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Genetic variation in early maturing European maize germplasm for resistance to ear rots and mycotoxin contamination caused by *Fusarium* spp.

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Contents

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1. General introduction

The cultivation of maize (*Zea mays* L.) in Europe has expanded dramatically since the introduction of hybrid varieties during the last 50 years. In 2006, 13.5 million ha of maize were grown for grain use and 7.8 million ha for forage and silage in Europe (FAOSTAT 2008). Along with the expansion of acreage of maize in Central Europe, the incidence of maize ear rots caused by *Fusarium* spp. has increased and this disease complex has emerged as a major threat to maize cultivation. These ear rots adversely affect the quantity and quality of grain production (Vigier et al., 2001). However, the main implication is the contamination of grain and stover with mycotoxins which can lead to reduced economic return besides serious intoxications in humans and animals. In fact, maize has the highest mycotoxin contamination among all important grain cereals (Munkvold, 2003a).

Fusarium species and mycotoxins associated with maize ear rot in Central Europe

Fusarium spp. cause two distinct ear rots of maize, Gibberella ear rot and Fusarium ear rot. Gibberella ear rot in Europe is caused by *F. graminearum* Schwabe (teleomorph *Gibberella zea* (Schw.) Petch) and *F. culmorum*, the former being the main causal agent. It predominates in cooler areas (Görtz et al., 2008; Dorn et al., 2009) and requires high humidity from silking to harvest for its development (Bottalico, 1998; Stewart et al., 2002). Symptoms of Gibberella ear rot typically initiate from the tip and cover the ear and husks with a red or pink mold (Munkvold, 2003b) (Fig. 1). Main inoculum sources of *F. graminearum* are macroconidia, ascospores or mycelia which are dispersed through wind, rain, insects or birds. The most important mode of disease infection is via the spores that land on the silks. These spores germinate and mycelia grow down the silk channel to the kernels and cob (Sutton, 1982). However, infection is also caused by spores entering through kernel wounds caused by insects, such as the European corn borer (*Ostrinia nubilalis* Hübner), birds or hail.

Gibberella ear rot leads to contamination with deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA). When infected grain is fed to livestock, DON causes vomiting (“vomitoxin”), feed refusal, decreased weight gain and it acts as an immunosuppressant (Pestka, 2007; Miller, 2008). ZEA causes reproductive problems, such

as reduced litter size, swine estrogenic symptom and male infertility (Morgavi and Riley, 2007).

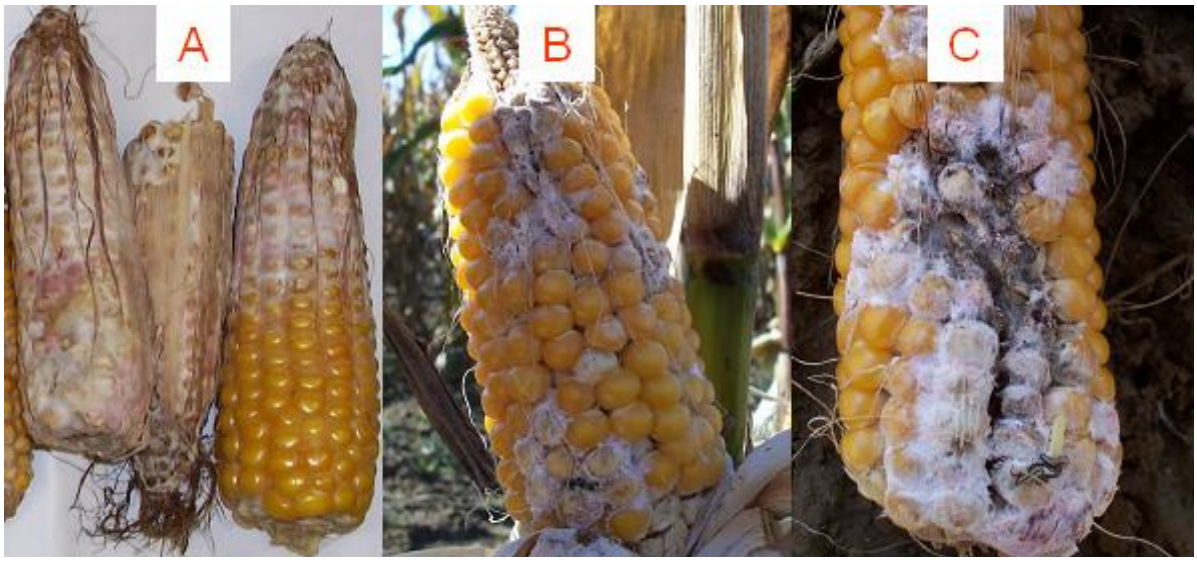

Figure 1. Typical symptoms of Gibberella ear rot caused by *Fusarium graminearum* (A), Fusarium ear rot caused by *F. verticillioides* (B), and development of Fusarium ear rot following damage of the European corn borer (*Ostrinia nubilalis*, C).

In Europe, Fusarium ear rot is primarily caused by *F. verticillioides* (Sacc.) Nirenberg (Syn. = *F. moniliforme* Sheldon) (teleomorph *G. fujikuroi* (Sawada) Wr.), but *F. subglutinans* and *F. proliferatum* are also frequently isolated from plant tissue (Logrieco et al., 2002). Fusarium ear rot was observed to predominate in warmer and drier areas/years compared to *F. graminearum* (Bottalico, 1998; Görtz et al. 2008; Dorn et al., 2009). Microconidia are the main inoculum source of *F. verticillioides*. These disperse in a similar manner as *F. graminearum*. Infection pathways include infection through the silk and entering through wounds of the kernels. The white or light pink mold of *F. verticillioides* typically occurs on random kernels, groups of kernels or physically injured kernels (Munkvold, 2003b) (Fig. 1). *Fusarium verticillioides* is known to cause symptomless infection of kernels and systemic growth in maize plants. Moreover, abiotic stress factors like nitrogen deficiency and drought were reported to enhance the severity of Fusarium ear rot (Shelby et al., 1994; Miller, 2001; Bacon et al., 2008).

Infection of maize ears with *F. verticillioides* leads to accumulation of fumonisins (FUM), mainly FB1. The toxin was reported to cause equine leukoencephalomalacia, porcine pulmonary edema, liver cancer in rats and neural tube defects in mice. There is

also evidence that it is associated with human esophageal cancer (Voss et al., 2007; Miller, 2008).

Due to the potential serious impacts on the health of farm animals and humans following the consumption of contaminated maize grain and related products, the EU (Commission Regulation (EC) No 1126/2007 of September 2007) has released legally enforceable limits in unprocessed maize for DON, FUM, and ZEA (1.750, 4.000 and 350 μg kg^{-1}, respectively). For animal feeding, the limits of mycotoxin concentrations are between 2.000 and 8.000 μg kg^{-1} for DON and FUM and 250 to 500 μg kg^{-1} for ZEA, depending on the animal. Amongst livestock, swine is the most sensitive animal to mycotoxin intake, whereas poultry is the least (Morgavi and Riley, 2007). Furthermore, mycotoxins also affect the health of farmers, grain handlers and producers through their exposure to contaminated dust, which is formed during harvesting of the crop and processing of infected maize grain.

Control of ear rots caused by *Fusarium* spp. and contamination by mycotoxins

Severe damage of *Fusarium* spp. to maize is often associated with continuous maize monoculture or in crop rotations where maize is followed by wheat and vice versa. Sources of inoculum are seed, soil, and infected plant residues. Therefore, disease management practices are based on extended crop rotation with non-host crops and the reduction of inoculum sources, mainly by plowing of infected residues. However, these practices are only of preventive character and do not provide the desired level of disease control. Moreover, they have only limited applicability in many maize cultivating areas due to economic considerations. There are practically no chemical treatments that are effective in preventing these ear rots. Maize that has been modified to have a Bt (*Bacillus thuringiensis*) endotoxin was reported to reduce the contamination with *Fusarium* spp. and the resultant mycotoxins in maize grain indirectly through protection of the plants against insect damage (Munkvold et al., 1997, 1999; Bakan et al., 2002; Schaafsma et al., 2002; Magg et al., 2003). However, Magg et al (2002) and Papst et al. (2005) observed reduced concentration only of some mycotoxins but not of others. Efforts have also been made in genetic engineering to minimize mycotoxin concentrations through detoxification *in vivo* (Duvick, 2001) but no efficient technology based on this approach is available. Furthermore, prospects for the commercial cultivation of genetically modified plants are

doubtful in most of the EU countries and especially in Germany. Therefore, the most promising approach to minimize ear rot damage and mycotoxin contamination in the field is the development of maize genotypes endowed with genetic resistance. Resistant maize hybrids have wide acceptance by growers and consumers, and their cultivation is economically rewarding. However, most maize genotypes are highly susceptible and sources of good resistance are rare (Reid et al., 2009).

Breeding for resistance to Gibberella and Fusarium ear rots

The most important prerequisites for a resistance breeding program are (1) genetic variation for resistance traits in the breeding material and (2) efficient and reliable germplasm screening methods to identify resistant genotypes. Because of the sporadic nature of epidemics, a technique allowing a large-scale artificial infection is required to select resistant genotypes in large sets of breeding material. According to the two major modes of fungal entry into the maize ear, two distinct inoculation methods are mainly used. One technique simulates fungal entry through the silk by injecting a conidial suspension into the silk channel of maize ears, while the other technique simulates fungal entry into kernel wounds by injecting a conidial suspension into artificially wounded kernels (Ullstrup, 1970; Chungu et al., 1996a; Reid et al., 1996a). These two main fungal entry modes, i.e., silk and kernel infection showed no or only moderate but inconsistent association with the response of maize genotypes (Lemmens 1999; Presello et al., 2004; Schaafsma et al., 2006).

Studies have been conducted to assess variability for resistance in maize breeding germplasm, showing genotypic differences for both Gibberella ear rot resistance and DON contamination in Canada (Reid et al., 1993, 1996a; Schaafsma et al., 1997), as well as for Fusarium ear rot and FUM contamination in the US Corn-Belt (White et al., 2002; Clements et al., 2004; Kleinschmidt et al., 2005). Resistances to both, Gibberella and Fusarium ear rots and the resulting mycotoxin contaminations are quantitatively inherited and there is no evidence of the presence of major genes or of completely resistant genotypes. Additive and dominance genetic effects as well as digenic (additive x dominance) interaction effects were reported to control the inheritance of resistance traits (Gendloff et al., 1986; Chungu et al., 1996b). This was confirmed by the few QTL studies that have been conducted so far on resistance to Gibberella ear rot (Ali et al., 2005) and on

Fusarium ear rot (Pérez-Brito et al., 2001; Robertson-Hoyt et al., 2006; Ding et al., 2008). Numerous QTL with only small to moderate effects were detected, and their effects showed limited consistency across environments. Therefore, marker-assisted selection for resistance to these ear rots can not be recommended with the present level of genetic information.

Depending on the location and environmental conditions, which vary from year to year, maize varieties may be affected by both Gibberella and Fusarium ear rots. Therefore, it is of interest to know if the mechanisms of resistance are specific to one of the pathogens, or genetic improvement of resistance is effective in controlling disease severity of and mycotoxin contamination by one or more pathogens belonging to *Fusarium* spp. Only a few studies examined the association between Gibberella and Fusarium ear rots, showing little evidence of general resistance mechanisms to both of them (Presello et al., 2004; Schaafsma et al., 2006).

In hybrid maize breeding, selection is carried out for line *per se* performance during inbreeding and on the hybrid level in testcrosses and experimental hybrids. Nevertheless, the ultimate goal is the superior performance of inbred lines in hybrid combinations. As generating and evaluating of testcrosses is expensive and labor intensive, indirect improvement of testcross performance by line *per se* selection would be advantageous in regard of saving time and resources. However, for indirect selection to be effective, a high correlation between line *per se* and testcross performance is required. This correlation was shown to vary considerably depending on the trait and the stage of inbreeding of the tested lines (Hallauer and Miranda, 1988). So far, little is known about the correlation between line *per se* and testcross performance for resistance to ear rots caused by *Fusarium* spp. and the related mycotoxin contaminations.

The relationship of the amount of symptomatic tissue and mycotoxin production is a key element in the design and implementation of resistance breeding programs. Field evaluation of ear rot symptoms is cheaper and faster than chemical determination of mycotoxin concentrations. If visible disease rating is an appropriate trait to predict mycotoxin contamination, it would enable increased testing capacity and maximize selection gain in a resistance breeding program. Strong correlations have been observed for ear rot symptoms of Gibberella ear rot and DON in Canada after artificial inoculation (Reid et al., 1996b, 1996c), but no clear trend was reported between disease symptoms and

ZEA production (Cullen et al., 1983; Hart et al., 1984; Bakan et al., 2002). For Fusarium ear rot, a high correlation between the symptoms and FUM has been observed in the US corn belt (Robertson et al., 2006).

The present study was conducted to establish basic concepts for an efficient resistance breeding to Gibberella and Fusarium ear rots in maize breeding programs in Central Europe which are based on early maturing germplasm adapted to the prevalent climatic conditions. In contrast to US and Canadian materials, little information on genetic variation for resistance to Gibberella and Fusarium ear rots and the mechanisms of resistance are publicly available for early European maize materials.

Objectives

The specific objectives of this study were to:

(1) evaluate a set of early maturing European elite inbred lines for resistance to ear rots and mycotoxins contamination caused by *F. graminearum* and *F. verticillioides*,
(2) estimate genetic and genotype-environment interaction variances and heritabilities for ear rot ratings and mycotoxin concentrations,
(3) determine genotypic and phenotypic correlations of ear rot ratings with mycotoxin concentrations,
(4) determine correlations between line *per se* and testcross performance for Gibberella ear rot rating and DON concentration,
(5) examine the aggressiveness of, and mycotoxin production by different isolates of *F. graminearum* and *F. verticillioides*, and
(6) evaluate the potential of near infrared spectroscopy to estimate concentrations of DON and FUM in maize grains under artificial inoculation.

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2. Paper 1: Genetic variation for resistance to ear rots and mycotoxins contamination in early European maize inbred lines

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Abstract. Maize ear rots caused by *Fusarium* spp. are of major concern in all maize-growing areas of Europe. Our objectives were to (1) evaluate early maturing European elite inbred lines for resistance to *F. graminearum* and *F. verticillioides* and mycotoxin production, (2) estimate genetic and genotype-environment interaction variances and heritabilities, and (3) examine the relationships among these traits. Two field experiments were conducted under artificial inoculation across different environments. In Experiment 1, 42 inbreds were evaluated for resistance to *F. graminearum*. In Experiment 2, 21 inbreds (a subset of inbreds tested in Experiment 1) were evaluated for resistance to *F. verticillioides*. Data were recorded on severity of Gibberella (AGER) and Fusarium ear rots and accumulation of Deoxynivalenol (DON), Zearalenone, and Fumonisins. Artificial inoculation was effective in promoting both diseases, particularly Gibberella ear rot. Genotypic and genotype-environment interaction variances were generally significant.

Heritability estimates were moderate to high. Disease severity had strong correlations with respective mycotoxin concentrations, being highest between AGER and DON (0.94). Selection for resistance to both ear rots is expected to result in favorable correlated response for the respective mycotoxin concentrations, particularly for DON through selection for resistance to Gibberella ear rot. We recommend to conduct initial selection on the basis of visual rating and to evaluate the selected elite material for mycotoxin concentration.

3. Paper 2: Determination of mycotoxin concentration by ELISA and Near Infrared Spectroscopy in fusarium-inocualted maize

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Cereal Res. Commun. 37: 521-529 (2009).

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Abstract. Maize ear rots caused by *Fusarium* ssp. cause reduction in grain yield and contamination with mycotoxins which are harmful to humans and animals. To develop resistant maize cultivars, reliable large-scale phenotyping is essential. Our objectives were to (1) examine the precision of enzyme-linked immunosorbent assays (ELISA) for determination of deoxynivalenol (DON) and fumonisins (FUM), (2) to evaluate the potential of near-infrared reflectance spectroscopy (NIRS) to estimate concentrations of DON and FUM in grain produced in artificially inoculated maize plants, and (3) to compare the efficiency of ELISA, NIRS, and visual rating of disease severity for the estimation of mycotoxin concentrations. Insignificant variation was observed between duplicate evaluations of DON and FUM by ELISA, showing very high accuracy of this method. The DON and FUM determinations by ELISA were more closely correlated with the concentrations predicted through NIRS than with visual rating of disease severity. For the prediction of DON, NIRS had very high magnitude of the coefficients of determination

of calibration and cross validation (R^2 = 0.82-0.90). Thus, the adoption of NIRS has promising potential to predict DON concentrations in grain samples of inoculated maize ears in resistance breeding programs.

4. Paper 3: 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

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Abstract. The most important pathogens causing ear rot of maize in Central Europe are *Fusarium graminearum* and *F. verticillioides*. Our objectives were to (1) compare eight isolates of each species on two susceptible inbred lines for their variation in ear rot rating and mycotoxin production across three years, and (2) examine two susceptible and three resistant inbred lines for potential isolate x line interactions across two years under inoculation of the silk channel. Ear rot rating, zearalenon (ZEA) and deoxynivalenol (DON) concentrations were evaluated for all *F. graminearum* isolates, additionally nivalenol (NIV) concentrations were analyzed for two NIV producers. Fumonisin concentrations (FUM) were evaluated for all *F. verticillioides* isolates. Mean ear rot severity was highest for DON producers of *F. graminearum* (62.9% of the ear covered by

mycelium), followed by NIV producers of the same species (24.2%) and lowest for *F. verticillioides* isolates (9.8%). For the latter species, ear rot severities highly differed among years (2006: 24%, 2007: 3%, 2008: 7%). Mycotoxin concentrations among isolates showed a broad range (DON: 100-284 mg kg^{-1}, NIV: 15-38 mg kg^{-1}, ZEA: 1.1-49.5 mg kg^{-1}, FUM: 14.5-57.5 mg kg^{-1}). Significant genotypic variances were found for isolates and inbred lines in all traits and both species. Isolate x line interactions were significant only for ear rot rating ($P<0.01$) and DON concentration ($P<0.05$) of the *F. graminearum* isolates. However, no rank reversals occurred. Most isolates were capable of differentiating the susceptible from the resistant lines. For resistance screening, a sufficiently aggressive isolate should be used to warrant maximal differentiation among inbred lines. High FUM concentrations of grain with minimal disease symptoms must be considered in *F. verticillioides* infections.

5. Paper 4: Genetic variation in testcrosses and relationship between line per se and testcross performance for resistance to Gibberella ear rot in maize

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Abstract. Gibberella ear rot (GER) caused by *Fusarium* spp. is a major concern in maize production in Central Europe. Thus, the development of hybrid cultivars having resistance to GER is an important breeding goal. The objectives of our study were to (1) evaluate the variation in testcross performance (TP) of European maize germplasm for GER resistance and deoxynivalenol (DON) contamination, (2) estimate variance components, heritabilities, and correlations of these resistance traits, and (3) examine the relationship between line *per se* (LP) and TP. Sixty testcrosses of 30 diverse flint inbred lines with two dent inbred testers were evaluated in four environments under artificial inoculation. Data were recorded on severity of GER and concentration of DON. Significant estimates of genotypic and genotype-environment interaction variances were found for testcrosses with both testers. Genotypic variances were in general higher for LP than for TP in each set of

testcrosses. Phenotypic correlations between LP and TP were moderate ($\hat{r}_p \leq 0.57$) for resistance traits. We suggest a multi-stage selection procedure to develop GER resistant maize hybrids based on multi-environment tests under artificial inoculation starting with selection for LP, followed by selection for TP. In both cases, the tested genotypess should first be scored for GER resistance and only the most promising evaluated for DON concentration.

6. General discussion

The most cost effective, environmental friendly and publicly acceptable method of controlling ear rots and mycotoxin concentrations in maize caused by *Fusarium* spp. is growing of cultivars endowed with genetic resistance. So far, maize breeding materials in Europe have not been subjected to long-term selection pressure for ear rot resistance and low mycotoxin contamination. Ear rots caused by *Fusarium* spp. were not considered to be an economic threat until the EU released legally enforceable limits in unprocessed maize grain for the mycotoxins deoxynivalenol (DON), fumonisins (FUM), and zearalenone (ZEA). In our study, we focused on *F. graminearum*, causing Gibberella ear rot (GER), and *F. verticillioides*, causing Fusarium ear rot (FER). These species have been reported to be the predominant causative agents of the two diseases under Central European conditions (Logrieco et al. 2002; Görtz et al. 2008; Dorn et al. 2009). Gibberella ear rot causes contamination with DON, nivalenol and ZEA, and Fusarium ear rot causes contamination with FUM.

Improving the level of resistance in the adapted breeding material requires sound knowledge of the epidemiology of the pathogen species, effective screening methods and reliable estimates of the quantitative-genetic parameters for resistance traits. Currently, this information can only be gained from field experiments, as there are no laboratory tests or seedling assays available and testing under greenhouse conditions is not practicable in large scale breeding programs.

Screening techniques

To investigate genetic differences in the breeding material for their response to ear rot severity and mycotoxin contamination, an appropriate infection pressure of the relevant pathogen(s) is required. The predominance of pathogen species and the severity of ear rot symptoms are strongly influenced by environmental conditions (Görtz et al. 2008), and therefore may change among locations and years. Moreover, disease pressure within one location can be highly variable due to variable occurrence and distribution of inoculum sources like infected debris or neighboring crops. We recorded ear rot severity under natural infection in four environments (EWE06, HOH06, EWE07, and HOH07). No significant genotypic differences were observed in the tested material even in EWE06, the

environment with the highest natural ear rot severity. This leads to the conclusion that under the conditions prevailing in Southwest Germany, artificial inoculation is necessary to warrant adequate and uniform infection pressure and, thus, to facilitate selection for resistance traits in a breeding program.

Artificial inoculation is carried out to provide the required dose of inoculum at the appropriate time and at the right place (organ of the plant). This has several advantages compared to natural disease pressure: (i) it assures testing for the desired resistance traits as the predominant and relevant pathogen is used for inoculation, (ii) all plots and plants in an experiment are exposed to the same inoculum pressure, leading to higher heritability by decreasing the plot-to-plot random variability, and (iii) it circumvents escape reactions in the plant-pathogen interaction, which can mask useable genetic resistance.

There are two major modes of fungal entry and, accordingly, resistance to ear rots caused by *Fusarium* spp. is divided into two components: (i) silk resistance (or resistance to initial penetration) and (ii) kernel resistance (or resistance to the spread of the pathogen in the host tissue). Consequently, two inoculation techniques have been developed: without and with mechanical injury of the kernel tissue (Ullstrup, 1970; Chungu et al., 1996a; Reid et al., 1996). The correlation between both types of resistance was found to be only low to moderate. Therefore, some experts are of the view that testing for only one type may not provide the information required in a resistance breeding program (Reid et al., 1996; Lemmens, 1999).

Inoculation methods with mechanical injury of the kernel tissue simulate insect feeding to some degree. Although high levels of infection are obtained by this method, it is of limited value because important morphological barriers are bypassed (Drepper and Renfro, 1990). Therefore, wound-type inoculations might be of superior importance in geographic regions that are facing high pressure from insect feeding on ears, especially the European corn borer (*Ostrinia nubilalis*). However, under such conditions it might be worthwhile to select for resistance to insect feeding, rather than tackling the resulting secondary damage. Further, for both, *F. graminearum* and *F. verticillioides*, the predominant pathway described in the literature for infecting maize ears is via the silks (Nelson 1992; Reid et al., 1996; Munkvold et al. 1997b; Desjardins et al., 2002). Hence, we conducted our study by applying the silk channel inoculation as described by Reid et al. (1996). This inoculation method was reported to be very promising in generating

information on resistance to the most important infection pathway combined with high consistency compared to other methods (Ullstrup, 1970; Chungu et al., 1996a). Furthermore, it also seems to be an appropriate method to combine testing for resistance to initial penetration and spread of the pathogens in the host tissue.

In our study, artificial silk channel inoculation proved to be effective in promoting disease development of *F. verticillioides* (AFER) in inbred lines and of *F. graminearum* (AGER) in both inbred lines as well as hybrids (Bolduan et al., 2009a, 2010; Miedaner et al., 2010). In general, ear rot severities and mycotoxin concentrations were much higher following inoculation than under natural conditions. Inoculation with *F. graminearum* consistently resulted in higher AGER severity at the location with cooler crop season, i.e., HOH compared with EWE (Bolduan et al., 2009a; 2010). This may be due to low temperature stress conditions for maize that weakened the host. Therefore, screening for AGER should be avoided in chilly environments as the expected heavy disease development might not allow for any differentiation between genotypes. The ability to differentiate genotypes might also be reduced if the volume and/or the concentration of the inoculum are too high (Reid et al., 1996). Excessively high volumes of inoculum will be forced down the silks and will directly infect cob and kernels which does not occur in nature. The natural barrier of the silk channel would be bypassed and genotypes with useful resistance are rated as susceptible. High inoculum concentrations would even increase disease severity in environments which are already conducive to fungal growth. Therefore, it might be worthwhile to adjust the inoculum dose to the environmental conditions, as well as to the genotypes (inbreds vs. hybrids) and the average resistance level of the material to be tested to allow for an optimal differentiation of the test candidates.

Moreover, care has to be taken to select representative plants for inoculation in order to obtain more consistent and reliable results in the screening experiments. Plants that are weakened by biotic and/or abiotic stress may show increased susceptibility to infection, which masks their expression of genetic resistance. Ears of a given genotype should be inoculated at the same time to ensure similar weather conditions for infection and disease development. On the other hand, disease severity is affected by the flowering date (physiological stage of development) of the host plant; inoculations are not optimally effective if made too early or too late (Reid et al. 1996).

Silk channel inoculation and visual assessment of ear rot severity are labor intensive and time consuming. Therefore, it would be worthwhile to evaluate phenotypic traits that can be easily assessed without prior inoculation but are related to resistance traits. This would help to eliminate the most susceptible genotypes, and thereby to minimize the number of genotypes that are to be evaluated under artificial inoculation. Traits considered to be related with ear rot resistance were days to silking (DTS), husk covering (Butrón et al., 2006; Warfield and Davis, 1996) as well as length of the silk channel. However, in our study, DTS showed no significant correlation to the resistance traits. Silk channel length had only a moderate phenotypic correlation with AFER ($\hat{r}_p$ = -0.54). Further, we observed no significant genotypic variation for husk covering and husk tightness in the tested inbred lines (Bolduan et al., 2009a). Therefore, our study gives no support to perform selection based on these agronomic traits in order to improve resistance to ear rots in maize.

Fusarium species and isolates

Fusarium infection of maize ears is caused by two distinct diseases that show overlappings but also differ in some of their epidemiological characteristics. Under Central European conditions, *F. graminearum* and *F. verticillioides* have been reported to be the predominant causative agents of Gibberella and Fusarium ear rot, respectively (Logrieco et al. 2002; Görtz et al. 2008; Dorn et al. 2009). However, changes in environmental conditions, agricultural practices, and crop rotations may result in a shift in the predominance of the pathogen species. For example, *F. culmorum,* the causal organism of Fusarium head blight of wheat has been almost completely replaced by *F. graminearum* during the last decades in Europe (Waalwijk et al., 2003). Furthermore, resistance responses to different *Fusarium* spp. are only moderately correlated with each other (Presello et al., 2004; Schaafsma et al., 2006; Bolduan et al., 2009a). Consequently, monitoring the abundances of *Fusarium* species causing ear rots of maize is essential to adapt resistance breeding programs to the pathogen species predominant in the target environment.

Silk channel inoculation with *F. graminearum* was generally more effective than with *F. verticillioides* for disease development and accumulation of the main mycotoxins

DON and FUM, respectively (Paper 1 and 3). This can not only be explained by the growth conditions in Central Europe, which are less favorable for *F. verticillioides* than for *F. graminearum* (Bottalico 1998). Several studies showed that inoculation with *F. verticillioides* leads to relatively low infection severity even under warmer growth conditions (Reid et al. 2002) combined with very high inoculation pressure (Clements et al. 2004; Robertson et al. 2006). However, FUM concentrations in our experiments were much higher than expected from the relatively low levels of visible infection. This is in accordance to other studies, where appreciable FUM concentrations were found even in the absence of symptoms (Munkvold et al., 1997a; Desjardins et al., 1998). The variable response to inoculation is most likely attributable to the different epidemiology of *F. verticillioides* compared to *F. graminearum*.

Modern maize cultivars are generally infected with symptomless endophytic colonizations by *F. verticilliodes* but disease symptoms are rarely exhibited under non-stress environments (Bakan et al., 2002; Dorn et al., 2009). It was reported that under extreme drought or other conditions stressing plant growth, the fungus is not in a balanced state with the plant, resulting in different degrees of pathological responses (Schulz et al., 1999). This illustrates that the effects of infection by *F. verticillioides* are more multifaceted and less understood than those of *F. graminearum*. Therefore, selection for Fusarium ear rot and FUM concentration seems to be more difficult due to more complex host-pathogen-environment interactions. Furthermore, the error of visual assessment of AFER is higher compared to AGER due to the random distribution of single infected kernels, which reduces heritabilities.

We observed moderate positive correlations between AGER and AFER (r_p = 0.63, r_g = 0.88) and between DON and FUM (r_p = 0.59; r_g = 0.77) (Bolduan et al., 2009a) as also reported in earlier studies (Presello et al., 2004; Schaafsma et al., 2006). These results suggest that there may be some common genes and mechanisms for resistance to both *Fusarium* species. Therefore, selection for resistance to one species is expected to result in correlated response for resistance to the other. In our study, genotypes with low AGER and DON values also showed low AFER and FUM values but not necessarily vice versa. For this reason, pre-selection for resistance to *F. graminearum* can be recommended if resources are limited. Nevertheless, evaluation of resistance to both pathogens is essential as the testing of the germplasm advances in each breeding cycle. Further research on

Fusarium ear rot is necessary to get a better understanding of the complex interactions between *F. verticillioides* and maize to develop hybrids with low FUM concentrations.

For developing hybrids with stable resistance in the field, the breeder has not only to work on the predominant pathogen species in the target region but must also select aggressive isolates of the pathogen for inoculation. We evaluated eight isolates, each of *F. graminearum* and *F. verticillioides*, for analyzing variation of ear rot severity and mycotoxin production following silk channel inoculation of two susceptible and three resistant inbred lines. The isolates differed in their geographical origin and for *F. graminearum* also for the host from which they were originally obtained. Significant variation among isolates was found for ear rot severity and mycotoxin concentrations in *F. graminearum* and *F. verticillioides*. Further, all isolates produced ear rot severity and mycotoxin concentrations that enabled differentiation between susceptible and resistant inbreds in Southwest Germany, irrespective of their geographic origin (Miedaner et al., 2010). More importantly, the *F. graminearum* isolate (Fg1) that originated from maize did not show higher aggressiveness than the isolates originating from wheat. This corroborates the low pathogenic specialisation of *F. graminearum* reported earlier by Miedaner (2008).

Environmental conditions had higher influence on disease severity for *F. verticillioides* than for *F. graminearum* (Bolduan et al., 2009a; Miedaner et al., 2010). The least aggressive isolates of both pathogen species were the most sensitive to varying environmental conditions (Miedaner et al., 2010). Moreover, differences between susceptible and resistant inbreds were smaller for the less aggressive isolates. No major changes in ranking were found among resistant and susceptible inbreds in our study, when inoculated with different isolates of *F. graminearum* or *F. verticillioides* (Paper 3). Therefore, inoculation with a single environmentally stable and sufficiently aggressive isolate is efficient for reliable identification of genotypes with useful resistance.

Carter et al. (2002) and Maier et al. (2006) stated that NIV-producing isolates show especially high aggressiveness on maize. Unlike these reports, the two NIV-producing isolates tested in our study were among the least aggressive isolates (Miedaner et al., 2010). Therefore, a proper choice is to use DON producing isolates in resistance screenings, considering the importance of DON due to the legally enforceable limits released by the EU. Further, all tested *F. graminearum* isolates produced considerable amounts of ZEA. This is in contrast to the study of Hart et al. (1982), where only one out

of three isolates produced ZEA in the inoculated maize ears. Information about ZEA in maize is much less than for DON. Owing to its estrogenic behaviour and its high toxigenicity, this toxin should be subject to future research in maize

Variation for resistance traits

Significant estimates of σ_g^2 ($P < 0.01$) and h^2 (≥ 0.65) were found for ear rot ratings and mycotoxin concentrations following silk channel inoculation in the tested inbred lines and their testcrosses for Gibberella ear rot (Bolduan et al., 2009a; Bolduan et al., 2010) and for inbred lines for Fusarium ear rot (Bolduan et al., 2009a). These results are especially promising as the majority of the tested germplasm belonged to the European flint pool which confers early vigor and chilling tolerance to the dent x flint hybrids. Compared to the broad variation in the heterotic pool of the dent material, flint germplasm has a comparatively narrow genetic basis (Reif et al., 2005). Further, our study suggests that selection will be effective for improving resistance to ear rots caused by *Fusarium* spp. in elite flint inbreds with superior agronomic performance. This is of great importance as the relationship between resistance traits and grain yield influences the feasibility of developing cultivars with improved resistance, especially if the sources of resistance are unadapted and/or exotic. Negative genetic relationships between disease resistance and agronomic traits may occur due to negative pleiotropy or to repulsion phase linkages between favorable alleles at disease resistance loci and genes affecting agronomic traits. For example, the US Corn Belt line CG1 had good performance for AFER and FUM, but did not even develop ears in HOH07 and showed considerable lodging in all environments. Even with marker assisted backcrossing it would take at least three to four years to introgress one QTL for resistance from unadapted (exotic) germplasm into elite breeding material due to linkage drag, whereas resistance loci from adapted germplasm can be directly used for recurrent selection or forward breeding.

Significant estimates of σ_{ge}^2 were found for all traits, except for FUM following artificial inoculation with both pathogens (Bolduan et al., 2009a, 2010). Hence, for effective selection, resistance traits need to be evaluated in more than one environment. On the other hand, inoculation procedures are labour-intensive and require time-consuming visits to off-station sites during flowering time. Therefore, initial selection among inbred

lines may be conducted for ear rot rating in only one environment with the objective to eliminate only the highly susceptible ones. Subsequently, the selected lines can be tested in two to three environments for line *per se* performance (LP) and/or testcross performance (TP).

For AGER we obtained lower estimates of σ_g^2 and h^2 values for TP than for LP. This was in accordance with theoretical quantitative-genetic expectations under the assumption of additive gene action (Wricke and Weber, 1986). Thus, multi-location testing is more important for TP than for LP (Bolduan et al., 2010). Further, assuming similar selection intensities, the expected response to selection for LP should be higher than for TP. However, due to moderate correlations between LP and TP for AGER and DON, selection based on LP is not sufficient because the ultimate goal is to develop resistant hybrids.

Estimates of σ_g^2 for TP, were distinctly smaller than half $\hat{\sigma}_g^2$ of LP, particularly with the susceptible tester D23. Further, the overall mean AGER was similar for TP and LP of the resistant tester P006. This most likely reflects the presence of non-additive effects in the inheritance of resistance traits. The presence of dominant resistance genes in each tester is also supported by the moderate correlations (r_g, r_p) of LP with TP in each set of testcrosses for AGER and DON. Our findings are in accordance with the results of Gendloff et al. (1986) and Chungu et al. (1996b), who also found the presence of additive as well as non-additive gene action in the inheritance of resistance to GER.

High estimates of r_g between TP with the two different testers suggested that general combining ability is of greater importance than specific combining ability. Further, correlations (r_g, r_p) between LP with TP in both sets of testcrosses were similar. Therefore, the use of a single elite inbred tester should be effective for identifying lines with high general combining ability for resistance traits.

Estimates of σ_g^2 and h^2 for AGER and DON were smaller for TP with the susceptible tester D23 than with the resistant tester P006. Based on our findings, the resistance level of the inbred tester should be moderate to high as the use of a highly susceptible tester may lead to low differentiation between the tested lines due to very high disease levels. However, care must be exercised in the genetic interpretation of our data. We applied doubled volume of inoculum to testcrosses than to inbred lines to

counterbalance the higher vigor of hybrids. This may have reduced $\hat{\sigma}_g^2$ in testcrosses of the susceptible tester D23 due to a higher disease severity.

Efficiency of selection for resistance traits

For a successful breeding program it is essential to optimally allocate the available resources in order to maximize selection gain. The reduction of mycotoxin concentrations in the grain of hybrids can be seen as the major goal in improving maize for resistance to Gibberella and Fusarium ear rots. However, quantitative analysis of mycotoxins by HPLC is expensive and very time-consuming. Even for the ELISA method, currently the cheapest alternative, large resources are required for extensive germplasm evaluation as is the case in breeding programs. Significant and high estimates of r_p ($\geq$ 0.70) and r_g ($\geq$ 0.73) were found between ear rot severity and mycotoxin concentrations for Giberella and Fusarium ear rots, respectively, following silk channel inoculation (Bolduan et al., 2009a, 2010). These results showed that indirect selection for low mycotoxin concentrations through selection for low disease severity is preferable (Bolduan et al., 2009a). Visual rating for disease severity is much cheaper and faster than any type of mycotoxin analysis. Assuming a fixed budget, indirect selection based on AGER/AFER allows for testing a larger number of genotypes more intensively over a range of environments, compared to direct selection based on mycotoxin concentrations. Hence, selection gain will be increased through higher heritabilities and stronger selection intensities. In addition, selection based on AGER/AFER can be immediately put into effect for the planting of a winter nursery. In contrast, this would not be feasible with lab analysis of mycotoxins.

Heritability and selection gain can further be enhanced by increasing the number of test environments at the cost of the number of replications and plants per plot (Bolduan et al., 2009a). Our results suggest that it is sufficient to reduce the number of inoculated plants per plot to five if homogenous material is under test. This is the case in most of the breeding programs with the current shift towards production of doubled haploid lines. However, we recommend inoculating not fewer than five plants per plot to account for uncertainties of biological and environmental factors. Larger numbers of plants are required if segregating populations are to be tested.

Severity of ear rots in our study was rated as the percentage of visibly infected kernels (0 – 100%) on a single ear basis. This rating scale is most suitable if variance components and correlation coefficients have to be computed. Grades (1-5/9) based on classes are not as accurate and encounter limitations in their statistical analysis, whereas the assessment of grades in the field is faster.

As the testing progresses, genotypes selected for low disease severity need to be evaluated for mycotoxin concentrations to confirm the resistance reaction of the selected genotypes and discard "false positives". This is especially important if selection is based on AFER as large quantities of FUM were reported even in the case of symptomless infection (Bacon et al., 2008). Currently, the ELISA assay is the most suitable method for the evaluation of large sets of genotypes. It combines the advantages of ease of operation and high sample throughput compared to chemical analytical techniques like HPLC. In our study, we observed insignificant variation between duplicate evaluations of field plot samples for their DON or FUM concentrations, showing the high repeatability of this method (Bolduan et al., 2009b). Hence, proper ranking of genotypes for their DON and FUM concentrations can be based upon one ELISA determination per field plot.

As an alternative to the ELISA assay, we evaluated the potential of near infrared spectroscopy (NIRS) to estimate concentrations of DON and FUM. Maize flour was obtained by grinding the grain produce of artificially inoculated plants, which had higher than usual concentration of these mycotoxins. Ranking of genotypes by ELISA and NIRS was very similar but NIRS technology is remarkably cheaper, as no mycotoxin extractions and test kits are needed. Further, NIRS yielded higher R^2 for the prediction of DON and FUM than visual disease severity ratings. Thus, NIRS was shown to be superior to visual ratings of disease severity. However, visual rating is faster and cheaper than NIRS, as it does not involve shelling of the ears and grinding of the kernels. Therefore, we recommend performing initial selection on the basis of ear rot rating. Subsequently, concentration of mycotoxins in the selected fraction could be predicted with NIRS. Finally, those genotypes showing low ear rot ratings and predicted mycotoxin concentrations could be analyzed with ELISA.

The ultimate tool for the breeder would be NIRS measurements of the whole grain during the harvest process directly on the plot combine for non-destructive determination

of mycotoxins in the kernels. Although still destructive, our study provides a basis to draw preliminary conclusions about the potential use of NIRS in maize breeding programs.

Conclusions and outlook

The present study provides basic information about quantitative-genetic parameters and the inheritance of resistance to *F. graminearum* and *F. verticillioides* in early maturing European elite germplasm of maize. The presence of significant genetic variability along with the high estimates of heritability for all resistance traits seem to be promising for the development of high yielding hybrids with good agronomic performance in combination with low mycotoxin concentrations in the grain. Disease severity ratings showed strong correlations with the respective mycotoxin concentrations. Therefore, we recommend to perform initial selection on the basis of visual ratings and to evaluate only the selected elite material for mycotoxin concentrations. This helps the breeder to maximize selection gain for a given budget.

Based on the high estimates of h^2 and the moderate magnitude of correlation between LP and TP we recommend a multi-stage selection scheme to develop hybrids resistant to GER with low DON concentrations: (i) evaluation of agronomically promising lines for AGER in only one environment in order to eliminate only the highly susceptible lines, (ii) evaluation of TP of the selected lines for AGER with one tester of moderate to high resistance level from the opposite heterotic pool in two to three environments, (iii) evaluation for DON concentration in the elite fraction of testcrosses.

More reliable information needs to be generated especially about the complex relationship between *F. verticillioides* and the maize host as well as the inheritance of resistance traits for both, *F. verticillioides* and *F. graminearum*. For the latter, studies have been initiated at the University of Hohenheim for mapping quantitative trait loci to identify important genomic regions and determine the type of gene action involved in the inheritance of in GER resistance.

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7. Summary

Ear rots of maize, caused by *Fusarium* spp., are of major concern because they lead to losses in grain yield and contamination with mycotoxins which harm animals and humans. In the absence of other strategies, breeding maize for genetic resistance is currently the most promising avenue to control these rots and mycotoxin accumulation. The predominant pathogens in Central Europe are *F. graminearum*, the causative agent of Gibberella ear rot (GER), and *F. verticillioides*, the causative agent of Fusarium ear rot (FER). GER causes contamination with deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA), whereas FER causes contamination with fumonisins (FUM). Information on the resistance to GER and FER and mycotoxin contamination is lacking for maize adapted to the cooler climatic conditions of Central Europe.

In this study we investigated (1) the resistance of early maturing European elite inbred lines against GER and FER and contamination of mycotoxins, (2) the genetic variances and heritabilities for ear rot ratings and mycotoxin concentrations, (3) the correlations of ear rot ratings with mycotoxin concentrations, (4) the correlations between line *per se* (LP) and testcross performance (TP) for GER rating and DON concentration, (5) the aggressiveness of and mycotoxins produced by different isolates of *F. graminearum* and *F. verticillioides*, and (6) the potential of near infrared spectroscopy (NIRS) to estimate concentrations of DON and FUM in maize grains under artificial inoculation.

In total, four experiments were conducted. In Experiment 1, 42 inbred lines were inoculated with *F. graminearum* and evaluated for ear rot severity (AGER), DON, and ZEA accumulation in four environments. The material included 38 early-maturing elite inbreds developed by the University of Hohenheim and four Canadian inbreds with high resistance to GER and/or FER. Experiment 2 included 21 inbred lines, which were inoculated with *F. verticillioides* and tested for ear rot severity (AFER) and FUM contamination in three environments. In Experiment 3, testcrosses of 30 flint inbreds with two dent testers were inoculated with *F. graminearum* and evaluated for AGER and DON in four environments. In Experiment 4, five inbreds were inoculated with eight isolates each of *F. graminearum* and *F. verticillioides* and evaluated for ear rot ratings and mycotoxin concentrations in three environments. Maize flour obtained from inoculated plants of Experiment 1, 2 and 3 was used to develop NIRS calibrations for the prediction

of DON and FUM concentrations. The inbred lines included in Experiment 2, 3, and 4 were taken at random from those tested in Experiment 1.

Inoculation was carried out using the silk channel method. It resulted in higher ear rot severities and mycotoxin concentrations than under natural conditions. The study showed that the silk channel inoculation method was effective in promoting GER and FER and can be used for large-scale evaluation of breeding materials. In addition, our findings demonstrated that artificial inoculation is necessary under conditions prevailing in Southwest Germany to establish an uniformly high disease pressure and facilitate selection for ear rot resistance traits.

Significant genotypic variances and moderate to high heritabilities ($h^2 \geq 0.65$) were found for AGER, DON and ZEA among the inbred lines and for AGER and DON among the testcrosses, as well as for AFER and FUM among the inbred lines. Further, genotype x environment interaction variances were significant for all traits except FUM. Thus, the results underlined the presence of ample genotypic variation and the need to conduct multi-environment tests for reliable identification of resistant genotypes.

Ear rot ratings and mycotoxin production of eight isolates each of *F. graminearum* and *F. verticillioides* differed significantly. Even though, isolate x inbred interactions were significant only in the case of *F. graminearum*, and no rank reversals occurred among the tested inbred lines. Most isolates differentiated the susceptible inbreds from the resistant ones for severity ratings. However, the differences between the two groups were smaller for the less aggressive isolates. Therefore, we recommend using a single, environmentally stable and sufficiently aggressive isolate for resistance screenings under artificial inoculation.

Strong correlations between ear rot severity and mycotoxin concentrations ($r_g \geq 0.73$) indicated that selection for low ear rot severity under artificial inoculation will result in high correlated selection response for low mycotoxin concentration, particularly for AGER and DON ($r_g \geq 0.96$). Selection for ear rot severity is less resource-demanding and quicker than selection for mycotoxin concentration. Thus, it enables the breeder to maximize selection gain for a given budget. However, the selected elite material should be evaluated for mycotoxin concentrations in order to avoid “false positives”. In this regard, NIRS showed high potential to predict DON concentrations in grain obtained from artificially inoculated maize. Compared to the commonly employed ELISA assay, NIRS

assays are considerably cheaper, because no mycotoxin extractions and test kits are needed.

We observed moderate positive correlations between AGER and AFER ($r = 0.63$), and identified inbreds combining resistance to both ear rots. Therefore, selection for resistance to one pathogen is expected to result in indirect response to the other. Nevertheless, in advanced stages of each breeding cycle, lines preselected for other agronomically important traits should be evaluated for resistance to both pathogens.

Genotypic variances for AGER and DON were generally higher in LP than TP. Thus, assuming identical selection intensities for each scheme, the expected response to selection for LP should be higher than for TP. However, owing to moderate correlations between LP and TP for AGER and DON, selection based on LP is not sufficient, because the ultimate goal is to develop resistant hybrids. Therefore, a multi-stage selection procedure is recommended with evaluation of agronomically promising lines for AGER in only one environment in order to eliminate highly susceptible lines, followed by evaluation of TP of the selected lines for AGER with one tester of moderate to high resistance level from the opposite heterotic pool in two to three environments.

8. Zusammenfassung

Die durch Erreger der Gattung *Fusarium* hervorgerufenen Kolbenfäulen beim Mais sind von großer Bedeutung, da sie zu Ertragsverlusten und zur Kontamination des Erntegutes mit Mykotoxinen führen, welche die Gesundheit von Menschen und Tieren gefährden. Aufgrund fehlender geeigneter Bekämpfungsmöglichkeiten ist die Züchtung von genetisch resistentem Mais der derzeit aussichtsreichste Weg, die Kolbenfäulen sowie die damit einhergehende Kontamination mit Mykotoxinen zu bekämpfen. Die in Zentraleuropa vorherrschenden Erreger sind *F. graminearum* [engl. Gibberella ear rot (GER)] und *F. verticillioides* [engl. Fusarium ear rot (FER)]. GER führt zur Kontamination des Erntegutes mit Deoxynivalenol (DON), Nivalenol (NIV) und Zearalenon (ZEA), wohingegen FER zur Anreicherung mit Fumonisinen (FUM) führt. Für Maiszuchtmaterial, welches an die kühleren klimatischen Bedingungen in Zentraleuropa angepasst ist, liegen bislang keine verlässlichen Informationen über Resistenz gegen GER und FER und die entsprechenden Toxinkontaminationen vor.

In der vorliegenden Arbeit wurden (1) frühreife europäische Elite-Inzuchtlinien auf Resistenz gegen GER und FER sowie die entsprechenden Toxinkontaminationen getestet, (2) die genetischen Varianzen und Heritabilitäten für Befallsstärken und Toxinkonzentrationen geschätzt, (3) die Korrelationen zwischen Befallsstärken und Toxinkonzentrationen, sowie (4) die Korrelation zwischen Linieneigenleistung (LP) und Testkreuzungsleistung (TP) für GER-Befallsstärke und DON-Konzentrationen berechnet, (5) die Aggressivität und Toxinproduktion verschiedener Isolate von *F. graminearum* und *F. verticillioides* verglichen und (6) die Eignung der Nah-Infrarot Spektroskopie (NIRS) zur Schätzung von DON- und FUM-Konzentrationen in Maiskörnern nach künstlicher Inokulation untersucht.

Insgesamt wurden dazu vier Experimente durchgeführt. In Experiment 1 wurden 42 Inzuchtlinien mit *F. graminearum* inokuliert und auf die Befallsstärke (AGER) sowie die Anreicherung mit DON und ZEA in vier verschiedenen Umwelten untersucht. Der untersuchte Satz von Inzuchtlinien bestand aus 38 frühreifen Inzuchtlinien aus dem Zuchtprogramm der Universität Hohenheim sowie vier kanadischen Inzuchtlinien mit hoher Resistenz gegen GER und/oder FER. Experiment 2 beinhaltete 21 Inzuchtlinien, welche mit *F. verticillioides* inokuliert und auf Befallsstärke (AFER) sowie FUM-Konzentrationen in drei Umwelten untersucht wurden. In Experiment 3 wurden

Testkreuzungen von 30 Flint Inzuchtlinien mit zwei Dent Testern mit *F. graminearum* inokuliert und auf AGER sowie die Anreicherung mit DON in vier verschiedenen Umwelten untersucht. In Experiment 4 wurden fünf Inzuchtlinien mit jeweils acht verschiedenen Isolaten von *F. graminearum* und *F. verticillioides* inokuliert und auf Befallsstärke sowie Mykotoxinkonzentrationen in drei Umwelten untersucht. Für die Entwicklung einer NIRS-Kalibration zur Vorhersage von DON- und FUM-Konzentration wurde das Maismehl zuvor inokulierter Pflanzen aus den Experimenten 1, 2 und 3 verwendet. Die Inzuchtlinien, welche in den Experimenten 2, 3 und 4 untersucht wurden, stellten eine zufällige Auswahl aus den Inzuchtlinien in Experiment 1 dar.

Die Inokulationen wurden durch die Injektion einer Sporenlösung in den Narbenfadenkanal durchgeführt. Diese Methode führte generell zu höheren Befallsstärken und Mykotoxinkonzentrationen als unter natürlich auftretender Infektion. Die Ergebnisse dieser Untersuchung beweisen, dass die Inokulation des Narbenfadenkanals die Befallsstärke von GER sowie FER erhöht und für die breit angelegte Evaluierung von Zuchtmaterial empfohlen werden kann. Weiterhin zeigten die Ergebnisse, dass eine künstliche Inokulation unter den im Südwesten Deutschlands vorherrschenden klimatischen Bedingungen notwendig ist um einen gleichmäßig hohen Infektionsdruck und damit eine Selektion auf Resistenzmerkmale gegen Kolbenfäulen zu gewährleisten.

Signifikante genotypische Varianzen sowie mittlere bis hohe Heritabilitäten ($h^2 \geq$ 0.65) konnten für die Merkmale AGER, DON und ZEA zwischen den Inzuchtlinien und für AGER und DON zwischen den Testkreuzungen nachgewiesen werden. Gleiches gilt für AFER und FUM zwischen den Inzuchtlinien. Weiterhin waren die Genotyp x Umwelt Interaktionen signifikant für alle Merkmale mit Ausnahme von FUM. Die Ergebnisse sprechen somit für das Vorhandensein ausreichender genotypischer Variation sowie die Notwendigkeit einer Prüfung in mehreren Umwelten, um eine eindeutige Identifizierung von resistenten Genotypen zu gewährleisten.

Die Befallsstärken und Toxinproduktionen der je acht Isolate von *F. graminearum* und *F. verticillioides* unterschieden sich signifikant. Trotz signifikanter Isolat x Linien Interaktionen im Falle von *F. graminearum* konnten keine Rangumkehrungen zwischen den untersuchten Inzuchtlinien beobachtet werden. Wenngleich die meisten Isolate bezüglich der Befallsstärke geeignet waren zur Unterscheidung zwischen anfälligen und resistenten Inzuchtlinien, fielen diese Unterschiede bei weniger aggressiven Isolaten

geringer aus. Daher empfehlen wir für Resistenztests unter künstlicher Inokulation den Einsatz eines umweltstabilen Einzelisolates mit ausreichend hoher Aggressivität.

Starke Korrelationen zwischen Befallsstärke und Toxinkonzentrationen ($r_g \geq 0.73$) zeigten, dass eine Selektion auf geringe Befallsstärke unter künstlicher Inokulation zu einem korrelierten Selektionserfolg für niedrige Toxinkonzentrationen führt, insbesondere im Falle von AGER und DON ($r_g \geq 0.96$). Die Selektion auf Befallsstärke ist schneller und mit weniger Aufwand verbunden als die Selektion auf Toxinkonzentrationen. Dies ermöglicht dem Züchter die Maximierung des Selektionsgewinns im Rahmen eines vorgegebenen Budgets. Trotzdem sollte das selektierte Elitezuchtmaterial auch auf Toxinkonzentrationen untersucht werden, um die Selektion von „falsch Positiven" auszuschließen. In dieser Hinsicht zeigte der Einsatz von NIRS hohes Potential zur Vorhersage der DON-Konzentration in künstlich inokuliertem Mais. NIRS ist im Vergleich zu den üblicherweise genutzten ELISA-Assays bedeutend kostengünstiger, da keine Mykotoxin-Extraktion nötig ist und der Kauf von Testkits entfällt.

Eine mittlere positive Korrelation wurde zwischen AGER und AFER ($r = 0.63$) beobachtet und es konnten Inzuchtlinien identifiziert werden, welche eine Resistenz gegen beide Arten der Kolbenfäule vereinen. Folglich lässt die Selektion gegen einen der Erreger einen korrelierten Selektionserfolg für den anderen Erreger erwarten. Im fortgeschrittenen Stadium eines Selektionszyklus sollten jedoch solche Linien, welche bereits für andere agronomisch bedeutende Merkmale vorselektiert wurden, auf Resistenz gegen beide Erreger untersucht werden.

Die genotypischen Varianzen für AGER und DON waren für LP generell größer als für TP. Unter Annahme gleicher Selektionsintensität ist daher der erwartete Selektionsgewinn für LP höher als für TP. Im Hinblick auf den nur mäßigen Zusammenhang zwischen LP und TP für die Merkmale AGER und DON ist jedoch eine nur auf LP basierende Selektion nicht ausreichend, da das eigentliche Ziel die Entwicklung resistenter Hybriden ist. Wir empfehlen daher eine mehrstufige Selektion, beginnend mit dem Testen agronomisch vielversprechender Inzuchtlinien auf AGER in einer Umwelt, gefolgt von der Evaluierung der daraus selektierten Linien auf TP in zwei bis drei Umwelten, wobei ein Tester des anderen heterotischen Pools mit mittlerer bis hoher Resistenz für AGER verwendet werden könnte.

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Stuttgart, im September 2010

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Christof Bolduan