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Comparison of the Resistance of Winter Wheat to Fusarium Head Blight with *Fusarium graminearum* and *Fusarium sporotrichioides*

Diploma thesis

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Table of contents

Abbreviations

AE:	anther extrusion
ANOVA:	analysis of variance
AR:	anther retention
AUDPC:	area under disease progress curve
CH:	chasmogamous
CL:	cleistogamous
CWDE:	cell wall degrading enzymes
DAA:	days after anthesis
DAT:	anthesis date (days after the first of May)
DON:	Deoxynivalenol
FDK:	Fusarium damaged kernels
FHB:	Fusarium Head Blight
FG:	*Fusarium graminearum*
FSP:	*Fusarium sporotrichioides*
IFA:	Department for Agrobiotechnology, Institute of Plant Production
MIX:	mixed inoculum of *Fusarium graminearum* and *Fusarium sporotrichioides*
NIV:	Nivalenol
PH:	plant height
ZON:	Zearalenone

Abstract

Fusarium Head Blight (FHB) is a destructive fungal disease in wheat in many parts of the world. The objective of this study was to compare 96 different genotypes of winter wheat and one durum wheat for the resistance to FHB with the toxin producing species *Fusarium graminearum (FG)* and *Fusarium sporotrichioides (FSP)*, either alone or mixed together. Furthermore the author wanted to investigate associations between FHB severity and toxin production with the morphological plant traits anther extrusion (AE), plant height (PH) and flowering date (DAT). The wheat lines were grown in small plots in field experiments during the growing season of 2012. Trials were inoculated by spraying fungal spore suspensions during anthesis and FHB development was recorded by visual observations. In addition plant height, flowering date and the extent of anther extrusion were recorded at the field plots. After ripening plots were combine harvested and the seeds were evaluated for percentage of visually damaged grains (FDK) and for content of Fusarium mycotoxins. The evaluated wheat lines showed broad variation for FHB severity measured on field plots and on harvested grains. The three inoculation variants were correlated, supporting the non – species - specificity of *Fusarium* resistance in wheat. Selection for improved resistance of *F. graminearum* leads to correlated selection response resistance against *F. sporotrichioides*. Toxin content in the harvested grains was positively correlated with visual symptom severity. The extent of anther extrusion and plant height were negatively correlated with FHB severity and flowering date was positively correlated with FHB severity. Selection of lines with high anther extrusion and of tall lines should lead to an indirect selection gain for reduced FHB susceptibility.

Zusammenfassung

Titel: Vergleich der Resistenz von Winterweizen zu Ährenfusariose mit *Fusarium graminearum* und *Fusarium sporotrichioides*

Die weltweit bedeutende Pflanzenkrankheit Ährenfusariose (FHB) ist eine bedeutende Pilzkrankheit an Weizen. Ziel dieser Arbeit war es, 96 unterschiedliche Genotypen von Winterweizen auf deren Resistenz zu FHB durch die Toxin produzierenden Arten *Fusarium graminearum (FG)* und *Fusarium sporotrichioides (FSP)*, separat und ein Gemisch beider Arten zu prüfen. Außerdem wollte man Zusammenhänge zwischen FHB und Toxinproduktion mit den morphologischen Pflanzeneigenschaften Antherenausstoß (AE), Pflanzenhöhe (PH) und Blühdatum (DAT) analysieren. Die Weizenlinien wurden in kleinen Parzellen in Feldexperimenten während der Vegetationsphase 2012 ausgeführt. Die Parzellen wurden während der Blüte mittels Sprühinokulation von Sporensuspensionen inokuliert und die Entwicklung von FHB wurde durch Bonitierung festgestellt. Zusätzlich wurde die Pflanzenhöhe, Blühdatum und das Ausmaß an ausgestoßenen Antheren festgestellt. Nach der Vollreife wurden die Parzellen mit einem Parzellenmähdrescher geerntet, die Körner wurden auf prozentuellen Befall infizierter Samen (FDK) bonitiert und Toxinanalysen wurden durchgeführt. Die verwendeten Weizenlinien zeigten große Unterschiede in der FHB Anfälligkeit der bonitierten Feldparzellen und der geernteten Körner. Die drei Varianten korrelierten miteinander, wodurch die Nicht- Artenspezifizität der *Fusarium* Resistenz in Weizen unterstützt wird. Daher führt eine Selektion von verbesserter Resistenz von *F. graminearum* auch zu korrelierter Selektionsantwort von *F. sporotrichioides*. Die Toxingehalte der geernteten Körner waren positiv korreliert mit der Bonitierung der Anfälligkeit der Feldparzellen. Die Anzahl der ausgestoßenen Antheren und die Pflanzenhöhe waren negativ korreliert mit der FHB Anfälligkeit und das Blühdatum war positiv korreliert mit der FHB Anfälligkeit. Darum sollte die Selektion von Linien mit starkem Antherenausstoß und hohen Linien zu einer indirekten Selektion von reduzierter FHB Anfälligkeit führen.

1 Introduction and problem

The contamination of food with toxins is very important in our society and often a topic in today's media and public discussion. We all want a safe food supply for a healthy diet and therefore need to reduce the risk of eating contaminated food, nevertheless pathogens exist which play a big role in today's agronomy for human food and animal feeds. But there are only a couple of pathogens that have a big role in our today's agronomy for human foods and animal feeds. *Fusarium* species (spp.) is one of them and probably the most important one.

Its destructive function is found in many crops but in wheat (*Triticum aestivum L.*), one of the worldwide most severe diseases is Fusarium Head Blight (FHB). Besides negative effects on grain yield and the quality of wheat, FHB produces trichothecene mycotoxins like deoxynivalenol (DON) and nivalenol (NIV). These mycotoxins are toxic to humans and animals in very small amounts and may even lead to death. Therefore, plant scientists and especially breeders try to cope with this problem by decreasing FHB either in a chemical and agronomical way or by breeding. However, the use of fungicides and agronomy does not always lead to the decreased results of the amount of toxins. It only reduces yield loss and the negative effects on the quality. Breeding resistant and more tolerate seeds would be the better way of fighting FHB and reducing toxins in our food and feeds and so also became a main aim for breeders (DESJARDINS 2006).

Especially anther extrusion (AE) is suggested to play a big role in FHB and DON contamination as one of the main passive resistance mechanism in wheat and also became a significant factor in wheat breeding programmes. Therefore, the assumed correlation between AE and FHB bears potential for breeding new cultivars with reduced mycotoxin contamination (SKINNES ET AL. 2009).

Today there are several known FHB resistant genotypes and also some low susceptible cultivars. However, they still need to be combined with other disease resistances, desirable quality traits and agronomical performance.

1.1 Aim of this thesis

Objectives of this thesis were (1) to assess different genotypes of winter wheat for resistance of FHB, (2) to compare resistance of these winter wheat lines to two different *Fusarium* spp. - namely *Fusarium graminearum (F. graminearum)* and *Fusarium sporotrichioides (F. sporotrichioides)* and a mix of both and (3) to investigate associations of FHB severity and toxin production with other plant traits, such as extent of anther extrusion, plant height and flowering date.

1.2 Experimental Design and Procedure

This work was implemented in 2012 at the Department IFA - Tulln. A field experiment with six replications and three different treatments was planned in cooperation with the French seed companies

"Florimond Desprez" and "RAGT" who provided most of the winter wheat seeds. The experiment was a randomized complete block design with 96 different winter wheat lines, mainly French but also Austrian and Hungarian cultivars and breeding lines. In our experiment two different *Fusarium spp.* (*F. graminearum* and *F. sporotrichioides*, either alone or mixed together) were used to inoculate the wheat lines. FHB severity was visually scored six times in the field. In addition, the percentage of extruded anthers, the date of flowering and plant height were assessed on all entries. Also wilting symptoms, FHB – severity on harvested seeds (Fusarium damaged kernels), homogeneity and late tillers were determined. Additionally the toxin contents of the harvested wheat samples were measured.

2 Fusarium Head Blight in literature

2.1 Fusarium

2.1.1 The Pathogen

The work and studies with *Fusarium* began in 1809 by Johann Link with the identification of the genus and had its peak in 1961 with the determination of the trichothecene diacetoxyscirpenol structure until 1991 when scientists discovered the carcinogenicity of *Fusarium* and continues until now with genomic research (DESJARDINS 2006). Scientific studies have focussed their interests that much on *Fusarium* because of its combination of produced mycotoxins and its economic effect.

Fusarium fungi belong to the phylum of Ascomycota in the genera *Gibberella* and *Nectria* and are saprophytes and/ or facultative parasites. In general, *Fusarium* can be a pathogen in plenty of plants like banana, tomatoes, cucurbits, cereals, etc. and with diseases on all parts of those plants (SUMMERELL and LESLIE 2011). But only around 20 species can be associated with FHB which can be divided into four sections: Discolor (*F. graminearum (*teleomorph *Gibberella zeae), F. culmorum, F. crookwellense*), Roseum (*F. avenaceum*), Gibbusum (*F. equiseti, F. scirpi, F. acuminatum*) and Sporotrichiella (*F. poae, F. tricinctum, F. sporotrichioides*) (LIDDELL 2003). Nevertheless, in this thesis only *F. graminearum* and *F. sporotrichioides* were used as inoculum and hence are relevant for this work.

Both have a characteristic morphology to identify them for a trained eye but which is not always that easy. *F. graminearum* produces long and slightly curved to straight macroconidia with five to six septa. Those can be used for diagnostic identification with a white mycelium that becomes carmine red to rose and greyish when it ages. There are no microconidia. *F. graminearum* occurs worldwide in temperate climate and was the dominant *Fusarium* species of the swine feed refusal in the central United States in the 1970s and 1980s and the red mold disease (Akakabi- byo) from the 1950s to the 1970s in Japan (DESJARDINS 2006).

The macroconidia of *F. sporotrichioides* cannot be used to distinguish the species, only its microconidia with an ellipsoidal to oval shape and a more pink to brownish old mycelium and a usually deep red undersurface of the colony. Although *F. sporotrichioides* produces the known most toxic compounds of *Fusarium* spp., *F. graminearum* is much more aggressive and more severe on plants. *F. sporotrichioides* is more prevalent in cold, humid climate conditions and can be associated with the alimentary toxic aleukia in humans in Russia and Central Asia in the 1940s (DESJARDINS 2006; LIDDELL 2003).

2.1.2 Negative effects of FHB

Fusarium (lat. *fusus*= spindle) is a pathogen of plants with global importance with often devastating effects that produce a wide range of secondary metabolites (mycotoxins). These metabolites are associated with severe toxicoses of animals and humans and effect growth and development. In history, there are several cases of epidemics which display the effects of eating and feeding Fusarium mycotoxins, especially thrichothecenes. DESJARDINS (2006) writes about these well-known examples of humans and animals with the same symptoms all over the world: vomiting, food refusal, diarrhoea, nausea, skin irritation and necrotic lesions accompanied by trembling, dizziness, headache, hallucinations and even death. All these reported cases of Russia, Central and East Asia, Japan, Australia and North- and South America (Argentina) and Europe are associated with the consumption of toxic food, mainly all classes of wheat and other small grains (CARRANZA et al. 2007; DESJARDIN 2006; OBANOR et al. 2012).

This kind of food is infected with FHB which is caused by different *Fusarium* spp. FHB, also called "head scab" or "Fusarium ear blight", effects size and weight (smaller and shrivelled kernels), composition and the quality of seeds and therefore reduces yield by infection of the floret, poor seed germination and filling (ENGLE et al. 2004). Within a few weeks, FHB has the potential to negatively effect and also destroy fields of crops. This can be a problem for farmers and seed producers because it lowers the market grade and makes it difficult to process, export, market and feed (MCMULLEN et al. 1997).

In order to protect humans and animals from Fusarium mycotoxins maximum allowed levels for several toxins have been implemented by different authorities. For example, the European Union sets maximum levels for the Fusarium toxins, DON, Zearalenone and Fumonisin in all kinds of food which can be affected by FHB. For DON there is a maximum of 500 µg/kg in bread, pastries, biscuits and breakfast cereals, 1250 µg/kg in unprocessed cereals and 200 µg/kg in processed cereal- based food and baby food for infants and young children (COMMISSION REGULATION (EC) No 1126/2007). However, in less- developed countries the potential exposure to significant levels of trichothecenes is much higher, especially with favouring environmental and lacking storage conditions, but up to now reliable and proofing data are missing. Therefore better public education, awareness and management practices with contaminated food are needed (DESJARDINS 2006).

2.1.3 Infection and life cycle

The most important factor for natural infection in field is the persistence and multiplication in residues of crops after the harvest which is like a reservoir of inoculum. Warm and moist weather around crop anthesis favours and increases infection and is strongly associated with that, and especially the time of rainfall is a crucial factor, not the amount. But a few studies did not agree and confirmed that FHB causing fungi are less influenced by weather conditions because the environmental effect on the fungi

may differ between initial infection, subsequent colonization and between species (XU et al. 2007). Ascospores (sexual stage) are transported and flying around by wind with an optimum temperature of around 16°C. However, plants are mainly infected by conidia (asexual stage) that are splashed to the heads of plants via rain and water drops. These conidia germinate within 6 to 12 hours after inoculation and then form germ tubes with many hyphae after 12 to 24 hours after inoculation that colonize the spikelet. There are different studies and opinions about the primary penetration sites, including ovary and inner surface of lemma and palea, stomata etc. During the infection, the plant pathogenic fungi produces cell wall degrading enzymes (CWDE) and - following the hypothesis, which was not believed for a long time - it forms appressoria and infection cushions, to colonize the host tissue. After 48 to 76 hours it forms many intercellular and intracellular hyphae which grow inside and outside of the epidermal cells and via the cortex which also infects other spikelet. After this time the fungus is also able to complete one asexual cycle and produces conidiophores with macroconidia (BOENISCH and SCHÄFER 2011, BROWN et al. 2010, PRITSCH et al. 2000, WANYOIKE et al. 2002).

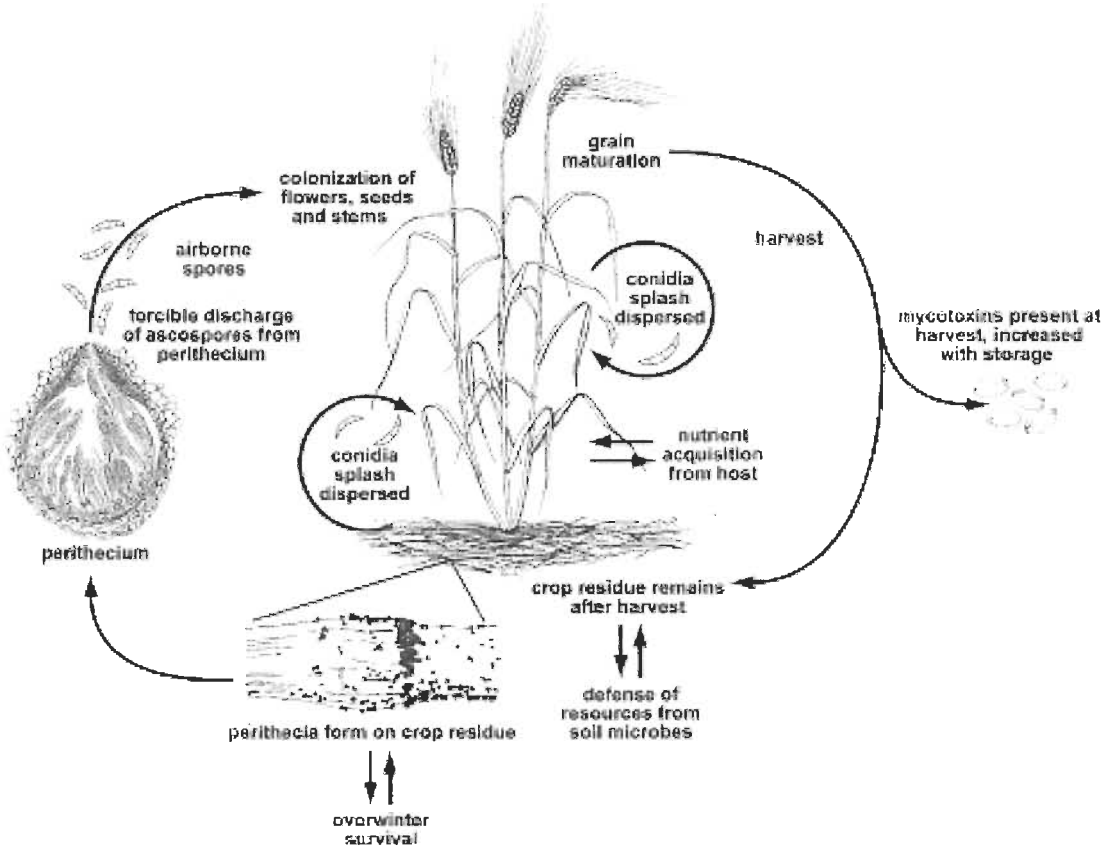

Figure 1: Infection cycle of *Fusarium* spp. in wheat (TRAIL 2009).

Infection of wheat florets takes place during flowering. Flowering morphology could therefore play a crucial role during the early phase of pathogen infection and establishment. But especially open florets (open flowering = chasmogamous, CH) seem to be important and provide good opportunities for infection compared to closed florets (closed flowering = cleistogamous, CL) (TRAIL 2009). Therefore, wheat floral compounds, like choline acetate and glycinebetaine, were first hypothesized to enhance infection but turned out to have no effect on the fungal growth (ENGLE 2004). However, the extent of AE or its mirror anther retention inside the florets has long been suggested as one major resistance mechanism, but a not well investigated one until now, could neither be negated nor yet proven. Therefore further investigations and experiments are still needed to verify the inheritance between AE and FHB (SKINNES et al. 2010). For example GILSINGER et al. (2005) could confirm that wider flower opening tend to stay open longer and therefore narrow opening flowering lines are able to reduce or even escape infection and so decrease FHB severity. KUBO et al. (2010) also approved such a result, namely that CL plants show better resistance to FHB infection in wheat. SKINNES et al. (2008) and LU et al. (2012) write that screening and selecting plants with low AE could also be a method for low FHB. This is also because of the advantageous fact that the toxin DON correlates with AE. However, KANG and BUCHENAUER (2000) write that plants could also be infected without anthers and that resistant cultivars are able to develop certain defence reactions against FHB and DON.

Moreover, YOSHIDA and NAKAJIMA (2010) write about the not ignorable late stages (20 days after anthesis) of toxin production and contamination with DON and NIV besides the early contamination at flowering time.

2.1.4 Symptoms

Field symptoms of FHB (Figure 2A) start as little brownish dots on the spikelet which get bigger and also increase with time, lead to prematurely whitened or bleached spikelet and also whole ear infections (whilting). The florets can also discolour pinkish in highly infected heads.

FHB symptoms on kernels (Figure 2B) are scabby kernels with orange to pinkish discolorations and a chalky appearance which look like bleached out and very often do not have their usual and shiny colour (MCMULLEN 1997).

Figure 2: Field symptoms of FHB on the spikelet (A) and on harvested grains (B).

2.1.5 Mycotoxins

Although FHB causes problems in agronomy, the most important factors are the consequences for health and economy with toxic contamination. The main problematic toxins are trichothecenes, fumonisins and zearalenone (Figure 3 (c) and (d)).

Table 1: *Fusarium* species, known sexual state (teleomorph), primary agronomic hosts, endemic regions, and major mycotoxins known to be produced GLENN (2007).

***Fusarium* species**	**Teleomorph**	**Hosts of primary concern**	**Region**	**Mycotoxin**
F. graminearum	*Gibberella zeae*	maize; small grains	worldwide	DON, ZON, NIV,FUS
F. sporotrichioides	unknown	small grains	worldwide	T2, HT2, BEA

2.1.6 Trichothecenes

Compared to all Fusarium toxins, trichothecenes are the most dangerous substances which cause chronic and fatal toxicoses of humans and animals. Generally *Fusarium* spp. produces more than 40 naturally occurring trichothecenes. Each of them can be produced by more than one *Fusarium* spp. and they can produce more than one trichothecene. Especially *F. graminearum* and its related species *F. crookwellense, F. culmorum, F. pseudograminearum* and *F. sporotrichioides* produce trichothecenes, but *F. graminearum* and *F. sporotrichioides* are the two model organisms for scientific trichothecene research. It also has to be noted that different isolates of the same species or region can vary

tremendously in their mycotoxin production and make the results of toxin related papers and studies of FHB interesting but difficult (KOKKONEN et al. 2010). They can occur in a wide variety of foods and feeds but the most important sources are cereal grains like wheat, corn and barley.

Fusarium trichothecenes are tricyclic sesquiterpenes, have a double bond between C- 9 and 10, for its toxicity a usual epoxide ring at C- 12 and C- 13 and are also called simple or nonmacrocyclic trichothecenes.

There are two groups: type A and type B (Figure 3 (b) and (c)) and the difference between them is a keto group at the C-8 position. The most important trichothecenes of type A are diacetoxyscirpenol (anguidine), beauverin, T-2 toxin (T2) or HT-2 toxin (HT2) and of type B nivalenol (NIV), DON (vomitoxin) or acetylnivalenol (Fusarenone - X), which have the keto group at the C-8 position. Type A trichothecenes, especially diacetoxyscirpenol and T2 toxin, are mainly produced by *F. sporotrichioides*. *F. graminearum* produces the much more aggressive DON, which predominates North and South America and Europe as well as the more toxic NIV of type B, which is more frequent in Europe and Asia (DESJARDINS 2006, HOREVAJ 2011, MESTERHAZY 2002).

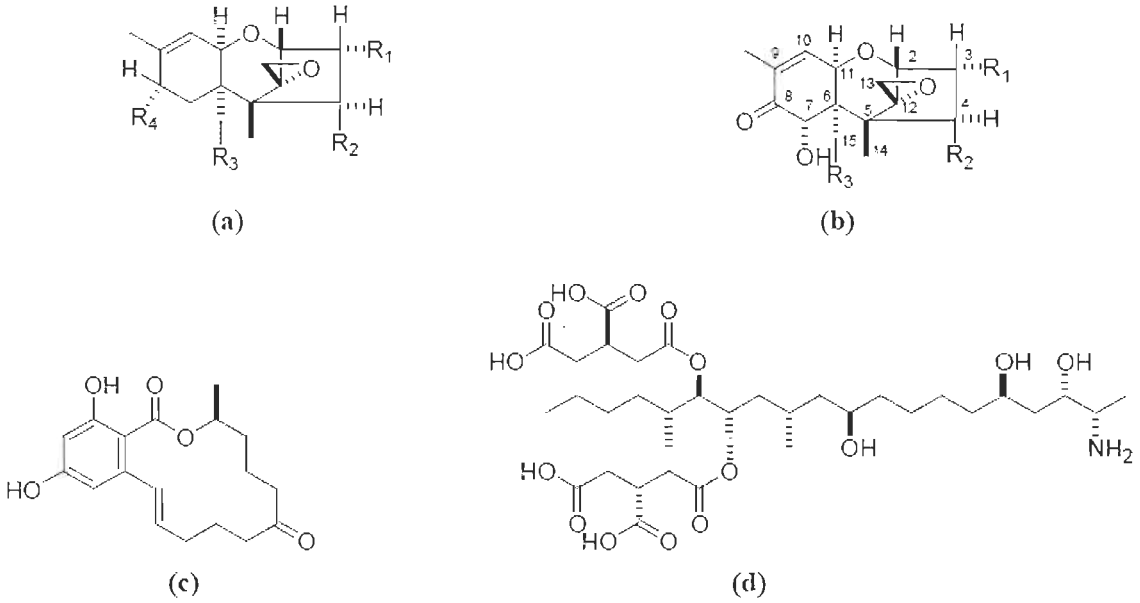

Figure 3: Chemical structures of major Fusarium mycotoxins. (a) type A trhichothecenes (b) type B trichothecenes (c) zearalenone (ZON) (d) fumonisin B1 (KUSHIRO 2008).

2.1.7 Deoxynivalenol

DON with the molecular formula $C_{15}H_{20}O_6$ can be produced by the FHB important fungi *F. graminearum* and *F. culmorum* and is the major and prevalent mycotoxin in scabby cereals. There is a direct relation between contamination with DON and the incidence of FHB. The susceptibility of cultivars, crop rotation, tillage and fungicide use affects the contents of DON significantly. Early

infected kernels show the highest DON, however also little mycelium can be expressed highly and therefore, even small infections have the potential for big DON amounts in little mycelium (HALLEN-ADAMS et al. 2011). LEMMENS et al. (2005) showed the ability to degrade and detoxify DON after production.

DON is a very stable compound and so thus, the regulation of the European Commission (see above) for foods and feeds exists. Although DON is reduced during processes like milling, because of the removal of bran and shorts, some sources write that it is stable after baking or even increases. This shows its heat stability, depending on temperature and time of cooking, pH, additives and other factors. DON is also highly water soluble and is reduced in boiling water. However, the main DON metabolite in wheat, Deoxynivalenol-3-β-D-glucopyranoside (D3G), remains stable through fermentation but decreases in baked breads (KUSHIRO 2008; SIMSEK et al. 2012).

Zearalenones are not correlated with any toxicoses in humans and animals and are no steroidal estrogenic toxins. They belong to a large family of metabolites and are structurally closely related to antibiotic metabolites (GLENN 2007).

2.2 Control of FHB

Controlling FHB is difficult and the methods are limited.

2.2.1 Chemical and biological methods

Registered wheat varieties often do not have the best resistance to FHB. This is because FHB is not such a big aim for breeders, but in epidemic years this can be a problem and then, only with the help of fungicides, it is possible to decrease the severity to an acceptable level and control the contamination with mycotoxins. However, even this method of controlling FHB has different success because wrong application, wrong timing and tricky environmental conditions need to be considered. Tebuconazole (Falcon and Folicur) is the best substance on the market right now. But when mistakes in application occur, even the good fungicides fail to protect from diseases, so spraying technology and the timing of application are very important. The heads should be covered on all sides with fungicide by using twin nozzles and the earliest time for applying is after the emergence of all heads. SZEGED (2008) could decrease FHB symptoms even by 80% with using Turbo Flood Jet nozzles and Prosaro fungicide which contains the substances tebuconazole and prothionocazole. Unfortunately, especially the efficacy of fungicides is not very high or shows different results and new ones are needed to be developed. Furthermore, the combination of fungicides is important to reduce the risk of developing resistant *Fusarium* lines (MESTERHAZY 2003).

There are also some methods of biological control with significant potentials. Some special species of yeast and bacteria which form endospores are used as antagonists. They use typical biocontrol

mechanisms like antibiosis, competition, parasitism, induced resistance and or inhibition of toxin synthesis for biological control of FHB, but further research and scientific development is still needed (CORIO DA LUZ 2003).

2.2.2 Agronomy methods

Although it is possible that agronomy methods are not fully effective, different agronomy methods exist. These methods are used to limit FHB contamination in crops which mainly need to stop an infection from crop residues. They are also needed because the available fungicides are not fully developed yet to control FHB and its mycotoxins. However, no tillage practice with plenty of unburied residues of the crops is worst and even increases FHB contamination. It is better to combine two or more agronomy methods in an integrated multiple system which favours a better control of DON contamination and including fungicides reduces FHB even more. Soil turning with ploughing and crop rotation with an advantageous preceding crop with a low amount of crop residues (best pre - crops of wheat would be any non - cereal like canola, potato, etc.) are very efficient management methods in agronomy and a key for reducing risks of severe damage. But choosing the right cultivar is compared to the best agronomy methods the easiest and most efficient way of controlling disease pressure (BLANDINO 2012; CHAMPEIL 2004).

2.2.3 Resistance

Choosing a good cultivar is the best way and important for preventing diseases in the field. Breeding varieties with FHB resistance is an obvious approach to minimize contaminations with toxins and also considered as the most effective, environmental and economic method to cope with this disease (LU et al. 2012).

But FHB was and still is not such a big topic and aim for breeders. The main reason for this might be that this disease does not appear naturally annually and depends on weather conditions. Therefore the variation in fields and between years makes it more difficult for breeding programs. Besides, there are different opinions about the relation between resistance to FHB and resistance to mycotoxin contamination because they are not always correlated. It is also controversial if vertical races of *Fusarium* spp. exist and the protection of breeding material would be needed, but most studies do not support this. However, aggressiveness and pathogenicity are most important for breeders because the ability to produce mycotoxins correlates closely with the aggressiveness and not the conidial productivity. But the cultivar's resistance also influences DON contamination very much. Resistant cultures have low mycotoxin contamination and vica verse. (BOUTIGNY et al. 2008, MESTERHAZY et al. 1999, MESTERHAZY 2002, MESTERHAZY 2003, MESTERHAZY et al. 2005).

Resistance is a complicated and still unclear factor and scientists need to put more effort into it. Present knowledge is that there are different active resistance types:

I. Resistance against the initial infection (SCHROEDER and CHRISTENSEN 1963);
II. Resistance against the spreading of the pathogen in the host tissue (SCHROEDER and CHRISTENSEN 1963);
III. Resistance against the kernel infection (MESTERHAZY 1995);
IV. Tolerating FHB and infection (MESTERHAZY 1995);
V. Resistance against toxins (MILLER et al. 1985).

Passive resistance is the independence of the plant's physiological status, or the morphological properties. One such mechanism is plant height which shows that as the height of plants increases, FHB decreases independently to active resistance (HILTON et al. 1999, LU et al. 2012). Small plants show an increased susceptibility to FHB which is also correlated to the *Rht-D1b* allele. That shows the need of at least two strong effecting genes to balance the negative effect of *Rht-D1b* to FHB (LU et al. 2011).

Another passive mechanism is the composition of wheat floral compounds. Choline acetate and glycinebetaine was thought to be stimulating for the growth of fungi but this did not turn out to be true (ENGLE 2004). The open or narrow flower opening in wheat is also such a hypothesized mechanism for future breeding research (GILSINGER et al. 2004).

Today, there are some known resistant genotypes (mainly spring wheat like Sumai 3 or Frontana) around the world and also some low susceptible cultivars (MESTERHAZY 2003). Results also have shown that it is possible to reduce the DON content and increase FHB resistance by selection starting in the F3 generation. The advantage of this would be to avoid using exotic resistant sources with genomic bottlenecks and by the way breeding to other diseases, yield, quality and other wanted features with a broaden genetic diversity (MESTERHAZY 2003, MIEDANER et al. 2003).

The biggest problem is to combine resistances of several diseases with other important quality traits and agronomic parameters like yield, protein, etc., and an intense and continuous work of breeders is required so that farmers can use such genotypes.

3 Material and methods

3.1 Plant material

An assortment of 96 genotypes was used for this experiment and tested for its resistance to FHB under field conditions. The genotypes were international but mainly European (French, Hungarian and Austrian) cultivars and lines which were either registered ones, local cultivars or selected experimental lines of companies and breeders which were still in progress. A wide range included some highly resistant and some very susceptible ones, some older, native, and also very new ones and some organic and conventional ones. All these genotypes of winter and one durum wheat are listed in Attachment 3.

3.2 Site of the field trial

The experiment was implemented from fall 2011 until the summer of 2012 on the experimental fields of the University of Natural Resources and Life Sciences Vienna (BOKU), Department for Agrobiotechnology (IFA), Institute for Biotechnology in Plant Production in Tulln. Tulln is a city at the Danube in Lower Austria with Pannonian climate and lies 180m above sea level with an average temperature of about 11°C and a yearly precipitation of 650mm. The soil is a meadow - chernozem and the previous crop was maize.

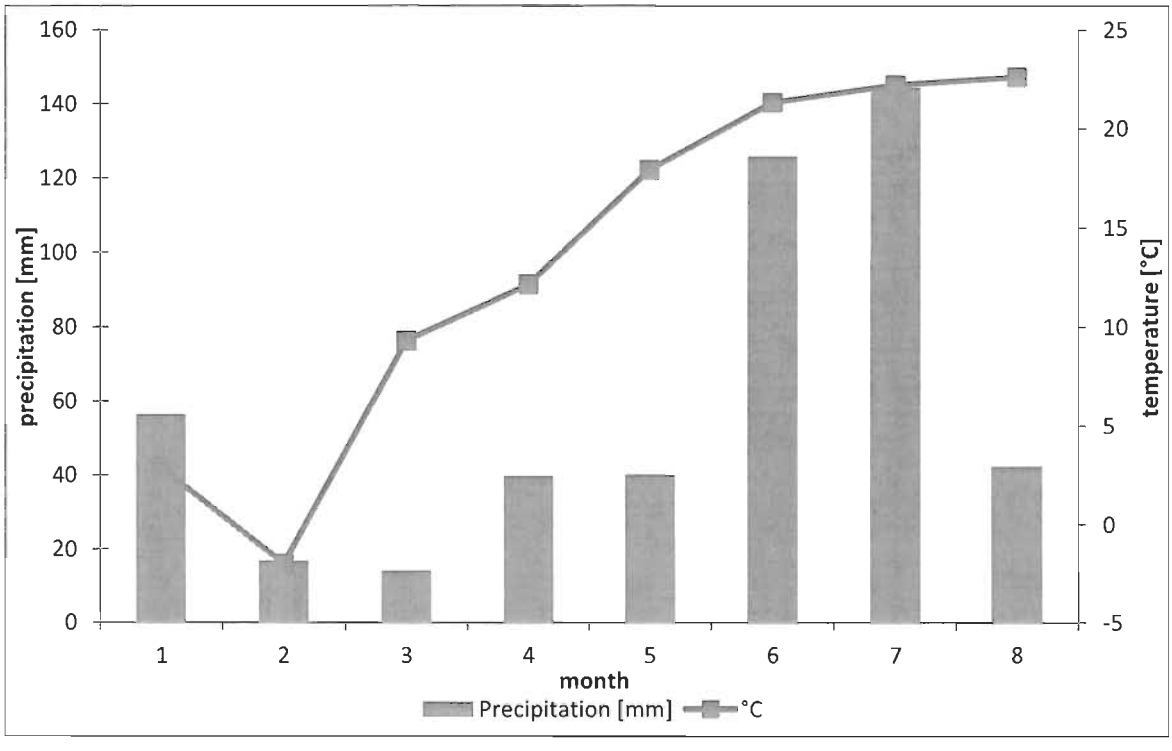

Figure 4: Mean monthly temperature [°C] and precipitation [mm] in Tulln from January until August 2012.

3.3 Experiment layout and agronomic measures

Three experiments were planted neighbouring each other. Each experiment was a randomized complete block design with two blocks. Experiment 1 was inoculated with *F. graminearum*, experiment 2 with *F. sporotrichioides* and experiment 3 with a mix of both species, which are abbreviated with *FG*, *FSP* and MIX in the following paragraphs, respectively. The sowing time was 17.11.2011. Each plot was a double row with a length of 60cm and a row spacing of 17cm in between the double rows and 33cm between them. One plot or double row so had 0,3m². 5g seed per double row were sown which is equivalent to about 170kg/ha. The seeds were treated with Celest Extra 050FS (2ml/kg) (Difenoconazol) and Gaucho 600FS (0,6ml/kg) (Imidacloprid) before sowing. Fertilizer was applied twice: on 03.04.2012 300kg/ha a mix of nitrogen, phosphorus, potassium and sulphur (NPK16:06:18+5S) and on 15.05.2012 200kg/ha of KAS with 27% of nitrogen. Herbicide was also applied twice: on 30.04.2012 with 1,5L/ha Andiamo Maxx (120 g/l Ioxynil, 120 g/l Bromoxynil and 360 g/l Mecoprop-P) and on 10.05.2012 with 1l/ha Puma Extra (69 g/l Fenoxaprop-P-ethyl and 75 g/l Mefenpyr-Diethyl as Safener). Insecticides or Fungicides were not used at any time.

The experiment was irrigated with a mist irrigation system from the 21.05.2012 to the 14.06.2012 every second day from 3 pm until 12 pm on the next day with a minimum break of 20 minutes and a misting duration of 10 seconds per irrigation cycle. The irrigation had a leaf - wetness sensor on the field and it turned on automatically when leaf - wetness dropped a pre - set value and minimum 20 minutes since the previous irrigation cycle. That means on dry days the irrigation was turned on about every 20 minutes during the day and at night about once per hour. So there were about 40 to 45 cycles of irrigation with an approximate amount of 5l/m² per day. With the irrigation system the plants were nearly always wet to secure a better environment for the fungi, especially a high humidity and plant wetness.

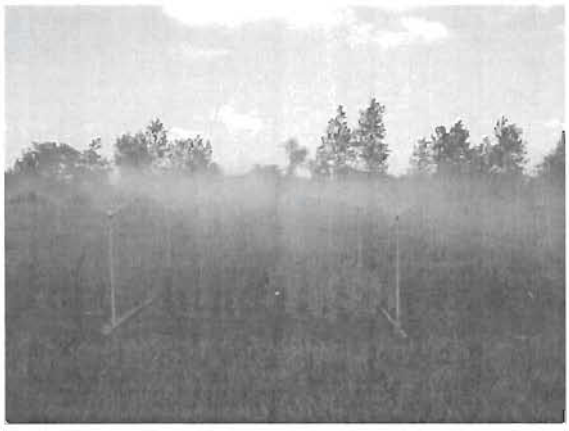

Figure 5: Mist irrigation device in action

3.4 Inoculum

Three different inocula were used: *F. graminearum, F. sporotrichioides* and a mix of both of them. For preparing the inoculum, 90g of *Vigna radiata* (mungbean) were cooked for 20-23 minutes (until the first ones broke), sieved, autoclaved and used as medium which was then inoculated with the wanted *Fusarium* spp. from prepared agar plates. For the exact production of the inoculum, see the attached Standard operating procedure (SOP 3-01) from the Department IFA - Tulln, Institute for

Biotechnology in Plant Production „Production of Inoculum of *Fusarium* spp. with Bubble Breeding" (Attachement 4).

For *F. graminearum* the isolate IFA 66 with a concentration of 20,000 conidias/ml was used, for *F. sporotrichioides* a mixture of the isolates IFA 198, 309, 475 and 488 with 40.000 conidias/ml and for the mix 10,000 conidias/ml of *F. graminearum* and 20,000 conidias/ml of *F. sporotrichioides*. The number of conidia was counted with a Bürker - Türk counting chamber, whereas the exact concentration was then made by adding water before spraying it on the field.

3.5 Inoculation

The inoculum was applied artificially by spray inoculation throughout the whole experiment. This was done with a small motorised crop sprayer for the back which had a volume of ten litres with three spray nozzles which reached over two double rows. For each of the three treatments (*FG, FSP* and MIX) the pre-prepared and frozen aliquots of the inocula were taken and mixed with ten liter of lukewarm tap water. The first inoculation started, when the first ears of the whole experiment were in flower, namely on May, 23rd. This was repeated every second day until at least two inoculations were done after the date of flowering and continued until June, 8th. Experiment 1 (*FG*) was inoculated with *F. graminearum*, experiment 2 (*FSP*) with *F. sporotrichioides* and experiment 3 (MIX) with the mix of both.

3.6 Disease assessment

The scoring or evaluation of kernel infection is important because FHB symptoms can be observed. However, this is only possible until the early stadiums of ripening. Later, scoring is more difficult because of the ripening and yellowing of the plants (heads) and scoring is also not that easy because of large differences. The scoring on symptoms of FHB started 10 days after blooming and was then done every fourth day, which means on day 10, 14, 18, 22, 26 and 30 after blooming. The scoring was done visually in each plot by scoring the percentage of infected spikelet, the severity, for type I and II resistance. Table 2 illustrates the applied scoring scheme.

Table 2: Used pattern for visual scoring of infected spikelet

% infected spikelet per plot	Symptom severity
0,1	at least one plant has symptoms
0,5	1 spikelet/10plants
1	1spikelet/5plants
5	1 spikelet/plant
10	2 spikelet/plant
15	3 spikelet/plant
20	4 spikelet/plant
25	5 spikelet/plant
30	6 spikelet/plant
35	7 spikelet/plant
40	8 spikelet/plant
45	9 spikelet/plant
50	10 spikelet/plant
55	11 spikelet/plant
60	12 spikelet/plant
65	13 spikelet/plant
70	14 spikelet/plant
75	15 spikelet/plant
80	16 spikelet/plant

Besides scoring FHB also the average height of the plants of each plot was measured. It was done by measuring from the ground to the top of the plant with a measuring stick excluding awns.

The date of flowering had to be determined for knowing when to start scoring for FHB because this was done 10 days afterwards. Wilting symptoms and homogeneity had to be established for possible effects on the later done toxin analysis and for knowing how the disease spreads in some seeds.

The probably most important determined factor for this thesis was the number of extruded anthers. Five ears and each time four spikelets from different plants of one plot were looked at, when they were ripe (white to light yellowish colour, but not green anymore) and counted how many anthers stayed inside the floret and retained, and how many were extruded. Anthers which were trapped between palea and lemma were also counted as retained. That means we scored a maximum of 20 florets per plot for anthers, either extruded or retained.

At ripening, all plots were harvested using a plot combine harvester (Wintersteiger Nursery Master Elite) set to low wind speed in order to retain also small and shrivelled seeds. A seed sample from each plot was filled into petri dishes and also scored, which gave us the percentage of Fusarium damaged kernels (%FDK), without looking at the strain or cultivar name to avoid favouring them and to get more objective results. The scoring of %FDKs was done visually by estimating the percentage

of diseased seeds which were either shrinked (shrivelled), bleached or had chlorosis, were smaller, and or had an unusual orange or pinkish - red colour.

Afterwards, two repetitions within each of the three experiments (*FG, FSP* and MIX) were mixed together and the toxins were analysed. This was done in a gas chromatography - mass spectrometer (GC - MS/ MS). DON, HT-2 - Toxin, T2 - Toxin and Zearalenone (µg/kg) were measured. Mycotoxin analysis was done by the service provider Capinov (Z.I. de Lanrinou, F-29206 LANDERNEAU Cedex, France, www.capinov.fr).

3.7 Statistical analysis

To compare the disease of the different genotypes better, AUDPC (=Area under disease progress curve) was calculated with the formula:

$$\text{AUDPC}= \sum_{i=1}^{n} [\frac{yi+ yi-1}{2} * (ti - ti - 1)]$$

n is the total amount of scalings;
yi is the grade of scaling

The analysed traits were: percent visually FHB damaged spikelets per plot in the field (%FHB), area under the disease progress curve over five visual field scores (AUDPC), plant height (PH), date of anthesis in days after May 1 (DAT), percent of extruded anthers (%AE), percent Fusarium damaged grains in the harvested samples (%FDK), content of toxins: dexynivalenol (DON), zearalenone (ZON), T2-toxin (T2) and HT-2 toxin (HT-2) in the harvested samples in µg/kg.

The values of the field and seed scorings were statistically analysed with the computer program R and R Commander, whereby the ANOVAS and the correlation coefficients were evaluated with Pearson product- moment correlation.

For the traits AUDPC the following linear model was applied:

$$x_{ijk} = \mu + r_i(e_j) + e_j + g_k + (eg)_{jk} + \varepsilon_{ijk}$$

Where x_{ijk} = the trait value observed for genotype (g) k in experiment (e) j and replication (r) i within experiment j.

μ = overall mean
$r_i(e_j)$ = effect of replication i nested within experiment j
e_j = effect of experiment j
g_k = effect of genotype k
$(eg)_{jk}$ = interaction of genotype k within experiment j
ε_{ijk} = random error term

All effects apart from ε_{ijk} were considered fixed.

For the traits DAT, PH and %AE the following simplified model was applied:

$$x_{ik} = \mu + r_i + g_k + \varepsilon_{ik}$$

Where x_{ik} = the trait value observed for genotype (k) in experiment (e) j and replication (r) i.

μ = overall mean
r_i = effect of replication i
g_k = effect of genotype k
ε_{ik} = random error term

All effects apart ε_{ik} from were considered fixed.

Correlations coefficients and scatterplots were calculated in R, histograms were drawn in Excel 2010.

4 Results

4.1 Field and laboratory observations for FHB severity

4.1.1 FHB severity for different *Fusarium* inoculation variants

Table 3 shows the average %FHB severity, AUDPC and %FDK across all tested lines of all three inoculation variants or experiments: *FG, FSP* and *MIX.* This table and especially the following Figure 6 show that all three used isolates led to FHB symptoms on wheat heads and visualize Fusarium damaged grains. Inoculation with *F. graminearum* alone caused the strongest average disease severity, followed by the mixed inoculation experiment. *F. sporotrichioides* alone led to much lower FHB severity.

Table 3: Means, minimum and maximum values of the three single experiments *FG, FSP* and MIX for %FHB, AUDPC and %FDK.

Trait	Experiment	Population mean	Population minimum	Population maximum
% FHB	*FG*	8,0	0,0	44,7
% FHB	*FSP*	3,4	0,0	23,3
% FHB	MIX	6,2	0,0	26,5
AUDPC	*FG*	191,7	0,5	1088,8
AUDPC	*FSP*	81,6	0,0	570,0
AUDPC	MIX	149,7	0,0	636,4
% FDK	*FG*	31,4	1,0	99,0
% FDK	*FSP*	16,8	1,8	90,0
% FDK	MIX	27,0	1,8	100,0

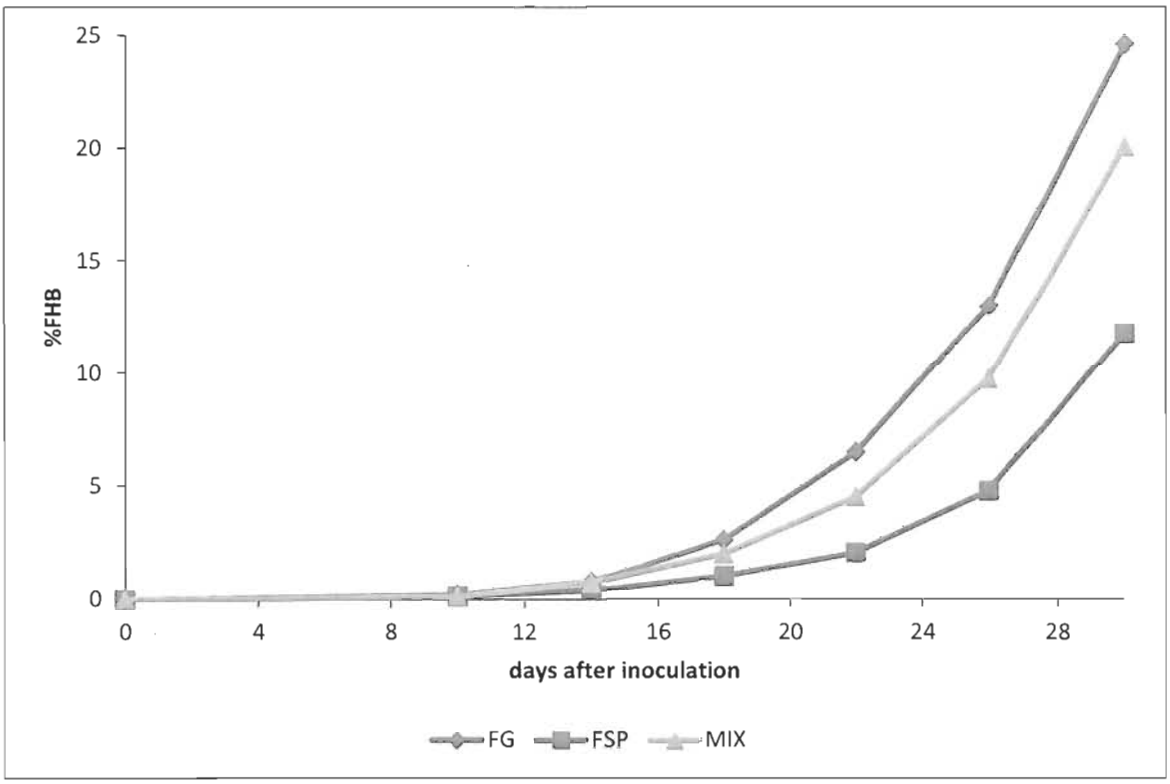

Figure 6: %FHB means 10, 14, 18, 22, 26 and 30 days after spray inoculation of the three experiments *FG, FSP* and MIX.

4.1.2 FHB severity on the tested wheat genotypes inoculated with different *Fusarium* isolates

All used isolates caused successful infection of the tested wheat lines. As an example, the disease progress curves measured by %FHB of four older and widely grown French cultivars with the three inoculation variants, also called experiments (*FG*, *FSP*, and the MIX), are illustrated in Figure 7 (A,B,C). As examples, the very susceptible cultivar "Charger", medium susceptible genotypes "Isengrain" and "Renan" and the resistant "Soissons" are shown. While for the *Fusarium* resistant cultivar "Renan" the maximum FHB severity 30 days after inoculation in the field was 0.5%FHB and 16 %FDK, the susceptible cultivar "Charger" reached nearly 70 %FHB severity and 37 %FDK.

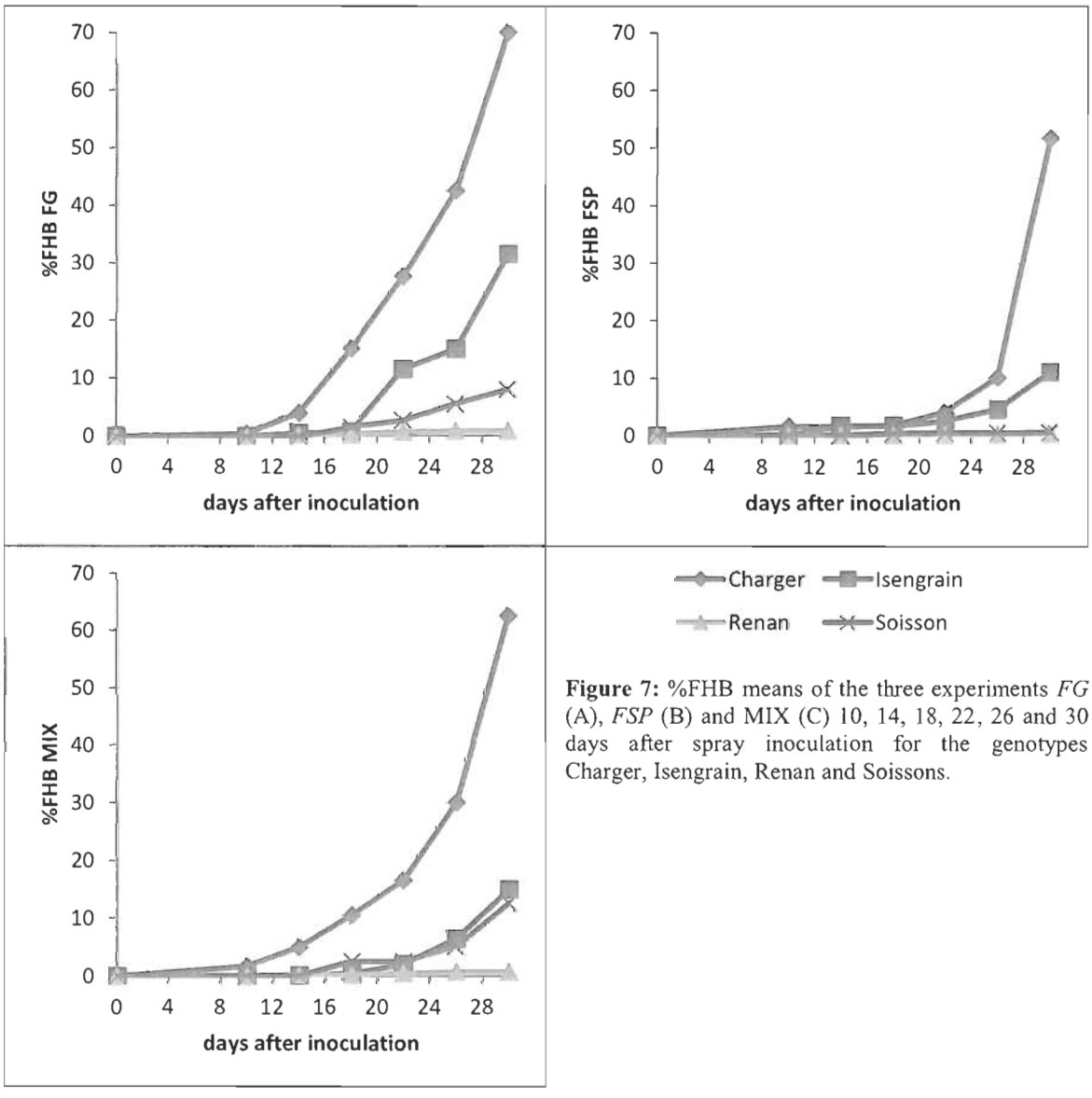

Figure 7: %FHB means of the three experiments *FG* (A), *FSP* (B) and MIX (C) 10, 14, 18, 22, 26 and 30 days after spray inoculation for the genotypes Charger, Isengrain, Renan and Soissons.

The genotypes indicated different variations of diseased spikelets for AUDPC. In Figure 8 the values for AUDPC of all tested observations are shown for each of the three following experiments.

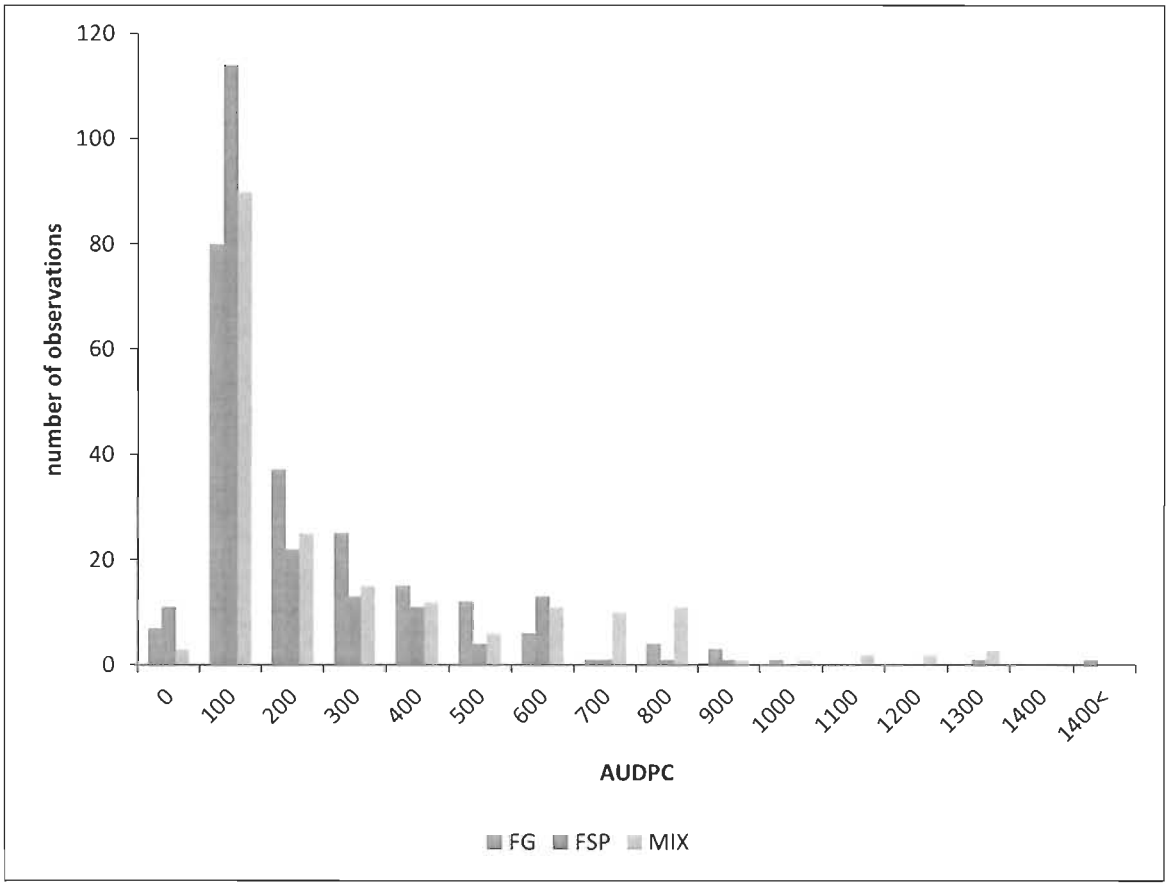

Figure 8: Histogram of AUDPC over all observations for the three experiments *FG, FSP* and MIX.

Analyses of Variance (ANOVA) was calculated for the trait AUDPC and is shown in Table 4. Normality, a requirement of the ANOVA, was tested with Shapiro - Wilk normality test. Experiments, genotypes and their interaction are highly significant, however, replications nested within experiments, are not significant.

Table 4: ANOVA for FHB severity measured by AUDPC.

Source	d.f.	SS	MS	F	Pr(>F)
Replication (Experiment)	3	32293	10764	1.30	0.27
Experiment	2	1185303	592651	71.85	<0.001
Genotype	95	13651746	143703	17.42	<0.001
Experiment*Genotype	190	2587640	13619	1.65	<0.001
Residuals	285	2350978	8249		

Table 5 exhibits Pearson correlation coefficients among the AUDPC and %FDK values for the three experiments *FG, FP* and the MIX. Correlations of AUDPC values between experiments were highly significant, somewhat lower for %FDK.

Table 5: Pearson correlation coefficients with p - values in brackets for AUDPC and %FDK for the experiments *FG, FSP* and MIX.

	AUDPC_FG	AUDPC_FSP	AUDPC_MIX	%FDK_FG	%FDK_FSP
AUDPC_FSP	0.81 (<0.001)				
AUDPC_MIX	0.84 (<0.001)	0.83 (<0.001)			
%FDK_FG	0.68 (0.986)	0.67 (<0.001)	0.71 (0.283)		
%FDK_FSP	0.55 (<0.001)	0.68 (<0.001)	0.58 (<0.001)	0.64 (0.960)	
%FDK_MIX	0.70 (<0.001)	0.73 (<0.001)	0.78 (<0.001)	0.77 (0.811)	0.68 (<0.001)

The next three Tables (6, 7 and 8) demonstrate the results for the ANOVAs of the AUDPC of each single experiment: *FG, FSP* and MIX. They all show that the genotypes are highly significant, but in the *FG* and *FSP* experiment there is no significant effect of replication.

Table 6: ANOVA for the experiment with *FG* measured by AUDPC.

Source	d.f.	SS	MS	F	Pr(>F)
Replication	1	382	382	0.03	0.86
Genotype	95	8112749	85397	6.98	<0.001
Residuals	95	1162928	12241		

Table 7: ANOVA for the experiment with *FSP* measured by AUDPC.

Source	d.f.	SS	MS	F	Pr(>F)
Replication	1	187	187.2	0.0425	0.84
Genotype	95	2422551	25500.5	5.79	<0.001
Residuals	95	418305	4403.2		

Table 8: ANOVA for the experiment with the MIX measured by AUDPC.

Source	d.f.	SS	MS	F	Pr(>F)
Replication	1	31724	31724	3.90	0.05
Genotype	95	5700352	60004	7.39	< 0.001
Residuals	95	773478	8142		

Examples for the significant positive correlations of the AUDPC values of wheat genotypes between the three experiments are shown in Figure 9 (A, B, C).

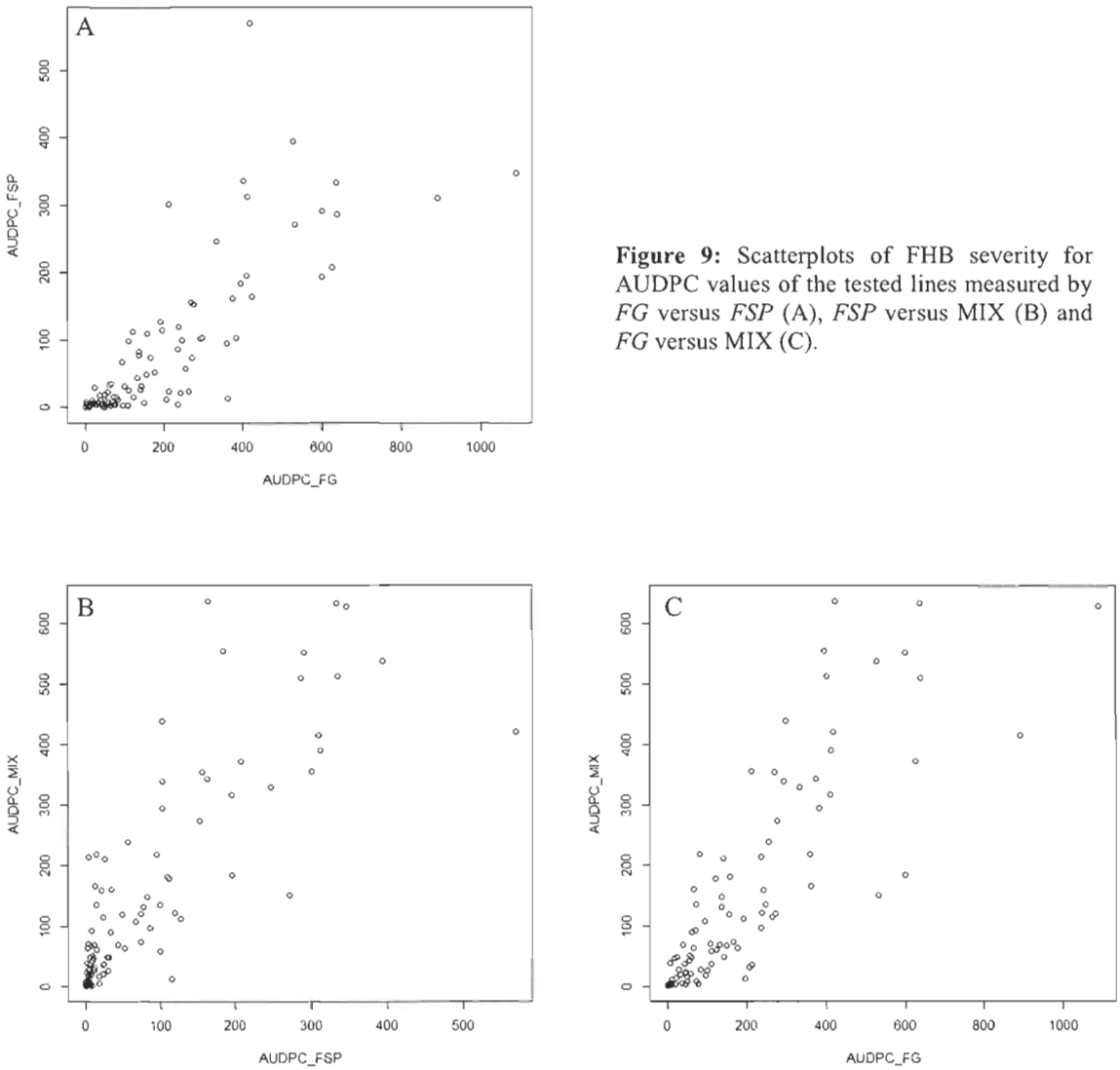

Figure 9: Scatterplots of FHB severity for AUDPC values of the tested lines measured by *FG* versus *FSP* (A), *FSP* versus MIX (B) and *FG* versus MIX (C).

4.2 Variation for developmental and morphological traits

Genetic variation was quite high among the tested genotypes. Means, minimum and maximum values are given for the traits plant height (PH), anthesis date (DAT) and percentage of anther extrusion (%AE) in Table 9.

Table 9: Means, minimum and maximum values for PH (cm), DAT (number of days after 1st of May) and %AE of all genotypes.

Trait	Population mean	Population minimum	Population maximum
PH	71	53	115
DAT	27.3	21	35
%AE	64.4	0	100

4.2.1 Anthesis date

Figure 10 indicates the mean values for DAT among the 96 genotypes shown in a frequency distribution diagram. It points out the big differences of the genotypes and its variations because the DAT varied in a time of two weeks.

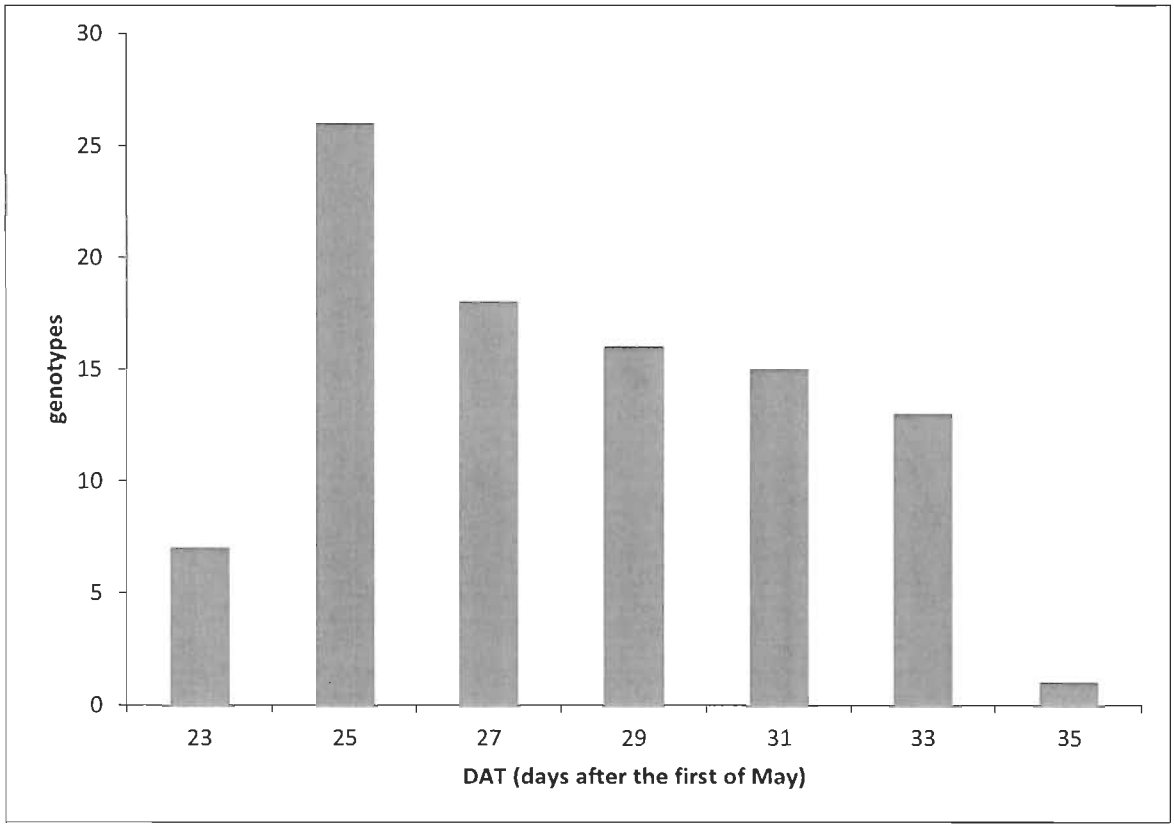

Figure 10: Frequency distribution of mean values for anthesis date (DAT in days after 1st of May) among 96 genotypes.

The ANOVA results for the trait DAT in Table 10 indicate that the genotype and the replication are highly significant.

Table 10: ANOVA for the trait flowering date in days after the first of May (DAT) across 6 replications.

Source	d.f.	SS	MS	F	Pr(>F)
Replication	5	26.23	5.25	4.22	0.0001
Genotype	95	5375.3	56.58	44.10	<0.001
Residuals	479	614.6	1.283		

4.2.2 Plant height

Figure 11 exhibits the frequency distribution for PH among the 96 genotypes. It shows the strong varying PHs with values ranging from 60 to more than 95cm.

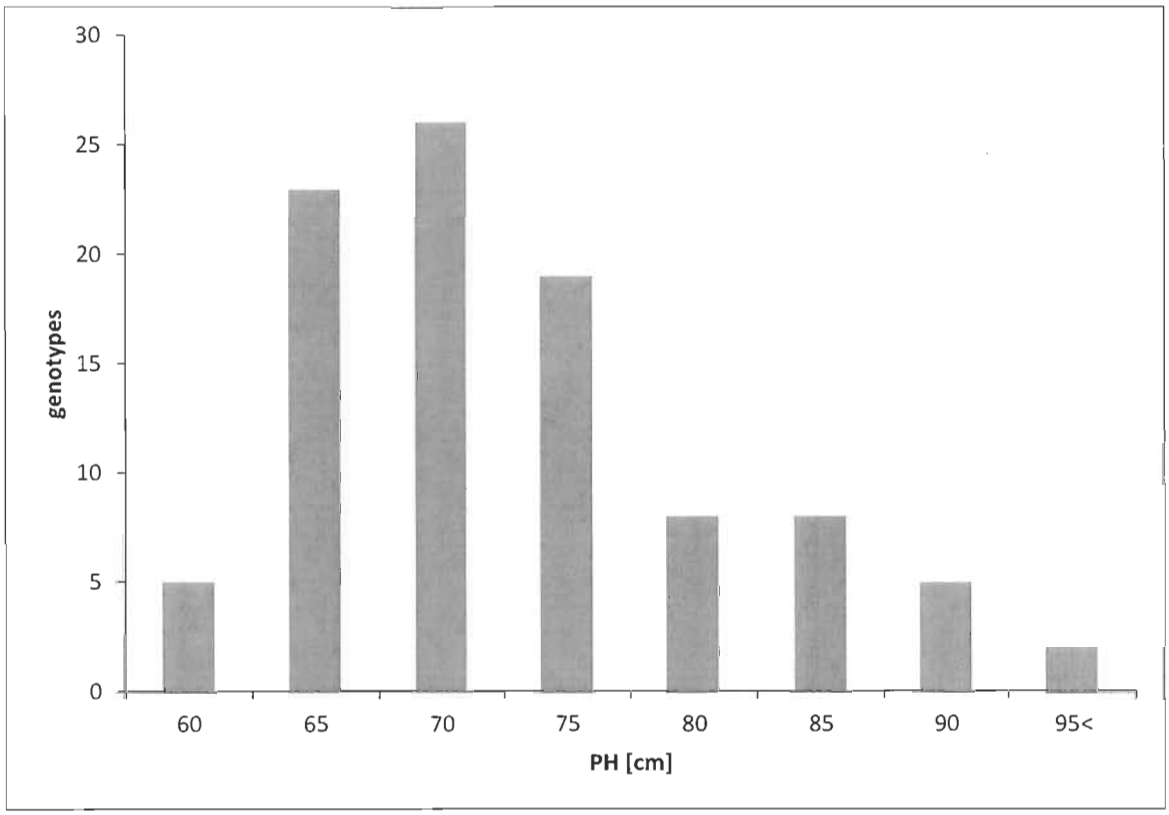

Figure 11: Frequency distribution of mean values for plant height (PH in cm) among 96 genotypes.

Table 11 shows the results of the ANOVA with the parameter PH. They indicate a significant difference between the genotypes and the factor replication.

Table 11: ANOVA for the trait plant height in cm (PH) across 6 replications.

Source	d.f.	SS	MS	F	Pr(>F)
Replication	5	436.81	87.36	6.38	<0.001
Genotype	95	45514	479.09	33.66	< 0.001
Residuals	479	6818	14.23		

4.2.3 Anther extrusion

Figure 12 shows the frequency distribution of %AE among the 96 genotypes. It shows the different amounts of extruded anthers and that a lot of genotypes extrude 100 percent of their anthers.

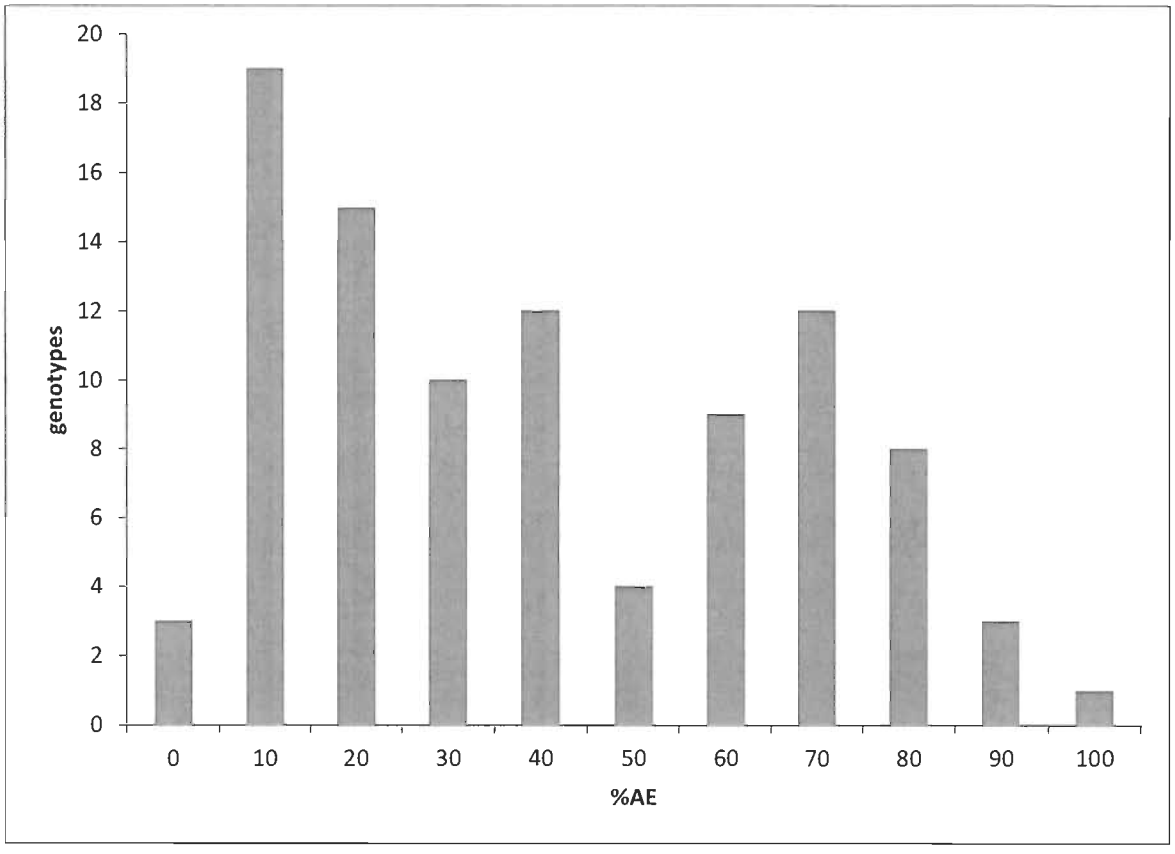

Figure 12: Frequency distribution of mean values for anther extrusion (AE in percent) among 96 genotypes.

ANOVA results for the parameter %AE (Table 12) indicate a significant difference between the genotypes, however none with replication.

Table 12: ANOVA for the trait anther extrusion in percent (%AE) across 6 replications.

Source	d.f.	SS	MS	F	Pr(>F)
Replication	5	12.54	2.51	1.19	0.31
Genotype	95	4096.79	43.124	20.17	<0.001
Residuals	479	102413	213.8		

4.3 Correlation coefficients and scatterplots for developmental and morphological traits

Table 13 displays correlation coefficients between the total means of the morphological traits %AE, DAT and PH, and the FHB traits AUDPC and %FDK of the experiments *FG, FSP* and MIX. Interesting and noticeable are the high negative correlations between %AE, AUDPC and %FDK and moderate high correlations between AUDPC, DAT and PH.

Table 133: Pearson correlation coefficients with p - values in brackets among %AE, DAT, PH, AUDPC and %FDK for the three experiments *FG, FSP* and MIX. Correlations of %AE, DAT and PH are for total mean values.

	%AE	DAT	PH
AUDPC *FG*	-0.64 (<0.001)	0.39 (<0.001)	-0.29 (0.005)
AUDPC *FSP*	-0.59 (<0.001)	0.30 (0.003)	-0.30 (0.003)
AUDPC MIX	-0.63 (<0.001)	0.40 (<0.001)	-0.32 (0.001)
%FDK *FG*	-0.54 (<0.001)	0.27 (0.008)	-0.42 (<0.001)
%FDK *FSP*	-0.37 (0.002)	0.20 (0.052)	-0.36 (0.001)
%FDK MIX	-0.55 (<0.001)	0.30 (0.003)	-0.37 (0.001)

In the next scatterplots (Figure 13, 14 and 15) the total mean morphological characteristics DAT, PH and %AE are compared with the AUDPC of the experiments *FG*, *FSP* and MIX.

Between total mean DAT and AUDPC of the three experiments there is a moderate positive correlation (Figure 13 A, B, C).

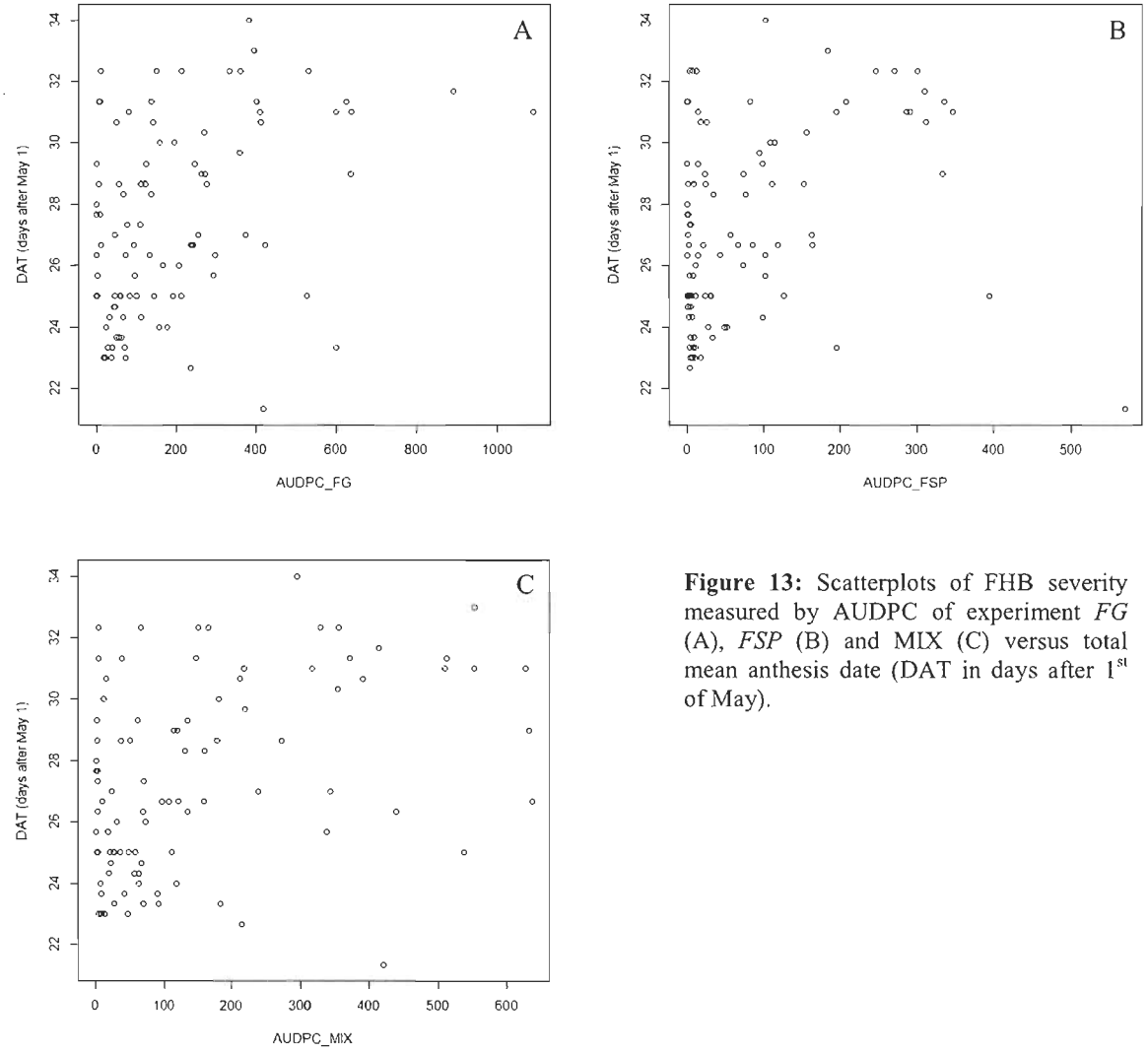

Figure 13: Scatterplots of FHB severity measured by AUDPC of experiment *FG* (A), *FSP* (B) and MIX (C) versus total mean anthesis date (DAT in days after 1st of May).

Moderate negative correlation was found between the experiments *FG*, *FSP* and MIX of AUDPC compared with total mean PH. This shows the tendency of high plants to be less susceptible to FHB. However, r - values were quite low. This is featured in Figure 14 (A, B, C).

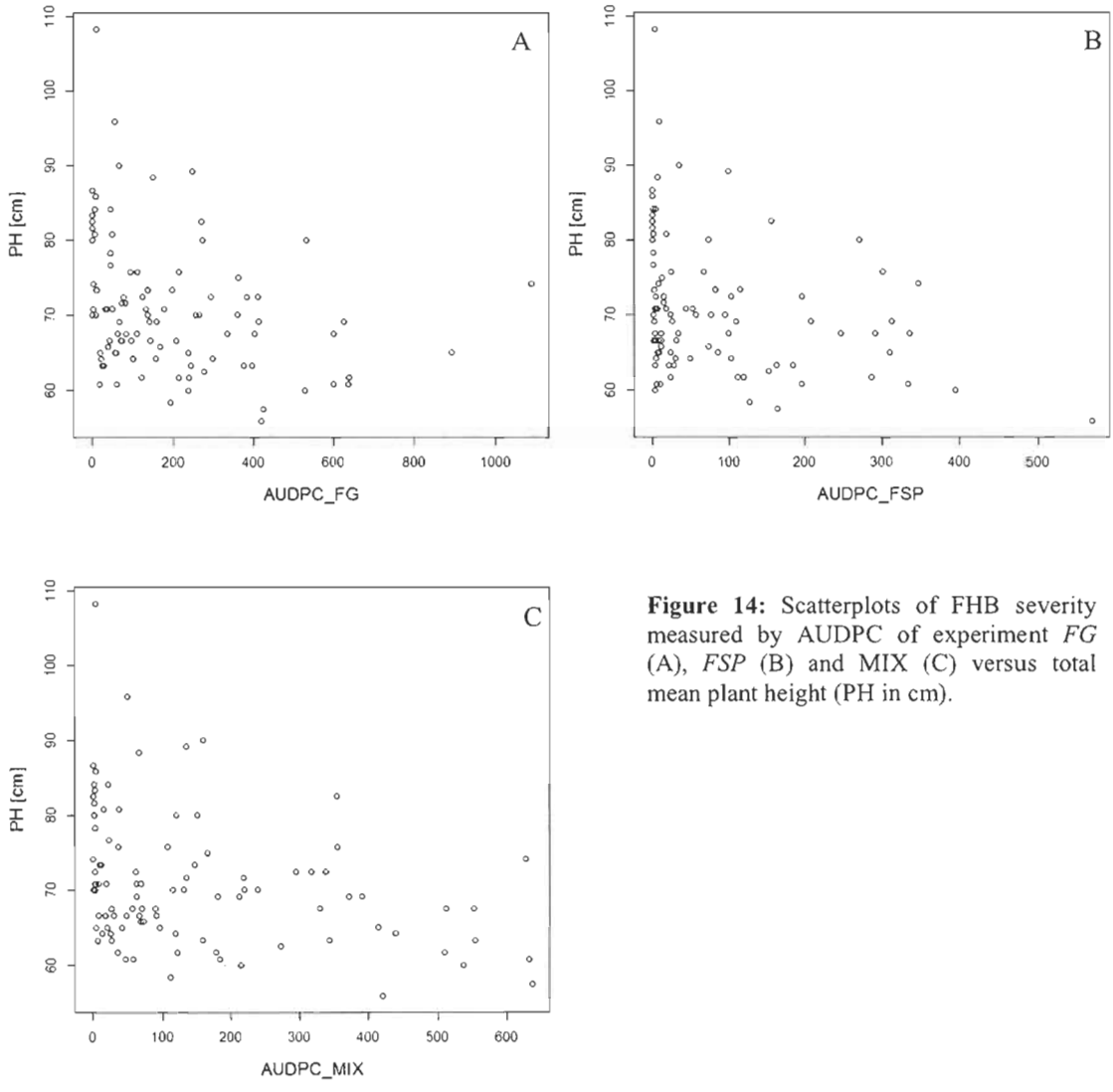

Figure 14: Scatterplots of FHB severity measured by AUDPC of experiment *FG* (A), *FSP* (B) and MIX (C) versus total mean plant height (PH in cm).

Correlations of AUDPC experiments *FG*, *FSP* and MIX versus total mean %AE is indicated significantly negative in Figure 15 and points out that genotypes with high AE tend to have a lower susceptibility to FHB.

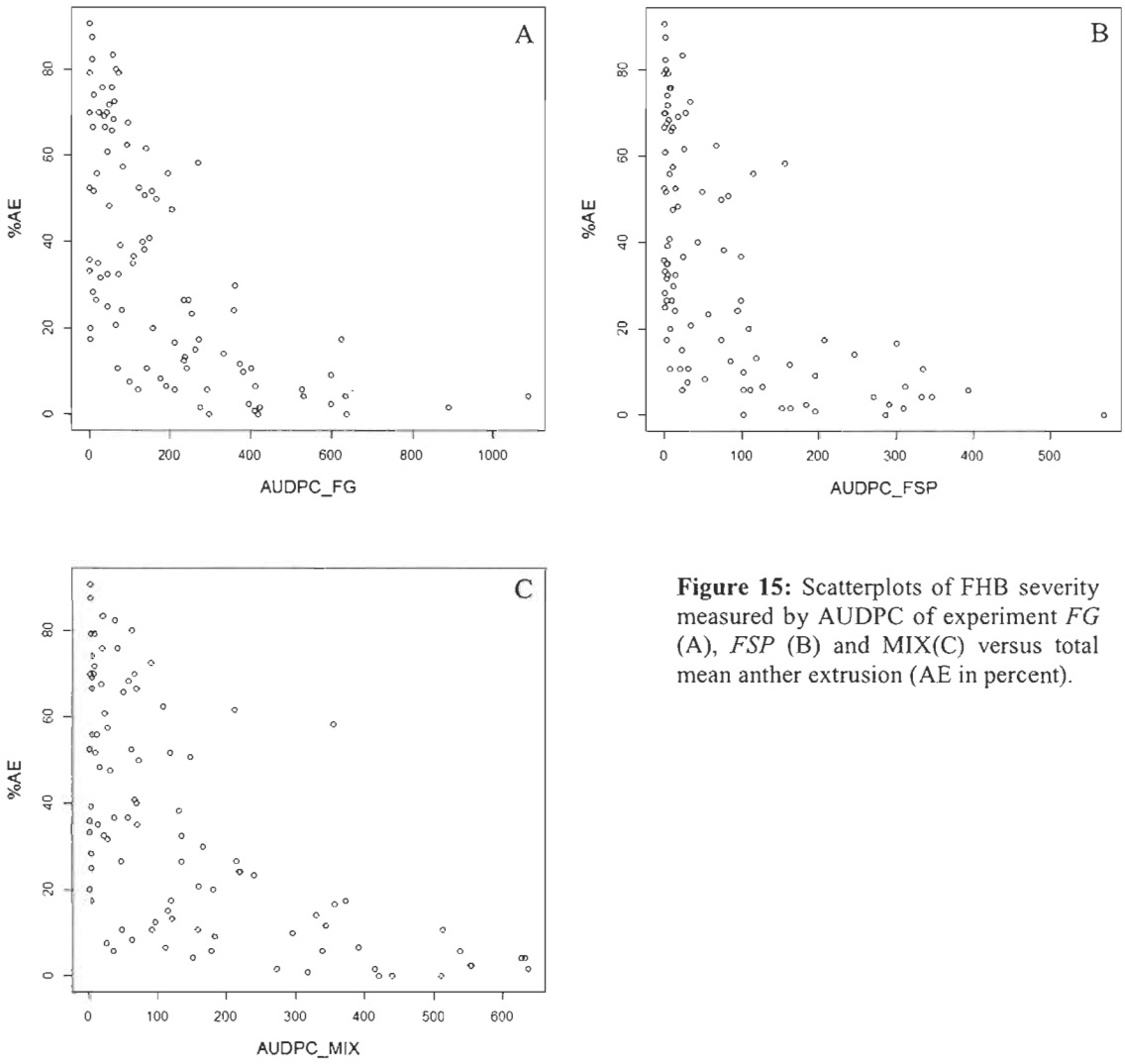

Figure 15: Scatterplots of FHB severity measured by AUDPC of experiment *FG* (A), *FSP* (B) and MIX(C) versus total mean anther extrusion (AE in percent).

4.4 Mycotoxin content in the harvested samples

Means, minimum and maximum toxin contents (µg/kg) of the three experiments *FG, FSP* and MIX are given in Table 14. Unexpectedly relatively high values for DON and ZON were found in the experiment of *FSP*.

Table 14: Means, minimum and maximum toxin contents (µg/kg) of the toxins DON, HT-2, T2 and ZON for the experiments *FG, FSP* and MIX.

Toxin (µg/kg)	Experiment	Population mean	Population minimum	Population maximum
DON	*FG*	8325	442	21800
HT-2	*FG*	- *	- *	- *
T2	*FG*	- *	- *	- *
ZON	*FG*	1472	28	6350
DON	*FSP*	2234	203	9060
HT-2	*FSP*	500	45	5280
T2	*FSP*	80	- *	1130
ZON	*FSP*	206	- *	1080
DON	MIX	6498	1210	14800
HT-2	MIX	234	26	929
T2	MIX	37	0	208
ZON	MIX	1138	33	5560

* below limit of detection (<10µg/kg)

Table 15 lists the correlation coefficients between AUDPC and %FDK versus the toxins (DON, HT-2, T2 and ZON) of the three single experiments (*FG, FSP* and MIX). Significance is given in the high AUDPC and %FDK results of *FG, FSP* and MIX. Interesting are the back - ground infections of the three used inoculation variants.

Table 15: Pearson correlation coefficients with p - values in brackets of AUPDC and %FDK for the experiments *FG, FSP* and MIX among the toxins DON, HT-2, T2 and ZON.

	DON	HT2	T2	ZON
AUDPC *FG*	0.62 (<0.001)	-	-	0.68 (<0.001)
AUDPC *FSP*	0.51 (<0.001)	0.68 (<0.001)	0.62 (<0.001)	0.68 (<0.001)
AUDPC MIX	0.71 (<0.001)	0.68 (<0.001)	0.60 (<0.001)	0.59 (<0.001)
%FDK *FG*	0.55 (<0.001)	-	-	0.69 (<0.001)
%FDK *FSP*	0.45 (<0.001)	0.50 (<0.001)	0.43 (<0.001)	0.56 (<0.001)
%FDK MIX	0.53 (<0.001)	0.48 (<0.001)	0.43 (0.003)	0.57 (<0.001)

The following scatterplots (Figure 16, 17, 18 and 19) visualize the correlations between AUDPC and the different toxins. Pearson product - moment correlation indicates significant positive association between the AUDPC of experiment *FG* and the toxins DON and ZON which is shown in Figure 16 (A, B).

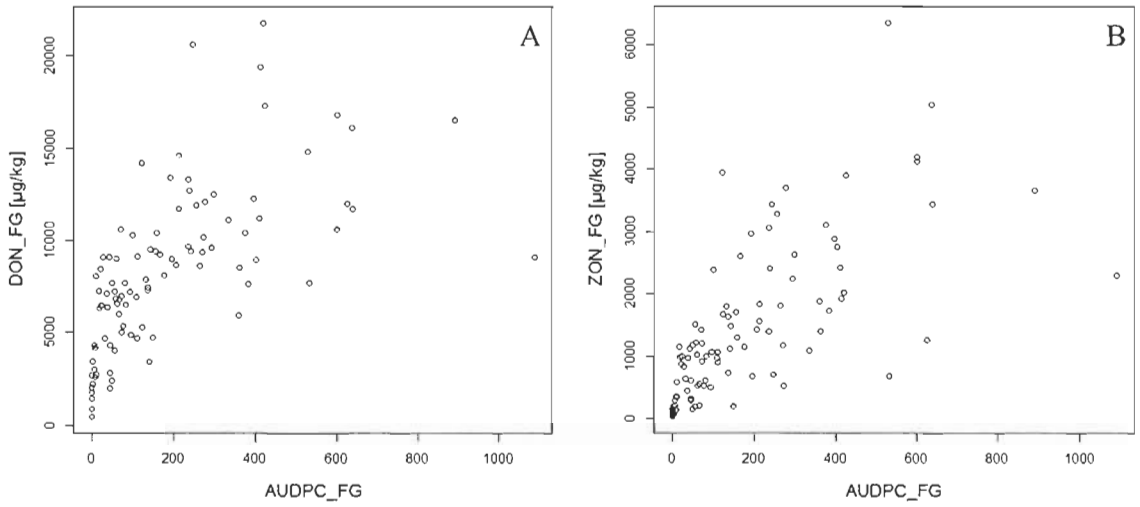

Figure 16: Scatterplot of FHB severity measured by AUPDC versus the toxins DON (A) and ZON (B) in µg/kg for the experiment *FG*.

Interesting is the relation between total mean %AE and the toxins because the scatterplots (Figure 17 A, B) show a significant negative correlation with DON and ZON.

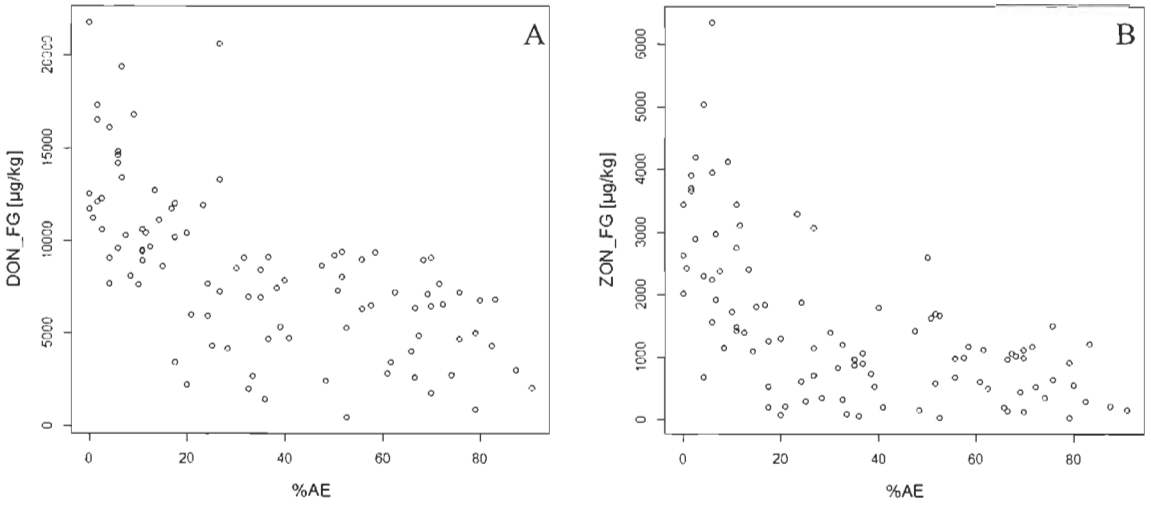

Figure 17: Scatterplot measured by total mean %AE versus the toxins DON (A) and ZON (B) in µg/kg for experiment *FG*.

Correlation between total mean %AE and the toxins of experiment *FSP* is lower, but DON and ZON again show the highest correlation coefficients.

FSP versus the toxins DON, HT-2, T2 and ZON also indicate positive correlations which are shown in Figure 18 (A, B, C, D).

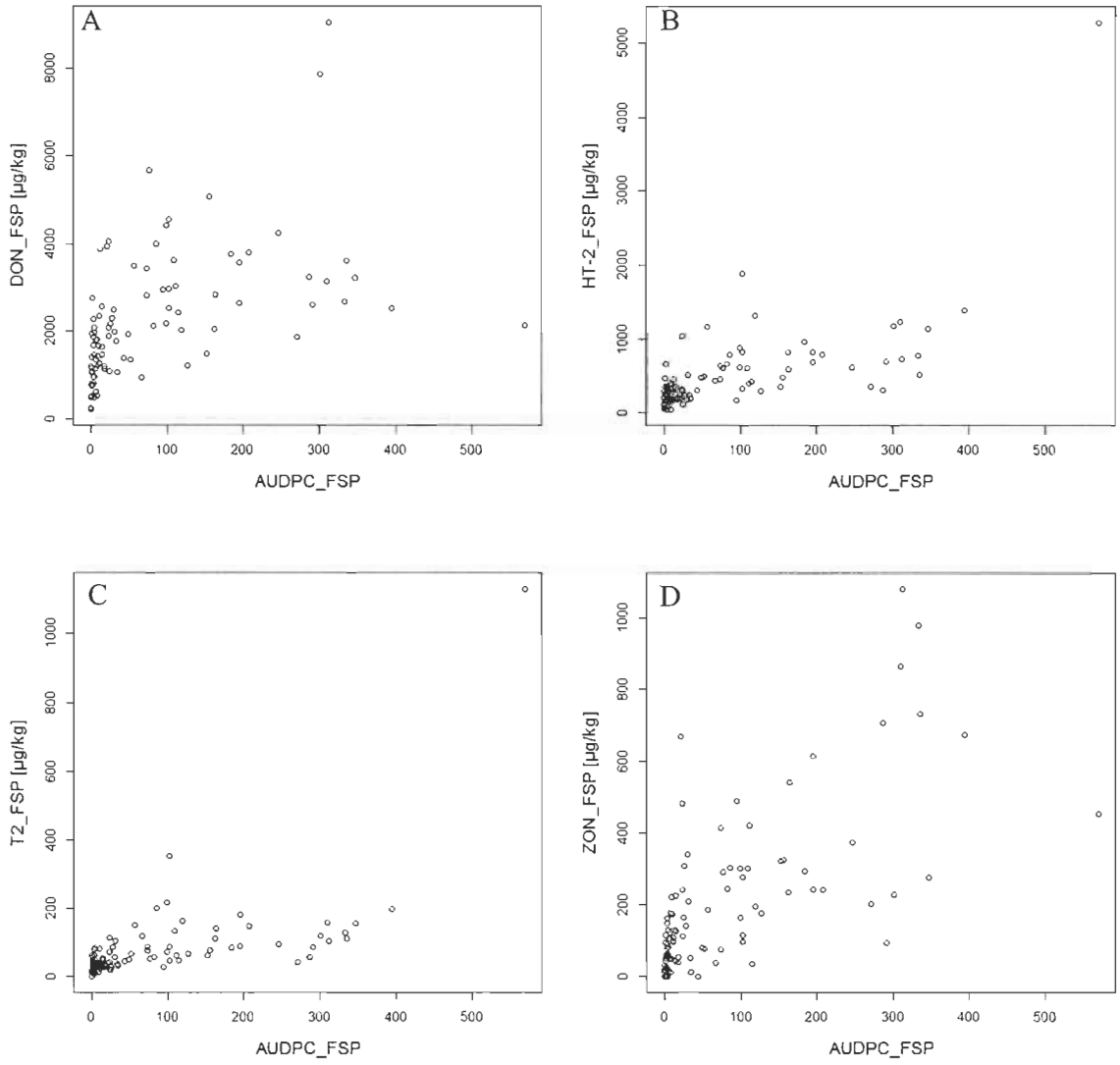

Figure 18: Scatterplot of FHB severity measured by AUPDC versus the toxins DON (A), HT-2 (B), T2 (C) and ZON (D) in µg/kg for experiment *FSP*.

The correlation coefficients indicate significant positive associations between the AUDPC of experiment MIX and the toxins DON, HT-2, T-2 and ZON which are shown in Figure 19 (A, B, C, and D).

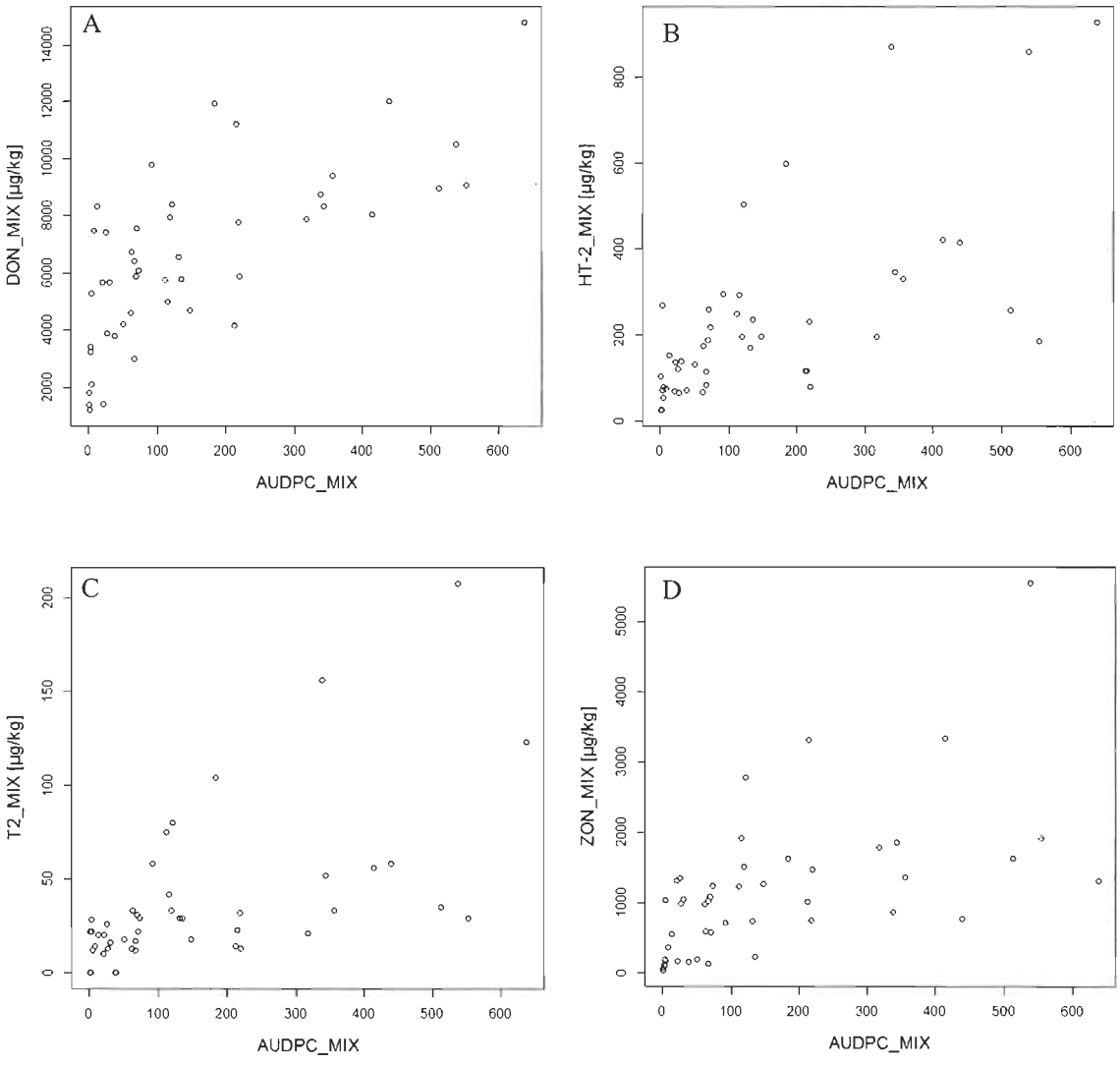

Figure 19: Scatterplot of FHB severity measured by AUDPC versus the toxins DON (A), HT-2 (B), T2 (C) and ZON (D) in µg/kg for experiment MIX.

5 Discussion

Reason for this study was to increase the knowledge of FHB resistance in wheat with the sources *Fusarium graminearum* and *Fusarium sporotrichioides*, their relation to mycotoxins and to show relations to morphological factors like plant height, days of anthesis or percentage of anther extrusion. Detection and evaluation of FHB resistance needs a lot of labour, time and costs because of the immense diversity of resistance and the big affection of environmental factors. For this, 96 genotypes with several replications were tested and investigated. Additionally, the mycotoxins of the three experiments were determined to show associations between gathered severity and toxins after artificial inoculation.

The scoring was done visually by estimating the percentage of infected spikelets of the whole inoculated plot, the severity, and the percentage of FDKs which highly correlated to each other, to evaluate phenotypically results. With such a representative value of combined type I and type II resistance, results could be gathered.

After successful inoculation, in the process of the experiment we soon saw the different and strongly varying susceptibilities of the genotypes. The scorings and toxin analyses then showed immense differences in the aggressiveness of the two isolates. The resistances of the genotypes varied from no symptoms to very susceptible ones, which showed a lot of symptoms after a short time. Of course, most of the genotypes were susceptible because of the good and efficient inoculation technique, but had very different levels of susceptibility. Surprisingly, especially some commonly used cultivars indicated good toxin resistances. Only the difficulty of scoring ripe spikelet or grains and the fact that we only scored until 30 days after inoculation, are possible sources for inaccuracy. At least another month lied in - between the last scoring and harvest where the fungi could spread. This could have influenced the visual scorings and toxin analysis of our experiments and therefore also the ANOVAs and correlations.

The results of the correlation analysis for the three variants exhibit high correlation coefficients. In the literature and especially in MESTERHÁZY et al. (2005) similar results are found. It shows that the susceptibility is related to many *Fusarium* spp. and not only to one specific and that breeding programs could select or breed by testing only one common isolate or *Fusarium* spp.

However, the environmental factors are always a big influence and important for infection in combination with the specific aggressiveness and adaptation to climates of the used isolates (DOOHAN et al. 2003). This probably explains the high significance of the ANOVA results for the three experiments.

The results of the ANOVA for the interaction between experiments and genotypes show significant differences and support the results of MESTERHÁZY et al. (2005) and LU et al. (2012). MESTERHÁZY et

al. (2005) were the first ones showing the non - specificity of *Fusarium* spp. with big significances of genotype * isolate interaction.

The severity of the mean *Fusarium graminearum* experiment was around 15 percent higher than the *Fusarium sporotrichioides* experiment which shows the AUDPC of all genotypes and the different distribution of the two isolates in Figure 6. *Fusarium sporotrichioides* causes a lower severity in a lot of genotypes compared to *Fusarium graminearum* which shows a higher severity and also develops much faster. The mix variant of those two isolates (MIX) indicates the middle way with very high AUDPC in many varieties. This means that using a mix of inocula is neither better nor worse than a single one and does not change the aggressiveness. Using more isolates parallel only helps getting acceptable results (MESTERHAZY 2003).

Confirmations for this are the significant positive correlation coefficients between AUDPCs and toxin levels. LEMMENS et al. (2005) and MESTERHÁZY (2002) indicated similar or even higher correlations coefficients between AUDPC, FDK and toxins, especially DON, than in this study.

Morphological resistance components are passive mechanisms and important for the natural resistances of plants. Plant height is one of such several conceived passive factors for resistance or better tolerance. Small plants show a higher susceptibility to FHB because of the dispersal of conidia with rain. Especially in fields with a lot of crop debris this is the main naturally spreading and infection reason of *Fusarium* spp. in cereals. And as plant height increases the FHB susceptibility decreases (HILTON et al. 1999, JOUANY 2007). In addition, there is also the possibility that the heads of higher plants may dry faster and decrease the requirements for the fungus. The increased susceptibility of small plants is also associated with dwarfing genes like *Rht-B1b* or *Rht-D1b* which are contained in a number of modern varieties (LU et al. 2012). Now the aim for breeders is to decrease the negative effects of those genes for FHB severity and compensate the relation between plant height and severity.

In this thesis similar results about plant height were obtained. However, the results have to be interpreted carefully because of the only moderate, but significant negative, correlation coefficient (r = -0,32) between AUDPC and plant height. In general, the differences of plant heights were big. Plants varied from 50 to more than 110cm, with a mean of 71cm. The low mean height of the used genotypes probably also shows the big spread of the before mentioned dwarfing genes in all common varieties and lines.

Flowering date also varied impressively. Early genotypes were in main flower 21 days after the 1st of May and late ones two weeks later. This probably results out of the different sowing times of the experiments and the genetic differences. The correlation between severity and date of anthesis showed moderate correlation coefficients with high significance.

Another important, but not well investigated, however, for this thesis the most relevant morphological factor is anther extrusion. In this study similar results with significant negative correlations between anther extrusion and FHB or toxins were investigated and we could indicate that anther extrusion is related to FHB severity and mycotoxin contamination. Also KUBO et al. (2013), LU et al. (2012) and SKINNES et al. (2009) received similar results. Therefore, it makes sense to select plants with high anther extrusion in wheat breeding programmes because correlation coefficients show big connections between anther extrusion and FHB or toxins and FDK. This morphological factor would be an easy way to reduce FHB in wheat and thereby also reduce mycotoxin contamination which is an important factor for wheat breeding programs and especially for food safety. Of course further investigations are needed because some varieties with only low anther extrusion and partially extruded anthers also show medium FHB and toxin contamination. It seems to be possible that FHB can also infect plants with many extruded anthers but then infect plants as fully and with high severity as plants with low anther extrusion. Therefore, anthers, which are stuck between glumes could be a relevant infection source. However, in combination with high plants a better resistance to FHB seems to be possible and probably reduces the risk of being infected with spores from crop debris (KUBO et al. 2013). In our study significant association with a moderate correlation coefficient ($r = 0,37$) was found between plant height and anther extrusion. Combining high anther extrusion with higher plant height would be a positive and possible aim in breeding.

To find such correlations was one main aim of the experiments for this thesis. By mist irrigation and field inoculation at full flowering, we tried to standardize and idealize the environment by simulating an epidemic under field conditions with consistent and permanent infection pressure to find genotypes with morphological and physiological advantages against FHB.

Another complex topic in wheat plants is toxin contamination. It is a factor which can vary immensely in some years and in environments with favouring conditions. Hence, comparisons with different years would be interesting for relations and statistical relevance because this year was a very dry one, although mist irrigation was used and FHB symptoms showed later and less severe than usual. Out of the visually scored results, AUDPC and %FDK of the three isolates correlate moderately to highly with the production of the toxins DON, T2, HT-2 and ZON. Therefore, the aggressiveness of the used isolates correlates with the production of toxins and is a relevant component for susceptibility. There is especially a strong correlation between aggressiveness and DON. Similar results were collected by MESTERHÁZY et al. (2005). However, also in resistant or tolerant varieties there are exceptions with low toxin production and an amount of toxins which is not zero or vice versa. Such genotypes might have much lower contaminations with mycotoxins than susceptible ones but still exceed regulations in food and feeds. For this reason, toxin amounts of naturally infection would be interesting and if they could also be as high as artificially inoculated ones. That makes the toxic burden complicated and it is even more difficult and time consuming to forecast the amount of toxins by field symptoms, but it is

the most important factor for breeders and scientists. These results offer the possibility of breeding for toxin resistance because this could lead to a higher resistance and tolerance to the disease.

Other interesting and not ignorable results of this thesis are the correlation coefficients between %FDK and toxins. The advantage of visually scoring the seeds (%FDK) is that the difficulty and possible inaccuracy of scoring ripe wheat plants and the fact that there may be plenty of time between last scoring and harvest is not present. However, scoring %FDK is also not easy, so that improving or finding a better and easier visually scoring or comparing system could help to detect toxins without accurate and expensive tests, because the results of the correlations, especially of *Fusarium graminearum* and *Fusarium sporotrichioides* indicate moderate and highly significant correlation coefficients.

In conclusion the results of this study illustrate significant differences in the resistance behaviour of the used genotypes. Statements about the infection and resistance mechanisms could not be made. Comparing the results of these varieties and lines with other years or locations would be interesting and could make better statistical and scientific statements. Therefore, further investigation about infection mechanisms (especially the genetic mechanisms) of *Fusarium* spp. and FHB resistances are needed to enhance resistances in wheat and other cereals and especially to verify associations between FHB, mycotoxins and morphological factors like anther extrusion and plant height. This thesis is part of a couple and perennial experiments around *Fusarium* spp. under field conditions and in the greenhouse. Besides that genetic mapping is done to increase resistance breeding against infestation of *Fusarium*.

6 List of Tables

7 List of Figures

8 References

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9 Attachments

Attachment 1: Mean data of AUDPC, flowering date (DAT), plant height (PH), percentage of extruded anthers (%AE) and percentage of Fusarium damaged kernels (%FDK) of the three experiments (*F. graminearum (FG), F. sporotrichioides (FSP)* and the mix of both isolates (MIX)) for all genotypes

Attachment 2: Toxin analysis data of DON, HT2, T2 and Zearalenone of the three experiments (*FG*, *FSP* and MIX) for all genotypes (µg/kg)

Attachment 3: List of genotypes

Attachment 4: Standard operating procedure SOP 3-01

Attachment 1: Mean data of AUDPC, flowering date (DAT), plant height (PH), percentage of extruded anthers (%AE) and percentage of Fusarium damaged kernels (%FDK) of the three experiments *F. graminearum (FG), F. sporotrichioides (FSP)* and the mix of both isolates (MIX) for all genotypes.

Genotype	AUDPC_FG	AUDPC_FSP	AUDPC_MIX	DAT_FG	DAT_FSP	DAT_MIX	PH_FG	PH_FSP	PH_MIX	%AE_FG	%AE_FSP	%AE_MIX	FDK_FG	FDK_FSP	FDK_MIX
04 CY BH FU 25	396,5	183,9	553,65	32	32	35	65	60	65	7,5	0	0	52,5	50	84
20568.1.3	0	0,2	2,2	25	25	25	80	80	85	87,5	100	85	1	1	1,5
20812.2.2	0	0,4	1,35	27	28	28	80	85	82,5	50	40	17,5	1,5	1,5	1
20818.1.2	1	0	1,35	27	28	29	85	87,5	87,5	55	57,5	45	3,5	2,5	8,5
A39.9.2.1	6,8	1,4	38,6	31	32	31	80	80	82,5	87,5	85	75	1,5	2,5	5,5
A40 22 1 2	7,6	1,4	2,8	28	29	29	80	87,5	85	90	92,5	80	1,5	1	0,5
Accroc	601,35	194,7	183,9	24	23	23	60	60	62,5	0	0	27,5	77,5	42,5	34
ADAGIO	21,55	5,2	13,2	23	23	23	60	67,5	65	55	32,5	17,5	11	1	5
Alchemy	382,6	103,2	294,95	33	34	35	70	72,5	75	7,5	5	17,5	75	27,5	84
Aligator	132,2	44,2	69,4	25	27	27	62,5	75	75	42,5	37,5	40	8	2	33
Alixan	73	14,6	135,4	26	26	27	65	72,5	77,5	25	40	32,5	30	5	15,5
Altigo	294,2	102,2	339,15	25	26	26	70	75	72,5	7,5	2,5	7,5	75	8	47,5
AMBELLO	100,8	30,75	25,8	25	25	25	65	65	62,5	12,5	2,5	7,5	4	5,5	9
Apache	57,4	23,2	20,75	25	25	25	65	65	65	82,5	85	82,5	3,5	25	6,5

Arlequin	166,35	74,15	72,8	27	26	25	67,5	67,5	62,5	40	57,5	52,5	70	55	54
As de cœur	123,15	14,75	61,2	30	29	29	75	72,5	70	60	37,5	60	45	3,5	6,5
ATHLON	112,15	98,95	57,15	25	24	24	65	70	67,5	42,5	27,5	40	6,5	3,5	6,5
AZZERTI	413,35	312,45	390,5	31	31	30	67,5	70	70	7,5	10	2,5	87,5	52,5	80
Azzuro	110,3	24,8	36,8	29	28	29	80	72,5	75	22,5	35	52,5	65	9,5	37,5
Bagou	375,85	162,35	343,35	29	26	26	62,5	65	62,5	0	0	35	77,5	35	54
Balance	402,3	335,75	512,7	31	31	32	65	67,5	70	25	2,5	5	70	67,5	70
Barok	109	3,35	70,6	27	27	28	67,5	65	70	30	17,5	57,5	25	5	7,5
Bermude	410,05	195,3	317,3	31	31	31	72,5	72,5	72,5	2,5	0	0	27,5	4	80
BIO 4036	37,6	18,2	4,6	23	23	23	70	72,5	70	57,5	70	80	3	3	1,5
BIO 5019	158,75	109,4	181,4	29	30	31	70	67,5	70	35	12,5	12,5	37,5	21,5	9
BIO 719	28,75	3,4	27,15	23	23	24	65	60	65	27,5	22,5	45	6,5	0	1
Bio110	206,2	12	31	27	26	25	62,5	70	67,5	47,5	37,5	57,5	12,5	1,5	6
Boregar	238,35	119,8	121,4	27	27	26	60	62,5	62,5	7,5	20	12,5	67,5	13	52,5
Capo	2,35	3,4	3,6	25	25	25	70	70	72,5	20	10	22,5	0,5	3	1,5
Centrum	148,95	6,6	66,95	33	32	32	87,5	90	87,5	37,5	47,5	37,5	10	8	9
CH761525	271	155,75	354,9	29	31	31	80	85	82,5	50	72,5	52,5	65	45	37,5
Charger	639,15	286,05	509,55	30	31	32	60	65	60	0	0	0	95	55	52,5

Chevalier	81,8	14,9	218,6	31	31	31	70	72,5	72,5	45	22,5	5	10	4	10
COMPIL	243	22	158,8	27	27	26	62,5	62,5	65	25	0	7,5	52,5	9	57
Cordiale	122,2	111,75	179	26	29	31	57,5	62,5	65	0	7,5	10	75	21,5	37,5
Dakter	418,7	570	421,25	21	21	22	52,5	57,5	57,5	0	0	0	90	80	100
E044/119-1	46,2	1,2	3,8	25	25	25	77,5	80	77,5	20	32,5	22,5	2,5	2,5	9
Enno	197,15	114,95	12,6	30	30	30	72,5	65	82,5	55	57,5	55	27,5	5,5	8
F046/16-1	44,4	5	21,8	25	24	25	82,5	87,5	82,5	17,5	27,5	52,5	1	4	3,5
FD 07170	156	48,8	119	24	25	23	65	62,5	65	50	75	30	8	2,5	2,5
FD 08114	256,4	57,2	238,95	27	27	27	72,5	70	67,5	27,5	25	17,5	27,5	9	21
FD12	263,55	24,15	115	30	27	30	70	70	70	10	22,5	12,5	32,5	6,5	57,5
FD3	626,85	207,4	372,1	32	31	31	67,5	70	70	20	15	17,5	40	37,5	96,5
FLUOR	46,2	1,2	23	27	26	28	75	77,5	77,5	50	60	72,5	32,5	4,5	2,5
Fr1E1_1	56,75	9,4	42	23	24	24	65	65	65	85	87,5	55	55	5	29
Fr1E2_2	72,2	4,35	8,4	23	23	23	67,5	67,5	65	77,5	77,5	82,5	7,5	3,5	11,5
Furore	273	74,15	120,4	29	29	29	80	80	80	20	12,5	20	10	3,5	12,5
Golden Spike	247,55	99,35	135,15	29	28	31	90	90	87,5	37,5	22,5	20	3	1,5	3,5
Goncourt	298,55	103,25	438,7	26	27	26	65	65	62,5	0	0	0	77,5	32,5	90
GRAINDOR	61,55	33,8	90,2	24	24	23	65	70	67,5	75	77,5	65	6	3	9

H5-169_B	56,4	9	50,8	28	29	29	95	95	97,5	75	65	57,5	4,5	2	2,5
H5-363_B	65,8	35,2	160,2	27	28	30	90	90	90	15	37,5	10	1,5	4	4
Illico	84,4	11,95	27,15	25	25	25	65	70	67,5	57,5	50	65	11	5	6,5
Isengrain	237,55	85,9	96,95	26	27	27	65	62,5	67,5	7,5	15	15	47,5	20	10
JB ASANO	532,35	270,85	150,8	33	32	32	80	80	80	0	0	12,5	17,5	2	6,5
K_9_21Ab	24,4	28	7,4	24	24	24	65	65	60	67,5	62,5	80	4,5	7,5	20
K_9_21aB	49,2	4,55	8,2	25	23	23	67,5	75	70	65	60	90	32,5	1,5	12,5
K_9_21ab	60,75	5,4	57,95	25	25	25	62,5	60	60	80	67,5	57,5	12,5	30	41
K_9_21AB	44,15	2	66,8	25	24	25	67,5	67,5	65	60	90	60	6,5	3	5
K11_16_AB.9	50,15	18,6	16,4	31	30	31	80	82,5	80	70	35	40	10,5	6,5	4
K6_7_AB.7	93,4	67,6	108	27	27	26	75	77,5	75	55	50	82,5	3,5	2,5	18
K9_21_AB.16	31,8	7,4	19,35	24	25	24	70	75	67,5	70	87,5	70	35	1	3
K9_21_AB.18	65,6	2,95	63,4	25	24	24	72,5	67,5	67,5	80	87,5	72,5	6,5	1,5	2
KALYSTAR	140,95	26,2	212,15	31	29	32	70	70	67,5	80	70	35	52,5	30	67,5
MANAGER	362,9	12,55	166,4	33	32	32	75	75	75	17,5	27,5	45	50	19	9
Mendel	277,75	152,7	273,5	29	28	29	62,5	60	65	5	0	0	85	21,5	52,5
Mercato	192	127,55	112	25	25	25	57,5	57,5	60	10	7,5	2,5	55	35	32,5
Midas	77,6	4,6	3,4	27	27	28	72,5	75	70	40	25	52,5	26	3	5

MIROIR	20,4	6,55	4,2	23	23	23	62,5	67,5	65	72,5	52,5	42,5	30	6,5	4
MUSIK	423,7	164,4	636,35	27	25	28	55	57,5	60	0	0	5	31,5	5,5	35
Nogal	236,1	4,1	214,7	22	23	23	60	60	60	30	42,5	7,5	26,5	15,5	30
Nuage	359,9	95,1	219,15	31	28	30	70	70	70	25	27,5	20	45	55	25
OXEBO	135,8	82,8	148,1	31	31	32	72,5	75	72,5	42,5	52,5	57,5	42,5	18	22,5
P1_1335.6	1	0,4	2,8	29	30	29	82,5	80	77,5	62,5	80	67,5	20	31,5	18
P2_1351.5	9,55	0,2	4,65	31	31	32	82,5	85	90	60	72,5	67,5	1,5	4	1,5
Pakito	142,35	31	48,55	25	25	25	65	67,5	67,5	15	10	7,5	2,5	7,5	11,5
PHARE	599,25	291,45	551,85	31	31	31	67,5	70	65	5	2,5	0	99	57,5	92,5
Player	137,75	77,2	131,15	29	27	29	70	70	70	35	47,5	32,5	47,5	11,5	55
Premio	214,2	24,15	35,95	25	25	25	60	62,5	62,5	12,5	0	5	40,5	36,5	15
Renan	11	2,6	10	27	26	27	67,5	77,5	75	45	42,5	67,5	6	41,5	5
RHT_216	0,8	1,2	1,4	28	27	28	67,5	72,5	70	42,5	20	37,5	0,5	1,5	2,5
RHT_269	8,55	1,2	3	27	28	28	70	70	70	32,5	25	27,5	5	11	3
RHT_423	2,4	8,35	0,6	26	27	24	72,5	75	75	25	10	25	8	2,5	2
Royssac	528,7	394,3	537,9	24	26	25	60	60	60	2,5	12,5	2,5	56,5	90	80
SAINT EX	18,35	10,2	47	23	23	23	60	62,5	60	55	20	5	4,5	8,5	12
SE11	1088,75	346,45	627,25	31	31	31	70	77,5	75	0	2,5	10	92,5	19	80

SE3 (Robigus)	335,3	246,9	329,55	32	32	33	65	70	67,5	10	17,5	15	52,5	10	12,5
Soissons	71	8,6	91,8	23	24	23	65	65	70	10	10	12,5	14	0	2,5
SOKAL	97,2	3,2	18	26	26	25	65	70	65	82,5	62,5	57,5	37,5	55	32,5
SY ALTEO	38,8	11,15	68,8	23	24	23	65	65	67,5	60	65	75	6,5	3,5	6,5
T.macha-A	10,35	3,4	5	33	33	31	107,5	107,5	110	85	65	72,5	1,5	0	0
Timber	892	310,25	414,85	34	31	30	62,5	65	67,5	2,5	2,5	0	44	67,5	67,5
TOBAK	213,4	301,1	355,7	31	33	33	72,5	80	75	10	2,5	37,5	47	49	86,5
Toisondor	636,5	333,4	632,35	29	29	29	60	62,5	60	10	2,5	0	65	3,5	70
TULIP	176,4	53,1	63,4	24	23	25	72,5	70	70	12,5	10	2,5	45	11	53
UNG-136.16.7.4.7	1,2	0,2	3	27	26	26	82,5	82,5	85	77,5	85	75	0,5	0	2,5

Attachment 2: Toxin analysis data of DON, HT2, T2 and Zearalenone of the three experiments (*FG, FSP* and MIX) for all genotypes (µg/kg).

Genotype	DON-FG	HT-2-FG	T-2-FG	ZON-FG	DON-FSP	HT-2-FSP	T-2-FSP	ZON-FSP	DON-MIX	HT-2-MIX	T-2-MIX	ZON-MIX
04 CY BH FU 25	12300	0	0	2880	3770	966	85	294				
20568.1.3	2050	0	0	155	478	101	14	20				
20812.2.2	1410	0	0	56	504	123	14	12	1400	26	0	33
20818.1.2	442	0	0	28	251	60	0	0				
A39.9.2.1	4340	0	0	287	1040	74	14	42	3790	72	0	160
A40 22 1 2	3020	0	0	207	513	65	10	24	1210	27	0	103
Accroc	16800	0	0	4130	2650	823	181	616	11900	598	104	1630
ADAGIO	8430	0	0	871	1970	341	47	84	8330	152	20	558
Alchemy	7620	0	0	1730	4550	824	87	117				
Aligator	7870	0	0	1800	1390	309	46	0	5860	188	31	1080
Alixan	6980	0	0	1200	1470	331	55	127	5790	236	29	228
Altigo	9590	0	0	2240	2960	1880	352	96	8740	872	156	863
AMBELLO	10300	0	0	2380	2480	188	58	341	7400	120	26	1350
Apache	6830	0	0	1220	2090	297	43	113	5650	69	10	1310
Arlequin	9210	0	0	2600	2810	462	77	414	6090	218	29	1240
As de cœur	5310	0	0	1670	1630	358	49	44	4600	67	13	979

ATHLON	9130	0	0	1070	2170	614	71	302				
AZZERTI	19400	0	0	1920	9060	734	103	1080				
Azzuro	4680	0	0	895	1080	116	20	166				
Bagou	10400	0	0	3100	2050	822	112	235	8320	345	52	1860
Balance	8940	0	0	2740	3600	516	111	734	8950	256	35	1630
Barok	6920	0	0	973	2270	311	32	149	7570	258	22	577
Bermude	11200	0	0	2410	3570	687	89	242	7880	195	21	1780
BIO 4036	7090	0	0	448	1130	180	34	54				
BIO 5019	10400	0	0	1300	3630	607	134	301				
BIO 719	9060	0	0	834	1670	245	63	162				
Bio110	8630	0	0	1430	2350	331	40	96	5660	138	16	1050
Boregar	12700	0	0	2400	2030	1320	164	195	8380	502	80	2780
Capo	3430	0	0	190	801	322	38	0				
Centrum	4760	0	0	199	1800	177	38	122	2990	84	17	129
CH761525	9340	0	0	1170	5070	485	77	326				
Charger	11700	0	0	3440	3230	312	58	708				
Chevalier	7650	0	0	609	2570	258	27	227	7770	232	32	747
COMPIL	9400	0	0	3430	3940	195	40	670				

Cordiale	14200	0	0	3950	3020	405	62	421				
Dakter	21800	0	12	2020	2140	5280	1130	456				
E044/119-1	4330	0	0	297	747	468	57	16	3230	269	28	104
Enno	8970	0	0	674	2420	420	48	36				
F046/16-1	1980	0	0	321	486	241	48	20	1430	137	20	163
FD 07170	9380	0	0	1700	1920	484	49	80	7950	196	33	1510
FD 08114	11900	0	0	3280	3490	1170	150	187				
FD12	8600	0	0	1810	1880	326	72	484	4980	292	42	1910
FD3	12000	0	0	1260	3800	790	148	243				
FLUOR	2850	0	0	612	1080	250	27	95				
Fr1E1_1	7220	0	0	1510	1420	51	12	223				
Fr1E2_2	5020	0	0	919	966	84	11	129	7470	76	14	359
Furore	10200	0	0	522	3440	646	86	76				
Golden Spike	20600	0	0	700	4410	876	217	166				
Goncourt	12500	0	0	2620	2520	338	48	278	12000	415	58	770
GRAINDOR	6570	0	0	533	1780	245	37	52				
H5-169_B	4030	0	0	200	1210	182	40	50	4200	130	18	188
H5-363_B	5990	0	0	208	1050	197	33	13				

Illico	6510	0	0	993	1250	168	33	108	3890	66	13	988
Isengrain	9680	0	0	1400	4000	785	201	305				
JB ASANO	7660	0	0	681	1860	354	42	202				
K_9_21Ab	6440	0	0	1000	2290	243	88	143				
K_9_21aB	7680	0	0	1170	1470	342	38	55				
K_9_21ab	8970	0	0	1020	1130	194	31	15				
K_9_21AB	9050	0	0	1120	1950	163	33	116	6400	115	12	1020
K11_16_AB.9	2410	0	0	151	1200	212	31	41				
K6_7_AB.7	7210	0	0	502	946	437	118	39				
K9_21_AB.16	4710	0	0	641	609	146	29	106				
K9_21_AB.18	6770	0	0	551	768	157	34	0				
KALYSTAR	3430	0	0	1120	2150	227	27	308	4140	117	14	1010
MANAGER	8510	0	0	1400	3870	455	29	131				
Mendel	12100	0	0	3700	1490	353	63	323				
Mercato	13400	0	0	2970	1210	296	67	178	5760	249	75	1230
Midas	5360	0	0	523	2090	283	85	60				
MIROIR	6320	0	0	987	1340	302	35	49	5290	79	12	1030
MUSIK	17300	0	0	3900	2830	593	142	543	14800	929	123	1310

Nogal	13300	0	0	3060	949	361	79	90	11200	117	23	3310
Nuage	5970	0	0	1880	2950	174	27	490	5860	80	13	1470
OXEBO	7310	0	0	1630	2110	668	58	245	4690	195	18	1260
P1_1335.6	1740	0	0	131	787	296	62	19				
P2_1351.5	2600	0	0	140	1200	109	16	31	2110	55	12	172
Pakito	9480	0	0	1480	1990	513	103	209				
PHARE	10600	0	0	4200	2600	695	86	94	9070	186	29	1910
Player	7430	0	0	730	5680	608	53	293	6550	171	29	730
Premio	14600	0	0	1560	4050	1040	115	243				
Renan	8060	0	0	587	2760	667	46	27				
RHT_216	2670	0	0	91	1150	354	47	59				
RHT_269	4170	0	0	342	1400	249	37	49	3400	71	22	192
RHT_423	2230	0	0	73	522	405	41	13	1810	104	22	75
Royssac	14800	0	0	6350	2520	1400	197	673	10500	859	208	5560
SAINT EX	7270	0	0	1150	1660	385	83	174				
SE11	9090	0	0	2290	3220	1140	155	277				
SE3 (Robigus)	11100	0	0	1090	4240	622	93	376				
Soissons	10600	0	0	1420	1810	198	41	176	9780	294	58	709

SOKAL	4870	0	0	1070	1870	340	43	79			
SY ALTEO	6380	0	0	964	1270	187	23	99			
T.macha-A	2730	0	0	355	1670	45	14	65			
Timber	16500	0	0	3660	3150	1240	159	865	8030	420	56	3340
TOBAK	11700	0	0	1830	7870	1180	118	230	9390	330	33	1360
Toisondor	16100	0	0	5030	2670	774	129	978			
TULIP	8090	0	0	1150	1340	497	68	79	6720	174	33	587
UNG-136.16.7.4.7	869	0	0	35	203	210	19	0			

Attachment 3: List of genotypes with contributor, company, admission and if known pedigree.

Name (Genotype)	Contributor	Company	Admission	Pedigree (if known)
Capo	IFA	Probstdorfer Saatzucht	2000	
Furore	IFA	Probstdorfer Saatzucht	2000	
Midas	IFA	Saatzucht Donau	2008	
CH761525	IFA	Breeding Line Switzerland		
Enno	IFA	Breeding Line Germany		
Golden Spike	IFA	Variety US (Utah Agricultural Experiment Station)		
Triticum macha-A	IFA	Machaweed		
UNG-136.16.7.4.7	IFA	Line Szeged, Hungary		Sagvari/Nobeokabozu//Mini-Mano/Sumai-3
20568.1.3	IFA	Line IFA-Tulln		Capo/Sumai-3
20812.2.2	IFA	Line IFA-Tulln		Capo/Sumai-3
20818.1.2	IFA	Line IFA-Tulln		Capo/Sumai-3
E044/119-1	IFA	Line IFA-Tulln		Globus/CM-82036
F046/16-1	IFA	Line IFA-Tulln		Globus/CM-82036
Fr1E1_1	IFA	Line IFA-Tulln		Frontana/Apache
Fr1E2_2	IFA	Line IFA-Tulln		Frontana/Apache
H5-169_B	IFA	Line IFA-Tulln		T.macha/Furore*3
H5-363_B	IFA	Line IFA-Tulln		T.macha/Furore*3
K_9_21Ab	IFA	Line IFA-Tulln		CM-82036/Apache*3
K_9_21aB	IFA	Line IFA-Tulln		CM-82036/Apache*3
K_9_21ab	IFA	Line IFA-Tulln		CM-82036/Apache*3
K_9_21AB	IFA	Line IFA-Tulln		CM-82036/Apache*3
K11_16_AB.9	IFA	Line IFA-Tulln		CM-82036/CH761525*3
K6_7_AB.7	IFA	Line IFA-Tulln		CM-82036/Enno*3
K9_21_AB.16	IFA	Line IFA-Tulln		CM-82036/Apache*3
K9_21_AB.18	IFA	Line IFA-Tulln		CM-82036/Apache*3
P1_1335.6	IFA	Line IFA-Tulln		20812.2.2/Hermann

P2_1351.5	IFA	Line IFA-Tulln		20812.2.2/Toras
RHT_216	IFA	Line IFA-Tulln		20812.2.2/Hermann*3
RHT_269	IFA	Line IFA-Tulln		20812.2.2/Toras*3
RHT_423	IFA	Line IFA-Tulln		20812.2.2/Hermann*3
ADAGIO	Arvalis	P.H. Peterson Saatzucht	1995	
Aligator	Arvalis	Unisigma	2010	
Alixan	Arvalis	Limagrain Verneuil Holding	2004	
Altigo	Arvalis	Limagrain Verneuil Holding	2007	
AMBELLO	Arvalis	RAGT	2010	
Apache	Arvalis	Limagrain Verneuil Holding	1999	
Arlequin	Arvalis	Limagrain Verneuil Holding	2007	
As de cœur	Arvalis			
ATHLON	Arvalis	Saaten Union Recherche	2010	
AZZERTI	Arvalis	Limagrain Belgium	2009	
Bagou	Arvalis	Saaten Union Recherche	2007	
Balance	Arvalis			
Barok	Arvalis	Agri-Obtentions	2009	
Bermude	Arvalis	Florimond Desprez	2007	
Boregar	Arvalis	RAGT	2007	
Charger	Arvalis	Limagrain Belgium	2002	
Chevalier	Arvalis	Deutsche Saatveredelung AG	2005	
Cordiale	Arvalis	KWS UK Limited	2003	
FLUOR	Arvalis	Unisigma	2002	
Goncourt	Arvalis	RAGT	2009	
GRAINDOR	Arvalis	Unisigma	2006	
Illico	Arvalis	Syngenta Seeds SAS	2010	
Isengrain	Arvalis	Florimond Desprez	2002	
KALYSTAR	Arvalis	Adrien Momont-Hennet	2010	
MANAGER	Arvalis	Semalliance Gie	2006	

Mendel	Arvalis	RAGT	2004
Mercato	Arvalis	RAGT	2005
MIROIR	Arvalis	Saaten Union Recherche	2010
MUSIK	Arvalis	Agri-Obtentions	2010
Nuage	Arvalis	RAGT	2006
OXEBO	Arvalis	Ets Jean Lemaire-Deffontaines	2002
Player	Arvalis		2010
Premio	Arvalis	RAGT	2007
Renan	Arvalis	Agri-Obtentions	2002
Royssac	Arvalis	RAGT	2003
SAINT EX	Arvalis	Secobra Recherches	2010
Soissons	Arvalis	Florimond Desprez	2002
SOKAL	Arvalis	Caussade Semences	2010
SY ALTEO	Arvalis	Syngenta Seeds SAS	2010
Timber	Arvalis		
Toisondor	Arvalis	RAGT	2004
TULIP	Arvalis	Saaten Union Recherche	2010
04 CY BH FU 25	Bioplante		
A39.9.2.1	Bioplante		
A40 22 1 2	Bioplante		
Alchemy	Bioplante	Limagrain UK Ltd	2004
Azzuro	Bioplante	Limagrain Verneuil Holding	2006
BIO 4036	Bioplante		
BIO 5019	Bioplante		
BIO 719	Bioplante		
Bio110	Bioplante		
Centrum	Bioplante		
COMPIL	Bioplante	Florimond Desprez	2002
Dakter	Bioplante	GIE Eurodur	2005

FD 07170	Bioplante		
FD 08114	Bioplante		
FD12	Bioplante		
FD3	Bioplante		
JB ASANO	Bioplante	Saatzucht Josef Breun GdbR	2008
Nogal	Bioplante	D. Desprez et Fils	2006
Pakito	Bioplante	RAGT	2010
PHARE	Bioplante	Florimond Desprez	2007
SE11	Bioplante		
SE3 (Robigus)	Bioplante	CPB Twyford Ltd	2002
TOBAK	Bioplante	W. von Boreis-Eckendorf GmbH & Co	2012
Accroc	Bioplante	RAGT	2010

Attachment 4: Standard operating procedure SOP 3-01.

BOKU	Page: 1 von 10 Valid per: 15.10.2007	Standard operating procedure SOP 3-01
	Revision: 0	Production of Inoculum of F. spp with Bubble Breeding

Contents:

	Page: 2 von 10 Valid per: 15.10.2007	Standard operating procedure SOP 3-01
	Revision: 0	Production of Inoculum of F. spp with Bubble Breeding

1. Purpose / Principle

This SOP regulates the production of inoculum for plant inoculation experiments with F spp. This SOP also regulates the handling of the inoculum and determination plus calculation of conidial concentrations, the area of application and the responsibility.

2. Area of application

Laboratory Fusarium Resistance Breeding, growth medium kitchen
IFA Tulln, Institut for Plant Production
responsible person for execution and calculation: Dipl.-HTL-Ing. Andrea Koutnik

3. Measurement principle/Basics

Under constant air supply into the mungbean-media during 5 days the preparation of (Macro-) conidia will be stimulated.

4. Procedure data / Validating

Not available

5. Equipment, Equipment settings and Material

5.1. Equipment

Autoclave Varioklav
LaminAir Heraeus
Incubator Kelvitron t
Labor compressed air
Cooker Alaska
Microscope Nikon Labophot 2

	Page: 3 von 10 Valid per: 15.10.2007	Standard operating procedure SOP 3-01
	Revision: 0	Production of Inoculum of F. spp with Bubble Breeding

5.2. Equipment settings

Autoclave Varioklav: for mungbean-media: 121°C, 60 min, "langsam"
For glas-tube and flexible-tube: 121°C, 20min "schnell"
LaminAir: switch on 20 min before use, disinfect with alcohol directly before use
Microscope: Objective : 10 / 0,25 (yellow) and 40 / 0,55 (light blue)
Incubator: 100°C (circulating air) for 2 days to sterilize the filter
Cooker: mungbeans in cooking water for 20-23 min.
Compressed-air line: Mannometer fixed at 1 bar, through the filter as low as needed

5.3. Material

Glas tube Ø 5mm, length ca. 50cm
Cotton wool and cord to fix the cotton wool on the glas tube
Flexible-tube Ø 5mm, length ca. 30cm (autoclaveable) and for every filter 3 x 10cm of this tubes
Paperbags 15cm x 21cm
Aluminium foil
Regulators for air pressure
Connections for the flexible tubes (T- or Y-formed)
Glasflask 10L
Filter: Erlenmayerflask filled with cotton wool, closed with a stopper with 2 tubes through it (1 x ca. 15-18cm, 1x ca. 5-8cm)
Mungbeans: 20g per L
Osmosewater
Sieves: 1x pore size 2-3mm, 1x pore size 0,5mm

6. Chemicals

Not available

7. Analysis Procedure

7.1. Sample collection

Take the required Fusarium culture from the existing master culture collection at the IFA Tulln (laboratory/resistance breeding room: BP/E/24 B) and scatter it on SNA (special Nirenberg agar). After app. 72 h the grown Fusarium shall be used for the inoculation of the mungbeansuspension.

7.2. Sample Preparation

Fill in 4,5L of osmotic water per pot (stainless steel) and cook it. Add 90g mungbeans per pot and cook them very softly 20-23min (until the first mungbeans break).
Filter it immediately through the sieves into the 10L flasks and fill it up to 9L with osmotic water.
Autoclave the flasks for 60 min at 121°C and 1bar.

Prepare the filter as followed: Put the 10cm flexible tube at the shorter glas tube, fix the connection at the end, add the other 2 flexible tubes (10cm) at the ends and at last put a connection at every end of the flexible tubes. Wrap this connections with alufoil and sterilize the whole filter in the incubator at 100°C for 2 days.
Connect the filter at the compressed-air line and blow it trough very soft for 2-3 hours.

Wrap the cotton wool round the glas tubes and fix it with cord. (this should be the stopper for the 10L flasks!). Wrap the prepared stopper plus glas tube in alufoil twice and autoclave it at 121°C for 20 min.
Also autoclave the 50cm flexible tubes wrapped in the paperbags and twice in alufoil.

Put the cooled down mungbean-suspension in the Laminair and inoculate every flask with the Fusarium (5x5mm piece from inoculated agar) and close it with the autoclaved cotton wool stopper. First connect the warm flexible tube with the warm glas tube. (leave the rest of the flexible tube in the paperbag).

Then connect the flexible tube from the paper bag with the filter – don't forget the regulators for air pressure!- and let the air bubble through permanent for 5 days.

After 5 days separate the flasks from the air, close them with normal stoppers (not to strong) and let the prepared conidia sink down in a cool room (at 4-8°C) over night.

Next day extract the mungbean suspension and most of the mycel with a water-jet-vacuum-pump. The sunken conidia will be processed as follows.

7.3. Calibration

Not available

7.4. Measurement

Counting of the conidia takes place in a Buerker - Tuerk - chamber.

App. 10 small chambers are counted out and the average value/chamber is calculated. A small chamber corresponds to $25x10^{-5}$ mm³ = $25x10^{-8}$ ml

x conidia are in $25x10^{-8}$ ml
in 1 ml → y

Example: on average 6 conidia are contained in 25 x 10^{-8} ml

6 / 25 x 10^{-8} ml = 0,24 x 10^{8} conidia / ml =

C = 24 Mio. conidia/ml

7.5. Evaluation

The dilution factor is calculated with the following formula:

$$C1 \times V1 = C2 \times V2$$

C1 = known concentration of conidia-suspension
V1 = unknown volume of conidia-suspension
C2 = known concentration of inoculum
V2 = known volume of inoculum

Example:
From the example mentioned in Pt. 7.4 10 litres of inoculum in a concentration of 50.000 conidia/ml should be prepared.

$$V1 = \frac{50.000 \text{ con/ml} \times 10000 \text{ ml}}{24 \times 1.000.000 \text{ con/ml}} = 20{,}8 \text{ ml}$$

Production of the desired inoculum:
20,8 ml from the counted conidia-suspension are filled up to 10 L.

7.6. Handling

The conidia-suspension is labelled as follows in the desired concentrations in appropriate PP-tubes and frozen (in our example 20,8 ml of conidia-suspension is frozen at -25 or at -80°C):

Fusarium xy / IFA-Nr Dissolved in xx L Is equivalent to yy.yyy K/ml

Example:

F.culmorum / IFA104 Dissolved in 10 L Is equivalent to 50.000 K/ml

The necessary quantity of frozen conidia-suspension tubes are thawed and vibrated in handwarm water and diluted accordingly to the data on the label.

7.7. Units of the results

The unit of concentration is K/ml (conidia/ml).

The exact dilution data are, as described in Pt. 7.6., on the label.

8. Applicable Documents

Production of growth media (Test- SOP 3 - 03)
Measurement of inoculum concentration with Bürker-Türk chamber (Test-SOP 3-S-06)
Manufacturing of master cultures
Laboratory order from 06 December 2005

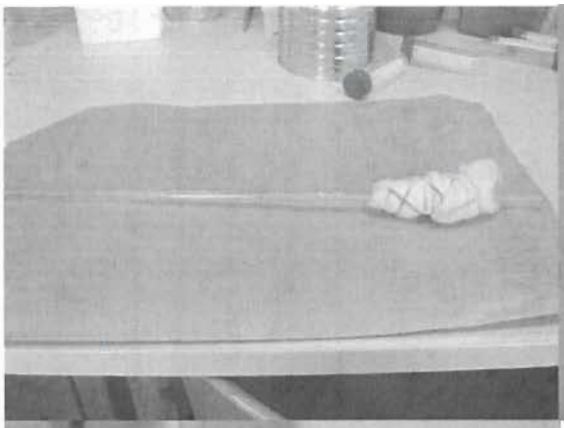
9. Literature

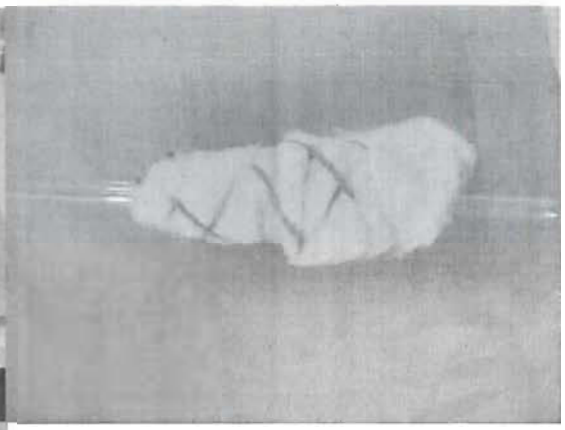
Not available

10. Attachments

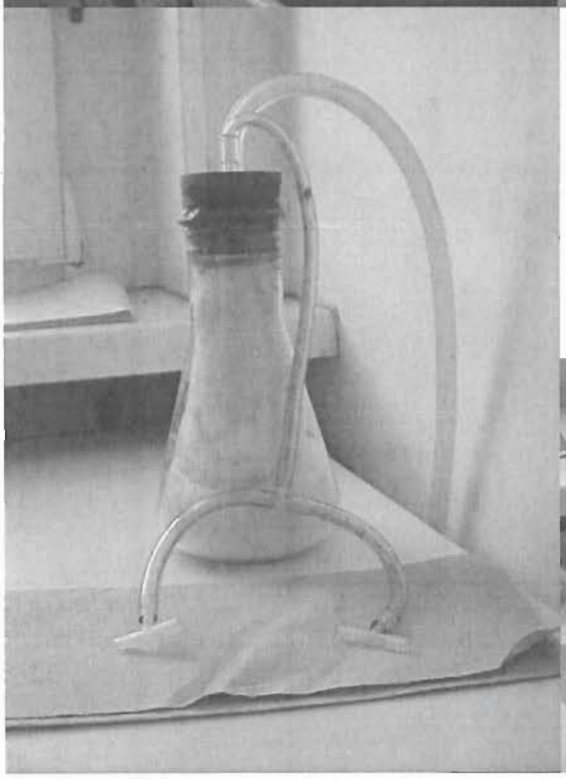
SOP-Validity proof
SOP-mailing list

BOKU	Page: 7 von 10 Valid per: 15.10.2007	Standard operating procedure SOP 3-01
	Revision: 0	Production of Inoculum of F. spp with Bubble Breeding

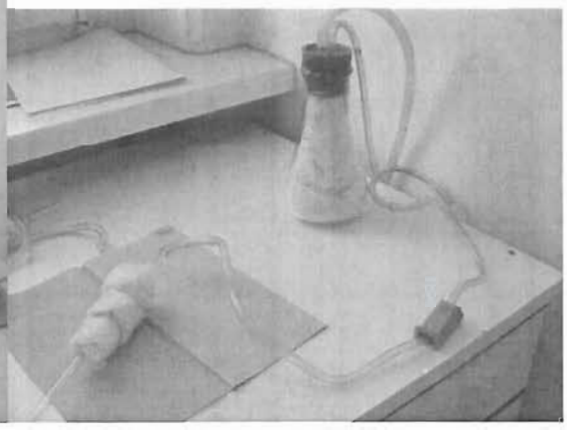




Preperation of the cotton wool stopper

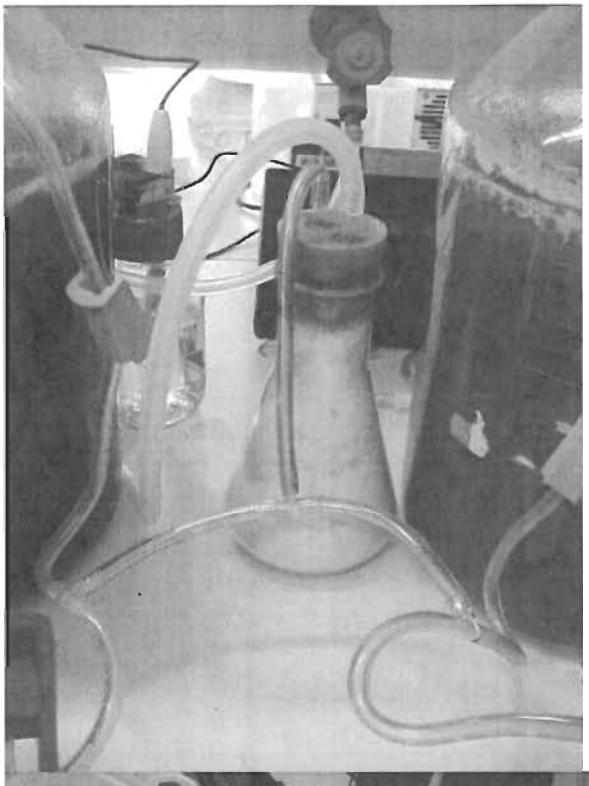


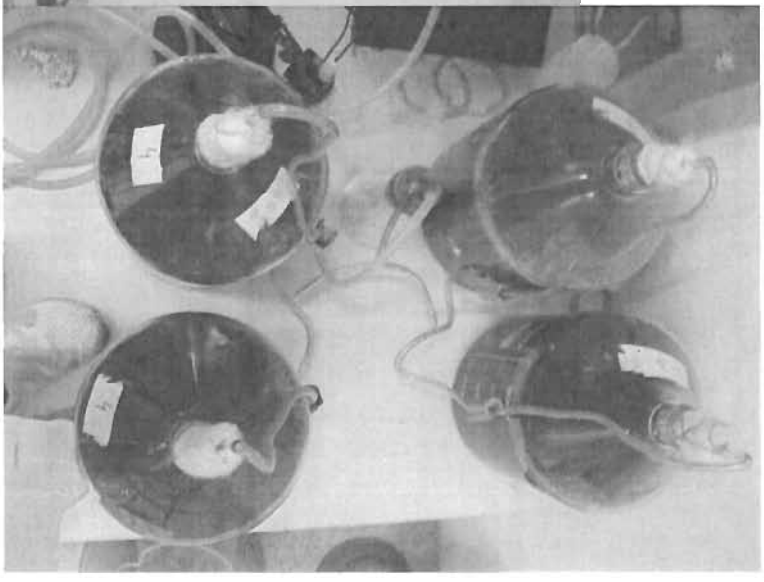
Sterilfilter



connection sterilfilter - cotton wool stopper

	Page: 8 von 10 Valid per: 15.10.2007	Standard operating procedure SOP 3-01
	Revision: 0	Production of Inoculum of F. spp with Bubble Breeding





Complete connection:
Stopper in flasks with inoculated mungbeanmedium
Connection to sterilfilter and compressed a