection over the silks) and kernel inoculation (simulating secondary infection after wounding) were appropriate since both caused similar ear rot severity. However, both inoculation methods should be tested separately due to only moderate correlations between them. The *F. graminearum* isolates caused high ear rot severity although most of them have been isolated from wheat indicating a low pathogenic specialization. Contrary, *F. verticillioides* and *F. proliferatum* isolates caused low severity which might be explained by their dual nature either as an endophyte or a pathogen. The selected isolates (IFA66; FV234/1) used for resistance evaluations were considered to be sufficient aggressive since they caused significant differences among genotypes. In the early maturity group resistance to *F. graminearum* or *F. verticillioides* should be tested separately due to moderate correlations.

Significant ($P<0.01$) genotypic variances in large sets and subsets of lines and also in testcrosses revealed that there is genetic variation in all maturity groups and also within heterotic groups. In the flint group less lines were resistant to *F. verticillioides* and *F. graminearum* than in dents indicating that resistance needs improvement, i.e. by introgression of resistance alleles followed by recurrent selection. Significant genotype × environment interactions may complicate selection and, therefore, multi-environmental trials are required for an accurate selection.

High genotypic correlations between ear rot rating and mycotoxin concentrations were found among lines and testcrosses ($r \geq 0.96$). The cost efficient indirect selection for mycotoxin concentrations based on ear rot rating could increase response to selection by testing more genotypes and/or in more test environments assuming a fixed budget. This should increase selection intensity and/or heritability. Indirectly selected genotypes should be additionally screened for FUM concentrations directly due to high concentrations sometimes found in visually less infected genotypes.

Moderate genotypic correlations between line and testcross performance were found ($r = 0.64 – 0.83$). One moderately to highly susceptible tester is sufficient due to high genotypic correlations between testcrosses of different testers. Both indicates a mainly additive gene action, but also non-additive gene action may play a role in some crosses. Selection for testcross performance based on line performance was less effective when calculating relative efficiencies. Different scenarios have been identified: (1) In Central Europe mainly resistance to ear rot in lines needs to be tested to ensure high seed quality, whereas resistance in testcrosses is not important due to low natural infection. (2) In Southern Europe, where high natural infections occur regularly, parallel selection for resistance to ear rot in lines and testcrosses is important. One susceptible tester should be used for creation of testcrosses. For selection in lines all parental lines should be inoculated but only lines selected out of testcrosses for agronomic traits would be rated afterwards saving resources. This is feasible due to later harvest date of lines than of testcrosses.

QTL meta-analyses revealed that a high number of MQTL existed in maize and wheat, but results in maize should be regarded with caution due to the low number of populations. In both species some MQTL specific to resistance to spread within ears or spikes could be extracted. In maize, some MQTL comprising only *F. verticillioides* resistance QTL were found. Both observations were supported by the moderate correlations between different inoculation methods and *Fusarium* species in maize. QTL meta-analyses were able to identify MQTL comprising either QTL of one origin or different origins and, therefore, could be a valuable tool for improving resistances by helping while the choice of the parents carrying different resistance alleles. Generally, it was shown that phenotypic selection should be effective to reduce mycotoxin concentrations in European maize, which could be enhanced by using results of QTL meta-analyses like shown for wheat.

8. Zusammenfassung

Infektionen der Kolben bzw. Ähren von Mais oder Weizen mit *Fusarium graminearum* führen zu Ertragsverlusten und, viel wichtiger, zu einer Kontaminierung der Ernte mit Mykotoxinen. Im Mais ist zusätzlich *F. verticillioides* ein ökonomisch wichtiger Erreger der Kolbenfäule. *F. verticillioides* produziert, neben anderen Mykotoxinen, die Gruppe der Fumonisine (FUM) und *F. graminearum* Deoxynivalenol (DON) und Zearalenon (ZEA). Diese Toxine sind für Mensch und Tier gesundheitsschädigend, weshalb die Europäische Union Höchstmengen hierfür festgelegt hat. In Europa gibt es allerdings kein wirksames zugelassenes Fungizid. Eine Alternative zur Toxinverringerung bietet die Züchtung und der Anbau *Fusarium*-resistenter Sorten. Jedoch ist bisher wenig über züchterische Parameter für Kolbenfusariosenresistenz in europäischem Maiszuchtmaterial bekannt. Im Gegensatz zum Mais wurden im Weizen bisher viele QTL-Studien (quantitative trait loci) veröffentlicht. Allerdings wurden die Informationen einzelner Studien noch nicht mithilfe einer Meta-Analyse zusammengefasst.

Das Hauptziel dieser Arbeit bestand darin, Schlussfolgerungen für die Züchtung der Resistenz gegen Ähren- oder Kolbenfusariosen und Mykotoxinbelastung zu ziehen, wobei das Hauptaugenmerk auf drei europäischen Maisreifegruppen lag. Hierzu wurden (1) künstliche Inokulation und natürliche Infektion verglichen, (2) die Aggressivität verschiedener *F. verticillioides*- und *F. graminearum*-Isolate evaluiert und (3) untersucht, ob getrenntes Testen der Resistenz gegen *F. verticillioides* und *F. graminearum* nötig ist. Außerdem wurden quantitativ-genetische Parameter wie Heritabilitäten und Korrelationen geschätzt, um Schlussfolgerungen bezüglich (4a) der genetischen Variation in Linien und Testkreuzungen, sowie den Beziehungen (4b) zwischen Bonitur und Toxinkonzentrationen und (4c) zwischen Linieneigen- und Testkreuzungsleistung ziehen zu können. Zusätzlich wurden (5) die Positionen von sogenannten Meta-QTL (MQTL) für Resistenz gegen Kolbenfäule im Mais und gegen Ährenfusariosen im Weizen mithilfe einer QTL Meta-Analyse analysiert.

Drei Reifegruppen (früh, mittelspät, spät) mit jeweils 150 Inzuchtlinien wurden auf Kolbenfäuleresistenz nach Inokulation mit *F. verticillioides* untersucht. In der frühen Reifegruppe wurden zusätzlich die gleichen Genotypen in getrennten, aber benachbarten Versuchen auf ihre Resistenz gegen *F. graminearum* untersucht. Die Untersuchungen fanden an zwei bis sechs Umwelten statt, wobei mit Narbenfadenkanalinokulation bzw. natürlicher Infektion infestiert wurde. In der späten Reifegruppe wurde zusätzlich die Korninokulation angewendet. Aus den 150 Linien wurden 50 – 60 Linien pro Reifegruppe mit zwei unverwandten Testern der jeweilig anderen heterotischen Gruppe gekreuzt. Mit Immunotests wurden die FUM-, DON- und ZEA-Konzentrationen der ausgewählten Linien als auch deren Testkreuzungen analysiert. Zusätzlich wurden noch acht *F. graminearum*- und jeweils vier bzw. drei *F. verticillioides*- und *F. proliferatum*-Isolate auf ihre Aggressivität an einem Standort in jedem Jahr getestet. QTL Meta-Analysen in Mais und Weizen wurden durchgeführt, indem (1) veröffentlichte QTL-Studien gesammelt, (2) Konsensuskarten erstellt und (3) die Positionen der QTL mithilfe des Softwarepakets „MetaQTL“ verglichen wurden.

Trotz signifikanter genotypischer Unterschiede zwischen den Linien nach Inokulation oder natürlicher Infektion, war die Inokulation überlegen, da die visuelle Differenzierung einfacher und die Präzision höher war. Deshalb sollte inokuliert werden. In der späten Reifegruppe waren sowohl Narbenfadenkanal- (simuliert Infektion über die Narbenfäden) als auch Korn-Inokulation (simuliert Sekundärinfektion nach Verwundung) geeignet, einen ähnlich hohen Kolbenfäulebefall hervorzurufen. Dennoch sollten beide Methoden aufgrund nur moderater Korrelationen getrennt getestet werden. Die *F. graminearum*-Isolate verursachten einen hohen Befall, obwohl diese überwiegend von Weizen isoliert wurden, was auf eine geringe Wirtsspezialisierung des Pathogens hinweist. Im Gegensatz zu *F. graminearum* verursachten *F. verticillioides*- und *F. proliferatum*-Isolate einen geringen Befall, welcher durch deren duale Natur entweder als Endophyt oder als Pathogen erklärt werden könnte. Die für die Resistenztests ausgewählten Isolate (IFA66; FV234/1) wurden als ausreichend aggressiv angesehen, da sie zu signifikanten Unterschieden zwischen den Genotypen führten. In der frühen Reifegruppe sollten Resistenzen gegen *F. graminearum* oder *F. verticillioides* aufgrund moderater Korrelationen getrennt getestet werden.

Signifikante ($P<0,01$) genotypische Varianzen in großen und kleinen Populationen sowie deren Testkreuzungen weisen auf genetische Variation für alle Merkmale in allen Reife- und heterotischen Gruppen hin. Im Flintpool wurden weniger resistente Linien gefunden als im Dentpool, was auf eine geringere Resistenz hindeutet. Deshalb sollte der Flintpool via Introgression von Resistenzallelen aus anderem Zuchtmaterial verbessert werden. Aufgrund der signifikanten Genotyp × Umwelt-Interaktionen sollten Resistenztests in mehreren Umwelten durchgeführt werden, um eine akkurate Selektion zu sichern.

Hohe genetische Korrelationen zwischen Bonitur und Toxinkonzentrationen wurden sowohl in den Linien als auch in den Testkreuzungen gefunden ($r \geq 0{,}96$). Die kostengünstigere indirekte Selektion auf verringerte Toxinkonzentrationen anhand der Bonitur ist unter der Annahme eines fixen Budgets effizienter, da der Selektionserfolg der indirekten Selektion durch Testen von mehr Genotypen und in mehr Umwelten erhöht werden könnte. Dies sollte die Selektionsintensität und die Heritabilität erhöhen. Jedoch sollten indirekt selektierte Genotypen direkt auf FUM-Konzentrationen mit Immunotests geprüft werden, da trotz geringem visuellen Befall teilweise hohe FUM-Konzentrationen gefunden werden konnten.

Moderate genetische Korrelationen zwischen Linien- und Testkreuzungsleistung wurden gefunden ($r = 0{,}64 – 0{,}83$). Ein moderat bis hoch anfälliger Tester ist ausreichend für Topcrosstests, da die genetischen Korrelationen zwischen Testkreuzungen verschiedener Tester hoch waren. Beides weist auf eine überwiegend additive Vererbung hin, wobei aber auch eine nicht-additive Vererbung in manchen Kreuzungen eine Rolle spielen könnte. Die Selektion anhand der Linien für Testkreuzungsleistung ist nicht effektiv, da die relativen Effizienzen kleiner als eins waren. Unterschiedliche Szenarien für die Züchtung wurden festgestellt: (1) In Mitteleuropa ist hauptsächlich die Resistenz in den Linien wichtig, um eine hohe Saatgutqualität zu sichern, jedoch in den Testkreuzungen nicht, da hier nur eine geringe natürliche Infektion vorkommt. (2) In Südeuropa, wo regelmäßig eine hohe natürliche Infektion vorkommt, ist die Selektion auf Resistenz sowohl in den Linien als auch in den Testkreuzungen wichtig. Für die Erstellung der Testkreuzungen ist ein anfälliger Tester ausreichend. Für die Selektion in den Linien sollten alle Elternlinien inokuliert werden. Allerdings werden nur die Linien bonitiert, welche zuvor anhand von Testkreuzungen für agronomische Merkmale wie Ertrag selektiert wurden. Dies ist möglich, da Testkreuzungen früher geerntet werden können, als die Bonitur der Linien gemacht werden muss.

Eine hohe Anzahl an MQTL in Mais und Weizen konnte mithilfe der QTL Meta-Analysen gefunden werden, allerdings sollten die Ergebnisse im Mais aufgrund der geringen Anzahl an Populationen mit Vorsicht betrachtet werden. In beiden Kulturarten wurden MQTL gefunden, welche resistenzspezifisch zur Ausbreitungsresistenz im Kolben bzw. in der Ähre waren. Im Mais konnten MQTL gefunden werden, welche nur *F. verticillioides*-Resistenz-QTL beinhalteten. Beide Ergebnisse werden durch die mittleren Korrelationen aus den Resistenztests gestützt. Mithilfe der QTL Meta-Analysen konnten MQTL gefunden werden, welche QTL einer oder mehrerer Herkünfte vereinten. Deshalb könnten Meta-Analysen ein wertvolles Werkzeug zur Verbesserung von Resistenzen sein, indem Eltern mit unterschiedlichen Resistenzallelen selektiert und gekreuzt werden können. Insgesamt zeigte sich, dass phänotypische Selektion zur Verringerung von Mykotoxinkonzentrationen in europäischem Mais effektiv sein sollte, wobei der Selektionsfortschritt mithilfe von QTL Meta-Analysen beschleunigt werden könnte.

9. Acknowledgements

Aber rühmen wir nicht den Weisen
dessen Name auf dem Buch prangt
denn man muss dem Weisen
seine Weisheit erst entreißen
darum sei denen auch gedankt
welche sie ihm abverlangt

(Frei nach Bertold Brecht)

My sincere thanks to my academic supervisor apl. Prof. Dr. T. Miedaner for giving me the opportunity to conduct my Ph.D. thesis with the institute and for his advices, suggestions and support during this thesis work.

I am very grateful to Dr. B. Kessel and Dr. M. Ouzunova, KWS Saat AG, Einbeck, for organization of field trials, conducting myctoxin analyses and the excellent collaboration.

Sincere thanks to Prof. Dr. C.-C. Schön for her scientific contribution to one publication and for serving on my examination committee.

Many thanks to the State Plant Breeding Institute, Univesität Hohenheim, and to KWS Saat AG, Einbeck, for providing facilities, material and manpower.

Many thanks to D. Seyfang, S. Burkardt and all the staffs at the locations for collection of data of the field trials.

Sincere thanks to M. Takács, B. Lieberherr and all the other helping hands while conducting mycotoxin analyses.

Thanks a lot to C. Bolduan for his introduction into *Fusarium* resistance in maize and the enlightening discussions.

Furthermore I thank all my co-doctorate candidates, especially Maren and Peter, for creating a pleasant working environment.

The financial support by the Bundesministerium für Bildung und Forschung (BMBF, Bonn) and the KWS SAAT AG within the German-French-Spanish CEREHEALTH Consortium (Project no: 0313992A) is highly appreciated.

Most sincere thanks to my family and Gabi, who encouraged and supported me throughout the years. Thank you very much.

Curriculum vitae

Name: Martin Messerschmidt, née Löffler

Date of birth: 10.07.1980

Place of birth: Göppingen, Germany

Education

05.07 – 03.10	Doctorate candidate – State Plant Breeding Institute, Universität Hohenheim, Stuttgart, Germany.
04.05 – 04.07	Agriculture studies, Universität Hohenheim, Stuttgart, Germany. Master of Science, April 2007
10.01 – 03.05	Agriculture studies, Universität Hohenheim, Stuttgart, Germany. Bachelor of Science, March 2005
06.2000	Abitur, Rechberg-Gymnasium, Donzdorf

Community service

07.00 – 04.01	Klinik am Eichert, Göppingen

Agriculture experiences

02.06 – 03.06	KWS SAAT AG, Institute of sugar beet breeding, Einbeck, Germany
07.05 – 09.05	KWS SAAT AG, Institute of sugar beet breeding, Einbeck, Germany
04.04 – 09.04	Institute of Plant Breeding, Seed Sciences and Quantitative Genetics, Stuttgart, Germany
11.03 – 12.03	Banana farm, Innisfail, Australia
09.03 – 11.03	Cattle farm, Beaudesert, Australia
07.02 – 10.02	Trial station ”Höfchen”, Bayer Crop Science AG, Burscheid, Germany
07.01 – 10.01	Reiherhof, Stuttgart, Germany

Employment record

Since 03.10	KWS SAAT AG, Institute of sugar beet breeding, Einbeck, Germany
05.07 – 03.10	State Plant Breeding Institute, Universität Hohenheim, Stuttgart, Germany

Erklärung

Hiermit erkläre ich an Eides statt, dass die vorliegende Arbeit von mir selbst verfasst und lediglich unter Zuhilfenahme der angegebenen Quellen und Hilfsmittel angefertigt wurde. Wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.

Die vorliegende Arbeit wurde in gleicher oder ähnlicher Form noch keiner anderen Institution oder Prüfungsbehörde vorgelegt.

Insbesondere erkläre ich, dass ich nicht früher oder gleichzeitig einen Antrag auf Eröffnung eines Promotionsverfahrens unter Vorlage der hier eingereichten Dissertation gestellt habe.

Stuttgart, im Februar 2010

Martin Löffler