Translated Version

Resistance Mechanisms against European Corn Borer *(Ostrinia nubilalis* Hb.) in Early-Maturing Dent Maize Germplasm

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Abstract

The continual spreading of the European corn borer (ECB, *Ostrinia nubilalis* Hb.) during the past decade underlines the importance of resistance breeding in maize. Polygenically inherited natural host plant resistance (HPR) would provide a better method of plant protection in contrast to monogenically controlled *Bt* (*Bacillus thuringiensis*) resistance, which may be easily overcome by the insect. The objective of the present study was to determine the correlation between plant characteristics and ECB resistance. Based on an evaluation of 230 $F_{2:3}$ lines, two sets of genotypes were selected, each comprising five "resistant" and five "susceptible" lines, regarding stalk damage ratings (SDR) or tunnel length (TL) of larvae feeding. They were evaluated for resistance traits SDR and TL using manual infestation with ECB larvae. Plant characteristic traits were analyzed for plants of insecticide-protected plots. Thereby, leaf toughness (LT) in July and stalk toughness (ST) as well as stalk diameter (SD) were recorded in August, September and October. At each harvest date, dry matter content of stover (DMCS), as well as the quality traits cellulase digestibility of organic matter (CDOM), crude fiber content (CF), crude protein content (CP) and water-soluble carbohydrates (WSC) were determined by NIRS (Near Infrared Reflection Spectroscopy). In addition, leaf material was harvested at the beginning of July for isolation of DIMBOA (2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3-(4H)-one) and ferulic (FER) as well as *p*-coumaric (*p*-CUM) acids. Field experiments were conducted at Freising in 2000 and 2001. Significant differences between the "resistant" and "susceptible" group were found for ST in September and October above and below the primary ear. The "susceptible" group showed consistently lower toughness. In addition, significant group differences were observed for CDOM in July, August and September. CDOM reached higher levels in the "susceptible" group. Increased resistance seems associated with a greater ST and a lower digestibility. However, selection for ECB resistance based on measurements of ST is very cost- and time-consuming and would only be a small advantage compared to ECB trials with mandatory infestations. A more promising method for selection would be offered by NIRS analysis, e.g., for CDOM or other cell wall components.

Introduction

The European corn borer (ECB, *Ostrinia nubilalis* Hbn.) is of increasing importance due to its continuous spreading (Eder, personal communication, Bavarian State Research Centre for Agriculture). Tunneling of larvae in maize stalks and ears is often followed by stalk breakage and ear loss. Grain yield reductions of up to 30 dt ha^{-1} were observed in Germany (Bohn et al. 1998).

Methods to control ECB include the application of pyrethroids or the toxin produced by the soil bacterium *Bacillus thuringiensis*. In addition, during the last ten years genetically modified maize hybrids producing the toxin of *B. thuringiensis* (*Bt* hybrids) have been developed (Koziel et al. 1993, Estruch et al. 1997, Archer et al. 2000, Magg et al. 2001). Nevertheless, the cultivation of transgenic hybrids is not permitted in Germany because of the continual discussion about possible risks of genetic engineering. Natural host plant resistance (HPR) represents a more eco-friendly control method against ECB larvae. HPR consists of three resistance mechanisms: non-preference, tolerance and antibiosis. Non-preference is characterized by the low attractiveness of maize plants for the moth. Tolerance is the ability of maize plants to withstand the feeding of ECB larvae. Antibiosis decreases larval development as well as the number of larvae per plant.

Several authors reported that quality traits such as digestibility, contents of lignin and phenolic acids, as well as leaf and stalk toughness were significantly correlated with ECB resistance (Buendgen et al. 1990, Bergvinson et al. 1994a, 1994b, Groh et al. 1998). Most of the studies were performed in the USA and Mexico with tropical and subtropical material, or germplasm of the US Corn Belt. In these studies, not only resistance against the ECB but also against *Diatraea* species was analyzed. In contrast to Central Europe, the ECB occurs in the US Corn Belt with two to four generations per year, referred to as first and second generation. The damage caused by the second generation is comparable to the situation in Central Europe (Kreps et al. 1997).

The objective of the present study was to evaluate the relationship between the level of resistance and plant ingredients, as well as quality traits. Resistant and susceptible $F_{2:3}$ families of the population (D06 × D408) were studied for stalk and leaf toughness, quality traits (e.g. digestibility), and for contents of phenolic acids and DIMBOA (2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3-(4H)-one). The plants were analyzed at

four harvest dates to observe changes during the vegetation period. Non-preference and tolerance were not analyzed because of artificial infestation and difficulties to determine yield reduction in $F_{2:3}$ families for assessment of tolerance.

Material and Methods

Plant materials

A population with 230 $F_{2:3}$ lines was evaluated for resistance at two locations in 1995 (Bohn et al. 2000). Based on these results, two sets of genotypes were selected, each comprising five "resistant" and five "susceptible" lines, regarding stalk damage ratings (SDR) or tunnel length (TL) of larvae feeding (Table 1).

Table 1 Means of the resistance traits stalk damage ratings (SDR) and tunnel length (TL) of larvae feeding for the different groups.

Group	SDR	TL
	1-9 Scale	cm
Resistant	1.61[†]	2.34
Susceptible	4.78	9.90
Susceptible - Resistant	3.17	7.60

[†] Data from experiments in 1995 (Bohn et al. 2000).

Field data

Experiments were conducted at Freising (Germany) in 2000 and 2001. The experimental design was a 5×4 a-design with two replications, containing infested and protected whole plots. An experimental unit was a two-row subplot with 50 plants (in 2000) or a three-row subplot with 75 plants (in 2001), of 4 m length, and 0.75 m row spacing. Trials were over-planted and later thinned to a final plant density of 8 plants m^{-2}.

The insecticide-protected whole plots were treated with FASTAC SC©, applied three times in 10 to 14 day intervals starting at the end of June. All plants of the remaining subplots were infested with freshly hatched ECB larvae to ensure a uniform infestation. An average number of 20 neonate ECB larvae was applied three times at weekly intervals for a total of about 60 larvae per plant. The manual infestation was synchronized with the natural

occurrence of ECB moths between end of June and mid-July. Egg masses for manual infestation were provided by Dr. P. Aupinel, INRA, Le Mangeraud, France.

Harvest date and sample preparation

For measurements and analyses, only plants of the protected plots were harvested. Thus, larvae feeding affecting the resistance mechanisms could be eliminated. Harvesting was done four times in monthly intervals, starting from the middle of July. One replication was always harvested one day, and the second replication the following day. Tissue for toughness measurements was stored in water to prevent desiccation and bias of measurements, with samples being stored not longer than five hours after harvesting. For the quality analyses of the stover (whole plant without ear), the remaining plant parts of one genotype were sampled and chopped. Three (in 2000) or ten (in 2001) plants per genotype were pooled.

ECB Resistance traits

Before the last harvesting date in October, the level of resistance was determined from the infested plots. Stalk damage ratings were recorded according to the rating scale of Hudon and Chiang (1991) (1 = intact plant, 9 = breakage below the ear or ear dropped-down). In addition, all plants were split longitudinally and the tunnel length below the ears was measured.

Measurements of leaf and stalk toughness

Toughness of leaves and stalks was measured with a standard INSTRON instrument (Model 4302), equipped with a 100 N Load Cell (INSTRON Static Load Cell 100 N UK 1045). Power appearing at the load cell (in Newton) was recorded during the whole measurement, and only the maximum power appearing shortly before penetration of the probe into the tissue was used for statistical analyses.

Leaf toughness (LT) was recorded at the first harvest date in mid-July. Samples were obtained from the first two-thirds-exposed leaf from the top of the whorl. According to Bergvinson et al. (1994a, 1994b), the measurements were taken from the undersurface of the leaves. The probe was positioned about 25 cm below the tip of a leaf and in about 2 cm distance from the main vein, between secondary veins. Two measurements on both sides of

the main vein were conducted on each leaf. The concave probe had a diameter of 0.8 mm and was moved with a speed of 1 cm min^{-1}. In order to avoid deformation, the leaf was manually fixed with a bolt washer (external diameter 24 mm, internal diameter 8 mm) (Figure 1).

Figure 1 Measurement of leaf toughness with a standard INSTRON instrument; the leaf is fixed for measurement with a washer.

Stalk toughness (ST) was measured at the remaining three harvest dates: mid-August, mid-September and mid-October. Segments for analyses were selected regarding feeding behavior of the larvae. The measurement in August was taken on the internode below the tassel, in September on the internode directly above the primary ear, and in October on the internode below the primary ear. The sample was held in a V-shaped high-grade steel groove. The probe was a flat-bottomed cylinder with a diameter of 1.2 mm. It entered the sample through the flat side of the stalk (Figure 2), in about 5 cm above the internode (Figure 3). The speed was set to 10 cm min^{-1}. Two measurements at 2 cm distance were performed on each sample and pooled for statistical analyses. In addition, the stalk diameter (SD) at the measuring point was also determined.

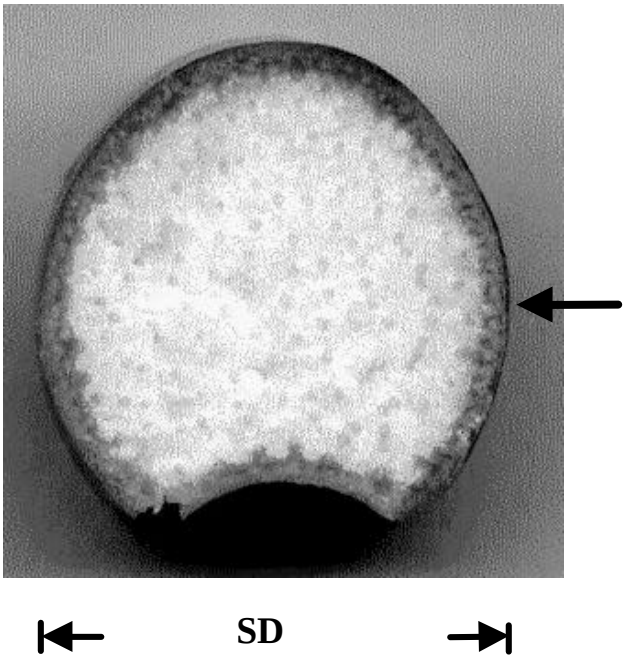

Figure 2 Measuring point (arrow) for stalk toughness and stalk diameter (SD).

Figure 3 Measurement of stalk toughness with a standard INSTRON instrument.

Silage quality traits

Approximately 1.5 kg of the pooled and chopped material was taken from each genotype and dried at 35°C. Preparation of samples for NIRS (Near Infrared Reflection Spectroscopy) analysis was performed according to the method of Degenhardt (1996). Dry matter content of stover (DMCS in %), as well as the quality traits cellulase digestibility of organic matter (CDOM, de Boever et al. 1986), crude fiber content (CF, Weender Futtermittelanalyse, Naumann and Bassler 1998), crude protein content (CP, Kjeldahl 1883) and water-soluble carbohydrates (WSC, Luff-Schoorl 1929) in g kg^{-1} dry matter were determined by NIRS. In October, WSC could not be estimated. The NIRS calibration was kindly provided by KWS SAAT AG, Einbeck.

DIMBOA and phenolic acid content

Leaf material was harvested at the second date of infestation at the beginning of July. For DIMBOA isolation, immature leaf tissue within the whorl that had not been exposed to light was harvested. The leaf material was cooled down in liquid nitrogen and stored

at - 80°C. DIMBOA was extracted with ethylacetate (EtOAc). The organic phase was evaporated at room temperature, the remaining pellet was dissolved in methanol (MeOH) and stored at -20°C until analysis by HPLC (High Pressure Liquid Chromatography).

Ferulic (FER) and *p*-coumaric (*p*-CUM) acids were isolated from the latest fully exposed dark-green leaves from the whorl. The leaves were freeze-dried and micro-milled. In the first step, the soluble phenolic acids were extracted from the dry matter with MeOH. The cell-wall-bound phenolics were derived from the remaining residue. The extraction was performed with EtOAc. After evaporation, the samples were dissolved in MeOH and kept at -20 °C until HPLC analysis.

Statistical analyses

For the measurements of plant toughness and level of resistance, data of individual plants of each subplot were averaged. Data of DIMBOA and phenolic acids analyses were log-transformed to follow a normal distribution. In the statistical models, genotypes and environments were considered fixed effects. Mean values and least significant differences (LSD 5%) were separately calculated for both locations and combined in a multi-factorial analysis. Resistance factors were assessed as relevant when significant differences between the “resistant” and “susceptible” groups were found (F-test). Calculations were performed with the PLABSTAT software (Utz 2001).

Results

Resistance traits

In 2000 and 2001, the genotypes selected according to SDR showed a significant group difference between “resistant” and “susceptible” to ECB larvae feeding (Table 2). In contrast, genotypes selected according to TL showed no significant group difference between “resistant” and “susceptible” to ECB larvae feeding. Thus, the classification for TL based on the experiments in 1995 could not be confirmed by the present study, and data of these ten genotypes were not used for further analyses.

Table 2 Means of groups and least significant differences (LSD 5%) for the resistance traits SDR (stalk damage ratings) and TL (tunnel length) analysed in 2000 and 2001.

Group	SDR	TL
	1-9 Scale	cm
Resistant	2.76	16.42
Susceptible	4.39	18.03
Susceptible - Resistant	1.63*	1.61
LSD 5%	0.55	4.35

* Group differences significant at $P = 0.05$.

Measurements of toughness

For leaf toughness no significant group difference between "resistant" and "susceptible" was calculated. Stalk toughness in September and October, as well as the stalk diameter in October, showed significant group differences between "resistant" and "susceptible" (Table 3). The susceptible $F_{2:3}$ lines were characterized by a lower stalk toughness and a reduced stalk diameter.

Table 3 Means of groups and least significant differences (LSD 5%) for LT (leaf toughness), ST (stalk toughness) and SD (stalk diameter) analysed in 2000 and 2001.

Group	LT	ST			SD		
	July	August	September	October	August	September	October
	N	---------- N ----------			---------- cm ----------		
Resistant	0.73	27.07	39.31	51.78	0.53	1.28	1.85
Susceptible	0.75	26.31	35.78	47.03	0.56	1.21	1.72
Susceptible - Resistant	-0.02	-0.76	-3.53*	-4.75*	0.03	-0.07	-0.13*
LSD 5%	0.09	1.37	2.44	4.73	0.04	0.08	0.10

* Group differences significant at $P = 0.05$.

Quality traits

For some harvesting dates significant group differences for the quality traits CDOM, CF and WSC were found (Table 4). Although not all differences were significant, the "resistant" group generally showed lower levels of CDOM and WSC. The level of CF was higher and CP was lower in August, September and October for the "resistant" group. DMCS was lower for the "susceptible" group in September and October, indicating a later maturity.

Table 4 Means of groups and least significant differences (LSD 5%) for the quality parameters dry matter content of the residual plant (DMCS), cellulase digestibility (CDOM), crude fibre content (CF), crude protein content (CP) and content of water-soluble carbohydrates (WSC) analysed in 2000 and 2001.

Group	July	August	September	October
DMCS	---------- % ----------			
Resistant	10.63	16.57	23.58	26.80
Susceptible	10.99	16.72	23.57	27.49
Susceptible - Resistant	0.36	0.15	-0.01	0.69
LSD 5%	0.64	0.53	1.19	1.50
CDOM	---------- 10^{-1} g kg^{-1} ----------			
Resistant	57.49	57.23	58.22	57.27
Susceptible	59.07	59.24	60.17	59.31
Susceptible - Resistant	1.58*	2.01*	1.95*	2.04
LSD 5%	0.94	1.18	0.95	3.54
CF	---------- 10^{-1} g kg^{-1} ----------			
Resistant	28.62	28.03	26.00	25.32
Susceptible	28.11	27.32	25.38	24.57
Susceptible - Resistant	-0.51*	-0.71*	-0.62	-0.75
LSD 5%	0.30	0.64	0.75	2.13
CP	---------- 10^{-1} g kg^{-1} ----------			
Resistant	10.64	6.99	6.33	7.31
Susceptible	10.28	7.16	6.59	7.70
Susceptible - Resistant	-0.36	0.17	0.26	0.39
LSD 5%	0.37	0.64	0.49	0.57
WSC	---------- 10^{-1} g kg^{-1} ----------			
Resistant	14.87	22.85	24.99	-¶
Susceptible	16.39	24.01	25.63	-¶
Susceptible - Resistant	1.52*	1.16	0.64	-¶
LSD 5%	1.13	1.29	1.67	-¶

* Group differences significant at $P = 0.05$.
¶ WSC could not be analysed in October.

Contents of phenolic acids and DIMBOA

Two important cell-wall-bound as well as soluble phenolic acids influencing the cell wall toughness are *p*-CUM and FER. For the soluble phenolic acids, most of the samples were under the detection limit (data not shown). Moreover, for the cell-wall-bound acids and DIMBOA contents, no significant group differences could be found (Table 5). Nevertheless, a tendency of a lower content of DIMBOA was observed in the "susceptible" group.

Table 5 Log-transformed means of groups and least significant differences (LSD 5%) for the cell-wall-bound phenolic acids *p*-coumaric acid (*p*-CUM) and ferulic acid (FER) as well as DIMBOA (DIM) analysed in 2000 and 2001.

Group	LN *p*-CUM	LN FER	LN DIM
	ppm	ppm	AU[¶] / g
Resistant	6.39	6.58	13.36
Susceptible	6.32	6.66	12.73
Susceptible - Resistant	-0.07	0.08	-0.63
LSD 5%	0.66	0.71	0.88

[¶] AU = absorption unit.

Discussion

The continual spreading of the ECB during the past decade underlines the importance of resistance breeding. Because the monogenically controlled *Bt* resistance may be easily overcome by the insect, polygenically inherited natural HPR provides a better method of plant protection. The major objective of the present study was to determine the correlation between plant characteristics and ECB resistance. Therefore groups of "resistant" and "susceptible" genotypes were compared.

Toughness and contents of phenolic acids and DIMBOA

Regarding the first and second instar larvae feeding on leaves and pollen, it was suggested that the LT might influence the survival rate of ECB larvae. In regions, where damage of the first generation of ECB larvae occurs (USA and Mexico), it was reported that not only LT but also leaf components like DIMBOA and phenolic acids were related to ECB

resistance (Bergvinson 1994b, 1997). These findings could not be verified in the present study. LT, contents of DIMBOA and soluble as well as cell-wall-bound phenolic acids do not seem to have an impact on the resistance against ECB. Furthermore, ST became more important in affecting resistance in Central Europe. In particular, a significant correlation was found between ST of the internodes above and below the primary ear and resistance. Experiments of Viereck (1981), showing a significant effect of ST on young larvae, are in agreement with our findings. Penetrating the stalk is the more difficult if the stalk is tougher or harder, and outside the stalks, the number of larvae can be reduced by several predators and environmental effects. Due to this considerations, Viereck (1981) detected a lower amount of larvae in the stalks for higher ST. A further effect of ST is the resistance against stalk breaking, which increases the tolerance to stalk damage by ECB. This was confirmed by recent studies of Flint-Garcia et al. (2002) and Martin et al. (2004). In addition, Viereck (1981) found both, ECB resistance and stalk breaking affected by the SD. In the present study, a significant correlation between SD below the primary ear and resistance was also observed. The SD in the "susceptible" group was about 5% lower than in the "resistant" group, indicating that stalk stability and, consequently, resistance to stalk breaking and damage increase with stalk diameter.

Quality traits

In many studies, a negative correlation was reported between resistance against ECB and date of flowering (Kreps et al. 1998, Bohn et al. 2000, Magg et al. 2001, Papst et al. 2001). A late date of flowering and a low DMC were associated together with a low SDR. In the present study, the negative correlation between DMCS and SDR was found for the harvest dates in July, August and October. In September, the "susceptible" group showed a lower DMCS. However, these results were not significant.

Buendgen et al. (1990) and Buxton et al. (1996) found a significant negative correlation between ECB resistance and silage maize quality. In the present study, a significant group difference between "resistant" and "susceptible" genotypes was observed for CDOM in July, August and September. The CDOM reached higher levels in the "susceptible" group than in the "resistant" group. The same trend occurred in October, although the difference was not significant.

In general, the digestibility is negatively linked to CF. We found a significant positive relation between CF and resistance only for the harvest dates in July and August. This finding may be due to antibiosis of the plants. With a high level of CF and a low level of CDOM, feed for larvae is not as available as in plants with low contents of CF or high levels of CDOM. Hence, these factors cause a higher mortality or emigration of larvae.

A significant correlation between SDR and CP could not be found, even though the "resistant" group showed lower levels of protein. Bergvinson et al. (1997) reported a higher level of resistance in combination with a lower content of CP in the pith. They concluded that a high level of protein, as nutrition component for the larvae, supported larvae development and survival rates.

At the end of the vegetation period most of the WSC, especially sugar, are transferred to the ears. Group differences between "resistant" and "susceptible" genotypes were significant in July, when the level of WSC was the highest. Even if the group differences for August and September were not significant, the susceptible group was always characterized by a higher content of WSC. It might be speculated that, due to sugar as nutrition for larvae, plants with a higher content of WSC show higher SDR.

Conclusions for resistance breeding

Significant associations among certain plant ingredients and plant characteristics to resistance against the ECB were found in early-maturing dent maize germplasm. Leaf characteristics showed no significant relationship with resistance, even though the larvae in the first developmental stages mainly feed on leaves and pollen. Based on these results, an increased resistance seems associated with a greater ST and a lower digestibility. Nevertheless, low digestibility is in contrast to breeding aims for silage maize. Although the ECB is more a problem in grain production than in silage maize, its importance may increase with the spreading of the insect. Thus, a good digestibility should be kept in mind when breeding new varieties. QTL studies have shown that regions for ECB resistance are located in adjacent intervals of genes for lignin synthesis (Bohn et al. 2000, Papst et al. 2001). Barrière et al. (2003) found that mostly the composition of lignin is responsible for digestibility. The proportion of two particular components, guaiacyl and syringyl, is the most important, but it does not necessarily affect the cell wall strength or ST

(Argillier et al. 1996). This is in accordance with results from a selection experiment for increased stalk toughness which did not adversely affect digestibility (Albrecht et al. 1986).

Indirect selection methods would be more helpful in breeding for resistance against the ECB than a direct selection with money- and time-consuming mandatory infestation. While measurements of stalk toughness showed a tight association to resistance, it is as time-consuming as ECB trials with artificial infestations. A more promising method for selection would be offered by NIRS analysis. Nevertheless, further analyses of indirect traits, such as cell wall components, lignin, and digestibility, are necessary with a wider range of genotypes for the development of a robust calibration.

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Mycotoxins Produced by *Fusarium* spp. in Isogenic Bt vs. non-Bt Maize Hybrids under European Corn Borer Pressure

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ABSTRACT

Stalk and ear rots caused by *Fusarium* subspecies are often related to mycotoxin accumulation in maize (*Zea mays* L.) kernels. Various mycotoxicoses in livestock and humans are triggered by the consumption of these toxins. The European corn borer (*Ostrinia nubilalis* Hübner) reportedly promotes the infection by *Fusarium* spp. The objectives of our study were to (i) evaluate the concentration of deoxinivalenol (DON), 3-acetyl-deoxynivalenol (3-A-DON), 15-acetyl-deoxynivalenol (15-A-DON), fumonisin (FUM), fusarenon-X (FUS-X), moniliformin (MON), and nivalenol (NIV) in kernels; (ii) determine the level of European corn borer (ECB) resistance; and (iii) investigate the association between the concentration of mycotoxins and ECB resistance. The study used early maturing European Bt (*Bacillus thuringiensis*) cultivars, their isogenic counterparts, and commercial hybrids. The field experiments were conducted at three locations in Germany. The mycotoxins most prevalent were DON, FUM, and MON. Plots infested by and protected from ECB differed significantly for DON and FUM concentrations. In addition, significant differences were found for concentrations of FUM between isogenic Bt and non-Bt hybrids. The two Bt events—Bt176 and Mon810—were also significantly different for FUM concentrations. Not all mycotoxins were related to ECB damage. Insect management and, therefore, the use of Bt cultivars may be a short-term solution to minimize toxins in kernels.

Fusarium subspecies cause ear and stalk rots in maize and produce mycotoxins that are often associated with chronic or acute mycotoxicoses in livestock and humans (Logrieco et al., 2002). Depending on the *Fusarium* spp., a wide array of mycotoxins are produced, including type B trichothecenes, such as DON and its precursors 3-A-DON and 15-A-DON, and FUM, FUS-X, MON, and NIV. These mycotoxins are suspected to cause immunosuppression, embryo abortions and deformation, swine endrogenic syndrome, porcine pulmonary edema, liver cancer in rats, and human esophageal cancer (Reid et al., 1999).

Fusarium spp. infect maize kernels through silks and kernel tissue wounds (Munkvold et al., 1997; Plienegger and Lemmens, 2002). European corn borer larvae cause physical injuries to stalks and ears and, therefore, are suspected to promote infections by *Fusarium* spp. In addition, ECB larvae can carry fungal inoculum from the plant surface into the kernels. In Austria, most of the maize samples analyzed were contaminated with *F. moniliforme* (J. Scheld.), *F. proliferatum* (T. Matsushima) Nirenberg, and *F. subglutinans* (Wollenweber and Reinking), whereas in southern Germany, *F. graminearum* Schwabe—the main producer of DON—prevailed (Lew, 1993; Botallico, 1998; Rintelen, 2000; Logrieco et al., 2002).

Maize cultivars carrying the Bt gene are highly resistant to ECB larval feeding. In addition, maize hybrids expressing the Bt gene in kernels were found to be less infected with *Fusarium* spp. and showed lower mycotoxin concentrations (Munkvold et al., 1997, 1999; Magg et al., 2002, 2003). The objectives of our study were to (i) evaluate the mycotoxin concentration in kernels of early maturing European maize hybrids, (ii) determine the level of ECB resistance of these cultivars, and (iii) investigate the association between the concentration of various mycotoxins produced by *Fusarium* spp. and ECB resistance.

MATERIALS AND METHODS

Plant Materials

Ten maize hybrids were evaluated for mycotoxin concentrations and their level of ECB resistance. This set of maize cultivars comprised four pairs of hybrids: two pairs of Bt cultivars carrying event Bt176 and their near-isogenic counterparts ('Pactol CB Bt'–'Pactol'; 'Valmont Bt'–'Prelude') and two pairs of Bt hybrids with event Mon810 and their near-isogenic counterparts ('Novelis Bt'–'Nobilis'; 'Monumental Bt'–'Monumental'). In addition, two commercial hybrids 'Symphony' and 'Clarica' were tested. The Bt hybrids and their near-isogenic counterparts were provided by Monsanto Agrar Deutschland GmbH (Düsseldorf, Germany) and Syngenta Seeds GmbH (Bad Salzuflen, Germany). All Bt hybrids carried the *CryIA(b)* gene. Event Bt176 contained two promoters regulating Bt gene expression in green plant tissues and pollen (Koziel et al., 1993). Transformation event Mon810 used a gene promotor that resulted in a season-long expression of the Bt toxin in all plant tissues (Archer et al., 2000).

Field Trials

The maize cultivars were evaluated for resistance to the univoltine ECB at three sites located in the main maize-growing regions of east (Seelow) and south (Freising, Heilbronn) Germany in 2001. All experimental sites are characterized by high natural occurrence of the univoltine ECB. The experimental design at each site was a split plot with two replications. Whole plots were assigned the "insecticide protected" and the "manually infested" treatments. The genotypes were assigned to subplots that were arranged according to a randomized

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Abbreviations: Bt, *Bacillus thuringiensis*; DON, deoxinivalenol; ECB, European corn borer; FUM, fumonisin; FUS-X, fusarenon-X; MON, moniliformin; NIV, nivalenol; PDE, percentage of damaged ears; SDR, stalk damage ratings; 3-A-DON, 3-acetyl-deoxynivalenol; 15-A-DON, 15-acetyl-deoxynivalenol.

complete block design. Each subplot consisted of a four-row plot with 25 plants per row, 4 m long, and a row spacing of 0.75 m. Trials were overplanted and later thinned to a final plant density of 8 plants m^{-2}.

European Corn Borer Treatments and Evaluation

All plants in the first three rows of each subplot in the manually infested treatment were infested with ECB larvae. The fourth row was not infested and used to prevent larval migration between plots. No artificial infestation was necessary at Seelow because of an exceptionally high ECB population density (Bohn et al., 1998). A total of approximately 60 neonate ECB larvae per plant was applied at three weekly intervals (20 ECB larvae per week) at all other locations. Freshly hatched larvae were mixed with corncob grits and placed into the whorl or leaf collar of maize plants using mechanical dispensers (Mihm, 1983). The manual infestation was synchronized with the natural occurrence of ECB moths between the end of June and mid-July. The plant development stages varied at infestation time from midwhorl stage to tasseling (VT) or silking (R1). Egg masses for manual infestation were provided by Dr. P. Aupinel, INRA, Le Mangeraud, France. The insecticide protected plots were treated with FASTAC SC (alphacypermethrin, BASF AG, Ludwigshafen, Germany) twice, starting at the end of June in 10- to 14-d intervals.

Resistance to ECB larval feeding was determined in the third row of infested plots using stalk damage ratings (SDR), where 1 = intact plants and 9 = dropped ears or breakage below the ear (Hudon and Chiang, 1991). In addition, ears were evaluated for ECB damage to determine the percentage of damaged ears (PDE).

Mycotoxin Analyses

Representative grain samples were taken from each treatment (subplot) within a plot. The concentrations of DON, 3-A-DON, 15-A-DON, NIV, and FUS-X were determined using Mycosep-Clean up and gas chromatography with electron capture detection (GC-ECD) validation. Concentration of FUM was assessed via IAS-Clean up and *o*-phthal-dialdehyd with high-pressure liquid chromatography (HPLC) fluorescence detection. The concentration of MON was assessed with an immunoassay and HPLC. The limits of detection for trichothecenes, FUM, and MON varied from 36 to 109 μg kg^{-1} (Schuhmacher et al., 1997; Weingärtner et al., 1997; Solfrizzo et al., 2001). The analyses were completed at the Institute for Agrobiotechnology, Tulln, Austria.

Statistical Analyses

Data of individual plants of each subplot were averaged to obtain a subplot mean for SDR. Analyses of variance were performed on subplot means of the 10 cultivars. Adjusted entry means and effective error mean squares were used to compute the combined analyses of variance across environments. All effects in the statistical model, except cultivars, were considered random. Orthogonal contrasts among cultivar groups (transgenic, isogenic, and commercial hybrids) were calculated and their significance was determined via Scheffé's test (Scheffé, 1959). If differences in mycotoxin concentrations between ECB treatments were not significant, differences between cultivar groups were calculated using the combined mean across infested and protected plots. The mycotoxin concentrations were log-transformed because their residual error terms did not follow a normal distribution. Phenotypic correlation coefficients ($\hat{r}_p$) were calculated among resistance and agronomic traits across all cultivars (Mode and Robinson, 1959). All calculations were done via PLABSTAT (Utz, 2001) and SAS (SAS Inst., 1996).

RESULTS

Mycotoxin Concentrations

Of all samples analyzed, 66% had detectable concentrations of DON, and 93% showed detectable concentrations of MON. Mycotoxins 3-A-DON, 15-A-DON, FUM, FUS, and NIV were detected in 13 (FUS) to 39% (15-A-DON) of all samples. The most prevalent mycotoxin across all locations and treatments was DON (1628 μg kg^{-1}), followed by FUM (1396 μg kg^{-1}). Contamination with NIV was relatively low, with an overall mean of 61 μg kg^{-1}. The coefficients of variation for the different mycotoxin concentrations across environments ranged from 128% for MON to 279% for FUM (Table 1).

Differences between the mean of mycotoxin concentrations in insecticide-protected and infested plots across locations were significant only for DON ($P < 0.05$) and FUM ($P < 0.01$) (data not shown). The mean FUM concentrations between transgenic and isogenic nontransgenic cultivars ($P < 0.01$), as well as between the two Bt events Bt176 and Mon810, were significantly ($P < 0.05$) different under ECB infestation.

For 3-A-DON, 15-A-DON, MON, and NIV, differences between ECB treatments were not significant, and differences between cultivar groups were calculated with means combined across infested and protected plots. Significant differences were found for MON ($P < 0.01$), NIV ($P < 0.01$), and 3-A-DON ($F_{1,120} = 3.02$, $P = 0.09$) (Table 2) between isogenic transgenic and nontransgenic cultivars and for 3-A-DON ($F_{1,120} = 3.31$, $P = 0.07$) and 15-A-DON ($P < 0.01$) between Bt events Bt176 and Mon810.

The mean concentrations of DON, its derivates 3-A-DON and 15-A-DON, and FUM across all cultivars were significantly ($P < 0.05$) different between locations. In Freising, concentrations of mycotoxins were low (Table 3). In Heilbronn, mycotoxin concentrations were low, except for FUM. At this location, significant ($P < 0.05$) differences existed between infested and protected plots for DON, FUM, MON, and NIV concentrations. The highest mycotoxin concentrations were found in Seelow. At this location, DON concentrations were more than 10 times higher than those in Freising and Heilbronn. A general trend was that mycotoxin

Table 1. Mean, standard deviation (SD), and coefficient of variation (CV) for mycotoxin concentrations across hybrids, treatments, and locations in 2001.

Mycotoxin†	Mean	SD	CV
	μg kg^{-1}		%
DON	1594	2900	178
3-A-DON	135	373	271
15-A-DON	387	654	165
FUM	1361	3898	279
MON	153	198	128
NIV	69	169	278

† DON, deoxynivalenol; 3-A-DON, 3-acetyldeoxynivalenol; 15-A-DON, 15-acetyl-deoxynivalenol; FUM, fumonisin; MON, moniliformin; NIV, nivalenol.

Table 2. Mean values for mycotoxin concentrations, stalk damage ratings (SDR), and percentage of damaged ears (PDE) for 10 maize hybrids comprising four early maturing European Bt cultivars (transgenic), their isogenic counterparts (isogenic), and two commercial hybrids (commercial) evaluated in three locations in 2001.

		Mycotoxin concentration across plots†				Mycotoxin concentration				Resistance traits infested plots	
						Infested plots‡		Protected plots			
Hybrid	Characterization	3-A-DON	15-A-DON	MON	NIV	DON	FUM	DON	FUM	SDR	PDE
		μg kg^{-1}								1–9 scale	%
Transgenic§											
Pactol CB Bt	Event176	102	342	123	24	1638	1345	1241	nd	1.61	3
Valmont Bt	Event176	87	573	125	50	1765	307	1236	nd	1.14	10
Novelis Bt	Mon810	43	346	67	nd	1140	625	645	nd	1.07	1
Monumental Bt	Mon810	20	104	85	8	781	nd	144	nd	1.28	0
Means		61a¶	223a	104a	16a	1331a	569a	817a	nda	1.28a	4a
Isogenic											
Pactol	non-Bt	171	445	220	321	2540	4773	1012	nd	2.59	17
Prelude	non-Bt	233	649	140	37	2670	5240	2776	nd	2.83	17
Nobilis	non-Bt	98	347	284	82	1708	4718	613	nd	4.30	54
Monumental	non-Bt	76	250	188	22	1041	4665	776	65	3.09	30
Means		123b	347a	204b	172b	1990ab	4849b	1294a	16a	3.20b	29b
Commercial											
Symphony	non-Bt	425	395	212	98	4495	4323	1164	63	4.39	60
Clarica	non-Bt	92	424	84	45	2155	1097	755	nd	2.11	33
Means		209b	386a	144b	108b	3325b	2710c	960a	32a	3.25b	47c

† 3-A-DON, 3-acetyldeoxynivalenol; 15-A-DON, 15-acetyl-deoxynivalenol; MON, moniliformin; NIV, nivalenol.
‡ DON, deoxynivalenol; FUM, fuminosin.
§ The isogenic (Bt, non-Bt) cultivar pairs were (Pactol CB Bt, Pactol), (Valmont Bt, Prelude), (Novelis Bt, Nobilis), and (Monumental Bt, Monumental).
¶ Means with different letters within a column are significantly different ($P < 0.10$).

concentrations in ECB-infested plots exceeded the mycotoxin concentrations found in the protected plots.

European Corn Borer Resistance

The SDR ($P < 0.01$) and PDE ($P < 0.01$) means were significantly lower for the Bt hybrids in contrast to their isogenic non-Bt counterparts and the commercial cultivars (Table 2). In addition, the Bt hybrids had significantly lower values for SDR ($P < 0.01$) and PDE ($P < 0.01$) than their isogenic non-Bt counterparts. A significant ($P < 0.01$) difference between isogenic hybrids and commercial hybrids could only be found for PDE but not for SDR. Differences between Bt events Bt176 and Mon810 were not significant for both resistance traits. In general, hybrids carrying event Mon810 showed lower PDE means and mycotoxin concentrations than hybrids with event Bt176, however.

Correlations

Across all cultivars ($N = 10$), significant genotypic associations were found between SDR and MON as well as SDR and FUM ($P < 0.01$) and between PDE and MON ($P < 0.05$) (see Fig. 1). Correlations between ECB resistance traits and mycotoxin concentrations were not significant when only non-Bt cultivars were taken into account (data not shown).

DISCUSSION

Toxins produced by fungi in maize kernels pose a serious threat to food security. The International Agency for Research on Cancer (IARC) classified FUM as possibly carcinogenic to humans. Fumonisin was reported to cause esophageal cancer in India, China, and southern Italy (Bottallico, 1998). Therefore, the demand for maize cultivars carrying resistance to *Fusarium* spp. as well as resistance to mycotoxin production is high. Because FUM may also be present in kernels from ears without ear-rot symptoms (Cotton and Munkvold, 1998; Munkvold et al., 1999; Clements et al., 2003) and because mycotoxin profiles depend on the composition of *Fusarium* spp. populations present in specific environments (Logrieco et al., 2002), we determined mycotoxin concentrations instead of solely ear-rot symptoms. Taking into account prior reports suggesting an association

Table 3. Mean mycotoxin concentrations across 10 maize hybrids evaluated at three locations in infested and protected plots.

	Mycotoxin concentrations†											
	DON		3-A-DON		15-A-DON		FUM		MON		NIV	
Location	Inf‡	Pro§	Inf	Pro	Inf	Pro	Inf	Pro	Inf	Pro	Inf	Pro
	μg kg^{-1}											
Freising	440	149	37	nd	71	22	513	nd	67	90	116	23
Heilbronn	257	72	nd	nd	9	nd	6980	20	304	80	139	13
Seelow	5283	2887	532	240	1280	944	636	19	311	57	67	57
Mean¶	1993a	1036b	189a	80a	453a	322a	2709a	13b	227a	78a	106a	31a

† DON, deoxynivalenol; 3-A-DON, 3-acetyldeoxynivalenol; 15-A-DON, 15-acetyl-deoxynivalenol; FUM, fumonisin; MON, moniliformin; NIV, nivalenol.
‡ Inf, infested subplots.
§ Pro, protected subplots.
¶ Means in adjacent rows with different letters are significantly different at probability level $P \leq 0.10$.

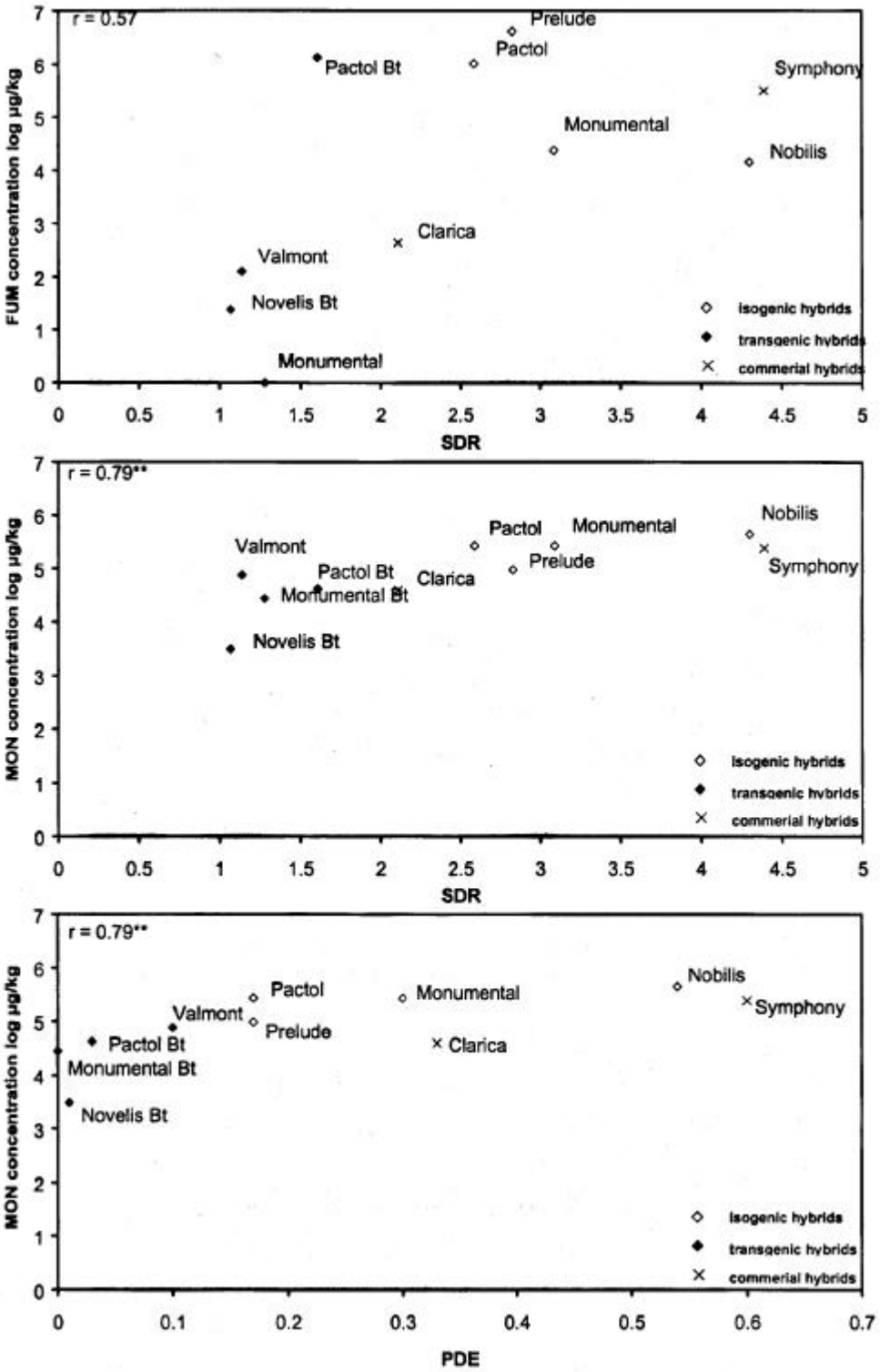

Fig. 1. Correlation between (i) stalk damage ratings (SDR) and mycotoxin concentrations of fuminosin (FUM), (ii) SDR and moniliformin (MON), and (iii) percentage of damaged ears (PDE) and FUM in infested plots.

between mycotoxin concentration and tissue damage caused by insect feeding, we evaluated Bt maize cultivars with resistance to ECB larvae feeding and their isogenic counterparts as well as commercial hybrids for the relationship between ECB resistance and mycotoxin level.

Agronomy Journal 97 (2005): 219-224

Mycotoxin Concentrations

Deoxinivalenol, FUM, and MON were more prevalent across all locations than other mycotoxins. Especially in Seelow, DON and 15-A-DON exceeded more than 10 times the mycotoxin concentrations found at the other two locations. These findings confirmed recent results reported by Magg et al. (2002), who evaluated 15 cultivars for mycotoxin concentrations in different German locations and also detected large environmental effects. These effects might have been caused by different climatic conditions impacting the inoculation success and composition of the endemic *Fusarium* spp. population. In the Seelow region, a close maize–wheat (*Triticum aestivum* L.) crop rotation with reduced tillage is practiced, which is known to favor a high natural inoculum of *Fusarium* spp., especially *F. graminearum* (Rintelen, 2000). In agreement with this hypothesis, a predominant occurrence of this species was noted at Seelow (G. Schröder, Agency of Brandenburg for Consumer Protection and Agriculture, personal communication, 2004). Therefore, the high DON concentrations found in Seelow can also be explained by the dominating occurrence of *F. graminearum* in this region.

Mycotoxin Concentrations and European Corn Borer Damage

A survey, conducted at Freising in 2002, of maize ears undamaged by ECB larvae found mainly contamination of kernels with *F. graminearum* (P. Büttner, Bavarian State Research Center for Agriculture, Freising, personal communication, 2004). The prevalence of *F. graminearum* was explained by its high aggressiveness caused by the immense and continuous dissemination on infected ears of this subspecies in contrast to *F. subglutinans* and *F. culmorum* (Plienegger, 2003). Characteristic mycotoxins of *F. graminearum* are DON and, with lower importance, FUS and NIV. In contrast, Lew (1993) and Logrieco et al. (2002) found maize ears not damaged by ECB larvae to be mostly inoculated with *F. graminearum*, *F. culmorum*, and *F. cerealis*, the main producers of DON and NIV. The authors concluded that ECB control might not be a viable tool to reduce contamination with DON. However, Valenta et al. (2002) determined DON concentrations in damaged and undamaged maize ears and found a positive relationship between DON concentrations and ECB larval feeding. A possible explanation for these contradictory results may be contrasting site-specific *Fusarium* spp. populations. If a *Fusarium* spp. population is mainly composed of *F. graminearum*, significant differences in DON concentration between damaged and undamaged ears are expected because *F. graminearum* may also take advantage of entry holes produced by ECB larval feeding without competition caused by other *Fusarium* spp. However, significant differences for DON concentration between damaged and undamaged ears may not occur if the population composition is more complex and non-DON producers occur at high frequency and, in addition, are favored by ECB larval feeding.

Fusarium subglutinans and *F. verticillioides* predominantly produce FUM and MON and are often found to be associated with ECB larvae feeding damage because larvae act as specific vectors for the dispersal of these fungi (Logrieco et al., 2002). Therefore, it may be possible to reduce FUM (especially fuminosin B1) and MON concentrations in maize kernels by managing ECB infestations (Munkvold et al., 1999; Magg et al., 2002). In our study, FUM concentration was also lower in protected plots than in infested plots across all locations. In addition, the relationship of FUM and MON concentrations with ECB damage was reflected in the difference between Bt and non-Bt cultivars, especially when the toxin was expressed in the kernel tissue, e.g., Mon810. The differences between the events were also shown in earlier studies of Munkvold et al. (1997) and Magg et al. (2002). In these studies, hybrids with the event Mon810 showed lower mycotoxin concentrations and lower kernel damage than hybrids with event Bt176. The drastically increased FUM concentrations at Heilbronn in ECB-infested plots may additionally be explained by environmental effects. This location was characterized by dry and hot growing conditions during the summer season, which favor *F. subglutinans* and *F. verticillioides* and the production of FUM (in particular fuminosin B1) (Schaafsma et al., 2002).

Concentrations of FUS and NIV were low at all locations. This finding is in agreement with the observations by Lepschy (2000), who described two different chemotypes of *F. graminearum*. In Europe and North America, mostly DON-producing strains are prevalent, whereas in Korea and Japan, primarily DON- and FUS-producing strains occur.

CONCLUSIONS

Mycotoxin composition and concentration depend on the *Fusarium* spp. present in a specific inoculum (Logrieco et al., 2002). The site-specific *Fusarium* spp. patterns are shaped by natural selection, i.e., environmental conditions, such as humidity and temperature, as well as site-specific agricultural practices, such as crop rotation and tillage (Logrieco et al., 2002; Schaafsma et al., 2002). Because of favorable conditions for *F. graminearum*, large concentrations of DON were found in Seelow and Freising (www.lfl.bayern.de/ipz/mais/05448; in German; verified 11 Oct. 2004). These concentrations exceed DON concentration thresholds already in place in the USA, Canada (1000 $\mu g\ kg^{-1}$), and Austria (500 $\mu g\ kg^{-1}$) (Lepschy, 2000). These high concentrations of DON underline the necessity for further research to improve *Fusarium* spp. resistance in maize. In the present study, we showed that ECB management reduced mycotoxins, such as FUM and MON, and, to a lesser extent, DON. The concentrations of other mycotoxins were not affected. Therefore, as a short-term solution, transgenic maize cultivars carrying the Bt gene may have the potential to decrease the concentration of specific *Fusarium* spp.–produced mycotoxins. However, *Fusarium* spp.–resistant maize cultivars with reduced mycotoxin concentrations are still necessary. For example, Vigier et al. (2001) reported maize genotypes that were resistant

to ear rot have a reduced DON concentration in maize kernels; the underlying resistance mechanisms are still unknown.

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General Discussion

Grain and silage yield, early-maturity, lodging resistance, as well as improved quality, e.g., digestibility of stover are major breeding aims for maize in Central Europe. In addition, the continuous spread of the European corn borer (ECB, *Ostrinia nubilalis* Hbn.) into northern maize growing regions of Europe and the invasion of the corn root worm (*Diabrotica virgifera virgifera* LeConte), a severe maize pest from North America, stress the importance of resistance breeding against insects in maize in Central Europe (Cate 2002).

Several chemical and biological methods are available for control of ECB larvae, but the application time for treatments is difficult to define because larvae migrate into maize stalks about three days after hatching and are then well protected against insecticides. The use of transgenic *Bt* hybrids would offer a further control method against ECB larvae feeding, but due to the high efficacy of *Bt* maize, it cannot be used without restrictions such as refuge strategies (Ostlie et al. 1997). Furthermore, the consumer's acceptance of transgenic cultivars is fairly uncertain in Central Europe. Therefore, the natural host plant resistance (HPR) would be a more valuable tool to control the occurrence of ECB larvae and minimize the economic damage of larvae feeding.

Resistance breeding against ECB

By using translocation stocks, Scott et al. (1966) demonstrated that resistance against the first generation of ECB was inherited by a relatively large number of genes on chromosomes 1, 2, 4, 6 and 8. Jennings et al. (1974) obtained similar results for resistance against second generation of ECB larvae and concluded that ECB resistance rests mainly on additive gene action. In both studies, recurrent selection was recommended to improve the degree of resistance in the breeding material. Hence, Guthrie and Russell (1989) used four cycles of recurrent selection to develop BS9, a population with improved resistance against first- and second- generation ECB. In Central Europe, ECB larvae occur univoltine and damage maize plants by stalk tunneling (Kreps et al. 1998). Therefore, resistance breeding in Central Europe concentrates only on resistance against the second generation.

Since the early 1990's the implementation of molecular marker techniques into breeding, as simple tools for genetic analyses, initiated many studies aiming at identifying quantitative trait loci (QTL) for resistance to stalk-tunneling insects. These studies confirmed the importance of additive gene action, even though some QTL displayed dominance and overdominance (Schön et al. 1993, Bohn et al. 1997, Cardinal et al. 1998, Kreps et al. 1998, Jampatong 1999, Bohn et al. 2000, Jampatong et al. 2002, Krakowsky et al. 2002).

Testcross (TC) performance is of main importance in maize breeding. Furthermore, for traits associated with ECB resistance, it is not possible to predict hybrid performance based on the performance of lines *per se*, due to the combination of additive, dominant or overdominant gene action. For improvement of polygenically inherited traits, selection of the most promising lines must be based on TC performance throughout the inbreeding process (Hallauer 1990). Likewise, the evaluation of TC performance is inevitable for marker assisted selection (MAS).

Consistency of QTL for ECB resistance

Since the early 1990's, many QTL mapping populations have been evaluated for QTL for maize stem borer resistance. The germplasm pools included stocks of the U.S. Corn Belt, European dent lines, as well as tropical and subtropical material. These populations were used to identify genomic regions involved in the resistance of maize against both ECB generations, as well as against tropical stem borers like the southwestern corn borer (*Diatraea grandiosella*) or sugarcane borer (*Diatraea saccharalis*). It is difficult to compare QTL results across populations, generations, and progeny types because different methodologies have often been used for the trials, causing a low congruency. Furthermore, environmental effects, varying population sizes, sampling effects, and different underlying resistance mechanisms in the germplasm pools used for the analyses influence the power of QTL detection in every population. Finally, the identification of common QTL positions is reduced if some QTL remain undetected (Khairallah et al. 1998).

QTL detection and estimation of their genetic effects depend on the progeny type of the population. In $F_{2:3}$ populations, QTL with both types of gene action, additive and dominant, can be detected. In recombinant inbred lines (RIL) and doubled haploids (DH), the power of QTL detection for alleles with additive gene action is increased due to the

increased homozygosity. In contrast, QTL mapping using TC progenies also reflects the interaction of parental alleles with the tester allele, which can decrease the power of QTL detection. In the special case of dominance or overdominance of the tester allele over both parental alleles, masking effects of the tester reduce the genotypic variance and, therefore, decrease the power of QTL detection.

Consistency across populations

In the present study, two European dent populations were tested for their *per se* performance regarding ECB resistance and agronomic traits. The populations consisted of $F_{2:3}$ families, derived from the crosses 1396A (resistant) × F478 (susceptible) (Population A) and D06 (resistant) × D408 (susceptible) (Population B, see Bohn et al. 2000). Both populations showed no common QTL position for tunnel length (TL) and stalk damage ratings (SDR). This is in agreement with Bohn et al. (1997) who found low congruency of genomic regions for resistance to southwestern corn borer across two tropical and subtropical $F_{2:3}$ populations. Furthermore, the Population A showed only poor agreement with other studies: only one of four QTL detected for resistance was located in adjacent intervals of QTL for insect resistance. The QTL mapping for this population must be critically regarded for two reasons. First, only one environment could be analyzed because low temperatures during early development of maize plants in the second environment caused reduced plant growth and vigor. Therefore, it could be possible that some QTL remained undetected using data from a single environment. Second, large monomorphic regions on chromosomes 3, 4, and 7 were found in both parents, making QTL detection in these genome regions impossible. For final conclusions on the consistency and reliability of QTL, the results of the TC analysis of the present study must also be considered.

Consistency across line per se and TC performance

According to the recommendations of Utz et al. (2000), we employed cross validation (CV) for the TC evaluation of Population B, to minimize the bias for estimation of QTL effects and to obtain reliable estimates of the genotypic variance explained by all QTL (Q^2, Moreau et al. 1998). In the present study, Q^2 for resistance trait SDR was around 30%. In contrast, Q^2 estimated with the currently used method without CV for QTL analysis was

61% (data not shown), indicating a bias of about 50%. This result is in good agreement with Utz et al. (2000), where re-analyses of several QTL studies resulted in a bias between 30% and 70% for Q^2, depending on the number of environments and the population size used for CV. Since the small estimates of Q^2 indicate undetected QTL positions with probably smaller genetic effects, further research should be concentrated on plausible marker-QTL associations with the largest effect detected using CV.

In spite of the decreased power of QTL detection in TC performance, six QTL for SDR were detected in TC progenies. Three of them were already detected in lines *per se*, the remaining three were TC progeny-specific. This result can be explained by the use of a highly susceptible tester (Schulz et al. 1997) and may be attributed to the presence of dominant genes for ECB resistance in the tester, masking alleles of lines *per se* (Kreps et al. 1998).

Based on these results, a rather low to intermediate consistency across populations and progeny types can be expected. Nevertheless, QTL for resistance were often located in adjacent intervals of regions for second generation ECB resistance in tropical and temperate maize populations (Schön et al. 1993, Groh et al. 1998, Bohn et al. 2000, Cardinal et al. 2001, Jampatong et al. 2002, Krakowsky et al. 2002). As could be confirmed from the present study, a compilation of all known QTL regions based on bin locations (chromosomal segments of approximately 20 cM, Gardiner et al. 1993) for stem borer resistance showed that most of these QTL are located in clusters on chromosomes 1, 5, 6, 8, and 9. However, a comparison of QTL results across studies is difficult. For resistance against ECB larvae, neither major genes, explaining a high degree of genetic effects for resistance, nor common markers, defining QTL positions more accurately, have been found across studies.

Marker-assisted selection

The major goals of QTL studies for ECB resistance are to identify reliable chromosomal regions conferring resistance, and to confirm the consistency of these positions across populations, types of progenies, and locations. This information is also needed to set up MAS programs for resistance breeding. MAS would offer the selection of promising genotypes for resistance breeding without costly and mandatory infestation with ECB larvae, and time-consuming evaluation of resistance. The prospects of MAS are promising only for

traits with a relatively low heritability ($h^2 < 0.5$) combined with a high proportion of Q^2 (Lande and Thompson 1990). However, for traits with extremely low values of h^2, QTL detection is extremely inefficient (Melchinger and Utz 2002). Although a low h^2 increases the effectiveness of MAS, Austin et al. (2001) recommended to set up MAS just for traits with a high h^2, where a greater number of QTL across generations was consistent. On the other hand, for traits with a high h^2 MAS may not necessarily be competitive over conventional phenotypic selection (CPS), because the genotypic value is well reflected by the phenotype, and can, therefore, be selected reliably.

Bohn et al. (2000) found for the $F_{2:3}$ families of Population B a relative efficiency (proportion of genotypic variance explained by the respective QTL / heritability, *RE*) of 0.87 for SDR, indicating that CPS is superior over MAS. However, they suggested that the use of MAS may be profitable if cost-effective marker systems were available and costs of the infestation trials were high. Due to the low consistency of QTL for resistance across different studies and populations, MAS should be based on QTL with most-accurate information (highest LOD score) (Knapp 1998), and CV should be used to select reliable marker-QTL associations for MAS with the largest effect on the respective trait (Utz et al. 2000). However, the low consistency of QTL data across the present studies does not suggest MAS as a reliable tool for resistance breeding against ECB, although h^2 was relatively low for SDR ($0.35 \leq h^2 \leq 0.59$) and the costs for infestation and evaluation were high. For TC progenies in the present study, the relative efficiency for SDR was 0.47 indicating that CPS is considerably superior over MAS. Even if the combination of phenotypic selection and MAS would increase *RE* to 1.05, the improvement in resistance breeding for ECB by MAS would only be marginal. These findings are in harmony with the study of Willcox et al. (2002), who directly compared MAS and phenotypic selection in the same population and found no difference between both methods in their ability to improve resistance against the first generation of southwestern corn borer. However, with improved molecular methods and genome analysis, MAS may be competitive over CPS in the future. In this case, marker-assisted recurrent selection as well as genotype construction may present approaches for developing resistant genotypes. Nevertheless, further steps for MAS systems highly depend on the accuracy of QTL detection. In particular, the transmission of QTL locations and the respective loci is more difficult, if QTL locations are uncertain and large (Hospital and Charcosset 1997, Gallais et al. 2000). In addition, the probability for the

successful transmission of such a segment decreases with the number of segments being transferred simultaneously. Therefore, oligogenically inherited traits governed by approximately five genes may be more promising for genotype building than traits with a higher number of genes involved, such as ECB resistance. Regarding only the clusters for insect resistance, about 14 genomic regions are probably responsible for insect resistance. However, even if MAS is not promising in the present situation, QTL data may give basic information about the existence of clusters for ECB resistance and putative candidate genes (Bohn et al. 1996, Bohn et al. 2000, Krakowsky et al. 2004). Even though these clusters contain many genes, it may be possible to define candidate genes for underlying putative resistance mechanisms and their biochemical pathways. For example, important genes for lignin synthesis (*bm1*, *bm2*, *bm3*) are located in some of the clusters for stem borer resistance (Bohn et al. 2000, Flint-Garcia et al. 2003). This information may be a first step towards analyzing the physical and biochemical basis of ECB resistance.

Linkage between ECB resistance and maturity

In many studies, late-maturing genotypes could be found with resistance levels higher than in early-maturing material (Russell et al. 1974, Bohn et al. 2000, Magg et al. 2001). Krakowsky et al. (2004) showed that several QTL for stalk tunneling disappeared when the data were adjusted for anthesis. These findings may indicate that anthesis can bias the results for ECB resistance. In the present study of $F_{2:3}$ families from Population A and the TC performance from Population B, an association between maturity and ECB resistance was confirmed. Common QTL positions were detected for SDR and dry matter content of kernels, as well as days to anthesis. The phenotypic correlation between the traits was highly significant ($P \leq 0.01$) for both mapping populations. Hudon and Chiang (1991) explained the association of resistance with improved stalk stability of later maturing germplasm at harvesting time. However, based on graphical genotypes, a number of individuals combining early-flowering with high levels of ECB resistance was found in the analyses of Bohn et al. (2000). This suggests that tight linkage rather than pleiotropy seems responsible for the observed association. Combining the desired alleles for both traits into a single genotype will be difficult by conventional breeding methods, because many breeding cycles will be

necessary to find suitable genotypes with the desired recombination events. However, application of molecular marker techniques could assist in identifying the respective genotypes.

Resistance mechanisms

To find reliable explanations for underlying genes and biochemical pathways, as well as to suggest new methods to evaluate ECB resistance, studies on resistance mechanisms are important. Resistance against first and second generation of ECB larvae in temperate maize is based on different resistance mechanisms (Cardinal et al. 2001, Jampatong et al. 2002). In this material, the content of DIMBOA (2,3-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3-(4H)-one) was responsible for resistance against the first generation. Considering the decreasing DIMBOA level in later plant stages, it does not seem to be active against the second generation of ECB larvae (Hedin et al. 1984). In contrast, detergent fiber, lignin, and biogenic silica of leaf sheaths affecting the cell wall fortification were reported to be related to the feeding of the second generation of ECB (Rojanaridpiched et al. 1984, Coors 1987, Bergvinson et al. 1994, Beeghly et al. 1997). Furthermore, these factors also had an impact on the first generation of ECB in germplasm with generally low DIMBOA (Groh et al. 1998). In agreement with these considerations, no significant correlations between level of resistance and content of DIMBOA and phenolic acids in early leaf material were found in the present study. In addition, no association between leaf toughness and resistance was observed. In contrast, stalk strength and quality traits, especially the digestibility of organic matter, seemed to have an impact on the resistance against stalk tunneling of ECB larvae. Viereck (1981) found a significant relationship between stalk strength and development of early larvae stages. He concluded that penetration of larvae was more difficult in the case of increased stalk strength and, therefore, larvae were exposed to environmental stresses for a longer time. In addition, increased stalk strength was associated with a better resistance against stalk lodging before harvest (Flint-Garcia et al. 2003, Martin et al. 2004) and could therefore, also cause lower stalk damage ratings. Regarding the association of SDR with dry matter content and anthesis, studies on the resistance of "stay green" types would be of interest. Lower stalk stability and, therefore, high SDR for genotypes with high dry matter content were probably caused by decomposition processes. In our TC study, the QTL on chromosome 8 and a QTL positions for "stay green" (Beavis et al. 1994) were located in

adjacent intervals, possibly indicating an association between the traits. In addition, Flint-Garcia et al. (2003) found potential candidate genes for vegetative phase change linked to stalk strength. These candidate genes were also located in clusters for insect resistance.

Understanding the relationship between corn borer resistance and digestibility is of importance in silage maize breeding. As reported by Buendgen et al. (1990) and Buxton et al. (1996), plant material with a high digestibility was more susceptible to ECB larvae feeding. Analyses in the present study confirmed this hypothesis and showed a significant difference for cellulase digestibility of organic matter (CDOM) between a resistant and susceptible group of $F_{2:3}$ families derived from Population B. This can be explained with a better availability of nutrients for the larvae or a reduced cell wall fortification. Likewise, a higher content of water-soluble carbohydrates was linked to an increased susceptibility to ECB damage. In addition, the amount of lignin seems to be of relevance for resistance (Buendgen et al. 1990), whereas the composition of lignin is responsible for a higher level of digestibility (Albrecht et al. 1986, Argiller et al. 1996, Barrière et al. 2003). In maize, genes are known that directly influence lignin content in cell walls and its subunit structure. It can be speculated that specific alleles at these gene loci cause the production of lignin that compress a subunit composition in cell walls, which results in an increased CDOM without decreasing the cell wall strength. These genes might also improve digestibility without increased SDR. In this case, simultaneous improvement of digestibility and corn borer resistance seems to be possible.

Simple and cost-effective tools are necessary for maize breeding. Measurements of the stalk strength are time-intensive and laborious and, therefore, comparable to expensive mass rearing and infestation. In contrast, analyses with NIRS (near infrared reflection spectroscopy) for digestibility and lignin content or lignin composition may be an efficient alternative.

Mycotoxin analyses

A further aspect in dealing with the damage of ECB larvae is the contamination of kernels with mycotoxins. It was reported that at least some of the mycotoxins were related to the damage of ECB larvae (Lew et al. 1991, Valenta et al. 2001), because the larvae act as specific vectors for spores, particularly of *Fusarium subglutinans* and *F. verticillioides* that produce fuminosin (FUM) and moniliformin (MON). In general, mycotoxins are

produced by *Fusarium* and *Aspergillus* spp. and pose a threat to food security. They are often associated with intoxication in humans and livestock (Moreno and Kand 1999, Logrieco et al. 2002). Observations of esophageal cancer of humans, embryo abortions and deformations in livestock, emphasize the demand for *Fusarium* and *Aspergillus* resistant maize cultivars. In the present study, highly resistant *Bt* maize hybrids, their isogenic counterparts and commercial hybrids were evaluated for ECB resistance and mycotoxin levels in kernels. Mycotoxins deoxynivalenol (DON), FUM, and MON were most prevalent, whereas fusarenon-X (FUS) was only found in a few samples. The concentrations of DON, its derivates, and FUM were significantly ($P \leq 0.05$) different between the locations. Extremely high levels of DON (> 20 000 µg/kg), 3-A-DON and 15-A-DON were detected at one location (Seelow). Environmental conditions and agriculture practices usually have a huge impact on the amount and composition of *Fusarium* inoculum, as reported by Logrieco et al. (2002) and Schaafsma et al. (2002). For example, DON is mainly produced by *F. graminearum*, known to increase with a close maize-wheat crop rotation with reduced tillage, as is currently practiced in Seelow (Rintelen 2000).

A significant correlation between ECB damage and mycotoxin concentration was found for FUM ($P \leq 0.01$), which is in agreement with Magg et al. (2002, 2003). In addition, lower MON concentrations were observed for ECB resistant genotypes, but the correlation was not significant. Highly resistant *Bt* hybrids showed significantly reduced mycotoxin concentrations. This is in accordance with the studies of Munkvold et al. (1999), who found that Fuminosin B1 could be reduced by controlling ECB. In contrast, no significant correlation between concentration of DON and ECB larvae feeding was found over all locations, except at one location (Seelow), where significant effects ($P \leq 0.10$) were observed.

Based on the results of the present study, it was concluded that the use of *Bt* hybrids could reduce the concentration of specific mycotoxins like FUM. Nevertheless, DON is the main problem for the food and feed industry, and it is not reliably reduced by ECB management and transgenic cultivars (Logrieco et al. 2002, Magg et al. 2003). Considering a number of other pathways for the infection with *Fusarium* spp. independent from insect damage, the application of maize cultivars carrying the *Bt* gene is only a short-term solution for reducing specific mycotoxin concentrations. On the long run, maize cultivars resistant to

Fusarium ssp. or mycotoxin accumulation must be developed separately from ECB resistance. Promising findings were reported by Vigier et al. (2001), who identified maize genotypes with resistance against ear rot, and with reduced DON concentration in the kernels. At present, the biochemical basis for mycotoxin production is not completely understood and may be controlled by several strategies, as described for aflatoxins (Moreno and Kand 1999). For example, compounds inhibiting the mycotoxin production or compounds inhibiting growth of the fungus may be identified. However, further research is warranted to investigate the chemical pathway of mycotoxin production, for application in resistance breeding or plant protection.

Conclusions

Economically relevant grain yield losses, caused by ECB larvae feeding, as well as possible effects of ECB larvae on the promotion of specific *Fusarium* strains and their mycotoxins underline the importance of research aiming at the improved resistance of maize against ECB. As highly resistant transgenic cultivars are currently not accepted by the European consumer, research on natural HPR is indispensable for developing new lines and hybrids. In addition, the use of transgenic cultivars may only be a short-time solution to reduce the concentration of some mycotoxins significantly correlated to ECB damage, such as FUM and MON.

Based on the results of the present study, only a small gain in selection response can be expected by employing MAS for improving HPR against ECB damage. Most of the QTL detected for stem borer resistance seem to occur in clusters on most of the maize chromosomes. Even if the hypothesis of common QTL clusters for resistance needs to be confirmed by a meta-analysis gathering the data of different studies to obtain reliable QTL results (Goffinet and Gerber, 2000), these findings may pave the way for further research on candidate genes and association studies for resistance against ECB larvae feeding. The presented analysis of resistance mechanisms suggests that stalk strength (especially lignin content) and digestibility are associated with ECB resistance. Genotypes with an improved digestibility, without impairing cell wall strength and susceptibility against feeding by ECB larvae would be most promising.

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Summary

The European corn borer (*Ostrinia nubilalis* Hb., ECB) is an important pest in maize production in Europe and North America. Feeding of ECB larvae causes grain yield losses of up to 30% and promotes ear and stalk rots caused by *Fusarium* spp.. These fungi often produce mycotoxins, which might cause immunosuppression, porcine pulmonary edema, and liver cancer in rats, as well as esophageal cancer in humans. Maize cultivars carrying the *Bt* gene are highly resistant to ECB larvae feeding. However, the use of transgenic cultivars is controversially discussed, because of possible adverse effects of the *Bt* toxin on other non-pest organisms in the ecosystem. In addition, the development of *Bt* resistant ECB larvae is expected due to the high efficacy of the *Bt* toxin and its monogenic inheritance. In contrast, the natural host plant resistance (HPR), which is quantitatively inherited, is regarded as more durable. The main objective of this study was to identify quantitative trait loci (QTL) for HPR against ECB and to draw conclusions about their usefulness in marker-assisted selection (MAS). The specific research questions were: (1) Where are QTL for ECB resistance and related agronomic traits located in the maize genome and what are their genetic effects? (2) How consistent are QTL detected across unrelated populations? (3) How consistent are QTL detected for line *per se* and testcross performance? (4) Which physiological mechanisms underlie the resistance against ECB larvae feeding? (5) What is the association between ECB resistance and mycotoxin concentrations in grain maize?

Two unrelated early-maturing dent populations were derived from the crosses 1396A (resistant) × F478 (susceptible) (Population A) and D06 (resistant) × D408 (susceptible) (Population B). For each population, 230 $F_{2:3}$ families were developed (Experiment 1). All $F_{2:3}$ families of Population B were testcrossed with D171, a susceptible tester line from the flint pool (Experiment 2). Two sets of $F_{2:3}$ families from Population B, each comprising the five most resistant and the five most susceptible lines, were selected based on stalk damage ratings (SDR) (Set 1) or tunnel length (TL) (Set 2) of larvae feeding (Experiment 3). Experiment 1 was evaluated for line *per se* performance for ECB resistance at two locations in the Upper Rhine Valley in 1995. Experiment 2 and Experiment 3 were grown at one loca-

tion in Bavaria in 2000 and 2001. In Experiment 4, 10 maize cultivars consisting of four pairs of transgenic hybrids and their isogenic counterparts, including two hybrids with *Bt* event *Bt*176 and two hybrids with event Mon810, were used to determine the association between mycotoxin concentration and ECB resistance. The hybrids were evaluated at three locations in East and South Germany in 2001. All entries in Experiment 4 were analyzed for mycotoxin concentration of deoxynivalenol (DON), fumonisin (FUM), fusarenon-X (FUS), moniliformin (MON) and nivalenol (NIV) in grain samples obtained from infested and insecticide protected plots. In all four experiments, resistance to ECB larvae feeding was evaluated using manual infestation with ECB larvae. Resistance scoring involved SDR in all experiments. Furthermore, TL, yield reduction under infestation, and the number of larvae per ear were recorded in Experiments 1 and 4. Experiment 3 was additionally evaluated for silage quality traits of stover (digestibility, contents of fiber, protein, and water soluble carbohydrates) using Near Infrared Reflection Spectroscopy (NIRS).

In Experiment 1, two QTL for SDR and two QTL for TL were detected in Population A, both explaining about 25% of the genotypic variance. For agronomic traits, one to three QTL were found, which explained between 2% and 12% of the genotypic variance. No common QTL for resistance traits was found across Populations A and B. Two QTL for *in vitro*-digestibility of organic matter and dry matter concentration were in common among both populations. Possible explanations for the low consistency of QTL across populations are a low power of QTL detection caused by small population sizes, sampling, and environmental effects. Furthermore, population-specific QTL regions cannot be ruled out.

In Experiment 2, six QTL for SDR explaining 27% of the genotypic variance were found for testcross performance. Three common QTL for SDR were detected for line *per se* and testcross performance. Phenotypic as well as genotypic correlations between line *per se* and testcross performance were low for SDR, indicating a moderate consistency across the different types of progeny. The low consistency across both types of progeny is presumably attributable to the low power of QTL detection in TC progenies caused by a decreased genotypic variance, masking effects of the tester allele, specific interactions of the parental alleles with the tester allele, and QTL × environment interactions. Despite the low consistency

of QTL across populations and progenies in the present study, a comparison with other reports from the literature revealed that most of the QTL occurred in clusters. Given the low percentage of genotypic variance explained by QTL-marker associations, we conclude that MAS will not be efficient for resistance breeding against ECB with the current molecular marker techniques.

In Experiment 3, significant correlations were observed between resistance and quality traits, such as digestibility and stalk strength. These findings confirm the importance of increased cell-wall fortification for resistance against ECB larvae feeding, and support the hypothesis that candidate genes for resistance are involved in lignin biosynthesis.

The analyses of mycotoxin concentrations in Experiment 4 showed that DON, FUM, and MON were the most prevalent mycotoxins in maize kernels. The concentration of DON reached alarming levels of more than 20 000 µg/kg. Differences between protected and infested plots were only significant for DON and FUM. For the infested plots, significant differences were found between *Bt* and non-*Bt* hybrids, as well as between the two events *Bt*176 and Mon810. Transgenic *Bt* hybrids, especially those carrying event Mon810, showed lower mycotoxin concentrations in kernels than the other hybrids. However, only low correlations were found between ECB resistance and mycotoxin concentrations across all 10 hybrids. Therefore, selection for ECB resistance does not necessarily reduce mycotoxin concentration, suggesting that each complex of characters must be improved simultaneously by breeding.

In conclusion, the possible severe effect of ECB larvae feeding on maize yield and quality underlines the need for a continued research on ECB management systems, including improved HPR of maize against ECB. Even if MAS for resistance against the ECB does not seem promising at the moment, the information about QTL regions may be a first step for further research on possible candidate genes, e.g., brown midrib genes located in the common QTL regions with effects on the lignin biosynthesis. Genotypes with an improved digestibility, without impairing ECB resistance by reduced cell-wall strength, would be most promising.

Zusammenfassung

Sowohl in Europa als auch in Nordamerika ist der Europäische Maiszünsler (*Ostrinia nubilalis* Hb.) einer der bedeutendsten Schädlinge im Maisanbau. Die Fraßtätigkeit der Larven kann bei Körnermais Ertragsausfälle bis zu 30 % verursachen und begünstigt außerdem das Auftreten von Kolben- und Stängelfäule durch *Fusarium* Pilze. Diese Pilze bilden häufig Mykotoxine, die im Verdacht stehen bei Schweinen zu Immunsuppressionen und zu Lungenödemen zu führen. Außerdem lösen sie möglicherweise bei Ratten Leberkrebs sowie bei Menschen Speiseröhrenkrebs aus. Der Anbau von transgenen Maissorten, die ein Gen zur Bildung des *Bacillus thuringiensis* (*Bt*)-Toxins enthalten, wird kontrovers diskutiert, obwohl diese Hybriden über eine sehr gute Resistenz gegen den Maiszünsler verfügen. Zum einen ist bisher die Wirkung des *Bt* Toxins auf Nützlinge und andere Nicht-Schadorganismen nicht vollständig geklärt, zum anderen kann aufgrund der hohen Wirksamkeit des Toxins und seiner monogenen Vererbung nicht ausgeschlossen werden, dass sich *Bt*-resistente Maiszünslerlarven entwickeln. Die natürliche Resistenz der Pflanze stellt im Gegensatz dazu eine wesentlich nachhaltigere Lösung zur Schädlingsbekämpfung dar. Ziel der Studie war es „quantitative trait loci“ (QTL) zu detektieren, die mit der Vererbung der natürlichen Resistenz zusammenhängen. Dabei sollten folgende Versuchsfragestellungen beantwortet werden: (1) Wo liegen die QTL für Maiszünslerresistenz bzw. damit verbundene agronomische Merkmale und wie groß ist ihr genetischer Effekt? (2) Gibt es Übereinstimmungen von QTL zwischen nicht verwandten Populationen? (3) Wie groß ist die Übereinstimmung der QTL zwischen Linien *per se* und Testkreuzungsnachkommen? (4) Welche physiologischen Mechanismen liegen der Resistenz gegen den Maiszünsler in der Pflanze zu Grunde? (5) Welchen Zusammenhang gibt es zwischen Maiszünslerresistenz und Mykotoxinbildung?

Für den Populationsvergleich wurden zwei nicht verwandte frühreife Dent-Populationen aus folgenden Kreuzungen aufgebaut: Population A: 1396A (resistent) × F478 (anfällig) und Population B: D06 (resistent) × D408 (anfällig). Aus jeder der Kreuzungen gingen 230 $F_{2:3}$ Familien hervor. In Experiment 1 wurden die $F_{2:3}$ Familien der Population A

hinsichtlich der Resistenzausprägung untersucht. Für Experiment 2 wurden die Nachkommen der Population B mit dem Tester D171, einer anfälligen Linie aus dem Flint Pool, gekreuzt und die Testkreuzungsnachkommen analysiert. Experiment 3 umfasste die Untersuchung von zwei Gruppen mit jeweils fünf extrem anfälligen und fünf extrem resistenten Genotypen der $F_{2:3}$ Familien aus Population B. Die Auswahl der Genotypen wurde hinsichtlich der Schadbilder Stängelbruch und Fraßganglänge getroffen. Die Daten für Experiment 1 wurden 1995 an zwei Standorten im oberen Rheintal erhoben. Experiment 2 und Experiment 3 wurden in den Jahren 2000 und 2001 an jeweils einem Standort in Bayern untersucht. Für Experiment 4 wurden zehn Maishybriden hinsichtlich der Beziehung zwischen Mykotoxinbildung und Maiszünslerresistenz untersucht. Das Sortenspektrum umfasste vier Paare transgener und isogener Sorten sowie zwei Standardhybriden. Jeweils zwei der transgenen Hybriden trugen das *Bt*-Konstrukt *Bt*176 bzw. Mon810. Die Versuche wurden im Jahr 2001 an drei Standorten in Ost- bzw. Süddeutschland durchgeführt. Alle Versuchsglieder aus Experiment 4 wurden auf die Mykotoxine Deoxynivalenol (DON), Fuminosin (FUM), Fusarenon (FUS), Moniliformin (MON) und Nivalenol (NIV) untersucht. Die Proben wurden sowohl aus den Nullparzellen (insektizid-geschützt) als auch unter künstlichem Befallsdruck (Ausbringung von Larven) genommen. In allen vier Experimenten wurde die Resistenz mittels künstlichem Befallsdruck ermittelt. Dazu wurde in jedem Fall der Stängelbruch erhoben, zusätzlich wurden in Experiment 1 und 4 Tunnellänge, Ertragsreduktion unter Befallsdruck oder Anzahl der Larven je Pflanze ausgewertet. Die Proben aus Experiment 3 wurden außerdem noch mittels NIRS (Nah-Infrarot-Reflexions-Spektroskopie) hinsichtlich der Silomais-Qualitätsparameter der Restpflanze (Verdaulichkeit, Gehalt an Rohfaser, Rohprotein und wasserlöslichen Kohlenhydraten) untersucht.

In Experiment 1 konnten zwei QTL für Stängelbruch und zwei QTL für Fraßganglänge detektiert werden, die jeweils 25 % der genotypischen Varianz erklärten. Für die agronomischen Merkmale wurden zwischen einem und drei QTL gefunden, die zwischen 2 und 12 % der genotypischen Varianz erklärten. Für die beiden Populationen A und B wurden keine gemeinsamen QTL für Resistenz gefunden. Allerdings waren zwei QTL für *in vitro* Verdaulichkeit der organischen Masse und für Trockenmasse in beiden Populationen identisch. Mögliche Erklärungen für die schlechte Übereinstimmung in beiden Populationen

können die niedrige Güte (Power) der QTL Detektion und populationsspezifische QTL sein. Die Güte der Detektion wird durch kleine Populationsgrößen, Stichprobeneffekte bei der Auswahl der Genotypen (sampling effects) und Umwelteinwirkungen negativ beeinflusst.

In Experiment 2 wurden für die Testkreuzungsnachkommen sechs QTL für Stängelbruch gefunden, die 27 % der genotypischen Varianz erklärten. Drei davon waren bereits auch für die $F_{2:3}$ Familien *per se* detektiert worden. Die phänotypische sowie die genotypische Korrelation zwischen $F_{2:3}$ Familien *per se* und den Testkreuzungsnachkommen waren für das Merkmal Stängelbruch niedrig. Es lag also nur eine mittelmäßige Übereinstimmung zwischen den Nachkommenschaften vor. Dies kann zum einen auf die niedrige Güte der QTL Detektion, als auch auf eine niedrige genotypische Varianz bei Testkreuzungsnachkommen zurückzuführen sein. Ebenso sind auch Maskierungseffekte durch die Testerallele, spezifische Interaktionen der elterlichen Allele mit den Testerallelen sowie QTL × Umwelt - Effekte als weitere Gründe anzuführen. Auch wenn in der vorliegenden Studie nur geringe Übereinstimmungen von QTL zwischen Populationen und zwischen Nachkommen gefunden wurden, deutet ein übergreifender Vergleich von QTL Studien darauf hin, dass die meisten QTL für Insektenresistenz in Clustern liegen. Da jedoch nur ein niedriger Anteil der genotypischen Varianz durch die QTL erklärt wurde, scheint die markergestützte Selektion zum jetzigen Zeitpunkt für die Resistenzzüchtung gegen den Maiszünsler noch nicht sinnvoll einsetzbar.

In Experiment 3 wurden signifikante Zusammenhänge zwischen Resistenz und Qualitätseigenschaften, wie Verdaulichkeit und Stängelhärte gefunden. Dadurch gewinnt eine erhöhte Zellwandfestigkeit für die Resistenz gegen den Maiszünsler an Bedeutung und es scheint sich die Hypothese zu bestätigen, dass Kandidatengene für die Resistenz möglicherweise mit der Ligninbiosynthese zusammen hängen.

Die Mykotoxinanalyse in Experiment 4 zeigte, dass in den meisten Fällen DON, FUM und MON in Maiskörnern zu finden waren. DON erreichte in den Versuchen alarmierende Konzentrationen von über 20.000 µg/kg. Zwischen den insektizid-geschützten Parzellen und den Parzellen unter Befallsdruck waren nur für die Mykotoxine DON und FUM signifikante

Unterschiede zu verzeichnen. Außerdem unterschieden sich *Bt* und nicht-*Bt* Hybriden, sowie die beiden Konstrukte *Bt*176 und Mon810 unter künstlichem Befallsdruck signifikant. Grundsätzlich zeigten die transgenen Hybriden, insbesondere Hybriden mit dem Konstrukt Mon810 eine niedrigere Mykotoxinkonzentration in den Körnern als die anderen Hybriden. Trotzdem war die Korrelation zwischen Maiszünslerresistenz und Mykotoxinkonzentration insgesamt nur niedrig. Demnach müssen Genotypen mit einer guten Resistenz gegen den Maiszünsler nicht zwingend über eine niedrige Mykotoxinbelastung verfügen. Im Züchtungsprozess müssen die beiden Eigenschaften daher gleichzeitig verbessert werden.

Der potenzielle Schaden des Maiszünslers auf den Ertrag und die Qualität ist so groß, dass die Entwicklung geeigneter Pflanzenschutzmaßnahmen gegen den Maiszünsler, einschließlich der Verbesserung der natürlichen Resistenz, notwendig ist. Auch wenn eine markergestützte Selektion für die Maiszünslerresistenz momentan noch nicht erfolgversprechend erscheint, kann die Information über die QTL Regionen ein erster Schritt auf der Suche nach Kandidatengenen sein. Beispielsweise liegen Gene für den „brown midrib type“, also für Pflanzen mit einem niedrigeren Ligninbildungsvermögen an Genorten, an denen sich auch einige Cluster für Insektenresistenz befinden. Für die Züchtung wären v.a. Genotypen wertvoll, die zwar über eine verbesserte Verdaulichkeit verfügen, bei denen jedoch die Resistenz gegen den Maiszünsler nicht durch eine verringerte Zellwandstabilität herabgesetzt ist.

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