Aspects of resistance to Fusarium head blight caused by *Fusarium culmorum* in wheat

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Aspects of resistance to Fusarium head blight caused by *Fusarium culmorum* in wheat

Proefschrift
ter verkrijging van de graad van
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STELLINGEN

1. Om te voorkómen dat Fusarium toxinen in graanproducten vóórkomen is epidemiologisch onderzoek onontbeerlijk.

2. Een acceptabel niveau van partiële resistentie berust veelal op een oligogene basis en niet op een polygene basis.

3. Het stapelen van resistentiegenen ter verhoging van de duurzaamheid van de resistentie zal bij tarweveredeling alleen worden toegepast wanneer moleculaire merkers voorhanden zijn.

4. Het routinematig stoppen van routineonderzoek getuigt van ondoordachtheid.

5. Het gebruik van overgevoeligheidsresistentiegenen in tarwerassen betekent dat de pathogeenpopulatie permanent bewaakt moet worden ten aanzien van verschuivingen in de frequenties van virulentiegenen: een routinematig onderzoek.

6. De benaming 'secundaire metaboliet' houdt niet in dat de metaboliet van secundair belang of waarde is.

7. Het consumeren van 'Quorn' (Marlow Foods), bestaande uit geconcentreerde, in steriele voedingsoplossing gekweekte schimmelmassa van *Fusarium graminearum*, als hoogwaardig gezondheidsproduct zonder cholesterol of onnodige calorieën maar mét vezels en 12 gram eiwit per ons (Anon., 1989), moet sterk worden ontraden.

   Anonymous, 1989. Vegetariër zijn in Nederland is niet lastig. NRC-handelsblad, mei 1989

8. De door Zeven (1990) geciteerde bewering dat het gebruik van de woorden *tarwe* en *weit* (met hun dialectische vormen) voor broodtarwe in Nederland geografisch verdeeld is door de meridiaan 'voormalige Zuiderzee - provincie Utrecht', waarbij ten westen van deze meridiaan *tarwe* gebruikt wordt en ten oosten *weit*, is onjuist.

   Zeven, A.C., 1990. Landraces and improved cultivars of bread wheat and other wheat types grown in the Netherlands up to 1944. Wageningen Agricultural University Papers 90.2

9. Het is zeer aanbevelingswaardig om het in productie nemen van bio-ethanol op basis van tarwe te onderzoeken.

   NRLO-Werkgroep 'Biomassa ten behoeve van energie', 1990. Het gebruik van agrarische producten voor transportbrandstoffen. NRLO-rapport 90/14.

10. "Let fame, that all hunt after in their lives,
    Live register'd upon our brazen tombs,
    And then grace us in the disgrace of death;
    When, spite of cormorant devouring Time,
    Th' endeavour of this present breath may buy
    That honour which shall bate his scythe's keen edge,
    And make us heirs of all eternity."

    Shakespeare. Love's labour's lost.

    is een metafoor voor
    "Publish or perish".

11. Brood, daar zit wat in!

12. De grootte van een stuk vlaai neemt binnen Nederland lineair af met de hoogte van de breedtegraad.

Stellingen behorende bij het proefschrift van Charles H.A. Snijders, getiteld 'Aspects of resistance to Fusarium head blight caused by *Fusarium culmorum* in wheat', te verdedigen op 9 oktober 1990 in de Aula van de Landbouwuniversiteit te Wageningen.

Author's abstract

In the Netherlands, Fusarium head blight of wheat is predominantly caused by *Fusarium culmorum*. A low infection level leads to important yield losses and contaminates the grain with mycotoxins, particularly deoxynivalenol. This mycotoxin is suggested to have toxic effects on the wheat plant. Genetic variation for resistance to Fusarium head blight appears to be very large. The resistance is quantitative; complete resistance does not exist. The estimated number of resistance genes in the studied winter wheats was small, the individual genes had large effects on resistance and inherited mainly additive. Transgression for resistance was observed in progenies. It was possible to select plants on a single plant base. No indications were found for *Fusarium* strain-specific resistance. Systemic fungal growth in wheat stems and variation in resistance for this trait was also demonstrated. There is a large potential for breeders to develop cultivars with a high level of resistance to Fusarium head blight.

"... if any fungus were to emigrate to another planet, *Fusarium*, as a most adaptable, versatile and pioneering genus, would be a good candidate for establishment out there."

W.C. Snyder, 1981. In: *Fusarium*: diseases, biology and taxonomy. Ed.: P.E. Nelson, T.A. Toussoun & R.J. Cook. Pennsylvania State University. pp.3-8.

VOORWOORD

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GENERAL INTRODUCTION

In August 1985, the Foundation for Agricultural Plant Breeding (SVP) and the Netherlands Grain Centre (NGC) initiated two research projects in wheat with the aim to develop selection methods for and identify sources of resistance to (1) head diseases caused by Fusarium head blight and *Septoria nodorum* Berk. (teleomorph: *Phaeosphaeria nodorum* (Müller) Hedjaroude) glume blotch, and (2) leaf blotch caused by *Septoria nodorum* and *Septoria tritici* Rob. ex. Desm. (teleomorph: *Mycosphaerella graminicola* (Fückel) Schroeter). In the period 1986 to 1988 the research was carried out under contract of NGC. After the season 1985-1986, the research was concentrated on resistance to Fusarium head blight caused by *Fusarium culmorum* (W.G. Smith), and the research program was extended to form the basis of this thesis. After the contract with NGC expired, a permanent position at the SVP made it possible to continue the research and finish this thesis on resistance to Fusarium head blight.

Fusarium head blight

Taxonomy and nomenclature of *Fusarium* species causing head blight is confusing. In this thesis the nomenclature used follows the system given in Nelson *et al.* (1983).

Fusarium head blight is a fungal disease of wheat found in both temperate and semi-tropical regions. A number of species of *Fusarium* may be responsible but generally *F. graminearum* Schwabe, with perfect state *Gibberella zeae* (Schw.), and *F. culmorum* (W.G. Smith), perfect state unknown, predominate. In the cooler climates of north-western Europe, including the Netherlands, *F. culmorum* becomes dominant. Both *F. graminearum* and *F. culmorum* cause root rot, foot rot, crown rot, stem rot and head blight in wheat (Nelson *et al.*, 1981). Both fungi also cause stalk rot and ear rot in maize and infect other cereals such as barley, oats and triticale (Booth, 1971). Heavy damage to wheat often occurs in areas of continuous cultivation or where wheat is planted after maize. Damage from head blight is caused in the form of reduced kernel set and kernel weight, destroyed starch granules and storage proteins, and seed infection (Bechtel *et al.*, 1985).

The Fusarium head blight fungi produce a large number of secondary metabolites, not all of which have been characterized, including the trichothecenes deoxynivalenol (vomitoxin) and nivalenol. These toxins have a range of chronic and acute effects on humans and animals that consume infected grain (Marasas *et al.*, 1984).

The pathogen originates from seed, soil, and from inoculum on host residues, including those of maize, small-grained cereals and certain grasses (Khonga & Sutton, 1988). Practice for suppressing initial inoculum, notably rotation of wheat

with non-host crops and ploughing of infested residues have long been recommended for managing Fusarium head blight in wheat. There are no practical chemical treatments that are effective in preventing this disease. The only and best way to eliminate this problem is to introduce adapted cultivars that are resistant to Fusarium head blight.

This thesis

The purpose of this thesis was to investigate the host-pathogen relationship between winter wheat and *Fusarium culmorum*, to identify sources of resistance to Fusarium head blight, to evaluate the nature and genetic aspects of resistance, and to study the relation between disease incidence and damage.

At the time this study was initiated, information on the host-pathogen relationship between wheat and *Fusarium* spp. was scarce, especially regarding *Fusarium culmorum*. The relevant literature is summarized in the concerning chapters.

This study was mainly carried out with SVP wheat breeding lines and wheat cultivars registered in the Netherlands, which had been evaluated for resistance at the SVP in the years 1982-1984. Based on these unpublished results, in 1985 a collection of standard winter wheat genotypes was chosen which covered the range from most resistant to most susceptible. However, during the following years, genotypes were identified which showed resistance or susceptibility levels that surpassed the extremes in the collection of standards.

During the first season, the reaction of wheat heads to *Fusarium* spp. was studied with isolates of *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium nivale* (Fr.) Ces. (perfect state: *Monographella nivalis* (Schaffnit) Müller), all collected in the Netherlands. However, the pathogenicity of *F. graminearum* and *Fusarium nivale* isolates in field and glasshouse trials in 1986 was low. Also inoculum production of these last two species caused problems. As the cool summers in the Netherlands cause *F. culmorum* to be the predominant Fusarium head blight species, research was further limited to isolates of this last *Fusarium* species.

The thesis is divided in chapters, the first eight prepared for individual publication. Chapter 9 contains the general discussion. The first eight chapters are published in scientific journals (or in press). Chapter 8 is published in a shortened version. Chapter 1 of the thesis contains a review of the aspects of the *Fusarium* toxin deoxynivalenol and discusses the natural occurrence of this mycotoxin in relation to Fusarium head blight epidemics. Chapter 2 discusses the effect of Fusarium head blight caused by *Fusarium culmorum* on deoxynivalenol content and kernel number and weight. Chapter 3 demonstrates the existence of systemic fungal growth of *Fusarium culmorum* in wheat stem tissue and discusses its consequence for Fusarium head blight. Chapter 4 addresses the question of the strain-specificity of

resistance, using four *F. culmorum* isolates and the collection of 17 winter wheats used as standards. In chapter 5 and 6 the genetic basis of the resistance to *F. culmorum* is investigated of 10 winter wheats with different levels of resistance. The response to selection for Fusarium head blight resistance in F_2 generations is presented in chapter 7. In chapter 8 sources of resistance to Fusarium head blight are identified in a collection of winter and spring wheats, and the relation between visual head blight symptoms and yield reduction is described.

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Chapter 1

FUSARIUM HEAD BLIGHT AND MYCOTOXIN CONTAMINATION OF WHEAT, A REVIEW

Summary

An infection of bread wheat by Fusarium head blight contaminates the crop with mycotoxins, particularly deoxynivalenol (DON) and nivalenol (NIV). The toxicity and natural occurrence of these mycotoxins in wheat are reviewed. Based on eight years data of Fusarium head blight epidemics of wheat in the Netherlands, DON contamination of the grain was estimated. Fusarium head blight ratings averaged an infection of 1.7% of all spikelets; estimates for DON contamination averaged 0.9 mg kg^{-1}. Taking a guideline level for DON in uncleaned bread wheat of 2 mg kg^{-1}, in 1979 and 1982 a wheat crop was produced with estimated DON concentrations above the limit of tolerance. Human and animal exposure to mycotoxins in the Netherlands appears to be small but chronic. The information presented in this paper illustrates the need for an annual evaluation of the crop for Fusarium head blight incidence and mycotoxin content, and the necessity of Fusarium head blight resistant wheat cultivars.

Introduction

In wet summers, Fusarium head blight can be an important problem in wheat. In the Netherlands, the species isolated from *Fusarium*-infected heads are mainly *F. culmorum* (W.G. Smith) Sacc. (teleomorph unknown) and *F. graminearum* Schwabe (teleomorph *Gibberella zeae* Schw.). Several forms of damage can be distinguished. An infection of the head by *Fusarium* can reduce kernel set and kernel weight, causing a yield reduction. Invasion of the kernel by *Fusarium* destroys the starch granules, storage proteins and cell walls, resulting in a poor quality product (Bechtel *et al.*, 1985; Meyer *et al.*, 1986). The embryo is usually not infected except in heavily invaded kernels. However, slightly infected kernels with apparently uninfected embryos exhibit reduced germination and vigour (Bechtel *et al.*, 1985). As a result, a *Fusarium* epidemic can be a serious problem for seed production.

Various *Fusarium* species including *F. culmorum* and *F. graminearum* are capable of producing mycotoxins in crops. The most notorious mycotoxins of *F. culmorum* and *F. graminearum* in wheat are of the trichothecene class: deoxynivalenol (DON, vomitoxin), acetyldeoxynivalenol (the isomers 3-ADON and 15-ADON) and nivalenol (NIV) (Kurata and Ueno, 1984; Marasas *et al.*, 1984), amongst which DON occurs most frequently. Quality loss because of mycotoxin content is an underestimated form of damage. Data on mycotoxin contents of West European wheat crops are scarce. The objective of this study was to review the toxicity and occurrence of DON and NIV in wheat, and to estimate the DON contamination of wheat grown in the Netherlands based on eight years data of Fusarium head blight epidemics.

Toxicity of *Fusarium* toxins

Fusarium toxins are harmful to human and animal health. The toxicity of the isomers 3-ADON and 15-ADON is comparable, and is about twice that of DON (Mirocha *et al.*, 1989). From the toxicity tests summarized by Joffe (1986), it can be concluded that NIV is about 10 times more toxic than DON.

Several papers report the effects on pigs fed on diets of DON-contaminated wheat (Bryden *et al.*, 1987; Friend *et al.*, 1986; Tobin, 1988). Swine diets of wheat naturally contaminated with *Fusarium* containing only 0.3 mg kg^{-1} DON resulted in decreased feed consumption and weight gain (Trenholm *et al.*, 1981). Foster *et al.* (1986) concluded that 4.7 mg kg^{-1} DON added in pure form to the diet did not have any lasting effect on feed consumption and weight gain, while a diet of naturally contaminated wheat with the same concentration of DON had significantly lower feed intake and weight gain values. Apparently other toxic metabolites in *Fusarium* contaminated feed contributed to the poor performance. The reason for pigs refusing the feed is not known; there is little evidence that smell, taste, or a combination of the two is responsible (Friend and Trenholm, 1988). Toxin contaminated feed will lead to poor performance and health, and as a consequence economic losses.

Poultry can tolerate larger concentrations of DON in their diet (Bryden *et al.*, 1987; Hamilton *et al.*, 1985; Manley *et al.*, 1988; Trenholm *et al.*, 1981). Young chickens and turkey poults can tolerate diets that contain DON up to at least 5 mg kg^{-1} from wheat (Hamilton *et al.*, 1985) .

Ill effects can occur also in humans because of the dietary intake of DON or its metabolites. The acute symptoms of trichothecene poisoning are characterized by: skin irritation, food refusal, vomiting, diarrhea, hemorrhages, neural disturbance, miscarriage and death (Joffe, 1986; Kuiper-Goodman, 1985). Human toxicoses due to ingestion of mycotoxin-contaminated food are well-documented (Bhat *et al.*, 1989). Chronic ingestion of small amounts of trichothecenes may result in an important secondary effect: the predisposition to infectious diseases through suppression of the immune system (Kuiper-Goodman, 1985; Miller and Atkinson, 1987). Precautions should be taken to avoid inhalation of mycotoxin-containing spores and dust, and direct skin contact with infected kernels (Trenholm *et al.*, 1989).

Carry over of DON into food for human consumption

Deoxynivalenol present in the rations of Leghorn chickens, laying hens and broiler chickens at dietary levels of 5 mg kg^{-1} was not detected in eggs or tissues at a detection limit of 10 μg kg^{-1} tissue (El Banna *et al.*, 1983). None of the eggs collected from White Leghorn hens given a DON-contaminated wheat diet (18 mg kg^{-1}) contained detectable quantities (detection limit: 10 μg kg^{-1}) of DON (Kubena *et al.*, 1987). Studies on the presence of DON-derived residues in milk of sheep indicated that only trace amounts (< 10 μg L^{-1}) were transmitted following either oral or intravenous administration of the toxin (Prelusky *et al.*, 1987).

Milling did not remove DON from naturally contaminated wheat (Lee *et al.*, 1987), but the toxin was distributed in the milling fractions (Scott *et al.*, 1983). On average, baking and cooking reduces DON in wheat or wheat products by 40% (Abbas *et al.*, 1988; Besling *et al.*, 1983; Carvajal *et al.*, 1987; Isohata *et al.*, 1986; Young *et al.*, 1984). Therefore, wheat products made from DON-contaminated wheat will still contain DON.

Current regulations

'Agriculture Canada' advises that animals should be fed diets containing less than 1 mg kg^{-1} DON and that clean grain should be fed to pregnant and lactating animals (J.D. Miller, Plant Research Centre Ottawa, personal communication). Sweden has a regulation advising a maximum of 0.5 mg kg^{-1} in the diets of swine and 2 mg kg^{-1} in cows (Pettersson and Wennberg, 1988).

For human nutrition, a tolerable daily DON intake of 3.0 μg kg^{-1} body weight for adults, and 1.5 μg kg^{-1} body weight for infants was established in Canada (Kuiper-Goodman, 1985). The guideline level for DON in uncleaned wheat and in the flour portion of finished foods made from bread wheat are therefore 2 mg kg^{-1} and

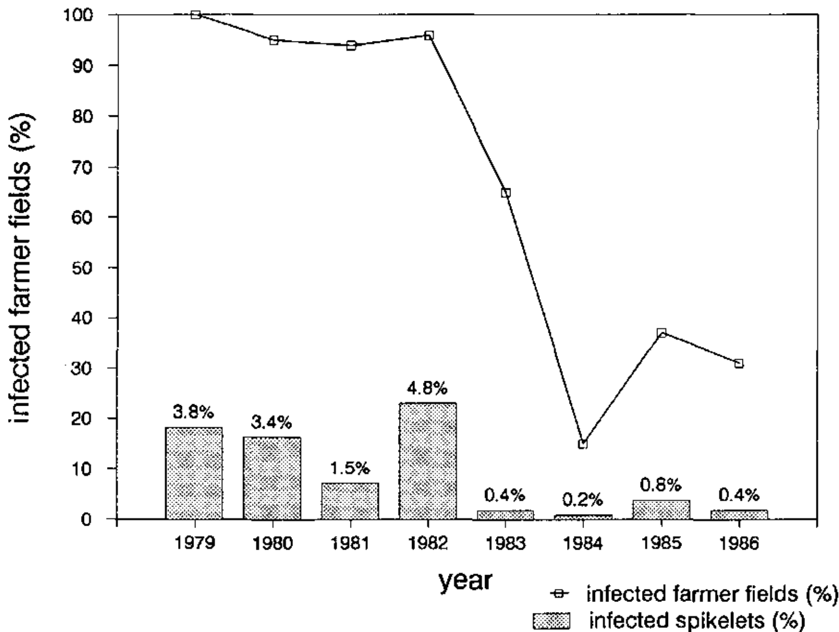

Figure 1.1: Fusarium head blight prevalence (percentage infected farmer fields) and percentage infected spikelets of affected fields in the Netherlands for the years 1979-1986. Source: EPIPRE (Anon. 1985b; 1987b; Daamen *et al.*, 1990).

1.2 mg kg^{-1}, respectively. For the U.S. the tolerance levels advised by 'Food and Drug Administration' are 2 and 1 mg kg^{-1}, respectively (Van Egmond, 1989). In Europe only Rumania and the U.S.S.R. have specified tolerance levels for DON: 0.005 mg kg^{-1} food and 0.5 mg kg^{-1} wheat, respectively (Van Egmond, 1989).

Fusarium head blight epidemics in the Netherlands

Figure 1.1 illustrates the Fusarium head blight occurrence in the Netherlands for the years 1979-1986, expressed in prevalence, i.e. the percentage of infected farmer fields, and the percentage infected spikelets of affected fields. The data were collected from the EPIPRE program (Anon. 1985b, 1987b; Daamen *et al.*, 1990) and illustrate the irregular pattern of epidemics over the years. The period 1979-1982 had a higher prevalence and heavier spikelet infections than the subsequent four years. As the relative acreage of cultivars grown did not change in the period 1979-1986 (Figure 1.2; Anon., 1987c), different resistance levels cannot explain the pattern of epidemics. Also, there was no qualitative change in the use of fungicides and seed coatings (Anon., 1979; 1981; 1985a; 1987a). Correlations coefficients for Fusarium head blight prevalence and percentage infected spikelets of affected fields versus weather conditions of the year concerned and the preceding year, and versus Fusarium head blight prevalence and percentage infected spikelets in the preceding year are given in Table 1.1. As in the Netherlands winter wheat (which covered in 1979-1986 92%

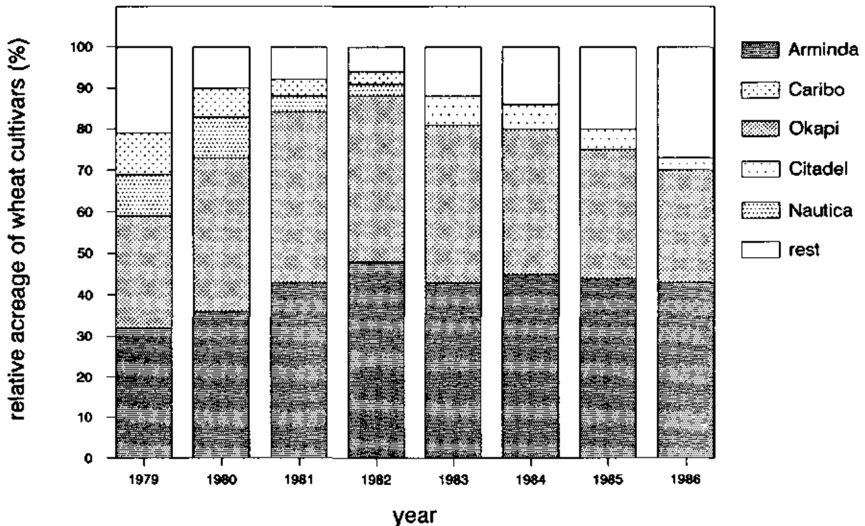

Figure 1.2. Relative acreage of winter wheat cultivars in the Netherlands in the years 1979-1986.

Table 1.1: Correlation coefficients for Fusarium head blight prevalence[a] and percentage infected spikelets of affected fields versus weather conditions[b] of the year concerned and the preceding year, and versus Fusarium head blight prevalence and percentage infected spikelets in the preceding year

	Fusarium head blight prevalence	percentage infected spikelets
year concerned		
temperature	-0.16	-0.24
relative humidity	0.64	0.67
precipitation	0.70	0.77*
preceding year		
temperature	-0.48	-0.34
relative humidity	0.74*	0.29
precipitation	0.48	-0.03
prevalence	0.76*	0.52
percentage infected spikelets	0.67	0.16

[a]Based on the percentage infected farmer fields.
[b]Temperature (sum of the daily average over the 30 days), relative humidity (sum of the daily average over the 30 days) and total precipitation in the period 11 June to 11 July of the years 1978-1986. Presented values are means over 5 stations in the Netherlands, viz De Kooy, Eelde, de Bilt, Vlissingen and Beek. Source: Anon. 1978-1986.
*Significant at P=0.05.

of the total wheat acreage) flowers in the second half of June, data on temperature, relative humidity and precipitation were gathered for the period 11 June - 11 July (Anon., 1978-1986). The positive correlation between Fusarium head blight prevalence and prevalence in the preceding year cannot be ascribed to the occurrence of soil-borne inoculum, as the crop rotation system prevents this. However, *Fusarium* winters on infected debris of cereals, corn or weeds, and wind and rain will further spread conidia and mycelium (Logrieco *et al.*, 1988; Sutton, 1982). Another explanation for the correlation might be that a high head blight prevalence resulted in seed infections of sowing-seed for the following year. Seed infection therefore could also explain the correlation between head blight prevalence and relative humidity of the period 11 June to 11 July in the preceding year (Table 1.1). The high correlation between percentage infected spikelets and precipitation in the period 11 June to 11 July is explained by the fact that *Fusarium culmorum* and *F. graminearum* are favoured by wet conditions (see also Daamen *et al.*, 1990). Above all, they are typical splash-borne pathogens (Sutton, 1982).

Natural occurrence of *Fusarium* toxins

Table 1.2 illustrates the natural occurrence of DON in wheat samples of recent years. All the wheat samples were randomly collected from several growing areas, grain-elevators or graded lots. Average DON concentrations varied from 0.03 mg kg^{-1} to 1.78 mg kg^{-1}. Maximum DON concentrations varied from 0.09 mg kg^{-1} to 8.53 mg kg^{-1}. For the Netherlands data exist only for the crops of 1982, 1983 and 1984 (Besling *et al.*, 1983; Besling, 1985; Tanaka *et al.*, 1990). Samples from Austria, Hungary and Poland were analyzed for 3-ADON, but none was detected (Ueno *et al.*, 1985). Some of the authors cited analyzed the wheat samples also for the more toxic nivalenol (NIV). Sundheim *et al.* (1988) detected NIV in 53 out of 53 Norwegian samples with

Table 1.2. Average and maximum deoxynivalenol contents (mg kg^{-1}) in positive samples from home-grown wheat

country	crop year	number of samples out of total	DON mean (mg kg^{-1})	DON max. (mg kg^{-1})	reference
Australia	1983	20/25	0.14	1.7	Blaney *et al.*, 1987
Austria	1984	3/4	0.36	0.91	Ueno *et al.*, 1985
Canada	1980	36/77	1.06	8.53	Trenholm *et al.*, 1983
	1986	8/8	0.27	0.45	Teich *et al.*, 1987
F.R.Germany	1982-1983	3/5	0.11	0.22	Besling *et al.*, 1983
	1984	5/9	0.06	0.09	Besling, 1985
France	1984	8/18	0.06	0.14	Besling, 1985
	1982-1983	22/25	0.09	0.27	Besling *et al.*, 1983
Hungary	1984	2/2	0.70	1.29	Ueno *et al.*, 1985
The Netherlands	1982-1983	12/18	0.05	0.16	Besling *et al.*, 1983
	1984	8/20	0.06	0.14	Besling, 1985
	1984	13/13	0.12	0.51	Tanaka *et al.*, 1990
Norway	1984	32/53	0.35	3.19	Sundheim *et al.*, 1988
Poland	1984	13/42	0.09	0.31	Ueno *et al.*, 1985
Sweden	1984	8/14	0.40	1.18	Pettersson *et al.*, 1986
U.K.	1984	20/31	0.03	0.31	Tanaka *et al.*, 1986
	1980-1982	32/200	0.20	0.40	Osborne & Willis,1984
U.S.	1984	7/11	0.06	0.14	Besling, 1985
	1982-1983	11/13	0.22	0.50	Besling *et al.*, 1983
	1982	31/33	1.78	5.50	Hagler *et al.*, 1984

an average of 0.06 mg kg^{-1} and a maximum of 0.89 mg kg^{-1}. Ueno *et al.* (1985) detected NIV in 4 out of 5 Austrian samples with an average of 0.02 mg kg^{-1} and a maximum of 0.04 mg kg^{-1}; in 37 out of 42 Polish samples an average of 0.05 mg kg^{-1} with a maximum of 0.35 mg kg^{-1} was detected. Tanaka *et al.* (1986) found NIV in 17 out of 31 U.K. samples with a mean of 0.10 and a maximum of 0.67 mg kg^{-1}. Tanaka *et al.* (1990) detected NIV in 12 out of 13 Dutch samples with a mean of 0.04 mg kg^{-1} and a maximum of 0.20 mg kg^{-1}. Osborne and Willis (1984) did not detect any NIV in U.K. samples at a detection limit of 0.05 mg kg^{-1}.

Lepschy-v. Gleissenthal *et al.* (1989) analyzed 'suspect' field samples of the 1987 Bavarian wheat crop for DON and NIV: 92 out of 106 DON-positive samples contained an average of 3.96 mg kg^{-1} with a maximum of 43.80 mg kg^{-1}. In samples with higher DON concentration NIV was detected with a concentration between 0.10 and 0.29 mg kg^{-1}.

From the Fusarium head blight prevalence PRE (%) and the percentage infected spikelets of affected fields SPI, a Fusarium head blight rating (%) can be calculated for each year as PRE × SPI / 100. For the years 1979-1986 the Fusarium head blight ratings averaged an infection of 1.7% of all spikelets. From the data reported by Snijders and Perkowski (1990) based on artificial inoculation of ten wheat genotypes with one pathogenic strain of *F. culmorum* (IPO 39-01), a linear regression of DON concentration (mg kg^{-1}) on head blight rating (%) was estimated with a regression coefficient β of 0.54 (constant=0; df=9; R^2=93%). The yearly Fusarium head blight ratings and the estimated DON levels in the kernels of the crops of the years 1979-1986 are given in Table 1.3. Estimates for DON contamination averaged 0.9 mg kg^{-1}. The estimates for kernel DON content in wheat grown in the Netherlands for the 1984 crop (Table 1.3) and the results from the toxin analysis (Table 1.2) both show low concentrations. Regarding the current regulations mentioned above, the estimated DON concentrations of the wheat crops of 1979 and 1982 were too high.

Estimates of DON-contaminated food intake in the Netherlands

During 1979-1986, the Netherlands produced annually an average of 946,276 tonnes of wheat, of which 23% was used for human consumption, 36% for feed and the rest for export and food aid (Anon., 1979-1987). Of the feed wheat, 55% was used for poultry and 39% for swine (Anon., 1988).

Indirect intake of DON by man through the consumption of animal tissues and produce from animals that were fed DON-contaminated feed is assumed to be essentially zero. Therefore, estimates of human exposure to DON are based on the direct intake by consumption of wheat products. Using a figure of 14.5 million people as the population of the Netherlands, it can be calculated that the human consumption of wheat grown in the Netherlands is ±16 kg/person/year. The total consumption of wheat flour per person in 1986 was 53 kg (Anon., 1988). Averaged over the years following the crops of 1979-1986, the sources of imported wheat used

Table 1.3. Fusarium head blight rating (percentage infected farmer fields × percentage infected spikelets of affected fields /100) and estimated kernel DON content for the wheat crop of the years 1979-1986 in the Netherlands. DON concentration was estimated by a linear regression of DON (mg kg^{-1}) on head blight rating (%) with β=0.54 and constant=0

crop year	Fusarium head blight rating (%)	DON content (mg kg^{-1})
1979	3.80	2.05
1980	3.23	1.74
1981	1.41	0.76
1982	4.61	2.49
1983	0.26	0.14
1984	0.03	0.02
1985	0.30	0.16
1986	0.12	0.07

Table 1.4. Percentage of imported wheat that was imported from E.C. countries (EC%), and estimated daily DON intake per person in the Netherlands for the years following the wheat crops of 1979-1986

crop year	EC%	intake/ person/day (μg)
1979	63	129.5
1980	67	114.2
1981	67	49.9
1982	76	176.3
1983	73	9.7
1984	83	1.2
1985	89	12.6
1986	95	5.5

in the Netherlands were: 77% imported from E.C. countries (Table 1.4) (of which 69% from France), 20% from the U.S. and 3% from Canada (Anon. 1979-1987). Assuming (*i*) 53 kg total consumption of wheat flour per person per year, of which 70% imported, (*ii*) all E.C.-grown wheat had the estimated DON concentration of the Netherlands home-grown wheat, (*iii*) the American and Canadian wheat contained no DON, and (*iv*) cooking or baking reduced DON by 40%, the daily intake of DON per person can be estimated (Table 1.4). Exposure to DON was small, but chronic. In the year following the crop of 1982 as much as 176.3 μg DON/person/day was consumed. Given the tolerable daily DON intake of 3 μg kg^{-1} body weight and a mean human body weight of 60 kg, the estimated daily intake in 1982 was about equal to the limit of tolerance advised in Canada and the U.S..

Conclusions

This analysis indicates the need for more information on the problem of *Fusarium* mycotoxins in wheat grown in Western Europe. More epidemiological data should be collected to develop a better model of the relation between climatic factors, Fusarium head blight infection and mycotoxin content. The irregular pattern of the Fusarium epidemics has led to an underestimation of the potential danger of a toxin contaminated wheat crop. In consideration of human and animal health, inspection of the crop and harvest is necessary. Breeding wheat for resistance to Fusarium head blight is of the utmost importance.

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Chapter 2

EFFECTS OF HEAD BLIGHT CAUSED BY *FUSARIUM CULMORUM* ON TOXIN CONTENT AND WEIGHT OF WHEAT KERNELS

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Summary

Ten winter wheat genotypes were inoculated with three strains of *Fusarium culmorum* (IPO 39-01, IPO 348-01 and IPO 436-01) isolated in the Netherlands. Seed samples were analyzed for several trichothecene mycotoxins and zearalenone. Deoxynivalenol was detected in concentrations ranging from 0 to 48 mg kg^{-1}. The mycotoxins 3-acetyldeoxynivalenol, nivalenol, fusarenon-X and zearalenone were not detected. Interactions between strains and genotypes were observed for head blight and kernel deoxynivalenol content. For each strain, high correlations were found between deoxynivalenol content and yield reduction. Path analysis suggested a relation between deoxynivalenol and kernel weight reduction. Infection by a highly pathogenic strain reduced yield in terms of kernel number reduction. In the case of two moderately pathogenic strains yield loss was ascribed to lower kernel weight. This is the first report on the relationships among head blight incidence caused by *Fusarium culmorum*, kernel toxin content and reduction of yield.

Introduction

In the Netherlands, Fusarium head blight in wheat is caused by *Fusarium culmorum* (W.G. Smith) Sacc. and to a lesser extent by *F. graminearum* Schwabe. These two pathogens are closely related, and plant resistance to *F. culmorum* is correlated with resistance to *F. graminearum* (Mesterhazy, 1983; 1987). Various *Fusarium* species including *F. culmorum* and *F. graminearum* are capable of producing mycotoxins in crops (Marasas *et al.*, 1984). The most notorious mycotoxins of *F. culmorum* and *F. graminearum* in wheat are deoxynivalenol (DON), and 3-acetyldeoxynivalenol (3-ADON) (Visconti *et al.*, 1986). These toxins are harmful to human and animal health. Canada allows a maximum DON concentration in unground wheat for human consumption of 1-2 mg kg^{-1}; in the United States this tolerance level is advised (Van Egmond, 1989).

Reports on the relationships among Fusarium head blight, toxin concentration and yield reduction are scarce. Head blight-susceptible cultivars of wheat contained much higher concentrations of DON in the kernels than resistant cultivars after inoculation with a single strain of *F. graminearum* (mean 6.56 versus 0.83 mg of DON kg^{-1}) (Miller *et al.*, 1985). From these data a correlation of 0.85 (8 df) between DON and spike weight reduction can be derived. No correlation was found between DON and kernel weight reduction. In a comparable study a correlation of 0.74 (12 df) was

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observed between Fusarium head blight and kernel DON content (Wang and Miller, 1988). From data on wheat heads enclosed in plastic bags after inoculation with *F. graminearum* and *F. culmorum* (Mesterhazy, 1983), a correlation between head blight and spike weight reduction can be derived of 0.88 (16 df), and a correlation between head blight and yield reduction can be derived of $r=0.91$. In commercial wheat fields infected by *F. graminearum*, correlations between head blight (percentage of heads with bleached spikelets 10 days after heading) and DON were found of 0.79 (20 df) (Teich and Hamilton, 1985) and 0.74 (23 df). No correlation was observed between blight and yield, and DON concentration and yield (Teich *et al.*, 1987).

This study aims to establish the relationships among head blight caused by *F. culmorum*, kernel toxin content, and reduction of yield. It is the first of its kind involving *F. culmorum*.

Materials and methods

Source of wheat kernels

Sixty seed samples from 10 genotypes inoculated with three strains of *F. culmorum* were selected for mycotoxin analysis. The 10 genotypes were drawn from a set of winter wheat cultivars and homozygous SVP-lines representing the whole range of Fusarium head blight susceptibility based on the SVP-data available in 1985. Ten strains, taken from monospore cultures of wheat-derived isolates of *F. culmorum* collected in the Netherlands, were prescreened for pathogenicity in the greenhouse. Two non-pathogenic strains were discarded. From the remaining eight strains three strains were drawn: IPO 39-01, IPO 348-01 and IPO 436-01, originating from isolations from a grain of seed, a head and a leaf sheath, respectively. The lyophilized strains are deposited at the Research Institute for Plant Protection (IPO), Wageningen. Conidiospores for inoculation were produced in 1 L Erlenmeyer flasks containing 250 ml sterilized wheat kernels of the cultivar Arminda. The cultures were incubated in darkness at 25°C for two weeks, followed by three weeks incubation at 5°C. To prepare spore suspensions, conidia were washed from the kernels with water.

On 22 November 1985, seeds were sown at a standard density of 330 seeds m^{-2} in rows 0.25 m apart. A split-plot design was established, with two blocks. Each plot, consisting of one genotype, was divided into subplots of 0.90×0.75 m across which the strains of *F. culmorum* and three controls were randomized so that the experimental subplots were separated from each other by border subplots of the same size.

Because wheat is most susceptible to Fusarium head blight at anthesis (Schroeder and Christensen, 1963), experimental inoculations were made at flowering time on 19, 24 or 25 June 1986, using a spore suspension of 250,000 spores ml^{-1}, at 1 L 10 m^{-2}. The spore suspension was sprayed from 0.25 m above the crop, for which a propane spray-gun was used. To ensure a high relative humidity during the nights

after inoculation, the field was sprinkled in the evening for 1 hr each day over a period of two weeks.

Incubation period, the period from inoculation to the appearance of first symptoms, was determined. These first symptoms consisted of light brown, watersoaked spots on the glumes. Soon, infected spikelets lost the water-soaked appearance, dried up, and assumed the colour of the ripe heads. On 15 July head blight values were determined as the product of the percentage of heads infected and the proportion of bleached spikelets per infected head. The third week of August, 25 leading tillers were randomly harvested per subplot. The heads were threshed by hand, and yield and kernel weight were assessed. Reduction of yield and 1000 kernel weight was assessed by calculating the difference between a subplot and the mean of the three control subplots for each main plot. This difference was expressed as a percentage of the mean value of the control subplots. To examine control plots for *Fusarium* contamination, seeds were plated out on selective *Fusarium* agar (SFA) (Burgess and Liddell, 1983).

In vitro production of mycotoxins

The toxigenic potential of the strains was determined by analyzing the mycotoxins (DON, 3-ADON and zearalenone) produced by *in vitro* cultures, growing for four weeks on wheat kernels ('Grana') of 45% moisture content at a temperature of 20°C. The procedure for this toxin analysis has been described (Bottalico *et al.*, 1983; Visconti *et al.*, 1986).

Chemicals and auxiliaries

All solvents were of analytical grade (Merck, Darmstadt, West-Germany). Charcoal columns were prepared as follows: a small ball of glass wool was placed in a filtration column (SPE C18 3 ml, Baker Chemicals, Deventer, The Netherlands), acid washed celite (0.15 g) (#545; Serva, Heidelberg, West-Germany) and a mixture containing 0.5 g alumina (Neutral activated; 70-230 mesh; Merck, Darmstadt, FRG), 0.9 g charcoal (SK-4; Serva), and 0.3 g celite was added. The column was connected to an assembly consisting of reservoir, filtration column, filter and stopcock (Baker Chemicals, Deventer, The Netherlands). For better separation, High Performance TLC-plates (#5633, Merck, Darmstadt, FRG) were activated for 1 hr at 110°C and then dipped in a solution of aluminum-chloride (Sigma, St.Louis, U.S.A)/water/methanol 10:15:90 (w/v/v). The plates were dried for 12 hr at room temperature followed by 1 hr at 40°C. Plates were washed by development with chloroform/acetone/2-propanol (70:15:15), air dried for 2 hr, and further washed by development with benzene/ acetone (60:35). The plates were dried as above. The trichothecene and zearalenone standards were obtained from Sigma, St.Louis, U.S.A. The rotary evaporator was a product of Büchi, Switzerland. Glass capillary micropipets were obtained from

Hirschmann Laborgeräte, FRG. A Vitatron TLD 100 fluorodensitometer was used.

Chemical analysis

In a first analysis the 60 samples of infected wheat kernels were analyzed for the trichothecene content of DON, 3-ADON, nivalenol (NIV), fusarenon-X (FUS-X), and zearalenone (ZEA) by thin layer chromatography (TLC), 2-dimensional TLC, and quantitative TLC, described by Visconti *et al.* (1986). The detection limit of this method was 0.1-0.5 mg kg^{-1}, depending on the toxin. Because initially only DON was found, only DON was analyzed in further chemical analyses. Samples were extracted with aqueous acetonitrile and cleaned up using charcoal columns (Eppley *et al.*, 1986; Tanaka *et al.* 1985; Trucksess *et al.*, 1984; Trucksess *et al.*, 1987). Each wheat sample (10 g) was ground and placed in a 200 ml Erlenmeyer flask with 40 ml acetonitrile/water (82:18). After being shaked vigorously for 15 min, the mixture was left for 12 hr and shaken again for 15 min. The samples were filtered under vacuum with a Buchner funnel on Whatman (#2) filter paper. The prepared columns were washed with 15 ml acetonitrile/water (82:18) mixture. After the vacuum had been disconnected, a clean filter flask was inserted and 20 ml of collected extract was put on the column. After 5 min, vacuum was again applied, providing a flow rate through the column of 1 ml min^{-1}. The extract volume was filtered to reach the glass wool in the bottom of the column and 30 ml acetonitrile/water (82:18) was added. The solution containing DON was transferred to a round-bottom flask. After the solvents had been evaporated under vacuum at 40°C, the residue was dissolved using two aliquots of 2 ml ethyl acetate and 1 ml chloroform/acetonitrile (4:1), and transferred to the vial. The solvent was evaporated under a stream of nitrogen. The dry residue was dissolved by sonification for 2 min in 200 μl of chloroform/acetonitrile (4:1). Extracts of 5 and 10 μl volume and the standard were applied to the prepared TLC plates by glass capillary micropipets. The plate was placed in an unsaturated tank containing chloroform/acetone/2-propanol (8:1:1). Each determination was carried out in five replicates. Blue fluorescent DON spots were quantified visually and via a fluorodensitometer using 20 μg of DON ml^{-1} as a working standard prepared in an ethyl-acetate/methanol (9:1) solution.

Recovery tests for DON (n=5) carried out according to the above method gave the following yields: at 0.5 mg kg^{-1} 88.5% ± 2.6; at 0.1 mg kg^{-1} 84.0% ± 3.0 and at 0.05 mg kg^{-1} 86.6% ± 3.9. The limit of detection for DON was 0.05 mg kg^{-1}, and minimum detection equalled 0.02 mg/spot. A measure for the repeatability of the DON analysis is the intraclass correlation (Steel and Torrie, 1981). Based on 10 genotypes (classes) and two observations per genotype, r_I had a value of 0.99.

Statistical analysis

Both wheat genotypes and *F. culmorum* strains were random samples from fixed sets.

For the analysis of variance a split-plot model with random effects was used (Steel and Torrie, 1981). For variance and regression analyses an angular transformation was applied for the ratings of Fusarium head blight, and the square root transformation for kernel DON content. From the mean squares of the variance analysis and the expected mean squares based on a random effect model (Steel and Torrie, 1981), the variance components were estimated. Because the variance component due to genotypes requires more than two mean squares for its estimation, an approximate F-test with quasi F-ratio was used (Winer, 1971), with the approximate degrees of freedom calculated by the method of Satterthwaite (Satterthwaite, 1946). The heritabilities were estimated on a phenotypic mean basis averaged over blocks and strains as follows:

$$h^2 = \frac{\sigma_g^2}{\frac{\sigma_{block}^2}{r} + \sigma_g^2 + \frac{\sigma_\gamma^2}{r} + \frac{\sigma_{strain}^2}{s} + \frac{\sigma_{g \times strain}^2}{s} + \frac{\sigma_\varepsilon^2}{rs}}$$

where σ_g^2 stands for the variance component due to genotypes within populations, while σ_{block}^2, σ_{strain}^2 and $\sigma_{g \times strain}^2$ stand for the variance components due to blocks, strains and genotype × strain, respectively. σ_γ^2 and σ_ε^2 are variance components due to genotype × block (whole-plot error) and block × genotype × strain (subplot error), while r and s stand for number of blocks (r=2) and strains (s=3), respectively. Path analysis (Sokal and Rohlf, 1981) was used to test a theoretical model for establishing possible causal relationships. A path coefficient is the standardized partial regression coefficient and estimates the strength of the relationship between cause X and effect Y. The indirect path coefficient between two variables is the sum of the product of the chain of path coefficients along all the indirect paths by which they are connected.

Results and discussion

Date of inoculation had no influence on head blight incidence or DON concentration. There was no visible head blight infection in the control plots. Seeds harvested from the control plots did not show any *Fusarium* infection. Therefore kernels harvested from the control plots were considered not to contain any DON (Miller *et al.*, 1985). As the control plots were free of *Fusarium*, cross-contamination was considered to be absent.

Head blight incidence and kernel DON concentration are shown in Table 2.1. Estimates for variance components, their relative importance in the total variance, and estimates for heritabilities are given in Table 2.2. The Fusarium head blight ratings varied from 0% in several subplots to 85% in a subplot consisting of SVP 73012-1-2-3 inoculated with strain IPO 39-01. For Fusarium head blight significant interactions existed between plant genotypes and *Fusarium* strains. Strain IPO 39-01 is a highly pathogenic strain, while IPO 348-01 and IPO 436-01 are of moderate pathogenicity (Table 2.1). The estimated h^2 of 0.67 (Table 2.2) shows that the proportion of the

Table 2.1. Fusarium head blight and kernel DON content of 10 wheat genotypes inoculated with three strains of *Fusarium culmorum*

genotype	*F. culmorum* strains IPO 39-01		IPO 348-01		IPO 436-01		mean	
	head blight[a,b] (%)	DON[a] (mg kg^{-1})	head blight (%)	DON (mg kg^{-1})	head blight (%)	DON (mg kg^{-1})	head blight (%)	DON (mg kg^{-1})
SVP 72017-17-5-10	2.0	4.6	3.0	0.8	1.5	0.6	2.2	2.0
SVP 77078-30	9.0	3.4	1.0	ND[c]	2.5	0.4	4.2	1.2
SVP 75059-28	11.0	3.4	3.0	ND	1.5	0.2	5.2	1.2
Saiga	4.5	4.2	4.5	0.6	9.0	2.1	6.0	2.3
SVP 72003-4-2-4	23.5	5.8	9.5	0.5	3.5	0.7	12.2	2.3
SVP 73030-8-1-1	60.0	33.2	7.5	1.9	9.0	6.8	25.5	13.9
SVP 75059-32	32.5	13.3	9.0	ND	25.0	1.4	28.0	4.9
SVP 73016-2-4	47.0	31.5	14.5	0.4	22.5	4.5	28.0	12.1
SVP 73012-1-2-3	67.5	34.0	17.5	1.5	17.0	8.4	34.0	14.6
SVP 72005-20-3-1	62.5	37.0	27.5	1.6	23.0	5.6	37.7	14.7
LSD (P=0.05)	29.6	17.1	9.0	2.2	10.7	5.4		
mean	32.0 ±8.8[d]	17.0 ±5.0	9.7 ±2.8	0.7 ±0.3	13.2 ±3.0	3.1 ±1.0	18.3	7.0

[a] Values shown are the means over two blocks.
[b] Head blight ratings were determined as the product of the percentage of heads infected and the proportion of infected spikelets per infected head.
[c] No deoxynivalenol detected.
[d] Standard deviation of the mean.

Table 2.2. Estimates of variance components[a] of the data in Table 2.1, absolute and as percentage of the total variance, and heritability[c] for head blight and kernel DON content

	head blight		DON	
	absolute	in % of total variance	absolute	in % of total variance
$\hat{\sigma}^2_g$	97.18**	38	0.79*	18
$\hat{\sigma}^2_{strain}$	68.20**	26	2.42**	56
$\hat{\sigma}^2_{g \times strain}$	39.54*	15	0.55*	13
$\hat{\sigma}^2_{block}$	13.38**	5	0[b]	0[b]
$\hat{\sigma}^2_{\gamma}$	0[b]	0[b]	0[b]	0[b]
$\hat{\sigma}^2_{e}$	40.28	16	0.57	13
$\hat{h}^2$	0.67		0.42	

[a] The indices g, γ and e stand for genotype, whole-plot error and subplot error, respectively.
[b] Based on F-statistics, computed from the split-plot analysis of variance, the estimate was not significantly different from 0.
[c] Heritability was estimated on a phenotypic mean basis averaged over blocks and strains.
* Significant at P=0.05.
** Significant at P=0.01.

total variability among wheat genotypes that was due to additive genetic causes was high.

DON was found in 53 of the 60 samples, with a maximum of 48 mg kg^{-1} in seeds of SVP 72005-20-3-1 inoculated with strain IPO 39-01. Strain IPO 39-01 had a very high DON producing capacity (Table 2.1). For statistical analysis, non detection (ND) was regarded as a concentration with a value of 0. For DON content, variance analysis revealed interactions between plant genotypes and *Fusarium* strains. Table 2.2 shows that $\hat{\sigma}^2_{g \times strain}$ is of about the same level as $\hat{\sigma}^2_g$. The largest part of the variance is determined by $\hat{\sigma}^2_{strain}$. Except for DON, no 3-ADON or any other trichothecene looked for was found. As DON can be formed by hydrolysis of 3-ADON or 15-ADON (Miller *et al.*, 1983; Yoshizawa and Morooka, 1975), there may have been 3-ADON in the samples in trace amounts below the detection limit. No ZEA was detected. Usually there has been little, if any, ZEA in *Fusarium* infected wheat (Miller and Young, 1985; Miller *et al.*, 1985; Visconti *et al.*, 1986). In the *in vitro* experiment, however, besides DON also 3-ADON and ZEA were produced (Table 2.3). As was the case in the field, IPO 39-01 had the highest toxigenic potential *in vitro*.

For incubation period (IP), reduction in 1000 kernel weight and yield, no significant interactions were found. The mean values for IP, 1000 kernel weight reduction and yield reduction are given in Table 2.4. For IP only the genotype effect accounted for the variation observed, the strain effect was not significant.

Because of the significant interactions between genotype and strains for head blight and for DON concentration, correlations between head blight, kernel DON content, kernel weight and yield reduction were calculated for each strain separately (Table 2.5). The significant negative correlations between head blight and incubation period show that the more resistant the genotype, the longer is the incubation period. This relation, which does not depend on the pathogenicity of the strain, makes the incubation period a potentially useful resistance component for selection. The correlations between DON concentration and kernel weight reduction and yield reduction, respectively, were high.

In Figure 2.1, a path diagram illustrates possible causal relationships for each strain separately. In comparison with the direct path (p_1) between head blight and yield reduction, for the pathogenic strain IPO 39-01 the calculated indirect path

Table 2.3. In vitro toxin production[a] of three strains of *Fusarium culmorum*

strain	zearalenone (mg kg^{-1})	3-ADON (mg kg^{-1})	DON (mg kg^{-1})
IPO 39-01	20	4.8	2.0
IPO 348-01	40	ND[b]	ND
IPO 436-01	1728	1.5	0.5

[a] Strains were cultured for four weeks on wheat grains (cultivar 'Grana') of 45% moisture content, at a temperature of 20°C.
[b] ND = non detected.

Table 2.4. The effect of inoculation of wheat by *Fusarium culmorum* on incubation period[a] (IP), 1000 kernel weight reduction[a] and yield reduction[a] in 10 different genotypes

genotype	IP[b] (days)	kernel weight reduction[c] (%)	yield reduction[c] (%)
SVP 72017-17-5-10	15.0	5.5	6.7
SVP 77078-30	16.0	3.5	2.7
SVP 75059-28	19.5	4.8	4.5
Saiga	10.2	6.5	7.4
SVP 72003-4-2-4	11.3	5.9	7.4
SVP 73030-8-1-1	10.0	19.5	26.2
SVP 75059-32	14.8	2.8	2.7
SVP 73016-2-4	8.7	12.8	27.6
SVP 73012-1-2-3	8.5	13.5	35.0
SVP 72005-20-3-1	9.5	22.4	29.1
LSD (P=0.05)	2.9	12.0	17.8
mean	12.4	9.7	15.6

[a] Results presented are mean values over three *F. culmorum* strains and two blocks.
[b] IP is the number of days from inoculation time to the appearance of first symptoms.
[c] Yield and 1000 kernel weight are based on 25 randomly harvested leading tillers. Reduction of yield and 1000 kernel weight was assessed by calculating the difference between an inoculated subplot and the mean of the three control subplots for each main plot. This difference was expressed as a percentage of the mean value of the control subplots.

Table 2.5. Correlations[a] between incubation period (IP), head blight, kernel DON content, kernel weight reduction and yield reduction, of 10 wheat genotypes after inoculation by *Fusarium culmorum*, for three different strains

		IP	head blight	kernel DON content	kernel weight reduction
head blight	IPO 39-01	-0.64*			
	IPO 348-01	-0.78**			
	IPO 436-01	-0.64*			
kernel DON content	IPO 39-01	-0.69*	0.96**		
	IPO 348-01	-0.77**	0.55		
	IPO 436-01	-0.82**	0.65*		
kernel weight reduction	IPO 39-01	-0.40	0.74*	0.75**	
	IPO 348-01	-0.81**	0.73*	0.83**	
	IPO 436-01	-0.73*	0.50	0.86**	
yield reduction	IPO 39-01	-0.69*	0.96**	0.97**	0.67*
	IPO 348-01	-0.69*	0.68*	0.73*	0.96**
	IPO 436-01	-0.83**	0.39	0.87**	0.91**

[a] Correlations are based on the means over two blocks, df=8.
* Significant at P=0.05
** *Significant at* P=0.01

($p_{1,indirect}$), via kernel weight reduction, is of minor importance. The yield reduction caused by strain IPO 39-01 must result from a lower kernel number. In contrast, for the two less pathogenic strains IPO 348-01 and IPO 436-01, the indirect path is more important; yield reduction is almost exclusively caused by a lower kernel weight. Comparison of the direct path (p_2) between head blight and kernel weight reduction with the indirect path ($p_{2,indirect}$), i.e. via DON, shows that for the strains IPO 39-01 and IPO 436-01 the path via DON ($p_{4\text{-}5}$) is more important than the direct path, while for IPO 348-01 p_2 and $p_{2,indirect}$ are about the same. For these three strains there appears to be a relation between DON and kernel weight reduction. If the measurement of DON is expressed on a per kernel basis, for p_2 the coefficients change into 0.98, 0.46 and 0.04 for IPO 39-01, IPO 348-01 and IPO 436-01, respectively. The coefficients $p_{2,indirect}$ change into -0.24, 0.27 and 0.46. In case of measuring DON on a per kernel basis the indirect path between head blight and kernel weight reduction via DON is only important for IPO 436-01.

Whether there is a causal, phytotoxic effect of DON on kernel weight reduction cannot be proved in this study, but is possible. There have been few reports concerning the phytotoxic action of DON. A DON concentration of 2×10^{-5}M (6 mg L^{-1}) strongly inhibited the growth of tomato seedlings (Bottalico *et al.*, 1980). Coleoptile tissue segments from some wheat cultivars would not grow at a DON concentration of 10^{-6}M (0.3 mg L^{-1}) (Wang and Miller, 1988). A retarded germination and growth of wheat was observed at 1.2×10^{-5}M DON (4 mg L^{-1}), while 4.8×10^{-5}M DON (14 mg L^{-1}) inhibited seedling growth completely (Snijders, 1988). The phytotoxic effect of DON can be explained by the fact that DON is a very potent

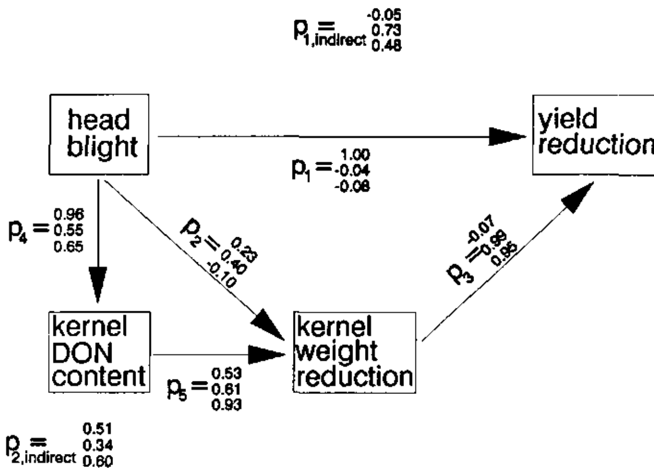

Figure 2.1: Path diagram illustrating possible causal relationships. The path coefficients (p_i) express the strength of the relationship for the strains IPO 39-01 (top figure in each column of three), IPO 348-01 (middle) and IPO 436-01 (bottom). The indirect path coefficient ($p_{i,indirect}$) between two variables expresses the strength of the relationship along all the indirect paths by which they are connected.

inhibitor of eukaryotic protein synthesis (Wang and Miller, 1988). Comparing the above data on phytotoxicity with the high concentrations of DON found for strain IPO 39-01 in the wheat kernels of the susceptible wheat genotypes, a phytotoxic effect of DON during development of the seed is imaginable.

From the data in Table 2.1, the head blight to DON ratio was calculated for each genotype-strain combination (Table 2.6). Within each strain, the extremes for head blight to DON ratios varied by a factor of 10. This corresponds with previous studies of ergosterol/DON ratios, where ergosterol was an index for estimating fungal biomass (Miller *et al.*, 1985). The head blight to DON ratios did not show significant correlations with any of the variates in Table 2.5. The correlations between head blight and DON in Table 2.5, and the variation in head blight to DON ratios in Table 2.6 confirm that besides the resistance mechanisms which determine the head blight severity (Schroeder and Christensen, 1963), there may be a second type of mechanism which influences kernel toxin content (Miller *et al.*, 1985). Several papers have reported mechanisms that may influence kernel DON content. The *Fusarium* resistant wheat cultivar Frontana degraded 18% of ^{14}C deoxynivalenol added to fragmented embryo callus cultures after 72 hr of incubation, while the susceptible wheat cultivar Casavant converted only 5% of the added DON (Miller and Arnison, 1986). Declines of DON *in vivo* have also been observed. In wheat spikes a maximum of 9.5 mg of DON kg^{-1} was found six weeks after inoculation with one strain of *F. graminearum*, after which time the concentration decreased to 2.5 mg kg^{-1} by week 9 (Miller and Young, 1985). In naturally infected wheat a decline in kernel DON content was observed from 1.56 mg kg^{-1} to 0.11 mg kg^{-1} in only 11 days' time (Scott *et al.*, 1984).

Infection by *Fusarium culmorum* must be minimized in order to prevent yield reduction and to produce a crop sufficiently mycotoxin free for human and animal consumption. Selection must focus both on resistance to infection and on mechanisms that either block synthesis or promote degradation of DON.

Table 2.6. Head blight to DON ratios[a] of 10 wheat genotypes inoculated with three strains of *Fusarium culmorum*

genotype	*F. culmorum* strains IPO 39-01	IPO 348-01	IPO 436-01
SVP 72017-17-5-10	0.4	3.8	2.5
SVP 77078-30	2.6	-[b]	6.3
SVP 75059-28	3.2	-	7.5
Saiga	1.1	7.5	4.3
SVP 72003-4-2-4	4.1	19.0	5.0
SVP 73030-8-1-1	1.8	3.9	1.3
SVP 75059-32	2.4	-	17.9
SVP 73016-2-4	1.5	36.3	5.0
SVP 73012-1-2-3	2.0	11.7	2.0
SVP 72005-20-3-1	1.7	17.2	4.1

[a] The values presented are based on means over two blocks.
[b] As no DON was detected, no ratio was calculable.

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Chapter 3

SYSTEMIC FUNGAL GROWTH OF *FUSARIUM CULMORUM* IN STEMS OF WINTER WHEAT

Summary

Systemic fungal growth of *Fusarium culmorum* in winter wheat was investigated under conditions precluding secondary infections by water splash. Growth of *F. culmorum* in stem tissue was found in both wounded and soil inoculated plants with both methods resulting in a high level of infection. Crown rot can therefore lead to infection of the higher stem internodes under conditions not suitable for *Fusarium* dispersal. However, no evidence was found for systemic fungal growth leading to infected heads. Existence of genetic variation for resistance to spread of *F. culmorum* in the host was found. This resistance was not correlated with resistance to Fusarium head blight.

Introduction

In the Netherlands Fusarium head blight in wheat (*Triticum aestivum* L.) is mainly caused by *Fusarium culmorum* (W.G. Smith) Sacc. and *F. graminearum* Schwabe. Whether *F. culmorum* or *F. graminearum* is the dominant pathogen is determined mainly by temperature (Cook, 1981). Inoculum is seed-borne or soil-borne and is produced on infected debris of cereals, corn or weeds. Besides head blight, both species cause seedling blight, and root, crown and foot rot (Atanasoff, 1924; Bennett, 1928; Cook, 1981). Stems and leaf sheaths can become infected due to the spread of infection from the crown. Completely rotted crowns and basal stems can lead to 'white' tillers with empty heads (Cook, 1968). Dispersal of spores by wind (Martin, 1988) and water splash leads to infection of the upper sheaths and heads (Atanasoff, 1924; Cook, 1981). Based on differences in morphology and perithecial production, *F. graminearum* isolates are subdivided into Group 1 and Group 2 (Burgess *et al.*, 1975; Cook, 1981; Francis and Burgess, 1977). Generally, isolates of *F. graminearum* Group 1 are associated with crown rot, while members of Group 2 cause head blight, although Group 1 can produce identical head blight symptoms to those of Group 2 (Burgess *et al.*, 1987).

Fusarium infection can remain latent during some plant growth stages. *F. culmorum* and *F. graminearum* were frequently isolated as endophytes from symptomless roots, culms, leaves, glumes and kernels of wheat (Sieber *et al.*, 1988). In plants with crown rot, *F. culmorum* was recovered up to two internodes above soil level (Atanasoff, 1924; Bennett, 1928; Cook, 1968; Purss, 1971). Wheat infected by soilborne *F. graminearum* showed foot rot symptoms which often extended to the second stem internode (Atanasoff, 1924; Purss, 1971). Purss (1966) reported that infection of the internodes by *F. graminearum* occurred as high up as the peduncle. Burgess *et al.* (1987) isolated *F. graminearum* Group 1 from all nodes and peduncles of head blight

infected plants. However, from no plant the fungus was isolated from all tiller nodes, even when the heads, lower stems and crowns of the plant were infected. Duben (1978) isolated several *Fusarium* species, including *F. culmorum* and *F. graminearum*, from higher internodes of plants infected by seed inoculation. Whether the infections of higher stem internodes originated from the fungus in the infected crown, or from leaf sheath infection was not clear in any of these studies. No convincing evidence for systemic fungal growth was found (Duben, 1978; Purss, 1966). For both *F. culmorum* and *F. graminearum* the possibility of colonization of heads by mycelial growth from crown and lower stem was excluded (Atanasoff, 1924; Bennett, 1928; Burgess *et al.*, 1987).

In this study, growth of *Fusarium culmorum* in winter wheat stem tissue was investigated under controlled conditions. Variation in resistance to systemic fungal growth between genotypes with different head blight resistance was also examined. This work had two purposes: (1) to evaluate the importance of internal spread of infections via the stems in relation to Fusarium head blight and (2) to evaluate the variation for resistance to spread of the pathogen in host tissue.

Materials and methods

Inoculum

In this study a strain of *Fusarium culmorum* with a high pathogenicity (IPO 39-01) was used. It was isolated from an infected wheat kernel collected in the Netherlands, single spored and deposited at the Research Institute for Plant Protection (IPO), Wageningen. Conidiospores were produced in 1 L Erlenmeyer flasks containing sterilized wheat kernels (cultivar 'Arminda') and oats (bulk) in a 3:1 ratio (Snijders and Perkowski, 1990).

Preliminary study

In a preliminary study tillers of wheat plants (bulk) in growth stage 45 (Zadoks *et al.*, 1974) were inoculated by two methods: (1) a toothpick contaminated with *F. culmorum* was stuck into the stem just above a node (Christensen and Wilcoxson, 1966) and (2) a needle, dipped in a spore suspension of 10^6 spores ml^{-1}, was pierced through the stem just above a node. There was one inoculation per tiller, just above the first, the second or the third node. After six weeks the inoculated tillers were cut at the nodes. The cut stems were then dipped in 70% alcohol for a few seconds, immersed in a 1% calcium hypochlorite solution for 10 minutes, followed by several rinses in sterile distilled water. The stem internodes were cut longitudinally. The inoculated internode and adjoining internodes above and below were placed on a modified Czapek-Dox medium, with the cut face up. The medium contained the

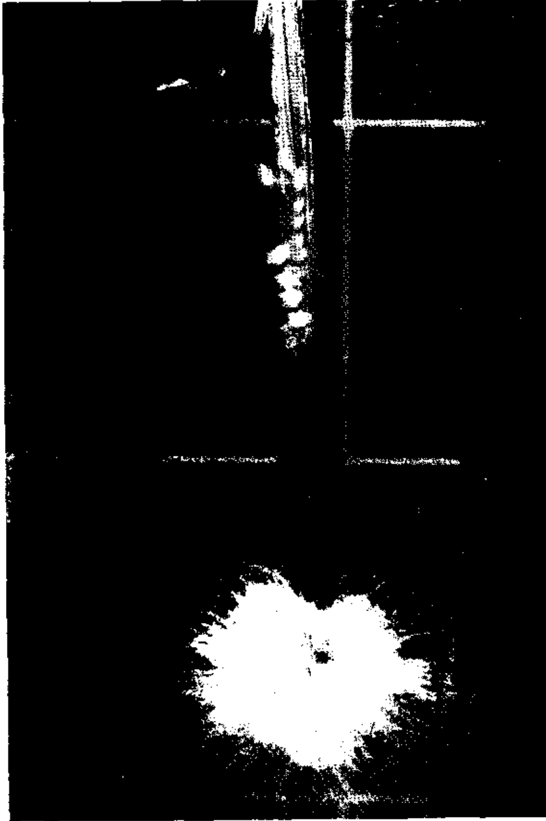

Figure 3.1. Young spike (arrow) infected by *Fusarium culmorum* while growing through the infected flag leaf sheath. Bar represents 1 cm.

following in one litre of water: 20 g dextrose, 0.5 g KH_2PO_4, 2 g $NaNO_3$, 0.5 g $MgSO_4.7H_2O$, 1 g concentrated yeast extract, 15 g agar and 50 mg tetracycline. After three days of incubation at 25°C and exposure to near U.V. light, the fungus sporulated on the infected plant parts and agar, and internodes could be examined for *F. culmorum* infection. All the inoculated internodes were infected. To a lesser extent, infections were observed in internodes above ánd below the inoculated internode. In one case, a young spike was infected by *F. culmorum* while growing through the infected flag leaf sheath (Figure 3.1).

Experiment 1

Surface-sterilized seeds of five genotypes were sown in water-saturated perlite. After vernalization the seminal roots of 20 seedlings per genotype were dipped in a spore suspension containing 10^6 spores ml^{-1}. Gelatine (2 g/L) was used to make the spores stick to the roots. The inoculated seedlings were planted in the greenhouse in containers with perlite (Agra-Perlite, gradation 0.6-2.5 mm, Pull, Rhenen, the Netherlands), saturated with a nutrient solution (Steiner, 1984). Immediately after planting, 1 ml of a spore suspension of 250,000 spores ml^{-1} was pipetted onto the perlite at the base of the plant. This was repeated one week later. Black plastic covering the perlite precluded watersplash and prevented growth of algae. A randomized block design with 20 blocks was used. Temperature was raised slowly starting at 15/12°C until a temperature of 22/17°C was reached at the end of three months.

At a growth stage varying from 55 to 65 on the Zadoks scale, the main stem and two primary tillers per plant were harvested. The stems were cut at the nodes. After surface sterilization the stem internodes were cut longitudinally and placed on the modified Czapek Dox medium as above. The internodes were numbered starting at the base of the culm. After four days of incubation at 25°C, the internodes were examined for infection. Plants were given a disease score which corresponded with the internode number to which the infection had reached.

Experiment 2

Seeds of 12 genotypes of winter wheat were sown in water-saturated perlite. After vernalization, 20 seedlings per genotype were inoculated as in experiment 1. After three months the main stems were harvested. Systemic fungal growth was assessed as above.

Results and discussion

After planting, the subcrown internode of the primary axis extended and the crown roots developed. A few weeks after the first inoculation, mycelial growth and brown, elongated lesions developed on the subcrown node, crown and stem base. This brown discoloration in the tiller base is one of the most commonly used indicators of crown rot (Purss, 1966). Almost all of the plants developed a crown rot and stem infection in the two experiments (99% and 91%, respectively). No head blight or sterile heads were observed in either experiment. Although in some plants the basal internodes were extremely short and could not be distinguished, all main stems and primary tillers were considered to consist of six internodes. The stems were solid at the nodes, but the internodes were hollow. In the case of infected stems, colonization of the internodes was continuous up to the highest internode to which the infection had reached. The means and LSD's for systemic fungal growth of the genotypes in the two

Table 3.1. Systemic fungal growth[a] of *Fusarium culmorum* in the main stem and primary tillers of plants with crown rot, for five genotypes in experiment 1

genotype	main stem[b]	primary tiller[c]
SVP 75059-28	3.5	4.0
* SVP 72017-17-5-10	4.1	4.4
* SVP 72005-20-3-1	4.7	4.6
SVP 73012-1-2-3	4.7	4.3
SVP 73030-8-1-1	4.8	5.0
mean	4.4	4.5
LSD (P=0.05)	1.0	0.7

[a] The 'number' of highest infected internode was used as a measure of systemic fungal growth.
[b] Values are means over 20 replicates.
[c] Values are means over 20 replicates and two primary tillers.
* Genotype tested in both experiment 1 and 2.

Table 3.2. Systemic fungal growth[a] of *Fusarium culmorum* in the main stem of plants with crown rot, for 12 genotypes in experiment 2

genotype	systemic fungal growth		plants[d]
	mean[b]	maximum[c]	
SVP 77076-1	2.1	3	18
SVP 77076-38	2.3	6	20
SVP 75059-32	2.4	6	18
SVP 77078-30	2.5	4	19
Arina	2.6	4	17
* SVP 72005-20-3-1	2.7	4	18
SVP 72003-4-2-4	2.7	4	19
SVP 73016-2-4	2.7	5	19
Saiga	2.9	6	17
SVP 77079-15	3.1	5	19
Nautica	3.2	5	18
* SVP 72017-17-5-10	3.6	6	17
mean	2.7		
LSD (P=0.05)	0.5		

[a] The 'number' of highest infected internode was used as a measure of systemic fungal growth.
[b] Values are means over 17-20 plants[d].
[c] Highest infected internode.
[d] Plants = number of plants with successful infection.
* Genotype tested in both experiment 1 and 2.

experiments are given in Tables 3.1 and 3.2. In experiment 1 for each genotype main stems and tillers were found with a *Fusarium* infection as high as the peduncle. For experiment 2 maximum height of *F. culmorum* infection is given in Table 3.2. The data for height of systemic growth were also expressed in cm. The results for the two experiments are illustrated in Figure 3.2.

According to Purss (1966), the subcrown tissue is the site of infection by soil-borne *F. graminearum*. Crown infections by soil-borne *F. culmorum* were caused by infections of crown roots or wounds during root formation (Cook, 1968). Sections of 3-day-old seedlings infected by *F. culmorum* showed both intercellular and intracellular hyphae in the hypocotyl from which hyphae traversed both upwards into the coleoptile and downwards into the root (Malalasekera *et al.*, 1973). In the experiments described in the present study the solid diaphragm between the internodes did not seem to be a barrier to *F. culmorum*. From the preliminary study it was concluded that the fungus can grow both acropetaly and basipetaly in the stem. Strausbaugh and Maloy (1986) observed that both *F. graminearum* and *F. culmorum* could attack wheat heads and grow down into the stem, with an average of 19.6 cm below the head. Atanasoff (1924) observed this feature for *F. graminearum*.

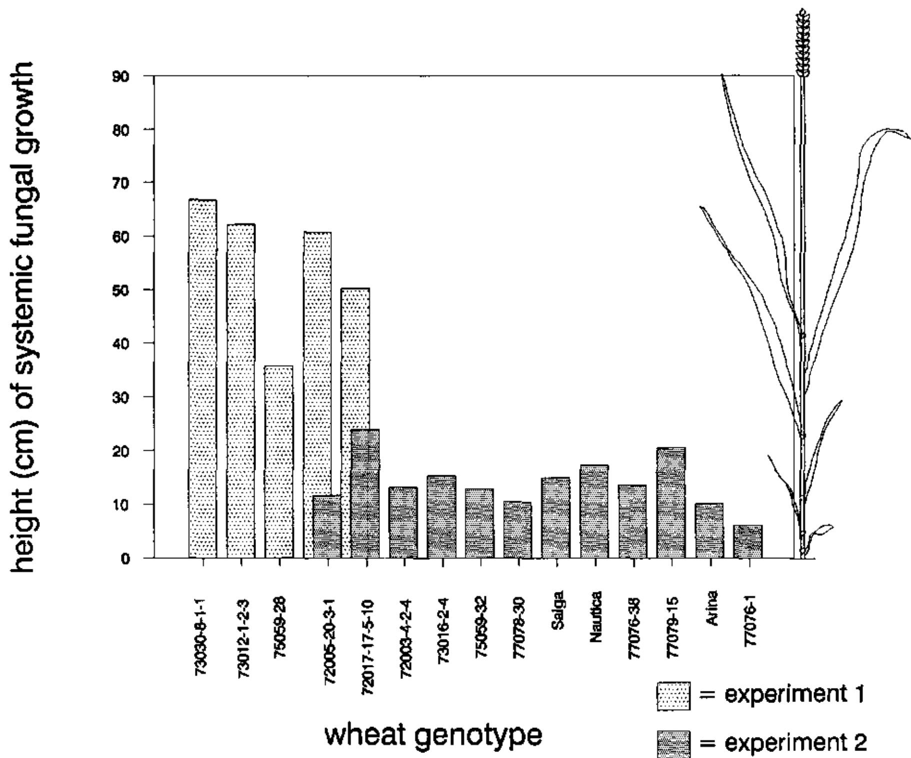

Figure 3.2. Systemic fungal growth of *Fusarium culmorum* in plants with crown rot, for a set of five wheat genotypes in experiment 1 and a set of 12 genotypes in experiment 2.

Growth of *F. culmorum* in stem tissue was found in both wounded and soil inoculated plants with both methods resulting in a high level of infection. Crown rot can therefore lead to infection of the higher stem internodes under conditions not suitable for *Fusarium* dispersal. However, no evidence was found for systemic fungal growth leading to infected heads. This generally confirms the findings of Bennett (1928): *F. culmorum* does not penetrate the central meristematic tissue. Normally, head blight does not occur as a result of stem infections during plant growth. In an infected internode, the hyphae can emerge through stomata between epidermal cells and produce spores in sporodochia (Malalasekera *et al.*, 1973). An infected internode near the head could increase the chance of head infection by water splash.

Existence of genetic variation for resistance to spread of *F. culmorum* in the host was found. For each genotype set the Spearman rank correlations between systemic fungal growth and Fusarium head blight ratings were estimated. The Fusarium head blight ratings were based on the means of field inoculation with strain IPO 39-01 over the years 1986, 1987 and 1988; the ratings were determined as the product of the percentage of heads infected and the proportion of infected spikelets per infected head (Snijders and Van Eeuwijk, 1990). No significant correlation was found with respect to all genotypes. However, SVP 75059-28 was significantly more resistant to systemic fungal growth than SVP 73030-8-1-1 ($P=0.05$); the former was also found to be more resistant to Fusarium head blight. Resistance to Fusarium head blight can be seen as consisting of two components: resistance to initial penetration (component I) and resistance to spread of the pathogen in host tissue (component II) (Schroeder and Christensen, 1963). Resistance to systemic fungal growth belongs to component II resistance.

This report gives some insight into the question of relation between the observation of stem and leaf sheath symptoms and Fusarium head blight attack. It illustrates the importance of the use of non-infected seed with respect to the prevention of a Fusarium head blight epidemic. Though seed treatment is indispensable, resistance to Fusarium head blight is the best way to prevent seed from infection.

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Fusarium isolate. However, interaction patterns were not stable over experiments and genotype ranking was only slightly influenced by the isolates. No evidence has been found for the occurrences of races of *Fusarium culmorum* or *F. graminearum* adapted to different wheat genotypes. Also in studies with *F. graminearum* in corn ear rot tests, significant but inconsistent isolate × genotype interaction patterns were found (Atlin *et al.*, 1983; Mesterhazy, 1982; Mesterhazy and Kovacs, 1986). However, large genotype rank reversals did not occur. This phenomenon is not restricted to *Fusarium* of wheat and corn. Environmental lability of interactions between wheat cultivars and isolates were also reported for *Cercosporella herpotrichoides* (Scott and Hollins, 1977).

In an initial study of *Fusarium culmorum* in wheat, a significant host genotype × pathogen strain interaction variance was observed (Snijders, 1987). The experiments were continued to investigate the consistency of the interaction patterns, i.e. whether strain-specific resistance to *Fusarium culmorum* head blight in wheat exists.

Materials and methods

Host and pathogen

A set of 17 winter wheat cultivars and SVP-lines was composed, representing the whole range of Fusarium head susceptibility based on data available in 1985. In field trials in three consecutive years this set was tested for resistance to Fusarium head blight. Ten strains taken from monospore cultures of isolates of *Fusarium culmorum*, collected in the Netherlands, were prescreened for pathogenicity in the glasshouse. Two non-pathogenic strains were discarded, and from the remaining strains four were drawn: IPO 39-01, IPO 329-01, IPO 348-01 and IPO 436-01, originating from isolations from a grain of seed, culm, head and leaf sheath, respectively. The lyophilized strains are deposited at the Research Institute for Plant Protection (IPO), Wageningen. Each year, conidiospores for inoculation were produced in 1 L Erlenmeyer flasks containing 250 ml sterilized cereal seeds: the first year wheat seeds (cultivar 'Arminda'), the next two years a wheat ('Arminda') and oat (bulk) seed mixture (3:1). A lyophilized strain was used as starting inoculum. The cultures were incubated in darkness at 25°C for two weeks, followed by three weeks incubation at 5°C. To prepare spore suspensions, conidia were washed from the kernels with water. Since wheat is most susceptible to Fusarium head blight at anthesis (Schroeder and Christensen, 1963), experimental inoculations were made at this time. The spore suspensions were applied at 100 ml m^{-2}. To ensure a high relative humidity during the nights after inoculation, the field was sprinkled in the evening for 1 hr each day over a period of two weeks. Head blight ratings were determined as the product of the percentage of heads infected and the proportion of infected spikelets per infected head (Snijders and Perkowski, 1990). In all experiments interplot interference was prevented.

Field trial 1986

On 22 November 1985, seeds were sown in sandy soil in Wageningen at a standard density of 330 seeds m^{-2} in rows 0.25 m apart. A split-plot design was established, with two blocks. Each main-plot, consisting of one genotype, was divided into subplots of 0.90 × 0.75 m over which the strains of *F. culmorum* were randomized so that the experimental subplots were separated from each other by border subplots of the same size. Further details are described in Snijders and Perkowski (1990). Fusarium head blight was assessed 26 days after first inoculation.

Field trial 1987

On 4 November 1986, seeds were sown in Flevoland in clay soil at a standard seed density of 330 seeds m^{-2} in rows 0.25 m apart. A split-plot design was established, with three blocks. The four strains of *Fusarium culmorum* were randomized over the main-plots. A distance of at least 4 m between the main-plots prevented interplot interference. The main-plots were divided into subplots of 2.00 × 0.75 m over which the wheat genotypes were randomized. On 25 June, when 30% of the wheat genotypes flowered, all genotypes were inoculated. At the time when 100% of the genotypes flowered, 2 July, a second inoculation was done. For the inoculation a spraying machine was used, which sprayed from 0.3 m above the crop. Spore concentrations varied from 25,000 to 250,000 spores ml^{-1}. On 21 July, 26 days after first inoculation, head blight was assessed. Observations were made on culm length.

Field trial 1988

On 10 November 1987, seeds were sown in Flevoland in clay soil. The same design was used as in Field trial 1987. Each subplot consisted of a hill plot (ø 0.25 m) seeded with 3 g seeds, at 0.5 m apart. Experimental inoculation was done on 2 June, when 30% of the wheat genotypes flowered, and repeated on 9 June and 16 June, by which time 100% of the genotypes flowered. For the inoculation a spraying machine was used. The spore suspensions had a concentration of 250,000 spores ml^{-1}. On 30 June, 28 days after the first inoculation, head blight was assessed. Observations were made on time of anthesis and culm length.

Statistical analysis

For the analysis of variance of Fusarium head blight ratings in the three consecutive experiments, the split-plot model with fixed effects was used (Steel and Torrie, 1981). For a description of the interactions, a Finlay-Wilkinson regression model (Finlay and

Wilkinson, 1963) and an Additive Main effects and Multiplicative Interaction (AMMI) model (Bradu and Gabriel, 1978; Gauch, 1988; Kempton, 1984; Zobel *et al.*, 1988) were used.

Results and discussion

As no head blight was observed in control and border plots, interplot interference was assumed to be absent. Inoculum concentration for individual inoculations and total amount of inoculum had no influence on the Fusarium head blight ratings. No significant correlations were found between Fusarium head blight and time of anthesis, and head blight and culm length. From preliminary analyses (not shown) it was concluded that within the experiments of 1986 and 1987 there was a statistically significant interaction between wheat genotypes and *Fusarium* strains, which could not be removed by transformation of the data to an angular or logarithmic scale. In 1988 there was no significant interaction between genotypes and strains, neither was there a significant strain effect.

The means over the replicates of the genotypical assessments per strain within each of the three years are presented in Table 4.1. This table shows the high pathogenicity of strain IPO 39-01. The non-additivity of the head blight ratings is

Table 4.1. Fusarium head blight incidence[ab] of 17 wheat genotypes for four *F. culmorum* strains and three years. Genotypes are presented in ascending order of incidence averaged over strains and years

	1986				1987				1988			
genotype	IPO 39-01	329-01	348-01	436-01	39-01	329-01	348-01	436-01	39-01	329-01	348-01	436-01
SVP 72017-17-5-10[c]	2.0	1.5	3.0	1.5	7.3	0.8	0.5	2.1	5.3	2.7	2.0	2.7
SVP 77076-4	9.0	1.0	3.0	1.5	13.5	0.3	0.1	0.2	7.0	1.7	3.3	3.7
Arina	8.0	2.5	5.0	4.0	12.0	0.3	0.1	2.8	6.3	1.0	2.0	3.3
SVP 77076-38	18.0	1.0	4.5	7.0	8.9	0.2	0.1	0.7	2.0	2.3	1.3	1.7
SVP 77076-1	6.0	3.0	1.0	1.5	11.1	0.1	0.4	2.7	7.0	4.7	5.0	8.3
Saiga	4.5	7.5	4.5	9.0	15.5	1.1	0.3	1.7	9.3	9.7	6.0	11.7
SVP 77078-30	9.0	13.0	1.0	2.5	17.8	0.6	0.4	8.4	15.7	5.3	3.3	4.7
SVP 72003-4-2-4	23.5	4.0	9.5	3.5	16.3	0.7	0.4	1.4	5.0	6.3	7.3	13.7
SVP 77079-15	27.5	4.5	2.5	8.5	35.2	1.9	0.5	5.8	3.3	6.3	4.0	8.3
SVP 75059-28	11.0	3.5	3.0	1.5	54.0	1.2	1.2	19.3	4.0	2.7	1.3	6.3
SVP 73030-8-1-1	60.0	7.0	7.5	9.0	36.3	3.0	5.0	7.5	13.0	4.7	5.0	5.7
SVP 73016-2-4	47.0	18.0	14.5	22.5	44.1	5.4	3.5	11.6	6.7	12.5	3.3	4.0
SVP 73012-1-2-3	67.5	16.0	17.5	17.0	34.2	7.0	2.5	9.3	11.0	9.0	6.0	25.7
SVP 75059-46	25.5	5.0	5.0	10.5	69.3	5.0	1.7	13.2	30.0	22.3	20.0	20.3
Nautica	62.5	20.5	20.0	30.5	32.2	1.3	0.8	4.8	40.0	25.0	18.0	20.3
SVP 75059-32	32.5	5.0	9.0	42.5	57.3	5.2	4.3	30.5	37.7	14.7	28.3	31.0
SVP 72005-20-3-1	62.5	16.5	27.5	23.0	58.5	3.7	2.7	21.7	36.7	26.3	13.3	20.3
mean	28.0	7.6	8.1	11.5	30.8	2.2	1.4	8.5	14.1	9.2	7.6	11.3

[a] Head blight ratings were determined as the product of the percentage of heads infected and the proportion of infected spikelets per infected head.

[b] Values presented are means over blocks.

[c] SVP-line code: the first two digits indicate the year of crossing, followed by three digits representing the crossing number. The number after each hyphen is a selection number.

striking. The head blight data of Table 4.1 were subjected to a Finlay-Wilkinson analysis, for which each strain-year combination was treated as a separate environment. The model may be written

$$Y_{ijk} = \mu + G_i + \beta_i E_j + I^*_{ij} + e_{ijk}$$

where μ is the mean value over all genotypes and environments, G_i is the effect of the i th genotype, the regression coefficient β_i is a measure of the stability of the i th genotype, E_j is the effect of the j th environment, I^*_{ij} is the residual interaction after allowing for differences in stability between the genotypes, and e_{ijk} is the error for the k th individual within the ij th genotype-environment. To fit the Finlay-Wilkinson model first the genotypical and environmental main effects are estimated in the way customary for ANOVA. Subsequently, the individual genotypical responses are regressed on the estimated environmental main effects to find estimates for the parameters β_i. The heterogeneity between regression lines has to account for the genotype × environment interaction. This approach is quite usual for yield data, but may seem somewhat unorthodox for disease incidences. Problems with respect to inference may be expected from failure of the assumptions for analysis of variance, like homogeneity of variance and normality. However, in this study the Finlay-Wilkinson model only served as a starting-point for a more appropriate model, and no ultimate conclusions are derived from the model itself. The results of the Finlay-Wilkinson analysis are shown in Table 4.2. The heterogeneity between lines accounted for 41% of the total interaction and the description seems to be acceptable. The plot of the fitted regression lines, Figure 4.1, approaches a special case of the Finlay-Wilkinson model, namely the situation where all regression lines intersect at the same point. This model is equivalent to the concurrence model (Mandel, 1969)

$$Y_{ijk} = \mu + G_i + E_j + cG_iE_j + I^*_{ij} + e_{ijk}$$

where μ, G_i, E_j, I^*_{ij} and e_{ijk} have the same interpretation as in the Finlay-Wilkinson model, while c is the only extra parameter needed for a description of the interaction. With this model 81% of the interaction that was explained by the heterogeneity of the

Table 4.2. Summary of the results from the Finlay-Wilkinson analysis

term	df	SS	MS
genotype	16	12368	773**
environment	11	15201	1382**
genotype × environment	176	13867 (100%)	79**
regressions	16	5662 (41%)	354**
concurrence	1	4586	4586**
deviations from concurrence	15	1076	72*
deviations from regressions	160	8205 (59%)	52*
error	>51		25

* Significant at P=0.01.
** Significant at P=0.001.

Finlay-Wilkinson fitted lines can be covered (Table 4.2). This means that the genotype × environment interaction as described by the Finlay-Wilkinson model consists mainly of a divergence of (centered) genotypical responses. This interpretation gains even more credibility from the strong associations existing between the evaluations of the genotypes over the set of environments as measured by Spearman rank-correlations (not shown). These were all positive; 58 out of 66 were significant at P=0.05.

At first sight this would seem an adequate explanation of the interaction. However, a first problem arises in the context of the deviations I^*_{ij} from the regressions. When tested against an error estimate of 25 with at least 51 degrees of freedom (Table 4.2), being the geometric mean of the error estimates for genotype-environment means over the three years, the deviations appear to be significant. This implies that in addition to the divergence of the regression lines, other factors are involved in the interaction. Obviously, the Finlay-Wilkinson model does not remove all pattern from the data. Furthermore, a plot of the residuals against the fitted values exhibited an increase of the variance with the mean.

A second problem is that a considerable part of the environmental range is not represented by actual measurements, invalidating an interpretation of the regression coefficients as stability measures. The regressions mainly express a contrast between the high disease incidences in the environments formed by IPO 39-01 in 1986 and 1987, and the rest of the strain-year combinations. To a major extent the slopes were determined by the two high incidence environments (Figure 4.1; Table 4.1). The influence of these two environments was investigated more closely by performing an analysis without them. The overall treatment sum of squares decreased dramatically from 41436 to 13579. However, the proportion of genotype × environment interaction remained more or less the same, 36% in the reduced set against 33% in the full set. Now a concurrence model gave an adequate description of the genotype × environment interaction, that is, deviations from the concurrence model were not significant anymore. However, the rank-order of the slopes showed some clear reversals in comparison to the rank-order derived from the Finlay-Wilkinson analysis for the full set of environments. This means that if circumstances had been such that only low disease pressures had occurred, an interaction analysis would have led to a concurrence model and the ranking of genotypes for stability would not have been predictive for situations with higher disease pressures.

It was evident that a description of the interaction in terms of a Finlay-Wilkinson model for the full set was not satisfactory. An alternative was a model with additive main effects and multiplicative interaction effects, an AMMI-model. This model may be written

$$Y_{ijk} = \mu + G_i + E_j + \sum_{n=1}^{N} \lambda_n a_{ni} b_{nj} + I^*_{ij} + e_{ijk}$$

where μ, G_i, E_j, I^*_{ij} and e_{ijk} have the same interpretation as above, while λ_n is the eigenvalue for axis n of the principal components analysis, and a_{ni} and b_{nj} are the corresponding genotypical and environmental scores. The a_{ni} may be interpreted as genotypical stabilities, while the b_{nj} may be seen as environmental characterizations. N denotes the number of multiplicative terms necessary for an adequate description of the interaction. The model can be fitted by first calculating additive main effects

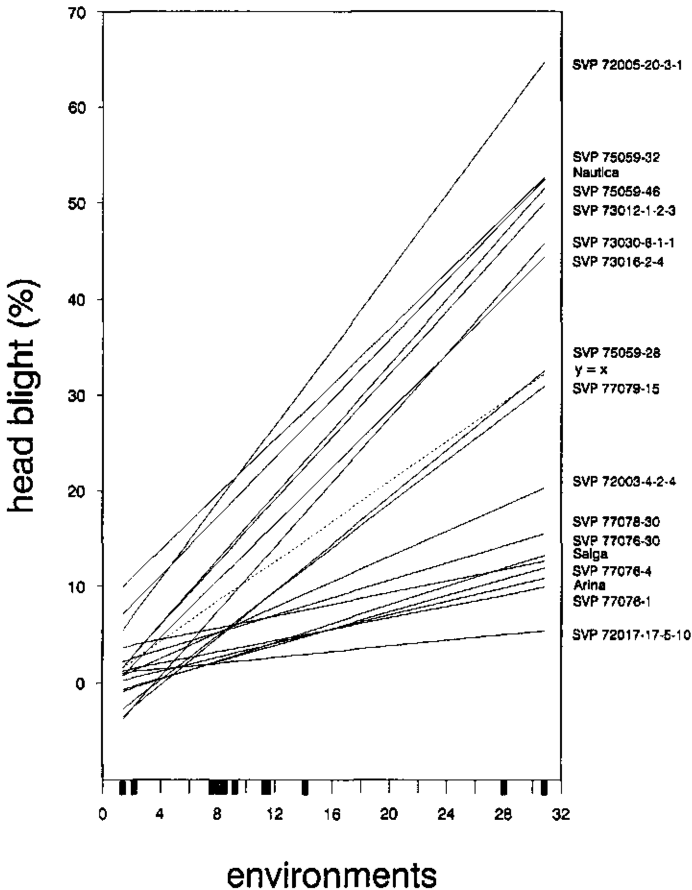

Figure 4.1. Regression lines of individual head blight ratings on mean head blight ratings per environment (formed by strain-year combinations) for 17 individual genotypes. The hanging symbols on the abscissa represent the environments, which are presented in Table 4.1.

for genotypes and environments, followed by a principal components analysis (singular value decomposition) of the matrix of the residuals (Gabriel, 1978).

With respect to the assessment of N two strategies are possible: (*i*) a strategy based on postdictive success, that is the ability of a model to fit its own data (e.g. traditional F-tests), and (*ii*) a strategy based on predictive success, the ability to predict validation data not used in constructing the model (Gauch, 1988; Gauch and Zobel, 1988). Because of the fact that in our experiment main- and subplot treatments changed over the years, assessment of predictive success was not straightforward. Therefore, model validation took place on postdictive grounds. Approximate F-tests were done after ascribing degrees of freedom to the eigenvalues following Mandel

(1969) and calculating the corresponding mean squares. A summary of the ANOVA for the AMMI-model is shown in Table 4.3. Three multiplicative terms seem necessary for an adequate description of the interaction. The rest of the terms is not significant when tested against the error estimate introduced above. The plot of residuals showed no gross failures of the assumptions. The interpretation of the components is as follows:

1 The first component provides genotypical scores, say 'stabilities' (Table 4.4), and environmental scores (Table 4.5) that are closely correlated with the stabilities and scores from the Finlay-Wilkinson model. From the environmental scores in Table 4.5 it can be seen that the first component is the contrast between IPO 39-01 in 1986 and 1987 on the one hand, and the rest of the strain-year combinations on the other hand. The proportion of variance explained by this component is 45% (for comparison 41% in the Finlay-Wilkinson model). It can be concluded that the first multiplicative term is more or less equivalent to the Finlay-Wilkinson regressions.
2 The second component arises from non-additivity of the genotypes SVP 75059-28, SVP 75059-32 and SVP 75059-46 inoculated with strain IPO 39-01 in 1987 (Table 4.4, 4.5). These three selections from the same cross had a far higher Fusarium head blight incidence in 1987 after inoculation with IPO 39-01 than may be expected from the genotypical and environmental main effects plus the Finlay-Wilkinson coefficients.
3 The third component results from genotype SVP 75059-28, with a far lower than expected incidence, and genotypes SVP 75059-32 and Nautica, with a higher than expected incidence. Again, this component is mainly due to an IPO 39-01 reaction, this time in 1988. The interpretation of this component is not easy. It probably represents merely noise, but as a consequence of a postdictive validation strategy, prone to lead to overfitting (Gauch, 1988), is not identified as such. An estimate for the amount of noise in the overall treatment sum of squares is the product of the treatment degrees of freedom with the error estimate: 203×25=5075.

Table 4.3. Summary of the results from the AMMI analysis

term	df	SS	MS
genotype	16	12368	773**
environment	11	15201	1382**
genotype × environment	176	13867 (100%)	79**
component 1	48[a]	6199 (45%)	129**
component 2	3	4061 (29%)	113**
component 3	27	1756 (13%)	65*
rest	65	1851 (13%)	17
error	>51		25

[a] The degrees of freedom for the components are calculated according to Mandel (1969).
* Significant at P=0.01.
** Significant at P=0.001.

Table 4.4. Genotypical scores (× 10^{-2}) from the AMMI-analysis, normalized at squared length 1

genotype	component		
	1	2	3
SVP 72017-17-5-10	-31	-13	1
SVP 77076-4	-21	-9	0
Arina	-23	-11	-1
SVP 77076-38	-12	-22	-3
SVP 77076-1	-27	-10	5
Saiga	-28	-7	12
SVP 77078-30	-23	-4	3
SVP 72003-4-2-4	-8	-18	-2
SVP 77079-15	4	6	-21
SVP 75059-28	-8	44*	-46*
SVP 73030-8-1-1	36	-13	-21
SVP 73016-2-4	23	-4	-32
SVP 73012-1-2-3	38	-27	-16
SVP 75059-46	5	57*	7
Nautica	32	-23	55*
SVP 75059-32	2	43*	49*
SVP 72005-20-3-1	38	12	11

*Genotypes with high scores used as a basis for component interpretation.

Table 4.5. Environmental scores (× 10^{-2}) from the AMMI-analysis, normalized at squared length 1

environment		component		
strain	year	1	2	3
IPO 39-01	1986	83*	-37	-8
IPO 329-01	1986	-10	-26	-19
IPO 348-01	1986	-2	-25	-11
IPO 436-01	1986	4	-2	35
IPO 39-01	1987	30*	79*	-31
IPO 329-01	1987	-25	-14	-31
IPO 348-01	1987	-28	-16	-31
IPO 436-01	1987	-15	21	-20
IPO 39-01	1988	0	13	63*
IPO 329-01	1988	-8	-2	12
IPO 348-01	1988	-19	6	24
IPO 436-01	1988	-10	1	18

* Environments with high scores used as a basis for component interpretation.

Acknowledging the fact that the noise will predominantly turn up in the higher axes, a strong argument for an interpretation of axis three in terms of noise is given.

The AMMI-model thus provides a good description of the data including the genotype × environment interaction and uncovers some features we were not able to disclose before. Altogether the interaction may be said to consist primarily of a divergence of the incidences at higher disease pressures, modified by genotype specific reactions in certain years. However, the modifications are on the whole not such that they heavily disrupt the rankings of the genotypes over the environments, although incidental changes occur. The divergence is mainly caused by the highly pathogenic strain IPO 39-01.

A last point concerns the scale of the measurement. Used was a percentage scale, as experience has shown that this is a convenient scale for resistance breeding research. For the purpose of genetic analyses the scale should preferably be one on which the analysis is as simple as possible, which means one on which interactions are small or absent. Various empirical transformations were tried. The most successful was the complementary log log transformation, which removed the genotype × strain × year interaction completely. However, genotype × year interaction and, to a lesser extent, genotype × strain interaction remained significant. The conclusions with respect to the status of resistance type, horizontal, did not change. The complementary log log transformation confers extra weight to the lower percentages. This seems unjustifiable in the light of the size of the measurement error. Therefore, the original percentage scale was retained. For non removable interactions Mather (1971) remarked that "we must always be prepared to bring interaction explicitly into an analysis".

Conclusions

The three environments with the highest disease pressure were the combinations of one particular strain (IPO 39-01) with the three years. No evidence was found for strain-specific resistance. The Fusarium head blight resistance in this study can be described as horizontal resistance in terms of Vanderplank (1984), with the exception of the lines selected from cross SVP 75059 which showed a 'strain-year combination' dependent resistance, ineffective in 1987. For large scale screening for resistance to Fusarium head blight using experimental inoculation, highly pathogenic strains should be used. The use of an AMMI-model for the description of genotype × strain interaction over years allows conclusions not obtainable by the additive models used in the studies reported in the introduction. It provides a means to check whether the environmental 'lability' of interaction in the aforementioned studies was really part of the pattern in the data and hence merits agricultural interpretation, or whether it was merely noise.

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Chapter 5

DIALLEL ANALYSIS OF RESISTANCE TO HEAD BLIGHT CAUSED BY *FUSARIUM CULMORUM* IN WINTER WHEAT

Summary

Ten homozygous winter wheat genotypes representing different levels of resistance to Fusarium head blight were crossed in all possible combinations excluding reciprocals. Parents, F_1 and F_2 were inoculated with one pathogenic strain of *Fusarium culmorum*. Data for head blight, observed 21 days after first inoculation (OBS-2), and for the area under the disease progress curve, based on observations 14, 21 and 28 days after first inoculation (AUDPC), were analyzed. The contrast between parents and F_1 crosses indicated dominance effects of the resistance genes. Diallel analysis according to Griffing's Method 4, Model 1 showed significant general combining ability (GCA) effects for both F_1 and F_2; specific combining ability effects were not significant. With the exception of one genotype for which general performance for Fusarium resistance was not in agreement with its GCA, the resistance to *F. culmorum* was uniformly transmitted to all offspring, and the parents can be described in terms of GCA. It is suggested that in the progenies with one of the awned lines as parent, one resistance gene was linked with the gene coding for presence of awns, located on chromosome 4B. A single observation date, taken at the right time, was as effective in assessing resistance as the AUDPC.

Introduction

Fusarium culmorum (W.G. Smith) Sacc. and *Fusarium graminearum* Schwabe are pathogens of wheat (*Triticum aestivum* L.) and cause Fusarium head blight. The use of wheat cultivars resistant to Fusarium head blight will limit yield loss under high *Fusarium* disease pressure. Moreover, a low head blight incidence will minimize the occurrence of Fusarium toxins, harmful to humans and animals (Marasas *et al.*, 1984). According to Schroeder and Christensen (1963) resistance to Fusarium head blight infection consists of two components: resistance to initial penetration (component I), and resistance to spreading of the pathogen within plant tissue (component II). Studies of Miller *et al.* (1985) and Snijders and Perkowski (1990) suggested a third component of resistance, based on the ability to degrade the phytotoxic mycotoxin deoxynivalenol.

There are few studies on the inheritance of the resistance in wheat to Fusarium head blight (Gu, 1983; Yu, 1982; Zhang and Pan, 1982; Zhou *et al.*, 1987). As (*i*) these studies give rather contradictory information and (*ii*) research has been limited to resistance to *Fusarium graminearum* in spring wheats, a study was initiated on the inheritance of the resistance to *Fusarium culmorum* in winter wheat (Snijders, 1990b; 1990c). In this paper the general and specific combining abilities of 10 winter wheat genotypes were estimated utilizing a half diallel cross. The aim of this diallel cross analysis for Fusarium head blight resistance was to investigate (*i*) if the parents could be described in terms of general combining ability (GCA) effects, and (*ii*) to what

extent these effects agreed with mean parent performance. If so, the progeny performances can be effectively estimated on the basis of resistance levels of the parents, as reaction to the pathogen will be uniformly transmitted to all offspring.

Materials and methods

The host

Ten homozygous winter wheat genotypes were selected, representing different levels of resistance to Fusarium head blight (Snijders and Perkowski, 1990; Snijders and Van Eeuwijk, 1990). Given in order of increasing susceptibility, the following nine SVP-lines and one cultivar were selected: SVP 75059-28, SVP 72017-17-5-10, SVP 77078-30, SVP 75059-32, Saiga, SVP 72003-4-2-4, SVP 73012-1-2-3, SVP 73016-2-4, SVP 72005-20-3-1 and SVP 73030-8-1-1. The genotypes SVP 75059-28 and SVP 75059-32 were awned, all other genotypes were awnless. The pedigrees of the SVP lines according to the standard method of Purdy *et al.* (1968) are given in Table 5.1. In 1987, all possible single crosses were made among the 10 genotypes, excluding reciprocals. F_1 and F_2 seed was produced in the greenhouse after vernalization of parents and F_1 plants. The F_2 seed was derived from selfed F_1 plants. In a field trial in 1988, a replicated plot design was established as follows: each plot consisted of up to 10 plants and there were 5 plots of each parent, 1-4 plots of each F_1, and 1-11 plots of each F_2 population, depending on the amount of seed. The distance between the plots was 35 cm, plant spacing within the plots was 12.5 cm. The plots were randomized over the field. Seeds were sown on 16 December 1987.

Table 5.1. Pedigrees[a] of the parents used for the diallel cross. Parents are given in descending order of resistance (OBS-2, see Table 5.6)

SVP 75059-28[b]	Petit Quinquin/3/Vogel 222//Halle 101/Comanche
SVP 72017-17-5-10	Marzotto//Dippes Triumph/Mironovskaja 808
SVP 77078-30	Norda//Dippes Triumph/Mironovskaja 808
SVP 75059-32	Petit Quinquin/3/Vogel 222//Halle 101/Comanche
Saiga	TCC/Felix//9208/3/Viking/Perdix
SVP 72003-4-2-4	Mariau//Dippes Triumph/Mironovskaja 808
SVP 73012-1-2-3	Knox 62/3/Strube 295/Halle 104//Vogel 222
SVP 73016-2-4	Starke/Pilote//Starke/Vogel 219
SVP 72005-20-3-1	Starke/Pilote//Mironovskaja 808
SVP 73030-8-1-1.	INRA B21-3-6-2//Heine VII/Varma/3/Dippes Triumph/Mironovskaja 808

[a] The pedigrees are given according the standard method of Purdy *et al.* (1968).
[b] SVP code: first two digits indicate the year of crossing, followed by three digits representing the crossing number. The number after each hyphen is a selection number.

Inoculation and disease assessment

After heading, the peduncles of three primary tillers of each plant were marked with non-phytotoxic paint. The heads of these tillers were inoculated at the time when 50% of the plants within the plot were flowering. Inoculation was repeated after four days. For inoculation one pathogenic strain of *Fusarium culmorum* (IPO 39-01) was selected and produced as described in Snijders and Van Eeuwijk (1990). Inoculum was applied with a hand sprayer at 250,000 spores ml^{-1} till run-off. From 3 June to 7 July a mist irrigation above the crop maintained a high relative humidity. Head blight, expressed as the mean percentage of infected spikelets of three heads per plant was observed 14, 21 and 28 days after first inoculation of the concerning plot. Symptoms were described in Snijders and Perkowski (1990). Time of anthesis, plant length and awning were recorded on a plant basis.

Statistical analyses

As the lines had deliberately been chosen on the basis of different resistance levels in earlier experiments, the genotypes were assumed to form a fixed set. No difference was assumed between reciprocal crosses. In addition to the head blight ratings at the second observation time (OBS-2), 21 days after first inoculation, the 'area under the disease progress curve' (AUDPC), derived from the observations on head blight at 14, 21 and 28 days after first inoculation was analyzed. This area was calculated as AUDPC = 3.5 × (OBS-1 + 2 × OBS-2 + OBS-3). For the F_1 generation, plant means were used in the analyses of variance. The minimum number of plants for an F_1 generation was three. For the F_2 generation the plot means were used for analysis. To test the null hypothesis that there were no genotypic differences among the parents, F_1 crosses and F_2 populations, a one-way analysis of variance was used (Steel and Torrie, 1981). Parents versus F_1 crosses as a source of variation tested the importance of heterosis effects.

For the diallel analysis of the F_1 the Experimental Method 4, Model 1 according to Griffing (1956) was chosen, for which one set of F_1s but neither parents nor reciprocal F_1s were included. The statistical model is

$$x_{ij} = u + g_i + g_j + s_{ij} \quad ,$$

where u is the population mean, g_i is the GCA effect of parent i, g_j is the GCA effect of parent j, and s_{ij} is the SCA effect for the combination of parent i and j. The restrictions $\sum_i g_i = 0$ and $\sum_{i \neq j} s_{ij} = 0$ are imposed on the combining ability effects. Overall means of all F_1 plants were used to estimate the general combining ability (GCA) and specific combining ability (SCA) variance components, and the individual combining ability effects. For estimation ordinary least squares were used. Due to missing parent combinations not all effects were estimated with the same precision. A similar diallel analysis was carried out for the F_2. To test the GCA effect, $F_{[(p-1),m]} = M_g/M_e'$ was used, where p is the number of parents, m is the degrees of freedom associated with the error, and M_g and M_e' are the GCA and error mean

squares. To test SCA effects $F_{[p(p-3)/2,m]} = M_s/M_e'$ was used, with M_s the SCA mean square (Griffing, 1956). The combining ability effects of F_1 and F_2 were compared by Spearman's rank correlation test, to test the importance of non-additive effects.

Results

Inundation during wintertime and hybrid necrosis decreased the number of plants of F_1 and F_2 plants considerably. If there were fewer than three plants per generation, the results were not included in the analyses. As a result of variation for time of anthesis, the inoculation period covered the period from 3 June to 21 June. Time

Table 5.2. Overall mean values of F_1s of a 10×10 diallel of winter wheat lines inoculated with one strain of *Fusarium culmorum* for head blight observed 21 days after first inoculation (OBS-2)[a], and the area under the disease progress curve (AUDPC)[b]; $x_{i.}$ is the average value among all crosses in which parent i is represented. Parents are given in descending order of mean Fusarium head blight resistance (OBS-2, see Table 5.6)

parent	parent number	variable	parent number									$x_{i.}$
			2	3	4	5	6	7	8	9	10	
SVP 75059-28	1	OBS-2	17.9	12.9	*	25.8	31.7	36.9	15.3	26.3	27.5	24.3
		AUDPC	261.9	243.6	*	408.3	463.2	497.0	271.7	393.5	362.1	362.7
SVP 72017-17-5-10	2	OBS-2		*	29.0	11.5	4.5	83.3	41.0	10.5	*	28.2
		AUDPC		*	431.6	206.5	95.4	1009.2	555.9	201.8	*	394.6
SVP 77078-30	3	OBS-2			40.0	*	*	*	73.6	39.5	58.5	44.9
		AUDPC			493.5	*	*	*	949.9	533.2	795.9	603.2
SVP 75059-32	4	OBS-2				*	29.5	*	42.6	*	*	35.3
		AUDPC				*	447.5	*	574.6	*	*	486.8
Saiga	5	OBS-2					30.0	*	46.7	*	59.0	34.6
		AUDPC					406.0	*	627.0	*	773.5	484.3
SVP 72003-4-2-4	6	OBS-2						41.6	*	*	*	27.5
		AUDPC						549.5	*	*	*	392.3
SVP 73012-1-2-3	7	OBS-2							*	73.4	76.4	62.3
		AUDPC							*	939.5	957.3	790.5
SVP 73016-2-4	8	OBS-2								70.0	70.4	51.4
		AUDPC								917.0	898.1	684.9
SVP 72005-20-3-1	9	OBS-2									*	43.9
		AUDPC									*	597.0
SVP 73030-8-1-1	10	OBS-2										58.3
		AUDPC										757.4
overall mean		OBS-2										40.2
		AUDPC										545.1

[a] Expressed as the mean percentage of infected spikelets of three heads per plant.
[b] Based on three head blight observations: 14, 21 and 28 days after first inoculation.
* Missing combination: less than 3 plants.

of anthesis and culm length did not show a significant correlation with OBS-2 or AUDPC. Overall head blight means for OBS-1, OBS-2 and OBS-3 were 14.0%, 44.8% and 69.3%, respectively.

For F_2 populations of crosses in which the awned parents SVP 75059-28 and SVP 75059-32 were used as a parent, a significant difference in resistance between the group with awned plants and the group of awnless plants was demonstrated (t-test; P=0.05): awned plants were more resistant. Presence and absence of awning showed a 1:3 segregation for all offspring (χ^2-test, P>0.99). As this means that awns were suppressed in the heterozygous condition, gene B1 on chromosome 4B must be responsible for the suppression of awns (Lupton, 1987).

The means for F_1 and F_2 generations for OBS-2 and AUDPC are given in Tables

Table 5.3. Overall mean values of F_2s of a 10×10 diallel of winter wheat lines inoculated with one strain of *Fusarium culmorum* for head blight observed 21 days after first inoculation (OBS-2)[a] and the area under the disease progress curve (AUDPC)[b]; $x_{i.}$ is the average value among all crosses in which parent i is represented. Parents are given in descending order of mean Fusarium head blight resistance (OBS-2, see Table 5.6)

parent	parent number	variable	parent number									$x_{i.}$
			2	3	4	5	6	7	8	9	10	
SVP 75059-28	1	OBS-2	28.1	24.5	21.2	25.2	39.2	73.4	43.9	52.2	*	38.5
		AUDPC	405.7	352.0	345.7	347.6	548.3	968.5	620.4	686.9	*	534.4
SVP 72017-17-5-10	2	OBS-2		47.7	26.9	23.8	41.8	*	41.1	59.2	65.1	41.7
		AUDPC		634.9	382.8	349.7	573.6	*	563.3	787.5	825.8	565.4
SVP 77078-30	3	OBS-2			37.8	37.8	26.3	66.3	47.6	52.3	47.4	43.1
		AUDPC			531.1	521.0	389.4	859.0	634.1	690.1	670.8	586.9
SVP 75059-32	4	OBS-2				39.2	28.6	47.2	46.9	55.1	38.8	38.0
		AUDPC				508.6	430.1	639.3	627.9	724.6	537.0	525.2
Saiga	5	OBS-2					34.0	*	52.8	48.0	*	37.2
		AUDPC					476.3	*	711.1	655.9	*	510.0
SVP 72003-4-2-4	6	OBS-2						*	42.9	44.7	53.7	38.9
		AUDPC						*	605.1	638.0	709.0	546.2
SVP 73012-1-2-3	7	OBS-2							72.1	63.6	*	64.5
		AUDPC							929.3	818.7	*	842.9
SVP 73016-2-4	8	OBS-2								68.2	67.1	53.6
		AUDPC								882.4	851.9	713.9
SVP 72005-20-3-1	9	OBS-2									74.6	57.5
		AUDPC									944.3	758.7
SVP 73030-8-1-1	10	OBS-2										57.8
		AUDPC										756.5
overall mean		OBS-2										46.3
		AUDPC										625.1

[a] Expressed as the mean percentage of infected spikelets of three heads per plant.
[b] Based on three head blight observations: 14, 21 and 28 days after first inoculation.
* Missing combination: less than 3 plants.

Table 5.4. Mean squares from analysis of variance of head blight reaction of F_1[a] and F_2[b] of a 10×10 diallel of winter wheat genotypes inoculated with one strain of *Fusarium culmorum*. df = degrees of freedom; MS = mean squares

F_1				F_2			
source	df	MS		source	df	MS	
		OBS-2[c]	AUDPC[d]			OBS-2	AUDPC
entries	37	9358.3*	1303579*	entries	48	1862.2*	258772*
parents	9	23593.7*	3238833*	parents	9	3551.9*	488227*
parents versus F_1	1	12185.2*	1754483*	parents versus F_2	1	367.7	53156
F_1	27	4508.4*	641793*	F_2	38	1501.3*	209839*
error	613	217.4	30020	error	283	206.5	29094

[a] Plant means were used in the analysis of variance.
[b] Plot means were used in the analysis of variance.
[c] Head blight percentage observed 21 days after first inoculation, expressed as the mean percentage of infected spikelets of three heads per plant.
[d] Area under the disease progress curve (AUDPC) based on three observations: 14, 21 and 28 days after first inoculation.
* Significant at a probability of 0.001.

Table 5.5. Mean squares from combining ability analysis of head blight reaction of F_1[a] and F_2[b] of a 10×10 diallel of winter wheat lines inoculated with one strain of *Fusarium culmorum*. df = degrees of freedom; MS = mean squares

source	F_1			F_2		
	df	MS		df	MS	
		OBS-2[c]	AUDPC[d]		OBS-2	AUDPC
general combining ability	9	1109.7*	151322*	9	744.9*	104789*
specific combining ability	18	209.2	29882	29	64.8	9086
error	317[e]	193.8	27270	244[f]	223.4	31174

[a] Means over all plants were used.
[b] Means over all plot means were used.
[c] Head blight observed 21 days after first inoculation, expressed as the mean percentage of infected spikelets of three heads per plant.
[d] Area under the disease progress curve (AUDPC) based on three observations: 14, 21 and 28 days after first inoculation.
[e] Error based on variance analysis of F_1 on plant means.
[f] Error based on variance analysis of F_2 on plot means.
* Significant at a probability of 0.001.

5.2 and 5.3. The means for the parents are given in Table 5.6. Overall generation means for OBS-2 were 47.4%, 40.2% and 46.3% for P, F_1 and F_2, respectively; for AUDPC the values were 643.7, 545.2 and 629.0. In the F_1s and F_2s for both OBS-2 and AUDPC, no systematic relation existed between means and standard deviations. For the homozygous parents, there remained significant differences among the standard deviations for OBS-2, which could not be eliminated by any scale transformation (Snijders, 1990b).

The analyses of variance for the F_1 and parents, and F_2 and parents are given in Table 5.4. The loss of a number of F_1 plants during winter time resulted in several empty plots. As the between plot variance for most parents was not significantly different from 0 (Snijders, 1990b), plant means were used for analysis of F_1, and plot variance was pooled with the error variance. Differences among parents, among F_1 crosses and F_2 populations were highly significant for both variables. The 'parents versus F_1 crosses' effect tests the mean deviation of the F_1s from their midparent value, and was highly significant. The 'parents versus F_2 populations' effect was not significant.

The combining ability analysis for F_1 and F_2 are given in Table 5.5. For both OBS-2 and AUDPC, GCA was the major component of variation among both F_1 and F_2 generations and was for both F_1 and F_2 significant (P=0.01). For both generations the SCA effect was of minor importance and not significant. In Table 5.6 the mean parent performance and GCA effects for F_1 and F_2 are given for each genotype. Weighted for the number of times parent i was used in a cross, $\Sigma g_i = 0$. Rank correlations between GCA effect of the F_1 and the GCA of the F_2 for OBS-2 and AUDPC were 0.76 and 0.73, respectively, which were significant at P=0.05.

Table 5.6. Mean parent performance[a] and general combining ability effects ($\hat{g}_i$) based on analysis of F_1 and F_2, of 10 parents for resistance to inoculation with one strain of *Fusarium culmorum*

parent	OBS-2[b]			AUDPC[c]		
	mean	$\hat{g}_i(F_1)$	$\hat{g}_i(F_2)$	mean	$\hat{g}_i(F_1)$	$\hat{g}_i(F_2)$
SVP 75059-28	8.3	-20.2	-8.5	160.9	-232.5	-98.0
SVP 72017-17-5-10	12.1	-12.9	-3.8	192.2	-163.7	-50.5
SVP 77078-30	29.9	3.7	-4.8	444.0	41.2	-56.1
SVP 75059-32	36.7	-3.5	-10.5	514.4	-40.2	-125.5
Saiga	37.7	-2.4	-6.8	549.1	-23.2	-89.5
SVP 72003-4-2-4	40.8	-10.5	-7.0	563.6	-123.4	-72.5
SVP 73012-1-2-3	66.6	27.9	19.2	898.9	312.5	229.0
SVP 73016-2-4	76.9	14.1	7.1	969.8	173.3	86.8
SVP 72005-20-3-1	79.8	1.2	11.5	986.9	25.7	137.1
SVP 73030-8-1-1	85.3	13.5	12.7	1067.6	158.0	144.9

[a] Means over all plants.
[b] Head blight observed 21 days after first inoculation, expressed as the mean percentage of infected spikelets of three heads per plant.
[c] Area under the disease progress curve (AUDPC) based on three observations: 14, 21 and 28 days after first inoculation.

Discussion

The inoculation time, chosen in accordance with time of anthesis, prevented escape from infection. As inoculation was done directly on the heads and an overhead mist was used, a possible role of plant length as an escaping mechanism (Mesterhazy, 1987) was also excluded.

Griffing's method estimates the GCA and SCA based on progeny performance alone. It does not provide any information on the average nor on any specific contribution of the parents to dominance and heterosis (Singh and Paroda, 1984). In the analysis of variance however, the 'parents versus F_1 crosses' effect tests the mean deviation of the F_1s from their midparent value. It reflects average heterosis contributed by all parents in the crosses and is attributable entirely to non-additive effects. For both OBS-2 and AUDPC the 'parents versus F_1 crosses' effect was significant. This means that there were dominance effects, predominantly in one direction (Mather and Jinks, 1982), viz the direction of resistance. Segregation in the F_2 resulted in a non-significant 'parents versus F_2 populations' effect.

As the differences among F_1 crosses and among F_2 populations were highly significant, it was relevant to further analyze the data for combining ability variance components. GCA includes mainly additive and additive × additive variance. From the combining ability analyses it was concluded that the GCA effects were the most important. Hence, it follows that the resistance to *Fusarium culmorum* is uniformly transmitted to all of the F_1s and F_2s. The SCA effect tests that part of the dominance deviation that is unique to each F_1 (Mather and Jinks, 1982). It is a measure of the deviation of crosses from the value expected on the basis of the performance of the parents and includes dominance, additive × dominance, dominance × dominance and higher order epistatic variance (Van Ginkel and Scharen, 1988). The non-significant SCA effects in the analysis of variance indicated that there were no specific combinations which had a resistance higher or lower than expected from the resistance level of the parent and GCA effect. This means that the most resistant progeny may be produced by crossing the two parents with the highest (negative) GCA (Table 5.6). For both OBS-2 and AUDPC, line SVP 75059-28 and line SVP 72017-17-5-10 had the highest resistance and the highest GCA. The high GCA of line SVP 72003-4-2-4 in both F_1 and F_2 was not in agreement with its resistance: offspring had higher levels of resistance than expected on basis of the resistance of this parent. In spite of the conclusion from the combining ability analysis that SCA effects were not significant, this can only be explained by epistasis.

Although the rank correlations between GCA of F_1 and F_2 were significant, they were not very high. GCA effects in the F_2 were smaller than in the F_1. One reason might be that in Griffing's model the GCA effects based on F_1 generations may also contain heterosis effects. Also the occurrence of missing parent combinations, more numerous in the F_1 than in the F_2, may have influenced the accuracy of the estimation of GCA effects. As the wheat genotypes were not random samples from some random mating parent population in Hardy-Weinberg and linkage equilibrium, the variance due to GCA and SCA cannot be interpreted in terms of additive and non-additive genetic variances (Griffing, 1956; Wright, 1985). However, the non-

significant SCA effects suggest that on the whole epistatic effects were unimportant.

The awned genotypes SVP 75059-28 and SVP 75059-32 were selections from the same cross. The conclusion that the awned plants were significantly more resistant than awnless plants is contradictory to the results of Mesterhazy (1987). Mesterhazy explained the higher susceptibility of the awned types by the fact that humidity, caused by dawn and rain, would last longer between awns, "providing longer optimal conditions for the pathogen". The regular mist irrigation used in this field trial aimed to exclude this predisposition. As in this study awning was based on absence of one dominant gene (1B) on chromosome 4B, and the awned plants were more resistant than awnless ones, it is possible that one resistance gene is linked with the awning suppression gene, with the association being in repulsion phase. Mesterhazy 's theory on predisposition contributes to this hypothesis, as it suggest natural selection for resistance in awned types.

OBS-2 and AUDPC were highly correlated. OBS-2 measured the resultant of both components of resistance I and II. AUDPC was analyzed on the grounds of being a possible tool for measuring the rate of spreading of the pathogen in the host (component II). However, the results were similar for both measurements, hence all conclusions were the same. The incubation period of Fusarium head blight under Netherlands conditions varies from 8 to 20 days (Snijders and Perkowski, 1990), and the latent period is at least 11 days (Snijders, unpublished data). As a result secondary infections may have occurred, causing AUDPC to be the resultant of both components of resistance, like OBS-2. That there is genetic variance for component II among these 10 genotypes was demonstrated in Snijders (1990a).

This study of *Fusarium culmorum* head blight resistance in winter wheat indicated a major role for general combining ability effects. Selection in a progeny from a cross between parents with high GCA for resistance should be successful. In the F_1 there were directional dominance effects of resistance, which however cannot be fixed in a homozygote line. In progenies with one of the awned line as parent, selection on awned heads will indirectly select for part of the resistance to Fusarium head blight.

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Chapter 6

THE INHERITANCE OF RESISTANCE TO HEAD BLIGHT CAUSED BY *FUSARIUM CULMORUM* IN WINTER WHEAT

Summary

Crosses were made among ten winter wheat genotypes representing different levels of resistance to Fusarium head blight to obtain F_1 and F_2 generations. Parents, F_1 and F_2 were inoculated with one strain of *Fusarium culmorum*. Data on incidence of head blight 21 days after first inoculation were analyzed. Broad-sense heritabilities averaged 0.39 and ranged from 0.05 to 0.89 in the individual F_2 families. The joint-scaling test indicated that the inheritance of Fusarium head blight resistance was adequately described by the additive-dominance model, with additive gene action being the most important factor of resistance. With respect to the non-additive effects, dominance of resistance predominated over recessiveness. The number of segregating genes governing resistance in the studied populations was estimated to vary between one and six. It was demonstrated that resistance genes differed between parents and affected resistance differently.

Introduction

Fusarium head blight of wheat (*Triticum aestivum* L.) is a destructive disease in the humid and semihumid wheat-growing regions of the world. It induces losses in various ways: by adversely affecting yield and grain quality, by mycotoxins in the grain, and by seedling blight as a result of seed infections. In the Netherlands, head blight is mainly caused by two *Fusarium* spp., viz *F. culmorum* (W.G. Smith) Sacc. (which is the dominant species) and *F. graminearum* Schwabe. Breeding for resistance to Fusarium head blight is hampered by lack of knowledge of the inheritance of the resistance. Studies of the inheritance of the resistance to Fusarium head blight in wheat are scarce and limited to resistance to *F. graminearum*. In various studies the resistance was inherited from partially dominant to overdominant, and was controlled by two to five genes (Gu, 1983; Yu, 1982; Zhang and Pan, 1982; Zhou *et al.*, 1987).

Since the aforementioned studies do not unequivocally state how many genes were involved and investigated resistance to *F. graminearum* in spring wheat only, a study was initiated to investigate the inheritance of the resistance to *F. culmorum* in winter wheat. In an earlier paper (Snijders, 1990a), the general and specific combining abilities for head blight resistance to *F. culmorum* of ten winter wheat genotypes were estimated using a set of diallel crosses. The results indicated that the general combining ability effects played a major role, whereas the specific combining ability effects were not statiscally significant. The objective of the present study was to investigate the inheritance of resistance to Fusarium head blight in winter wheat using a quantitative genetic approach, and to estimate the number of effective factors involved in reducing head blight.

Materials and methods

Host and pathogen

Ten homozygous winter wheat genotypes representing different levels of resistance to Fusarium head blight were crossed in all possible combinations, resulting in 45 crosses (Snijders, 1990a). Each F_1 was subsequently selfed. In 1988 a field trial with a replicated plot design was established, with no more than 10 plants per plot. The trial included 5 plots of each parent, 1-4 plots of each F_1 cross and 1-11 plots of each F_2 family, depending on the amount of seed available. Three marked heads of each plant were inoculated twice with *F. culmorum* strain IPO 39-01. Incidence of head blight, expressed as the percentage of infected spikelets in the three marked heads per plant, was recorded 21 days after first inoculation. For further details on materials and methods see Snijders (1990a).

Statistical analysis

Data on head blight were analyzed on a single plant basis. The variance components for each parental line P_i (with i=1..10) and each F_2 were estimated by equating observed mean squares with expected mean squares based on a one-way analysis of variance with unequal replication (Table 6.1; Steel and Torrie, 1981).
The variance of the observed within-plot mean square MS_W (Table 6.1) is estimated by

$$\hat{\sigma}^2_{MS_W} = \frac{2 \times (MS_W)^2}{(df_W + 2)}$$

where df_W stands for the degrees of freedom of MS_W (Wricke and Weber, 1986).
The weighted average within-plot variance due to environment $\bar{\sigma}^2_{eW}$ was estimated

Table 6.1. Estimates of mean squares for a one-way classification of an F_2 with unequal replication, where σ^2_g stands for the variance component due to genetic variation, σ^2_{eB} stands for the variance component due to environment between plots, $\bar{\sigma}^2_{eW}$ is the weighted within-plot variance due to environmental variation, p is the number of plots and r_j is the number of plants of plot j with j=1..p. SV=source of variation; MS=mean squares; EMS=expected mean squares

SV	MS	EMS
Between plots	MS_B	$\bar{\sigma}^2_{eW} + \sigma^2_g + \frac{1}{p-1}\left\{\Sigma r_j - \frac{\Sigma r_j^2}{\Sigma r_j}\right\}\sigma^2_{eB}$
Within plots	MS_W	$\bar{\sigma}^2_{eW} + \sigma^2_g$

from the σ^2_{eW} of the homozygous parents, taking as weight the reciprocals of the square root of the variance of the within-plot mean square $MS_{W(i)}$ for each parent P_i

$$\hat{\sigma}^2_e = \frac{\sum_i \left\{ \frac{1}{\hat{\sigma}_{MS_{W(i)}}} \times MS_{W(i)} \right\}}{\sum_i \left\{ \frac{1}{\hat{\sigma}_{MS_{W(i)}}} \right\}}$$

The variance of the weighted within-plot variance was estimated by:

$$\hat{\sigma}^2_{(\hat{\sigma}^2_e)} = \frac{\sum_i 1}{\left\{ \sum_i \frac{1}{\hat{\sigma}_{MS_{W(i)}}} \right\}^2}$$, with in this case $\sum_i 1 = 10$.

Genetic analysis, heritability

For overall mass selection of individual plants, ignoring classification in plots, the heritability in broad sense for each F_2 would be

$$h^2_{(F2)} = \frac{\sigma^2_g}{\sigma^2_g + \bar{\sigma}^2_{eW} + \sigma^2_{eB}} = \frac{MS_W - \bar{\sigma}^2_{eW}}{MS_W + \frac{MS_B - \bar{\sigma}^2_{eW}}{r'}}$$

where σ^2_g stands for the variance component due to genetic variation, σ^2_{eB} stands for the variance component due to environment between plots, $\bar{\sigma}^2_{eW}$ is the weighted within-plot variance due to environmental variation, r' is the coefficient corresponding to r, the number of plants per plot, for the average value of the within-plot mean square (Table 6.1), and MS_B and MS_W stand for the mean squares between and within plots. To obtain an approximate variance of h^2, the 'delta technique' was used (Bulmer, 1985: p.82):

$$\sigma^2 \left\{ \frac{x}{y} \right\} = \frac{\sigma^2_x}{x^2} + \frac{x^2 \times \sigma^2_y}{y^4}$$

The variance of h^2 can be approximated by:

$$\sigma^2_{h^2} = \frac{\frac{2(MS_W)^2}{(df_W + 2)} + \frac{\sigma^2}{(\sigma^2_e)}}{(\sigma^2_g)^2} + \frac{(\sigma^2_g)^2}{(\sigma^2_g + \sigma^2_{eW} + \sigma^2_{eB})^4} \times \left\{ \frac{2(MS_W)^2}{(df_W + 2)} + \frac{1}{(r')^2} \times \left\{ \frac{2(MS_W)^2}{(df_W + 2)} + \frac{2(MS_B)^2}{(df_B + 2)} \right\} \right\}$$

where df_B and df_W stand for the degrees of freedom of MS_B and MS_W, respectively.

Genetic analysis, gene action

Generation means were calculated from overall head blight means, irrespective of classification in plots. If differences among generation means are determined only by additive and dominance effects of the genes, i.e. if there is no epistasis and no differential viability or fertility, then the following four relationships should be true:

$$\bar{P}_1 = m + [d]$$
$$\bar{P}_2 = m - [d]$$
$$\bar{F}_1 = m + [h]$$
$$\bar{F}_2 = m + \tfrac{1}{2}[h]$$

with m = mid-parent value, [d] = pooled additive gene effects and [h] = pooled dominance effects (Mather and Jinks, 1982). The joint-scaling test (Mather and Jinks, 1982) was used to test the predicted relationships between generation means. The parameters m, [d] and [h] were estimated from the four generations by weighted least squares, using the reciprocals of the squared standard errors of each mean as weights. A comparison was made between the observed and expected means under the assumption that the sum of squares minimized in the fitting process was distributed as a χ^2 with three degrees of freedom less than the number of generation means available, in this case 4 - 3 = 1. A high value of χ^2 indicates epistasis.

Genetic analysis, number of genes

The minimum number of effective factors segregating in an F_2 family was estimated by the following equation (Wright, 1968):

$$n = \frac{0.25 \times (0.75 - h - h^2) \times R^2}{\sigma^2_g}$$

with n = minimum number of effective factors

h $= (\bar{F}_1 - \bar{P}_1)/(\bar{P}_2 - \bar{P}_1)$, the degree of dominance

R $= (\bar{P}_2 - \bar{P}_1)$, the range between extreme genotypes

$\bar{P}_1$ = mean of the parent with the lowest disease rating

$\bar{P}_2$ = mean of the parent with the highest disease rating

$\bar{F}_1$ = mean of the F_1 generation

σ^2_g = genetic variance among F_2 plants

For convenience the effective factors will be called 'genes'. The underlying assumptions of the equation are: (1) no systematic relation between mean and variance, (2) no linkage of genes, (3) no epistasis, (4) the relevant genes are of equal effect, (5) one parent supplies only plus alleles of those genes in which the two parents differ, whereas the other parent supplies only minus alleles, and (6) an equal degree of dominance for all plus alleles. The number of genes will be underestimated if the assumptions do not hold.

Results and discussion

Mean ratings and confidence intervals for head blight of the 10 parents are given in Table 6.2. Mean head blight ratings for F_1 and F_2 generations were given in Snijders (1990a). For various reasons (see Snijders, 1990a), the available data on head blight were restricted to 28 F_1 generations and 39 F_2 generations. On average, there were 13 F_1 plants per cross; the maximum was 28. The corresponding figures for F_2 plants were 50 and 80. Parents, F_1 and F_2 showed continuous distributions. In the F_1 and F_2 generations there was no systematic relation between means and total variances of head blight (r=0.07 and r=-0.06, respectively). For the parents however, there was a significant correlation between means and variance of r=0.67 (df=8; P=0.05). No improvement was obtained by transformation of the scale. Therefore the original percentage scale was retained. For five parents, σ^2_{eB} did not differ significantly from 0. From the high quotients of σ^2_{eW} and σ^2_{eB} for parents, F_1 and F_2 it was concluded that the field was homogeneous, which justifies an analysis based on overall means, irrespective of classification in plots. The estimated weighted within-plot variance due to environment $\hat{\sigma}^2_{eW}$ and its confidence interval (P=0.05) were 103.5 ± 17.6 .

Table 6.2. Mean ratings and confidence interval (P=0.05) for head blight incidence (%) in 10 parents after inoculation with *Fusarium culmorum* strain IPO 39-01 in 1988

parent	mean (%)
SVP 75059-28	8.3 ± 1.9
SVP 72017-17-5-10	12.1 ± 4.4
SVP 77078-30	29.9 ± 4.0
SVP 75059-32	36.7 ± 5.1
Saiga	37.7 ± 5.1
SVP 72003-4-2-4	40.8 ± 4.9
SVP 73012-1-2-3	66.6 ± 9.0
SVP 73016-2-4	76.9 ± 6.9
SVP 72005-20-3-1	79.8 ± 6.4
SVP 73030-8-1-1	85.3 ± 5.0

Heritability

The broad sense heritabilities and their standard errors for the head blight resistance of the 39 F_2 families are presented in Table 6.3. As no backcrosses were available, the additive variance could not be separated from the total genetic variance in the numerator of the broad-sense heritability equation. Estimates of broad-sense heritability ranged from 0.05 to 0.89, with an average of 0.39. Table 6.3 shows that the standard deviations were very large. This is not unexpected, because heritability estimates are based on estimates of components of variance with inherent large standard deviations.

Gene action

The significance of the deviation from zero of the equation $c = 4\bar{F}_2\text{-}2\bar{F}_1\text{-}\bar{P}_1\text{-}\bar{P}_2$ was tested for the 23 crosses for which both F_1 and F_2 generations were available. For 13 crosses c differed significantly from zero, implying that in these crosses there were epistatic gene effects. However, the various generation means did not have equal variances. Therefore, the predicted relationships between generation means were tested in the joint-scaling test, using weighted expectations. The estimates for midparent value m, pooled additive gene effects [d] and pooled dominance effects [h], and their standard deviations are given in Table 6.4. The joint-scaling test showed that the additive-dominance model fitted for all crosses except 8719 (Table 6.4). The additive gene effects were significant (P=0.05) for 16 crosses, and for 11 crosses they were accompanied by significant dominance effects. In 8 of these 11 crosses the estimated dominance effects were negative, indicating that in these hybrid combinations the levels of resistance were higher than those of the midparent. The degree of dominance varied considerably. The predominance of the negative dominance effects in number and degree agrees with results from studies of the

Table 6.3. Broad-sense heritabilities and standard errors of resistance to head blight caused by *Fusarium culmorum* strain IPO 39-01 for F_2 families of a 10×10 diallel of winter wheat genotypes. Parents are listed in descending order of level of resistance

parent	parent number	parent number								
		2	3	4	5	6	7	8	9	10
SVP 75059-28	1	0.15±0.34	0.55±0.31	0.89±0.64	0.27±0.39	0.21±0.34	0.22±1.49	0.27±0.27	0.14±0.34	-[a]
SVP 72017-17-5-10	2	..	0.62±0.32	0.53±0.31	0.27±0.59	0.37±0.31	-	0.33±0.32	0.34±0.30	0.54±0.27
SVP 77078-30	3	..	..	0.35±0.30	0.41±0.35	0.17±0.41	0.76±0.76	0.71±0.53	0.36±0.30	0.20±0.38
SVP 75059-32	4	..	..	..	0.70±0.37	0.35±0.45	0.20±0.53	0.07±0.90	0.49±0.29	0.26±0.27
Saiga	5	..	..	..	..	0.05±1.32	-	0.25±0.30	0.23±0.28	-
SVP 72003-4-2-4	6	..	..	..	..	..	-	0.20±0.45	0.67±0.50	0.81±0.45
SVP 73012-1-2-3	7	..	..	..	..	..	..	0.48±0.33	0.30±0.49	-
SVP 73016-2-4	8	..	..	..	..	..	..	..	0.59±0.40	0.44±0.47
SVP 72005-20-3-1	9	..	..	..	..	..	..	..	..	0.32±0.34
SVP 73030-8-1-1	10	..	..	..	..	..	..	..	..	..

[a] Missing combination.

Table 6.4. Values[a] and standard deviations for mid-parent values m, pooled additive gene effects [d] and pooled dominance effects [h], and χ^2 values based on the joint scaling test for 23 crosses among 10 winter wheat parents for head blight incidence due to experimental inoculation by *Fusarium culmorum* strain IPO 39-01. The number of independently segregating genes controlling the Fusarium head blight resistance was estimated by Wright's equation. Parents are listed in descending order of resistance

cross	cross number	m	[d]	[h]	χ^2 value	estimated number of genes
SVP 75059-28 ×						
SVP 72017-17-5-10	8718	10.7 ± 3.6	-2.2 ± 3.6	8.8 ± 7.4	0.30	0.1
SVP 77078-30	8735	19.7 ± 3.1	-11.2 ± 3.1*	-6.1 ± 4.5	0.21	0.3
Saiga	8743	23.7 ± 3.5	-15.2 ± 3.5*	-14.3 ± 5.6*	0.26	0.9
SVP 72003-4-2-4	8716	25.1 ± 3.3	-16.7 ± 3.3*	6.6 ± 3.5	0.20	0.8
SVP 73012-1-2-3	8719	49.4 ±26.9	-40.2 ±27.2	4.4 ±45.4	5.7 °	
SVP 73016-2-4	8720	43.5 ± 5.1	-35.1 ± 5.2*	-27.6 ± 6.8*	0.33	3.9
SVP 72005-20-3-1	8717	44.9 ± 5.3	-36.5 ± 5.3*	-16.0 ±10.6	0.26	3.6
SVP 72017-17-5-10 ×						
SVP 75059-32	8724	24.4 ± 0.1	-12.0 ± 0.1*	4.6 ± 0.1*	0.00	0.5
Saiga	8739	25.7 ± 2.8	-12.8 ± 2.9*	-12.9 ± 4.7*	0.10	1.5
SVP 72003-4-2-4	8702	29.0 ±11.0	-14.7 ±11.3	-24.4 ±11.3*	1.49	1.1
SVP 73016-2-4	8709	44.3 ± 0.9	-32.3 ± 0.9*	-3.6 ± 1.5*	0.00	2.7
SVP 72005-20-3-1	8703	50.7 ±16.6	-36.0 ±17.1*	-37.9 ±20.9	2.13	5.1
SVP 77078-30 ×						
SVP 75059-32	8736	33.4 ± 0.5	-3.4 ± 0.5*	6.7 ± 0.7*	0.00	0.1
SVP 73016-2-4	8733	51.1 ± 8.3	-22.7 ± 8.6*	22.3 ± 8.9*	0.63	1.3
SVP 72005-20-3-1	8730	55.3 ± 2.1	-25.1 ± 2.2*	-14.5 ± 4.1*	0.03	1.3
SVP 73030-8-1-1	8734	56.6 ± 4.4	-27.6 ± 4.5*	-0.9 ± 8.6	0.24	3.4
SVP 75059-32 ×						
SVP 72003-4-2-4	8722	38.0 ± 2.8	-2.1 ± 2.9	-11.0 ± 5.8*	0.09	1.2
SVP 73016-2-4	8726	56.5 ± 1.3	-20.0 ± 1.3*	-14.1 ± 1.8*	0.01	5.8
Saiga ×						
SVP 72003-4-2-4	8737	39.2 ± 0.3	-1.6 ± 0.4*	-9.3 ± 0.5*	0.00	1.0
SVP 73016-2-4	8741	57.4 ± 0.4	-19.6 ± 0.4*	-10.6 ± 0.6*	0.00	1.3
SVP 73012-1-2-3 ×						
SVP 72005-20-3-1	8705	70.1 ± 7.1	-7.2 ± 7.7	2.1 ± 8.9	0.24	0.2
SVP 73016-2-4 ×						
SVP 72005-20-3-1	8708	77.6 ± 2.8	-1.3 ± 2.9	-8.9 ± 4.9	0.05	0.0
SVP 73030-8-1-1	8715	80.0 ± 4.1	-4.5 ± 4.3	-11.2 ± 6.7	0.15	0.1

[a] As for head blight, m, [d] and [h] are expressed on a percentage scale.
* Significant at a probability of 5%.
° Family for which the additive-dominance model did not fit (P=0.01).

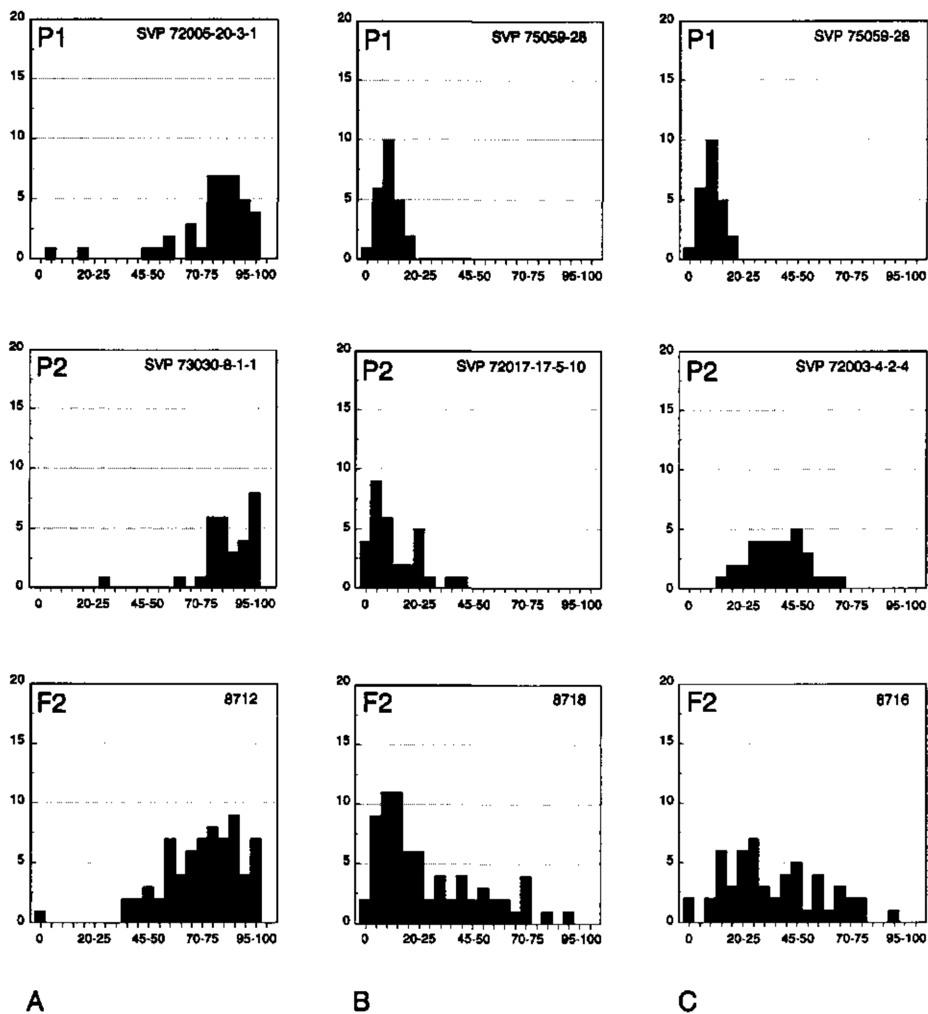

Figure 6.1. Distribution of frequencies of plants in 5% classes of Fusarium head blight incidence on a scale of 0 - 100%, for two parents (P_1 and P_2) and their F_2. Three crosses for which the F_2 distribution suggests transgression are shown. A: cross 8712, B: cross 8718 and C: cross 8716.

combining ability of this data set, where the mean deviation of the F_1s from the mean midparent values indicated heterosis for Fusarium head blight resistance (Snijders, 1990a). Despite the results of the joint-scaling test, the significant dominance effects in crosses 8702, 8722 and 8737 suggest epistasis. These crosses have SVP 72003-4-2-4 as common parent. An earlier paper (Snijders, 1990a) concluded that this genotype had a better general combining ability for resistance than expected from its resistance level. If the increased resistance is the result of an additive × additive epistatic component, it will be fixable in a pure inbred line (Snijders, 1990b).

On the whole, significant additive gene effects occurred more frequently and were larger than dominance effects. This indicates that additive effects predominantly determined the differences in resistance to Fusarium head blight within F_2 populations, and is in agreement with the finding that general combining ability effects were much more important than specific combining ability effects (Snijders, 1990a). Therefore, selecting for resistance on a single-plant basis in a segregating population could be successful if sufficient genetic variation is available.

Number of genes

The number of segregating genes involved in the resistance to Fusarium head blight was estimated for all crosses for which the joint-scaling test had shown that additive and dominance effects could explain the gene action (Table 6.4). The first three assumptions underlying Wright's equation were met. Of the two resistant parents, SVP 75059-28 differed from the susceptible SVP 72005-20-3-1 in 3.6 genes, and SVP 72017-17-5-10 differed from SVP 72005-20-3-1 in 5.1 genes. In addition to the higher level of resistance of SVP 75059-28, it is concluded that the resistance genes in SVP 72017-17-5-10 had less effect on the increase of resistance than the genes in SVP 75059-28. Crosses 8720, 8709, 8733 and 8726, all with SVP 73016-2-4 as susceptible parent, are even more striking examples of different gene effects. However, these findings do not exclude the possibility that within each partially resistant genotype the resistance genes had equal effects. Assumption 4 states that one parent supplies only plus factors of resistance, whereas the other parent supplies only minus factors. This could not be ascertained for any of the crosses. The F_2 progeny of the cross between the two most susceptible genotypes SVP 72005-20-3-1 and SVP 73030-8-1-1 showed transgression towards resistance (A in Figure 6.1). This indicates that even these two susceptible genotypes differed in at least one resistance gene. Apart from the cross between the two susceptible parents, transgressive segregation in the F_2 was only clearly observed for two crosses, 8718 and 8716, and only towards susceptibility (B and C in Figure 6.1). The parents of cross 8718 (SVP 75059-28 and SVP 72017-17-5-10) had the highest level of resistance. Although no significant gene effects were found (Table 6.4), the transgression indicated that some of the resistance genes in both parents must be different. This is not unexpected. The genes of the two parents differed in their effect on resistance and originated from different ancestors (Snijders, 1990a). Additional information on transgression of F_2 derived lines will be presented in a

subsequent paper (Snijders, 1990b).

In Table 6.4 it is shown that the [d] effects increased with the difference in level of resistance between the two parents. No trend was apparent for the [h] effects. Provided that non-allelic genes neither interact nor are linked, the total heritable variance given by *k* genes in F_2 will be the sum of the *k* individual contributions, namely $\frac{1}{2}\Sigma d^2 + \frac{1}{4}\Sigma h^2$ (Mather and Jinks, 1982). This would mean that the genetic variation has to increase with the difference in resistance levels between the parents, implying an increased heritability. However, heritability estimates did not increase with larger disparity between the parents ($r=-0.07$). This lack of correlation can be attributed to difference in resistance genes between parents as well as to the fact that the estimates for heritability were not accurate.

In Wright's equation R represents the range between the extreme genotypes for head blight resistance. If both parents in a cross show some resistance, but at different levels (as is suggested for several crosses in this study), each may contain plus factors not present in the other. If so, when calculating R it was wrong to assume that the difference in head blight resistance of the two parents was identical to the difference between the two extreme genotypes possible with the genes involved: as a result, the number of segregating genes was underestimated. Van Ginkel and Scharen (1988) postulated that the assumption of unidirectional gene distribution would be more closely adhered to if the extreme genotypes were selected from the F_2 and used to estimate R. However, in this study the large environmental variances and the continuous distributions of the progenies made it impossible to assign an F_2 plant with extreme resistance to a distinct class.

It may be concluded that the estimated number of segregating genes governing Fusarium head blight resistance in the populations studied varied between one and six. However, the results of the method used to calculate the number of genes should be viewed with caution. Besides the fact that Wright's equation is not a very accurate estimator, possibly also not all assumptions were met. The estimates of number of genes as shown in Table 6.4 are based on independent assortment. If linkage occurs, the number of genes really present could be higher than the estimated number. Nevertheless, it is clear that Fusarium head blight resistance is governed by several minor genes.

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Chapter 7

RESPONSE TO SELECTION IN F_2 GENERATIONS OF WINTER WHEAT FOR RESISTANCE TO HEAD BLIGHT CAUSED BY *FUSARIUM CULMORUM*

Summary

In a field trial, F_3 winter wheat lines from plants selected for Fusarium head blight resistance in F_2 generations of a set of crosses, composing a 10 × 10 half diallel, were tested with their parental lines for resistance to *Fusarium culmorum*. Selection responses averaged 3.7% on the head blight percentage scale and ranged from -22.0% to 27.1%. Realized heritabilities averaged 0.23 and ranged from 0 to 0.96. Significant transgression for resistance was observed which was suggested to be genetically fixed. It was estimated that resistant parents differed in one or two resistance genes. The possibility of accumulation of resistance genes was shown. The level of head blight resistance of the parental line appeared to be a good indicator of the potential resistance level of its crosses.

Introduction

In bread wheat (*Triticum aestivum* L.), head blight caused by *Fusarium* spp. is a serious problem in many countries with a temperate climate (Nelson *et al.*, 1981; Wang and Miller, 1987). In spite of efforts during the last few decades to breed wheat resistant to Fusarium head blight, progress has been slow. This is in part due to the lack of knowledge of the inheritance of the resistance. This report is one part of a study which was initiated to investigate the inheritance of the resistance to *Fusarium culmorum* (W.G. Smith) in winter wheat (Snijders, 1990a; 1990b).

Snijders (1990b) concluded from F_1 and F_2 analyses that resistance to head blight caused by *F. culmorum* in winter wheat was inherited predominantly in an additive way, although dominance effects were observed. Transgression occurred in some cases. The resistant parents differed in resistance genes, but the data were insufficient to determine the number of genes. In the present study the response to selection and transgression for resistance were studied. An attempt was made to estimate the number of resistance genes in which the resistant parents differed.

Materials and methods

Plant material

In a field trial in 1988 the F_2 generations of a set of crosses, composing a 10 × 10

half diallel of winter wheat lines, were inoculated twice with *Fusarium culmorum* strain IPO 39-01 (Snijders, 1990a). Incidence of head blight was assessed on a single plant basis as the percentage infected spikelets of three inoculated heads. A selection of 110 resistant plants out of 1951 F_2 plants (5.6%) was obtained by truncation selection. Truncation occurred at 10% head blight 21 days after first inoculation and at 25% head blight 28 days after first inoculation.

In a 1989 field trial, the 110 F_3 lines derived from the selected plants and the ten parental lines were tested for head blight resistance. The trial included three plots of each parental line consisting of up to 30 plants and one plot of each F_3 line consisting of up to 50 plants. The plots were randomized over the field. January 12, 1989, seeds were sown 0.12 m apart in rows 0.35 m apart, with no more than 10 seeds per row. As the seeds were harvested in 1988 from inoculated plots, all seeds were treated with methylmercury according to practice to prevent a seed-borne *Fusarium* infection.

Inoculation and disease assessment

For inoculation one pathogenic strain of *Fusarium culmorum* (IPO 39-01) was used and produced as described in Snijders and Van Eeuwijk (1990). Since wheat is most susceptible to infection at anthesis (Schroeder and Christensen, 1963), experimental inoculations were as far as possible carried out at this time. The heads of all plants of a plot were inoculated at the time when 50% of the plants within the plot were flowering. Inoculation was repeated after five days. The inoculum was sprayed with a propane spray-gun from 0.25 m above the crop at 250,000 spores ml^{-1}, in an amount of 100 ml m^{-2}. A regular mist irrigation above the crop maintained a high relative humidity during the period 14 June to 9 July. Incidence of head blight, expressed as the percentage of infected spikelets in the heads of the primary stem and primary tillers, was recorded on a single plant basis 21 days and 28 days after first inoculation (OBS-1 and OBS-2, respectively). Symptoms were described in Snijders and Perkowski (1990). Time of anthesis was recorded on a single plant basis.

Statistical and genetic analyses

Data on head blight were analyzed on a single plant basis. The weighted within-plot variance $\hat{\sigma}^2_{eW}$ was estimated from σ^2_{eW} of the homozygous parents, taking as weight the reciprocals of the standard error of the within plot mean square for each parent (Snijders, 1990b). The selection differential (S) was calculated as the difference in the mean head blight rating of the original F_2 population ($\bar{p}$) and that of the selected sample of resistant plants ($\bar{p}'$). The response to selection (R) was calculated as the difference between ($\bar{p}$) and the mean head blight rating of the progeny of the selected plants based on line means ($\bar{p}_1$). The realized heritability h^2_r was estimated by $h^2_r = R/S$.

Results

Due to differences in time of anthesis among F_3 lines and parental lines, the inoculation period lasted from 14 June to 26 June. For the 2638 plants observed, the correlation coefficient between the date of first inoculation and Fusarium head blight rating was 0.39, and the correlation coefficient between the period between anthesis and first inoculation and Fusarium head blight rating was 0.34. The low correlations coefficients lead to the conclusion that although the inoculation time was chosen in accordance with the average time of anthesis per plot and not per plant, escape from infection was not likely.

The number of plants tested per parental line and per F_3 line averaged 64 and 18, respectively. The mean head blight incidence over all plants was 8.2% for OBS-1 and 29.0% for OBS-2. The correlation coefficient between OBS-1 and OBS-2 was 0.74. OBS-2, the head blight ratings assessed 28 days after first inoculation, showed larger differences in resistance among parental lines than OBS-1 and were comparable with the mean head blight ratings of the parental lines assessed in 1988 (Snijders, 1990b). Therefore, from here on only OBS-2 ratings for head blight are considered. In Figure 7.1 the mean levels of head blight resistance of the parental lines of the 1989 trial are shown together with those of the 1988 trial where selection had taken place. Although

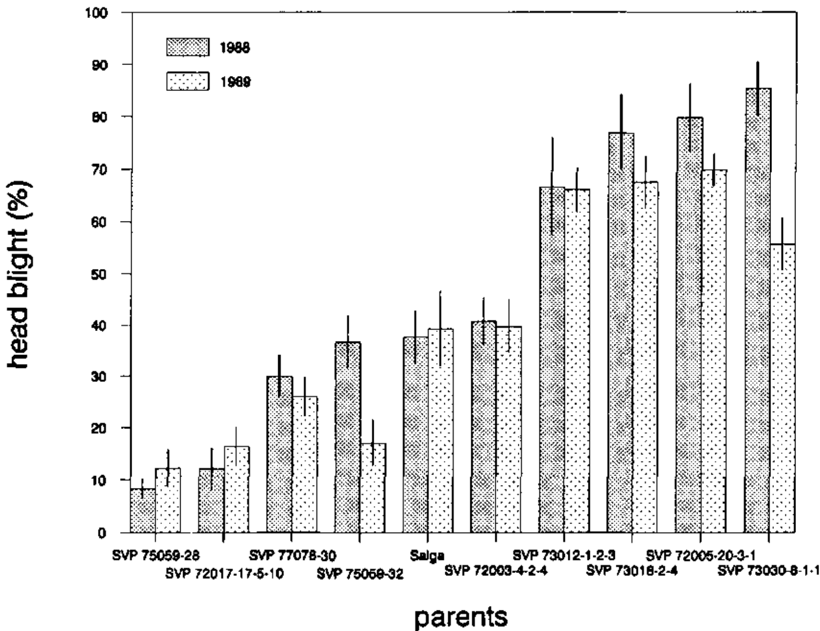

Figure 7.1. Levels of resistance to head blight and their confidence interval (P=0.05) in 10 parental lines after inoculation with *Fusarium culmorum* strain IPO 39-01 in 1988 and in 1989.

the most susceptible genotypes had a lower head blight rating in 1989 than in 1988, levels of resistance of the parental lines in the two years were closely related (r=0.93). It may be concluded that genotype × year interaction for Fusarium head blight was small. An exception was parent SVP 75059-32, which did not react the same as in 1988. Snijders and Van Eeuwijk (1990) concluded that the selections from cross SVP 75059, among them SVP 75059-32, showed a higher genotype × year

Table 7.1. Mean *Fusarium culmorum* (IPO 39-01) head blight rating (%) of the original 25 winter wheat F_2 families in 1988 ($\bar{p}$)[a], the mean head blight rating (%) in 1988 of the selected sample of resistant plants ($\bar{p}'$), the number of selected plants n($\bar{p}'$), the proportion (%) of selected plants out of the F_2 family, the selection differential S, the mean head blight rating (%) of the progeny produced by the selected plants based on F_3 line means ($\bar{p}_1$), the number of plants of the total progeny n($\bar{p}_1$), the selection response R, the realized heritability h^2_r and the number of F_3 lines which showed significant transgression for resistance (P=0.05). Parental lines are listed in descending order of their resistance in 1988

cross	cross number	1988					1989				
		$\bar{p}$	$\bar{p}'$	n($\bar{p}'$)	proportion (%)	S	$\bar{p}_1$	n($\bar{p}_1$)	R	h^2_r	transgression
SVP 75059-28 ×											
SVP 72017-17-5-10	8718	26.3	9.0	16	21.9	17.3	19.3	293	7.0	0.41	2
SVP 77078-30	8735	23.8	9.2	15	23.4	14.5	22.4	322	1.4	0.09	
SVP 75059-32	8728	21.2	4.8	3	33.3	16.4	43.2	43	-22.0	0.00	
Saiga	8743	24.3	9.4	11	19.0	14.9	22.7	284	1.6	0.11	
SVP 72003-4-2-4	8716	34.4	4.8	1	1.9	29.6	25.0	4	9.4	0.32	
SVP 73016-2-4	8720	38.8	8.7	5	6.3	30.1	34.0	112	4.8	0.16	
SVP 72005-20-3-1	8717	44.3	8.7	1	2.1	35.6	22.6	22	21.7	0.61	
SVP 72017-17-5-10 ×											
SVP 75059-32	8724	25.3	9.2	7	10.0	16.1	22.9	111	2.4	0.15	1
Saiga	8739	23.2	9.5	6	18.2	13.8	27.0	95	-3.8	0.00	
SVP 72003-4-2-4	8702	36.3	7.7	3	4.8	28.6	29.4	36	6.9	0.24	
SVP 73016-2-4	8709	36.3	8.1	5	8.8	28.2	45.9	123	-9.6	0.00	
SVP 72005-20-3-1	8703	50.6	4.8	2	2.8	45.8	60.0	11	-9.4	0.00	
SVP 73030-8-1-1	8713	54.5	4.8	1	1.4	49.6	42.5	4	12.0	0.24	
SVP 77078-30 ×											
SVP 75059-32	8736	34.1	7.4	3	4.1	26.7	26.2	59	7.9	0.29	1
Saiga	8745	34.1	6.4	1	1.5	27.7	19.5	17	14.6	0.53	
SVP 72003-4-2-4	8729	25.2	7.6	10	16.7	17.5	18.7	147	6.5	0.37	5
SVP 73016-2-4	8733	41.6	4.8	1	5.6	36.8	27.5	4	14.1	0.38	
SVP 72005-20-3-1	8730	45.3	6.4	1	1.8	38.9	45.8	26	-0.5	0.00	
SVP 75059-32 ×											
Saiga	8744	35.1	11.4	2	4.9	23.7	21.2	32	13.9	0.59	1
SVP 72003-4-2-4	8722	26.9	7.7	4	7.3	19.2	34.8	47	-7.9	0.00	
SVP 73016-2-4	8726	41.1	11.0	2	6.1	30.1	34.0	53	7.1	0.24	
SVP 72005-20-3-1	8723	47.4	6.8	2	3.4	40.7	50.2	10	-2.8	0.00	
SVP 73030-8-1-1	8727	34.8	7.9	5	6.4	26.9	30.3	53	4.5	0.17	
Saiga ×											
SVP 72003-4-2-4	8737	31.1	9.5	2	4.3	21.6	44.8	25	-13.7	0.00	
SVP 72003-4-2-4 ×											
SVP 73016-2-4	8707	38.4	10.3	1	4.0	28.1	11.3	8	27.1	0.96	1

[a] The data of 1988 were corrected by linear regression with a coefficient of 0.77 and a constant of 4.84.

interaction compared to other genotypes, including all other parents tested in this study. Therefore, one should be cautious with respect to conclusions for offspring of SVP 75059-28 and especially SVP 75059-32. The arithmetic mean head blight incidence of the 10 parental lines was lower in 1989 than in 1988. Therefore, the data of 1988 were corrected by linear regression with a coefficient of 0.77 and a constant of 4.84 before calculating $\bar{p}$, $\bar{p}'$, S and R.

For six parents, the environmental variance between-plots, σ^2_{eB}, was not significantly different from 0 (P=0.01). Three of the four parents with a significant σ^2_{eB} in 1989 also showed a significant σ^2_{eB} in 1988. These genotypes apparently show a higher genotype × environment interaction. The high value of the σ^2_{eW} to σ^2_{eB} ratio indicated that the field was homogeneous. The weighted environmental within-plot variance $\hat{\sigma}^2_{eW}$ was 214.9 ± 12.6. This is almost twice the environmental variance estimated for 1988. There was no correlation between mean head blight ratings and variances of the ten parental lines (r=0.09).

Table 7.1 presents for each family the following results: the mean head blight ratings of the original F_2 family in 1988 ($\bar{p}$), the mean head blight in 1988 of the sample of selected plants ($\bar{p}'$), the number of selected plants n($\bar{p}'$), the proportion (%) of selected plants out of the F_2 family, the selection differential S, the mean head blight of the progeny produced by the selected parents based on F_3 line means ($\bar{p}_1$), the number of plants of the total progeny n($\bar{p}_1$), the selection response R, the realized heritability h^2_r and the number of F_3 lines per cross which showed a transgression for resistance significant at P=0.05.

Discussion

Selection response

Selection responses averaged 3.7% on the head blight percentage scale and ranged from -22.0% to 27.1%. Realized heritabilities averaged 0.23 and ranged from 0 to 0.96 (Table 7.1). Negative responses must be explained by the fact that the selected plants obviously had escaped infection in 1988. Where a simple additive-dominance model is adequate, the response to selection (R) is equal to the product of the heritability in narrow sense h^2_n and the selection differential (S) (Mather and Jinks, 1982). For 17 out of the 25 families from which plants were selected in 1988 the additive-dominance model could be tested on basis of F_1 and F_2: the model satisfied for all 17 families (Snijders, 1990b). However, only estimates for heritabilities in broad sense h^2_b were obtained from that study. If we compare the realized heritabilities calculated in 1989 (Table 7.1) with the broad sense heritabilities estimated from the components of variance in 1988 (Snijders, 1990b), in 7 out of 25 families the h^2_r was higher than the h^2_b. This means that for these families the obtained gain in resistance was higher than expected from the estimates of components of variance in the F_2 generation.

However, it should not be overlooked that the estimates of h_b^2 were not very accurate (Snijders, 1990b). The average realized heritability $\bar{h}_r^2$ was 0.23 compared to a $\bar{h}_b^2$ of 0.39.

Transgression

Based on the F_3 line means, six out of 25 crosses showed significant transgressive segregation for a higher resistance (P=0.05; Table 7.1). Three resistant F_3 lines showed a resistance higher than that of the most resistant parental line, SVP 75059-28: two F_3 lines from cross 8718, the cross between the most resistant parents, with head blight levels and confidence intervals (P=0.05) of 4.6% ± 1.5 and 5.9% ± 2.2, respectively, and one F_3 line from cross 8729 with an infection level of 6.7% ± 3.0. In the 1988 trial, for the crosses with a moderately or highly resistant parent, only in the F_2s of 8718 and 8716 transgressive segregation was clearly observed, and for susceptibility only (Snijders, 1990b). In this study the transgression for resistance found for the F_3 lines gives evidence that the resistance genes in the resistant parents were partly different from one another. This confirms the suggestion in Snijders (1990b) and agrees with their pedigrees which had no ancestors in common (Snijders, 1990a). Accumulation of resistance genes is herewith shown to be possible.

Fixation of resistance genes

Single plant selection in the F_2 proved to be very successful. The speed of selective advance depends on the number of units of inheritance which contribute to the additive genetic variance and the non-additive genetic variance. The greater the proportion of additive heritable variation, the more effective is the selection (Mather and Jinks, 1982). The resistance to Fusarium head blight in the selected populations must be inherited mainly in an additive way, and the number of resistance genes in which the parents differ must be small. If there was mainly additive genetic variance, $\bar{h}_r^2$ should be equal to $\bar{h}_b^2$. The fact that $\bar{h}_r^2$ was lower than $\bar{h}_b^2$ might be explained by the important number of selected plants which were not resistant but had escaped infection in 1988.

It is obvious that there is a relationship between mean and variance of the F_3 lines (r=0.56; Figure 7.2). Analysis of variance of the head blight data of the parental lines had shown that there was no correlation between mean and environmental variance. In Figure 7.2, the dotted line indicates the level of weighted environmental variance $\hat{\sigma}_{eW}^2$. Within the F_3 lines with a high mean head blight resistance, and especially within those F_3 lines which were significantly more resistant than their most resistant parent, genetic variance was small or absent. These lines are probably fixed for Fusarium head blight resistance and will not segregate in later generations. The number of plants of the original F_2 for each family averaged 52, with a maximum of 80 plants. If the parents had differed in three independently segregating resistance genes, in the

F_2 only one individual in 64 would have been homozygous for each of the extreme genotypes. Not merely would the chance of immediate fixation under selection be very small, it is even unlikely that any extreme resistant genotype would have been picked up in an F_2 of this size. This would indicate that the parents of which the offspring showed transgression in the F_2 may differ in only one or two resistance genes.

General combining ability

In this study the response to the truncation selection was studied per F_2 family, in order to search among crosses for the better ones, and to concentrate on the elite crosses. The truncation selection over the whole F_2 population resulted in selection of plants from merely those families with one or two resistant parents (Table 7.1). Transgression for resistance of F_3 lines was also merely found in these families. In retrospect, it proved superfluous to study the many F_2 families of the 10 × 10 half diallel, as performance of the parent appeared to be a good indicator of the potential of their crosses for resistance breeding. This confirms the conclusion from the

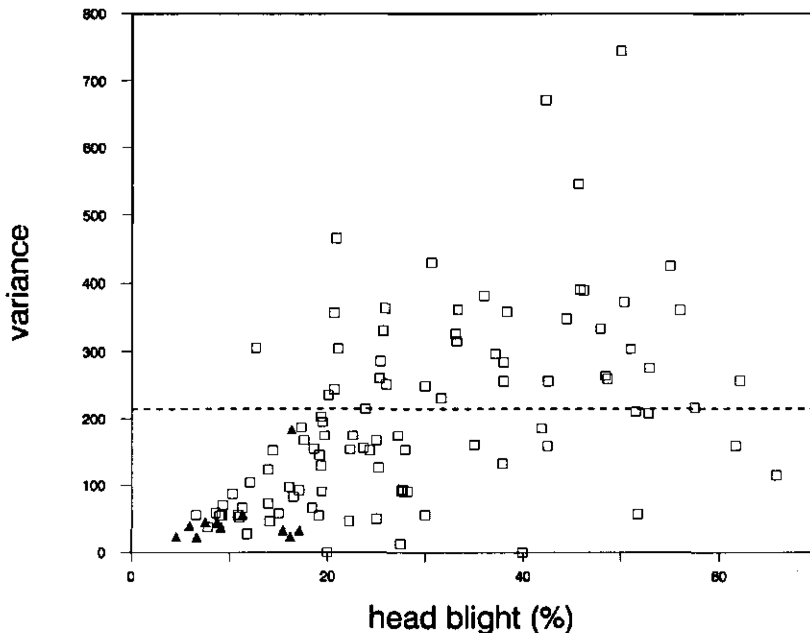

Figure 7.2. The relationship between the mean head blight reactions against their variances for all F_3 lines after inoculation with *Fusarium culmorum* strain IPO 39-01 in 1989. The horizontal dotted line indicates the weighted within-plot variance $\bar{\sigma}^2_{eW}$. ▲ indicates F_3 lines with significant transgression (P=0.05).

combining ability analysis of the 10 × 10 half diallel that specific combining ability effects were unimportant and general performance of the parent was in agreement with its general combining ability (Snijders, 1990a). However, one exception was made for genotype SVP 72003-4-2-4, which had a high general combining ability which was not in accordance with its resistance level. Also, Table 7.1 shows that crosses with this moderately susceptible genotype resulted in offspring with very high resistance levels and transgression for resistance. Snijders (1990b) suggested that in crosses with SVP 72003-4-2-4 an epistatic gene effect might be present. This could explain the high resistance levels of the offspring. As one F_3 line was even more resistant than the most resistant parent used in this study, and the variance within this line was negligible, epistasis might be of additive × additive gene interaction and fixed in this line.

Early generation selection

An important question in many breeding programs is in which generation selection should take place. Snijders (1990b) concluded that the two resistant parents SVP 75059-28 and SVP 72017-17-5-10 differed from the susceptible parent SVP 72005-20-3-1 in four and five genes, respectively. From this study it was concluded that the two resistant parents differ in one or two genes. This means that the fixed resistance from the selected F_3 lines may differ from a common, susceptible wheat genotype in as much as six genes. In the F_2 of this cross only one in 4^6 plants will contain all resistance genes. Thus, for early selection in the F_2 the population should be very large, and the selection pressure lenient. For a practical breeding program, it would be better to delay selection till the F_3 or F_4, where homozygotes have formed an increased proportion of the segregates and the likelyhood for advance under selection will be increased. However, selection will be only successful if there is a uniform and adequate disease pressure in the nursery where the selection takes place.

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Chapter 8

GENETIC VARIATION FOR RESISTANCE TO FUSARIUM HEAD BLIGHT IN BREAD WHEAT

Summary

During a four year period, a total of 258 winter and spring wheat genotypes were evaluated for resistance to head blight after inoculation with *Fusarium culmorum* strain IPO 39-01. It was concluded that genetic variation for resistance is very large. Spring wheat genotypes which had been reported to be resistant to head blight caused by *Fusarium graminearum* were also resistant to *F. culmorum*. The resistant germplasm was divided into three gene pools: winter wheats from Eastern Europe, spring wheats from China/Japan and spring wheats from Brazil. In 32 winter wheat genotypes in 1987, and 54 winter wheat genotypes in 1989, the percentage yield reduction depended on the square root of percentage head blight with an average regression coefficient of 6.6. Heritability estimates indicated that for selection for Fusarium head blight resistance, visually assessed head blight was a better selection criterion than yield reduction.

Introduction

Fusarium head blight in bread wheat (*Triticum aestivum* L.) is mainly caused by *Fusarium culmorum* (W.G. Smith) and *Fusarium graminearum* (Schwabe), of which *F. culmorum* is the dominant pathogen in north-western Europe (Mielke, 1988; Parry *et al.*, 1984; Rapilly *et al.*, 1971). Damage from head blight consists of reduced kernel set and kernel weight, destroyed starch granules and storage proteins, and infection and mycotoxin contamination of the seed (Snijders, 1990a). In general, bread wheat cultivars are susceptible to Fusarium head blight. During the last years the occurrence of epidemics has steadily increased in all wheat growing areas over the world (Anonymous, 1989; Mielke, 1988). There is little information about sources of resistance to Fusarium head blight. Parry *et al.* (1984) showed that English winter wheat cultivars differed significantly in their resistance to head blight caused by *Fusarium culmorum*. Based on 15 years screening for resistance to *F. culmorum*, Mielke (1988) did not find resistance in hundreds of *Triticum aestivum* cultivars and breeding lines, 18 other *Triticum* spp., 21 *Aegilops* spp. and 11 *Agropyrum* spp. Recently the Hungarian cultivar 'Ringó Sztár' was introduced with a resistance level high enough to withstand a severe natural Fusarium inoculation (Mesterhazy, 1987). Spring wheat cultivars carrying resistance to *F. graminearum* have been bred in Brazil ('Frontana' and 'Encruzilhada'), in Japan ('Nobeokabozu Komugi'), and in China ('Su Mai #3' and several 'Ning' selections) (Mesterhazy, 1987; Schroeder and Christensen, 1963; Wang and Miller, 1987). To what extent resistance to *F. culmorum* is related to resistance to *F. graminearum* is not clear. Mesterhazy (1987; 1988) found correlation coefficients of up to 0.90 between the reaction of wheat genotypes to

F. culmorum isolates and the reaction to *F. graminearum* isolates.

This study was designed to identify sources of resistance to Fusarium head blight in wheat by means of visually assessed head blight ratings after experimental inoculation with *F. culmorum.* In addition, the relationship between visually assessed head blight ratings, from here on described as head blight, and yield reduction was investigated.

Materials and methods

Head blight

During a four year period, 1986-1989, a total of 258 winter wheat and spring wheat entries were evaluated for resistance to Fusarium head blight. A total of 17 winter wheat genotypes with different resistance levels (Snijders and Van Eeuwijk, 1990) were tested each year and used as standards. The experimental design was a randomized complete block. Winter and spring type genotypes were tested in the same blocks, but because of difference in earliness grouped per type. Depending on the amount of seed, plots consisted either of field plots of 2.00 × 0.75 m, seeded at the standard seed density of 330 seeds m^{-2} in rows 0.25 m apart, or of hill plots (ϕ 0.25 m) seeded with 3 g, at 0.5 m apart. The winter wheat trial of 1986 was described in Snijders and Perkowski (1990). In 1987, 32 winter wheat genotypes were tested in field plots (as described in Snijders and Van Eeuwijk, 1990), and 19 winter wheat genotypes and 23 spring wheat genotypes were tested in hill plots; all genotypes were tested in three replications. In 1988, 52 winter wheat genotypes (as described in Snijders and Van Eeuwijk, 1990) together with 162 spring wheat genotypes were tested in hill plots in three replications. In 1989, 54 winter wheat genotypes were tested in field plots in six replications; 7 winter wheat and 7 spring wheat genotypes were tested in hill plots in three replications. One pathogenic strain of *Fusarium culmorum* (IPO 39-01) was used for inoculation. Inoculum production and inoculation methods have been described earlier (Snijders and Van Eeuwijk, 1990). With exception of 1986, inoculation was carried out simultaneously for all winter wheat genotypes, and likewise simultaneously for all spring wheat genotypes. Because of differences in day of anthesis among genotypes and among plants, in the years 1987-1989 inoculation was repeated two to four times. Since wheat is most susceptible to infection at anthesis (Schroeder and Christensen, 1963), the first inoculation took place when 30% of the genotypes within a group was flowering. Details of inoculation time are given in Table 8.1. Fusarium head blight was observed 25-28 days after first inoculation (Table 8.1). The first head blight symptoms consisted of light brown, watersoaked spots on the glumes. Soon, infected spikelets lost the water-soaked appearance, dried up, and assumed the colour of the ripe heads. Head blight ratings were determined as the product of the percentage of heads infected and the proportion of infected (bleached) spikelets per infected head.

Table 8.1. Time of inoculation and observation of field trials in 1986-1989, carried out to evaluate the resistance of bread wheat to head blight caused by experimental inoculation with *Fusarium culmorum* strain IPO 39-01

	1986		1987		1988		1989	
	winter wheat	spring wheat	winter wheat	spring wheat	winter wheat	spring wheat	winter wheat	spring wheat
month of inoculation	June	-	June	June	June	June	May	May
date of first inoculation	19, 24 or 25	-	25	25	2	16	25	25
number of inoculations	1	-	2	2	3	3	4	4
intervals (days) between inoculation	-	-	7	7	7	7	4	4
observation time in days after first inoculation	26	-	26	26	27	25	28	28

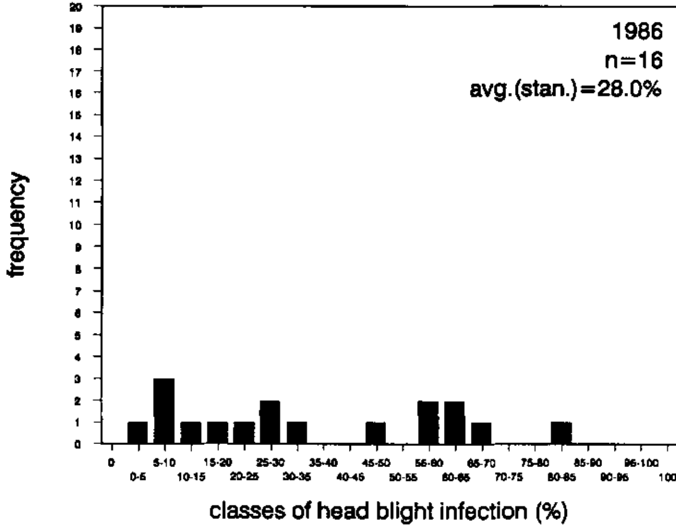
Yield reduction

In 1987 and 1989, the inoculated field plots of winter wheat were harvested with a small combine harvester (Seedmaster Universal, Wintersteiger, Ried, Austria). In each block also a non-inoculated control plot of each genotype was harvested. Of the 32 genotypes of 1987 and the 54 genotypes in 1989, 26 genotypes were tested in both years, including the 17 standards. Threshing and cleaning of the harvested seed was done with care to prevent the loss of small, shriveled grains. Reductions of kernel weight, kernel number and yield were determined per block as the difference between inoculated and non-inoculated plot, expressed as a percentage of the non-inoculated control plot.

The set of genotypes in 1987 and the set of 1989 were assumed to be random sets. From the mean squares of the variance analyses and the expected mean squares based on a randomized complete block design (Steel and Torrie, 1981), the variance components could be estimated for percentage infected heads, proportion infected spikelets, Fusarium head blight, kernel weight reduction, kernel number reduction and yield reduction. The heritabilities were estimated on a phenotypic mean basis averaged over blocks as follows:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{block}^2 + \sigma_e^2}{r}}$$

where σ_g^2, σ_{block}^2 and σ_e^2 stand for the variance components due to genotypes, blocks and error, respectively, while r stands for the number of blocks.



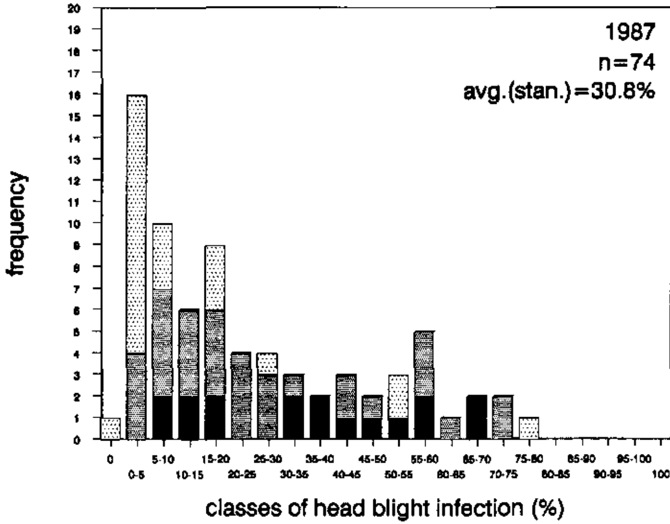
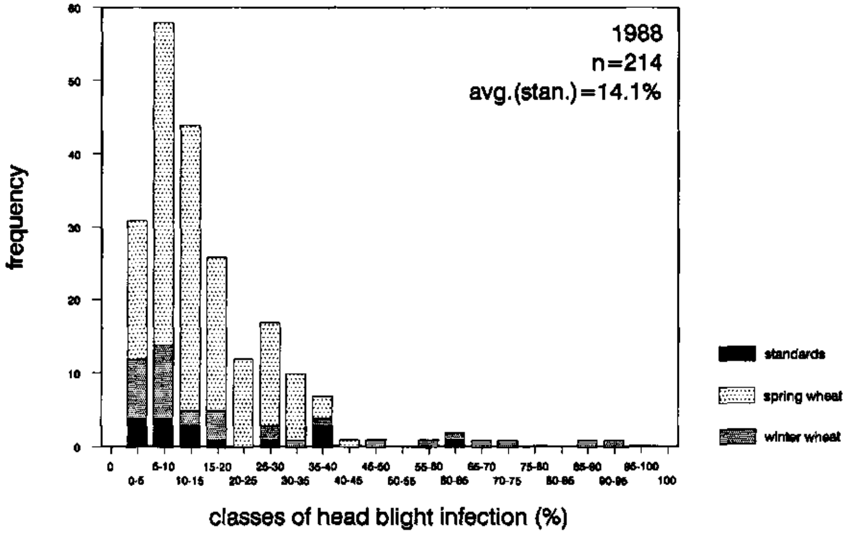


Figure 8.1. Distribution of frequencies of wheat genotypes in 5% classes of Fusarium head blight on a scale of 0-100%, after experimental inoculation by *Fusarium culmorum*, for field trials in 1986, 1987, 1988 and 1989. The group of standards consisted of 17 winter wheats with different levels of resistance.



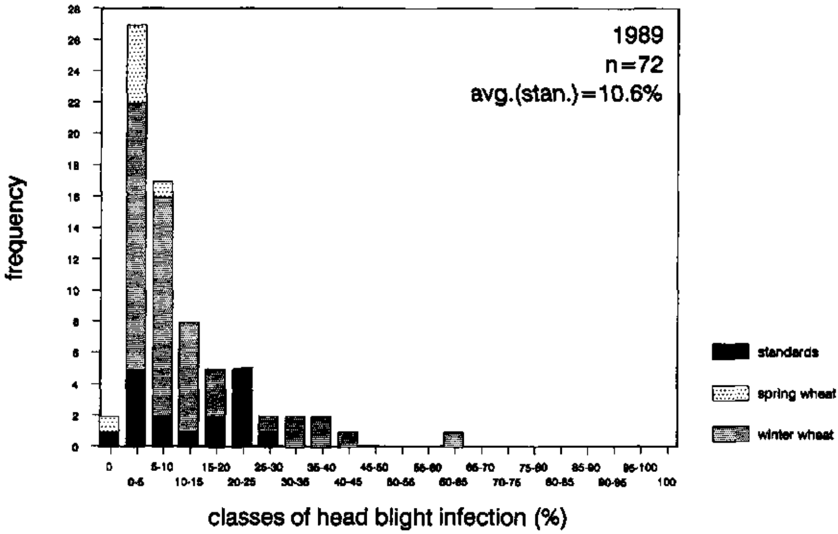


Figure 8.1. Continuation

Table 8.2. Resistance of 258 bread wheat genotypes to Fusarium head blight caused by *Fusarium culmorum* strain IPO 39-01 expressed as a FHB_{index}[a], the number of years the genotype was tested, the wheat type, the donor and the land of origin of the genotype. Genotypes are presented in descending order of resistance

cultivar/line	FHB_{index}	years	type	donor	land of origin
FT 83-326	0.9	2	spring	ABC[b]	Brazil
Frontana	1.3	2	spring	SVP[c]	Brazil
Ning 8343	1.4	3	spring	CIMMYT[d]	China
Praag 8	1.7	3	winter	SVP	Czechoslovakia
Wuhan #1	1.9	3	spring	CIMMYT	China
FT 83-371	1.9	2	spring	ABC	Brazil
CRI Ringó Sztár-Nobeoka B.(88/802/7)	2.4	1	winter	CRI[e]	Hungary
Nanjing 7840	3.1	3	spring	CIMMYT	China
Novokrumka 0102	3.4	3	winter	SVP	USSR
C 33	4.0	1	spring	EMUFP[f]	Brazil
Hadden	4.0	1	spring	EMUFP	USA
FT 83-268	4.0	2	spring	ABC	Brazil
FT 83-302	4.3	2	spring	ABC	Brazil
SVP 75066-28-1	4.4	3	winter	SVP	Netherlands
SVP 72017-17-5-10	4.7	4	winter	SVP	Netherlands
Fermo	5.0	3	winter	SVP	Switzerland
IAC 5 (Maringa)	5.4	3	spring	EMBRAPA[g]	Brazil
SVP 85046	5.4	2	winter	SVP	Netherlands
C 7918	5.5	1	spring	EMUFP	Brazil
Nobeokabozu Precoce	5.5	1	spring	EMUFP	Japan
CRI Zombor-Sumey-3 (88/823/3)	5.5	1	winter	CRI	Hungary
Ft 83-152	6.1	1	spring	ABC	Brazil
Kooperatorka	6.3	3	winter	SVP	USSR
Toropi	6.3	3	spring	EMBRAPA	Brazil
Peking 8	6.4	1	spring	EMUFP	China
SVP 77079-47	6.4	1	winter	SVP	Netherlands
SVP 77076-1	6.6	4	winter	SVP	Netherlands
Match	6.8	1	winter	SVP	France
YMI #6-11B-OY	6.8	1	spring	CIMMYT	Mexico
SVP 77076-38	6.8	4	winter	SVP	Netherlands
CRI Lili²-Ringó Sztár (88/832/1)	6.9	1	winter	CRI	Hungary
Shanghai #3	6.9	3	spring	CIMMYT	China
Arina	7.0	4	winter	SVP	Switzerland
SVP 77076-4	7.1	4	winter	SVP	Netherlands
CRI HD-CCM×HD-Bty/CCM (88)	7.3	1	winter	CRI	Hungary
Nyubai	7.4	1	spring	EMUFP	Japan

[a] $$FHB_{index} = \frac{0.75 \times FHB_{1986} + 0.68 \times FHB_{1987} + 1.48 \times FHB_{1988} + 1.97 \times FHB_{1989}}{\text{number of years the genotype was tested}}$$

[b]ABC = Fundação ABC para Assistência e Divulgação Técnica Agropecucária, Ponta Grossa, Brazil
[c]SVP = Foundation for Agricultural Plant Breeding, Wageningen, the Netherlands, now CPO
[d]CIMMYT = International Maize and Wheat Improvement Center, Mexico, D.F. Mexico
[e]CRI = Cereal Research Institute, Szeged, Hungary
[f]EMUFP = Elisieu Maciel Universidade Federal de Pelotas, Pelotas, Brazil
[g]EMBRAPA = Centro Nacional de Pesquisa de Trigo, Passo Fundo, Brazil

cultivar/line	FHB_{index}	years	type	donor	land of origin
PF 859002	7.4	1	spring	EMUFP	Brazil
Hulha Negra	7.4	1	spring	EMUFP	Brazil
PF 8016	7.4	1	spring	EMUFP	Brazil
Suzhoe F3#1[h]	7.4	1	spring	EMBRAPA	China
CEP 83116	7.8	1	spring	EMUFP	Brazil
Nobeokabozu komugi	8.0	3	spring	KNASF[i]	Japan
SVP 75018-22-1	8.3	2	winter	SVP	Netherlands
Bo Su 1	8.4	1	spring	EMUFP	China
Sabre	8.5	3	winter	SVP	France
SVP 85049	8.6	2	winter	SVP	Netherlands
Belocerkovskaja 198	8.7	1	winter	SVP	USSR
Ringó Sztár	8.8	2	winter	CRI	Hungary
PF 859139	8.9	1	spring	EMUFP	Brazil
Charrua	8.9	1	spring	EMUFP	Brazil
Nasu	8.9	1	spring	EMUFP	China
PF 8215	9.3	1	spring	EMUFP	Brazil
Klein Atlas	9.3	1	spring	EMUFP	Argentina
Encruzilhada	9.4	3	spring	EMBRAPA	Brazil
SVP 85047	9.7	2	winter	SVP	Netherlands
BR 5	9.9	1	spring	EMUFP	Brazil
Maris Huntsman	10.2	1	winter	PBI[j]	UK
Kaluzkaja 9	10.2	3	winter	SVP	USSR
PF 859115	10.4	1	spring	EMUFP	Brazil
Yecora 70	10.4	1	spring	EMUFP	Mexico
PF 859116	10.4	1	spring	EMUFP	Brazil
BW 20	10.4	1	spring	PRC[k]	Canada
Br 14 Embrapa	10.4	2	spring	ABC	Brazil
Bence(88/232)	10.4	1	winter	CRI	Hungary
PF 859122	10.8	1	spring	EMUFP	Brazil
Nobeokabozu komugi	10.8	1	spring	EMUFP	Japan
CNT 9	10.8	1	spring	EMUFP	Brazil
Nova Prata	10.8	1	spring	EMUFP	Brazil
B 7503	10.8	1	spring	EMUFP	Brazil
PF 859118	10.8	1	spring	EMUFP	Brazil
Nhupora	10.8	1	spring	EMUFP	Brazil
BH 1146	10.8	1	spring	ABC	Brazil
SVP 72003-4-2-4	11.1	4	winter	SVP	Netherlands
CRI HD528FD3-Brjk (88/137)	11.2	1	winter	CRI	Hungary
PF 859011	11.4	1	spring	EMUFP	Brazil
Monakinka	11.4	1	spring	VIR[l]	USSR
Galahad	11.5	1	winter	PBI	UK
SVP 77078-30	11.5	4	winter	SVP	Netherlands
SVP 85054	11.7	2	winter	SVP	Netherlands

[h]syn. Sushui, Hsü-shui
[i]KNASF = Kyushu National Agricultural Station, Fukuoka, Japan
[j]PBI = Plant Breeding Institute, Cambridge, UK
[k]PRC = Station de Recherches, Agriculture Canada, Sainte-Foy, Canada
[l]VIR = Vavilov All-Union Institute of Plant Industry, Leningrad, USSR

cultivar/line	FHB_{index}	years	type	donor	land of origin
Cotipora	11.8	1	spring	EMUFP	Brazil
Libellula	11.8	1	winter	SCAT[m]	Italy
PF 859008	12.3	1	spring	EMUFP	Brazil
Jacui	12.3	1	spring	EMUFP	Brazil
Turda 195	12.3	1	winter	SCAT	Rumania
Norin 34	12.9	1	spring	EMUFP	Japan
Minuano 82	12.9	1	spring	EMUFP	Brazil
PF 859074	12.9	1	spring	EMUFP	Brazil
Neepawa	12.9	1	spring	PRC	Canada
Van May 17	12.9	1	spring	EMUFP	China
Kraka	12.9	1	winter	SVP	Denmark
Zenith	13.3	2	winter	SVP	Switzerland
CEP 14 Tapes	13.3	1	spring	EMUFP	Brazil
PF 859146	13.3	1	spring	EMUFP	Brazil
Tobari 66	13.3	1	spring	SVP	Mexico
BR 6	13.3	1	spring	EMUFP	Brazil
Su May 2	13.3	1	spring	EMUFP	China
Pel 72393	13.3	1	spring	EMUFP	Brazil
CRI 80-1170×79-1/57 (88/910/6)	13.4	1	winter	CRI	Hungary
Saiga	13.5	4	winter	SVP	Netherlands
Pagode	13.6	1	winter	SVP	Netherlands
Ning 8401	13.6	1	spring	CIMMYT	China
Shanghai #4	13.6	1	spring	CIMMYT	China
Su Mai 3[n]	13.6	1	spring	EMBRAPA	China
SVP 77079-15	14.0	4	winter	SVP	Netherlands
PF 859114	14.3	1	spring	EMUFP	Brazil
Norrøna	14.3	1	spring	SVP	Norway
Livilla	14.7	1	winter	UW[o]	Poland
PF 859004	14.8	1	spring	EMUFP	Brazil
BR 14 (PF 79782)	14.8	1	spring	EMUFP	Brazil
PF 859030	15.2	1	spring	EMUFP	Brazil
PF 859147	15.2	1	spring	EMUFP	Brazil
PF 859173	15.2	1	spring	EMUFP	Brazil
SVP 67036-1-1-4	15.3	3	winter	SVP	Netherlands
Toropi	15.5	1	spring	EMUFP	Brazil
CNT 10	15.8	1	spring	EMUFP	Brazil
CEP 7951	15.8	1	spring	EMUFP	Brazil
Lin Su 3	15.8	1	spring	EMUFP	China
Florin	15.9	1	winter	SVP	France
CRI 81-50(88/37)	16.2	1	winter	CRI	Hungary
SVP 75059-28	16.2	4	winter	SVP	Netherlands
PF 859171	16.3	1	spring	EMUFP	Brazil
PF 859109	16.3	1	spring	EMUFP	Brazil
RS 1 Fenix	16.3	1	spring	EMUFP	Brazil
Abura komugi	16.7	1	spring	EMUFP	Japan
BR 4	16.7	1	spring	EMUFP	Brazil

[m]SCAT = Statiunea de Cercetari Agricole, Turda, Rumania
[n]syn. Su mei 3, Sumai 3, Su May 3, Soo-moo-3
[o]UW = University of Warsaw, Department of Plant Pathology, Warsaw, Poland

cultivar/line	FHB_{index}	years	type	donor	land of origin
SVP 77079-11	16.9	1	winter	SVP	Netherlands
Flanders	16.9	1	winter	NIAB[p]	UK
Granada	17.1	1	winter	SVP	Germany
Vacaria	17.3	1	spring	EMUFP	Brazil
Soissons	17.5	1	winter	SVP	France
CEP 82128	17.7	1	spring	EMUFP	Brazil
PF 859018	17.7	1	spring	EMUFP	Brazil
PF 85516	17.7	1	spring	EMUFP	Brazil
Alvarez 110	17.7	1	spring	EMUFP	Argentina
Peladinho	17.7	1	spring	EMUFP	Brazil
Arminda	17.7	3	winter	SVP	Netherlands
Pistou	18.1	1	winter	SVP	France
Londrina	18.2	1	spring	EMUFP	Brazil
PF 85440	18.2	1	spring	EMUFP	Brazil
Novosibirskaja	18.8	1	spring	VIR	USSR
BR 3	18.8	1	spring	EMUFP	Brazil
Santiago	19.2	1	spring	EMUFP	Brazil
PF 859149	19.2	1	spring	EMUFP	Brazil
PF 859113	19.2	1	spring	EMUFP	Brazil
SVP 85043	19.4	2	winter	SVP	Netherlands
CEP 11	19.7	1	spring	EMUFP	Brazil
Norrene	19.7	1	spring	VIR	Norway
PF 859142	19.7	1	spring	EMUFP	Brazil
Sinton	19.7	1	spring	PRC	Canada
Pel 74142	20.3	1	spring	EMUFP	Brazil
PF 859127	20.3	1	spring	EMUFP	Brazil
CWW 81-13	20.3	1	winter	PBI	UK
Suzhoe #1-5B-OY	20.3	1	spring	CIMMYT	China
Champtal	20.6	2	winter	SVP	France
PF 859143	20.7	1	spring	EMUFP	Brazil
PF 859140	20.7	1	spring	EMUFP	Brazil
Hu 77 (7)-27	21.1	1	spring	EMUFP	China
PF 85437	21.7	1	spring	EMUFP	Brazil
S 8017	21.7	1	spring	EMUFP	Brazil
PF 859022	22.6	1	spring	EMUFP	Brazil
PF 859105	22.6	1	spring	EMUFP	Brazil
PF 859012	23.7	1	spring	EMUFP	Brazil
PF 859134	23.7	1	spring	EMUFP	Brazil
N. Cebeco 8277	24.0	2	winter	SVP	Netherlands
Shinchunaga	24.1	1	spring	EMUFP	China
Tor.-N.B.	24.1	1	spring	EMUFP	Brazil
Yang May 1	24.7	1	spring	EMUFP	China
SVP 85042	24.8	2	winter	SVP	Netherlands
PF 84433	25.1	1	spring	EMUFP	Brazil
Transylvania	25.1	1	winter	SCAT	Rumenia
PF 859172	25.6	1	spring	EMUFP	Brazil
Mascarenha	25.6	1	spring	EMUFP	Brazil

[p]NIAB = National Institute for Agricultural Botany, Cambridge, UK

cultivar/line	FHB_{index}	years	type	donor	land of origin
Long Lin 112	26.2	1	spring	EMUFP	China
B 7944	26.6	1	spring	EMUFP	Brazil
Fan 415	27.1	1	spring	EMUFP	China
Br 14 (PF 79781)	27.7	1	spring	EMUFP	Brazil
Pel 73101	27.7	1	spring	EMUFP	Brazil
RS 3 Palmeiras	28.1	1	spring	EMUFP	Brazil
Pel 73007	28.5	1	spring	EMUFP	Brazil
RS 2 Sta. Maria	28.5	1	spring	EMUFP	Brazil
Stetson	28.7	1	winter	NIAB	UK
Pel 74099	29.1	1	spring	EMUFP	Brazil
Sprague	29.4	1	winter	WASH[q]	USA
BR 14 (PF 79767)	29.6	1	spring	EMUFP	Brazil
PAT 19	30.0	1	spring	EMUFP	Brazil
SVP 73016-2-4	30.6	4	winter	SVP	Netherlands
CEP 80131	30.6	1	spring	EMUFP	Brazil
SVP 73030-8-1-1	30.8	4	winter	SVP	Netherlands
CNT 8	31.1	1	spring	EMUFP	Brazil
CNT 1	31.1	1	spring	EMUFP	Brazil
Pel 73151	31.1	1	spring	EMUFP	Brazil
CWW 4055/3	31.8	3	winter	PBI	UK
RS 4 Ubiraiara	32.1	1	spring	EMUFP	Brazil
Marathon	32.3	1	winter	SVP	France
IAS 20	32.5	1	spring	EMUFP	Brazil
Aquila	32.7	1	winter	NIAB	UK
Butui	33.0	1	spring	EMUFP	Brazil
CRI 84-42	33.3	2	winter	CRI	Hungary
SB 7923	33.6	1	spring	EMUFP	Brazil
Pel 73081	34.0	1	spring	EMUFP	Brazil
SVP 73012-1-2-3	34.1	4	winter	SVP	Netherlands
PAT 7219	34.5	1	spring	EMUFP	Brazil
Lee	35.0	1	spring	SVP	USA
SVP 75059-32	35.4	4	winter	SVP	Netherlands
PF 859006	35.9	1	spring	EMUFP	Brazil
Kaláka	36.1	1	winter	CRI	Hungary
Cocoragne	37.3	1	spring	ABC	Brazil
PF 859106	37.4	1	spring	EMUFP	Brazil
PF 85512	38.0	1	spring	EMUFP	Brazil
Multiplicacion 14	38.0	1	spring	EMUFP	Uruguay
SVP 75059-46	38.0	4	winter	SVP	Netherlands
CRI 84-32	38.1	2	winter	CRI	Hungary
Creneau	38.2	2	winter	SVP	France
Avalon	38.4	1	winter	PBI	UK
PF 859090	38.4	1	spring	EMUFP	Brazil
CEP 8056	38.4	1	spring	EMUFP	Brazil
CEP 80153	39.2	1	spring	EMUFP	Brazil
BR 15	39.9	1	spring	EMUFP	Brazil
SVP 75059-48	40.2	1	winter	SVP	Netherlands
Oasis	40.4	1	spring	EMUFP	USA

[q]WASH = Washington State University, Department of Plant Pathology, Pullman, USA

cultivar/line	FHB_{index}	years	type	donor	land of origin
Grana	40.7	1	winter	UW	Poland
Herval	41.4	1	spring	EMUFP	Brazil
PF 8090	41.8	1	spring	EMUFP	Brazil
Japan 105	41.8	1	spring	EMUFP	Japan
PF 859028	41.8	1	spring	EMUFP	Brazil
SVP 73009-50-4	41.9	4	winter	SVP	Netherlands
Apexal	42.4	1	winter	SVP	France
Nautica	43.0	4	winter	SVP	Netherlands
Ralle	44.4	1	spring	SVP	Germany
RH 18	44.4	1	spring	EMUFP	Brazil
SVP 72005-20-3-1	44.4	4	winter	SVP	Netherlands
Van May 15	44.8	1	spring	EMUFP	China
CNT 7	44.8	1	spring	EMUFP	Brazil
PF 859112	45.4	1	spring	EMUFP	Brazil
BR 8	45.8	1	spring	EMUFP	Brazil
Gk Szemes	48.8	2	winter	CRI	Hungary
Decibel	49.1	1	winter	SVP	France
PAT 7392	49.2	1	spring	EMUFP	Brazil
Miller	49.7	1	winter	SVP	Netherlands
BR 14	50.7	1	spring	EMUFP	Brazil
PF 8049	51.8	1	spring	EMUFP	Brazil
RH 54	51.8	1	spring	EMUFP	Brazil
PF 859031	51.8	1	spring	EMUFP	Brazil
Anahuac	52.5	1	spring	ABC	Brazil
CRI 79-1#57	54.8	2	winter	CRI	Hungary
CRI Nobeoka Bozu-Lili (88/798/4)	55.2	1	winter	CRI	Hungary
SVP 77071-2-6	55.9	4	winter	SVP	Netherlands
PF 859029	56.2	1	spring	EMUFP	Brazil
PF 80271	58.7	1	spring	EMUFP	Brazil
BR 14 (PF 79780)	59.1	1	spring	EMUFP	Brazil
Csürös	65.0	1	winter	CRI	Hungary
CRI 85-84	65.1	2	winter	CRI	Hungary
B 7502	66.5	1	spring	EMUFP	Brazil
CRI 80-1/70	69.3	2	winter	CRI	Hungary
CRI 81-69	75.1	2	winter	CRI	Hungary
CRI 80-1/61	102.3	2	winter	CRI	Hungary
Balkan	106.4	2	winter	SVP	Bulgaria
But	127.7	1	winter	SVP	France

Results

Head blight

In Figure 8.1 the distributions of frequencies are given with which genotypes fell into classes of Fusarium head blight, each covering a range of 5% on a scale of 0-100%, for the four consecutive years. The distribution of the standards shows that the disease pressure was higher in 1986 and 1987 than in 1988 and 1989. The mean Fusarium head blight ratings for the 17 standards were 28.0%, 30.8%, 14.1% and 10.6% for 1986, 1987, 1988 and 1989, respectively, with an overall mean over the four years of 20.9%. Therefore, to compare the resistance levels of the 258 genotypes tested, a Fusarium head blight (FHB) index was calculated for each genotype, based on a weighted mean over the four years:

$$FHB_{index} = \frac{0.75 \times FHB_{1986} + 0.68 \times FHB_{1987} + 1.48 \times FHB_{1988} + 1.97 \times FHB_{1989}}{\text{number of years the genotype was tested}}$$

In Table 8.2 the FHB_{index}, the number of years the genotype was tested, the type of material, the donor and the land of origin of the genotype are presented for 258 genotypes. 'Nobeokabozu' was supplied by three donors and is presented in Table 8.2 as 'Nobeokabozu precoce' and two times as 'Nobeokabozu komugi'. The observed differences in resistance, and culm length and earliness (not shown) indicated that the three 'Nobeokabozu' cultivars are different selections from the original 'Nobeokabozu komugi'.

Yield reduction

As cross-infection was prevented by the lay-out of the experiments and border plots, there was no Fusarium head blight in the control plots. Figure 8.2 shows the relationship between the Fusarium head blight ratings and yield reduction for the field trial of 1987. A regression of yield reduction (y) on Fusarium head blight (x) was estimated by $y = 6\sqrt{x}$, with a coefficient of determination (R^2) of 49%. In Figure 8.3, the Fusarium head blight ratings are plotted against yield reduction for the field trial of 1989. A regression of yield reduction (y) on Fusarium head blight (x) was estimated by $y = 7.2\sqrt{x}$, with an R^2 of 63%.

In Table 8.3 the correlations between percentage infected heads, proportion infected spikelets, Fusarium head blight, kernel weight reduction, kernel number reduction and yield reduction are shown for 1987 and 1989, respectively. The variance components and heritabilities of these traits are shown in Table 8.4.

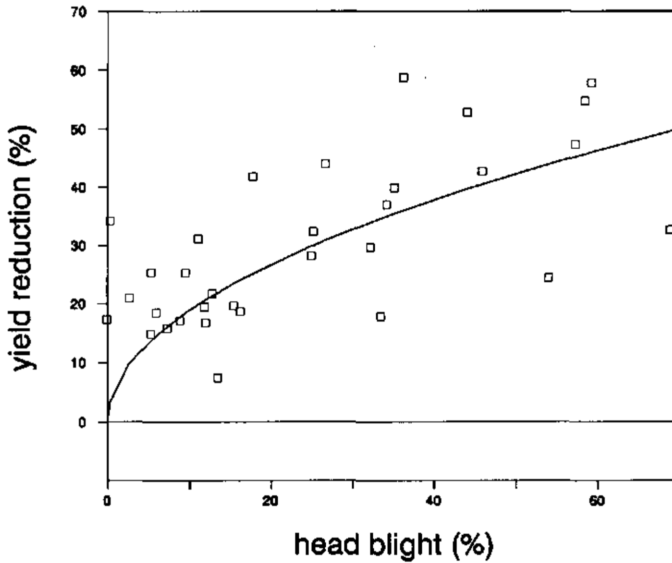


Figure 8.2. The relation between Fusarium head blight and yield reduction in a field trial in 1987, after inoculation by *Fusarium culmorum* strain IPO 39-01. The regression of yield reduction (y) on Fusarium head blight (x) is estimated by $y = 6\sqrt{x}$.

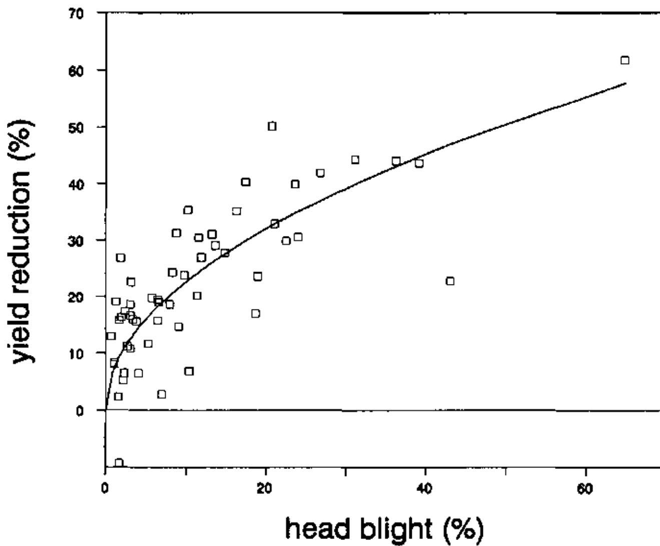


Figure 8.3. The relation between Fusarium head blight and yield reduction in a field trial in 1989, after inoculation by *Fusarium culmorum* strain IPO 39-01. The regression of yield reduction (y) on Fusarium head blight (x) is estimated by $y = 7.2\sqrt{x}$.

Discussion

Since the *Fusarium culmorum* strain, starting inoculum, inoculum preparation and inoculation method were the same for the four years of testing, the significant year effect (Figure 8.1) must be mainly a result of weather. This is supported by the contrast in the data of time of inoculation/anthesis. In 1986 and 1987 anthesis was late and the disease pressure was high; in 1988 and 1989 anthesis was early and the disease pressure was low (Table 8.1). In addition to the 17 standard winter wheats tested each year, new entries were added from 1987 onwards, which either had been reported to be resistant in literature, or were newly released commercial cultivars. New entries which showed Fusarium head blight susceptibility in the field trial were not included in the trial of the succeeding year. This explains the skewness of the distributions in Figure 8.1 towards resistance. Several genotypes showed high resistance to Fusarium head blight, but none were immune. From the distributions of frequency of resistance of genotypes in Figure 8.1, the FHB_{index} for all genotypes in Table 8.2, and the heritabilities given in Table 8.4, it is concluded that the genetic variation for resistance to head blight caused by *F. culmorum* is very large.

Particularly the spring wheats contributed to the high frequency of genotypes in the resistant classes (0-5% and 5-10% head blight). Spring wheat cultivars like 'Frontana', 'Ning 8343', 'Nanjing 7840' and 'Nobeokabozu', which had been reported to be resistant to head blight caused by *F. graminearum* (Mesterhazy, 1987; Schroeder and Christensen, 1963; Wang and Miller, 1987), also showed high resistance levels against

Table 8.3. Correlations between percentage infected heads, proportion infected spikelets, Fusarium head blight[a], kernel weight reduction[b], kernel number reduction and yield reduction, after inoculation by *Fusarium culmorum* strain IPO 39-01, based on 32 genotypes inoculated in 1987, and 54 genotypes inoculated in 1989. All correlation coefficients are significant at P=0.01

	year	% infected heads	proportion inf. spikelets	Fusarium head blight	yield reduction
kernel weight reduction	1987	0.54	0.65	0.66	0.89
	1989	0.86	0.69	0.82	0.90
kernel number reduction	1987	0.49	0.66	0.64	0.91
	1989	0.52	0.48	0.57	0.87
yield reduction	1987	0.57	0.72	0.72	....
	1989	0.78	0.64	0.77	....
proportion infected spikelets	1987	0.78	....	....	....
	1989	0.73	....	....	....

[a] Head blight ratings were assessed as a product of the percentage of heads infected and the proportion infected spikelets per infected head.

[b] Reductions of kernel weight, kernel number and yield were determined per block as the difference between inoculated and non-inoculated plot, expressed as a percentage of the non-inoculated control plot.

F. culmorum. For eight winter wheat cultivars and one spring wheat cultivar tested by Mielke (1988) which were also tested in the present study, lack of resistance was confirmed. The resistant germplasm is divided into three gene pools: resistant winter wheat genotypes from Eastern Europe, resistant spring wheat genotypes from China/Japan and resistant spring wheat genotypes from Brazil. In the pedigrees of the Fusarium head blight resistant SVP-breeding lines resistant germplasm from the East European gene pool was found.

In all field trials, head blight ratings were assessed as the product of the percentage of infected heads and the proportion of infected spikelets per infected head. Correlations between these two Fusarium head blight components were 0.78 and 0.73, for 1987 and 1989, respectively (Table 8.3). Both components showed significant correlations with yield reduction, and heritabilities were similar in both years (Table 8.4). These findings and multiple regression analysis (not shown) indicate that both the percentage infected heads and the proportion infected spikelets contributed to the yield reduction. Assessment of only the percentage infected heads, which is more practical and less laborious, would be a less accurate estimate of the resistance level than the product of the percentage infected heads and the proportion infected spikelets.

The estimated regressions of yield reduction on head blight for the 1987 and 1989 experiment were very similar. These regressions indicate that in the range from 0 to 5% head blight, the increment of yield reduction is very high. An epidemic with a Fusarium head blight rating of only 1% would lead to a yield reduction between 6

Table 8.4. Estimated variance components σ_g^2[a] and σ_e^2[b], and heritability of percentage infected heads, proportion infected spikelets, Fusarium head blight[c], kernel weight reduction[d], kernel number reduction and yield reduction after inoculation by *Fusarium culmorum* strain IPO 39-01, based on 32 genotypes inoculated in 1987, and 54 genotypes inoculated in 1989

	1987			1989		
	σ_g^2	σ_e^2	h^2	σ_g^2	σ_e^2	h^2
% infected heads	433.7	346.0	0.79	542.0	189.2	0.94
proportion infected spikelets	322.7	210.8	0.82	87.0	86.4	0.86
Fusarium head blight	364.8	191.5	0.85	151.8	50.0	0.95
kernel weight reduction	55.1	72.8	0.66	77.7	21.6	0.96
kernel number reduction	34.5	227.4	0.31	53.2	157.2	0.65
yield reduction	120.8	225.2	0.60	162.0	127.8	0.88

[a] Variance component due to genotypes.
[b] Variance component due to error.
[c] Head blight values were assessed as a product of the percentage of heads infected and the proportion infected spikelets per infected head.
[d] Reductions of kernel weight, kernel number and yield were determined per block as the difference between inoculated and non-inoculated plot, expressed as a percentage of the non-inoculated control plot.

and 7%. During the years 1979-1986, data on natural Fusarium head blight epidemics in wheat were collected in the Netherlands (Snijders, 1990a). Although the *Fusarium* epidemics caused maximal only 4.6% head blight and averaged 1.7% over the years, the regression estimates in the present study indicate that yield reduction might have been considerable.

The correlations in Table 8.3 show that Fusarium head blight has an effect on both kernel weight reduction and kernel number reduction, and both kernel weight and kernel number reduction influenced yield reduction. However, the variance components in Table 8.4 show that within each trial the genetic variation is higher for kernel weight reduction than for kernel number reduction.

The increase in number of blocks from three in field trial 1987 to six in field trial 1989 had its largest effects on the accuracy of the estimation of yield reduction and yield reduction components (Table 8.4). In both experiments Fusarium head blight showed a high heritability. With only three blocks the heritability for yield reduction is important smaller then for Fusarium head blight. Although the correlation between Fusarium head blight and yield reduction is higher than 0.70, visually assessed Fusarium head blight is a more reliable criterion for evaluation of resistance than yield reduction, the latter used for selection by Mesterhazy (1988).

The present study demonstrates that there is merit in searching for new sources of resistance to Fusarium head blight in winter and spring wheat. To prevent yield reduction and mycotoxin contamination of the crop (Snijders, 1990a; Snijders and Perkowski, 1990), cultivars are needed with a resistance level that is complete. As major genes coding for complete resistance have not been found until now, this can only be achieved by accumulation of resistance genes with small effects. Snijders (1990b) proved the possibility of accumulation of resistance genes in winter wheat. Therefore, the strategy in breeding for Fusarium head blight resistance should aim to accumulate resistance genes from spring and winter wheats in order to develop wheat cultivars with levels of resistance approaching immunity. Crosses between resistant genotypes from different gene pools will have to prove whether different resistance genes are involved in the three gene pools.

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Chapter 9

GENERAL DISCUSSION

Damage caused by Fusarium head blight

Snijders (1990a; 1990f) and Snijders and Perkowski (1990) suggest that damage, quantitative in the form of yield reduction and qualitative in the form of toxin contamination, is considerable with only slight *Fusarium culmorum* head blight infection. Further publications on epidemiological data of Fusarium head blight epidemics in relation to damage do not exist for north-western European countries. More information exists of Fusarium head blight epidemics in developing countries. Outbreaks of head blight have occurred on average every two years for the last 30 years in the Yangtze Valley of China (Anonymous, 1988; 1989). Up to 2.5 million tonnes of grain have been lost in years of heavy infection and the yield may be depressed 30-40% in some fields. The disease is also serious in parts of India and Pakistan, and in the southern hemisphere countries of South America. A major epidemic, in conjunction with other diseases, reduced Argentina's 1985 wheat crop by some 30%. Fusarium head blight is also important in many of the Andean countries, in Ethiopia, in Zambia and in parts of southern Africa. Large-scale human toxicoses (>50,000 people) have been reported in India and China (Anonymous, 1988; 1989; Bhat *et al.*, 1989). A major outbreak of this disease in eastern Canada during the humid summer of 1980 caused important economic losses in the cereal and livestock industries (Anonymous, 1989; Trenholm *et al.*, 1983). The irregular pattern of Fusarium epidemics in the Netherlands does not stimulate to pay much attention to Fusarium head blight and resistance to this disease. The present commercial cultivars registered in the Netherlands, UK and France do not contain any resistance (this thesis, chapter 8). This thesis has demonstrated that in the Netherlands a year with a Fusarium epidemic will cause an important loss of the wheat crop, in yield and quality.

The role of DON in pathogenesis

Deoxynivalenol (DON) is a pathogen-produced, non-hostspecific toxin. It affects plants in general (Snijders and Perkowski, 1990) and animals as well (Snijders, 1990a). The mycotoxins of the trichothecene class, including DON, are true secondary metabolites, which means that they are produced upon a specific nutrient limitation, in this case N (Miller and Blackwell, 1986). Till now the role of DON in pathogenesis is not decided. Commonly used criteria to evaluate toxin as a factor in pathogenesis were reported and criticized by Yoder (1980), and are here evaluated in relation to DON

produced by *Fusarium culmorum* and *F. graminearum*. The first thing to notice is that the toxin is non-specific and has a high molecular weight (M=296), both facts that are in contrast with those pathologically important toxins documented by Yoder (1980; 1981). A commonly used criterion to evaluate the involvement of toxin in pathogenesis is the isolation of toxin from diseased plants. Snijders and Perkowski (1990) found DON in infected plant tissue of wheat. However, it does not indicate that toxin is causing the disease; it may as well result from it. Therefore the kinetics of toxin production during disease development should be studied. A sample of 25×10^6 conidiospores of *Fusarium culmorum* strain IPO 39-01 contained no detectable DON (Snijders, unpublished results). Whether germinating conidia release DON to the leaf surface prior to penetration has so far not been studied. This means that there is no evidence that toxin action occurs during the penetration process. More indications exist on the involvement of the toxin in the colonization in plant tissue. "The invasion of corn kernels by *F. graminearum* viewed by histology demonstrated that the corn cells were killed in advance of invading mycelium (D. Wicklow, pers.com.)" (Miller and Greenhalg, 1988). There could be a role in this for DON, as DON is produced in the cells immediately adjacent to the hyphal tip (Miller and Greenhalg, 1988). DON causes physiological changes in infected tissue. Trichothecenes have been reported to increase electrolyte loss (Miller and Greenhalg, 1988). Cassale and Hart (1988) showed that low levels of DON inhibited protein synthesis in plants, since DON blocks various parts of the peptidyl transferase reaction (Miller, 1989). If the defense mechanism of the host relating to hyphal invasion after penetration requires protein synthesis (e.g. for the production of enzymes), and the cells around the place of infection are lacking this because of presence of DON, further colonization will not be inhibited. Also the reported reduction of root and coleoptile growth of seedlings exposed to a DON-containing medium (Bottalico *et al.*, 1980; Snijders, 1988a), the growth reduction of coleoptile segments when exposed to DON (Wang and Miller, 1988; Snijders, unpublished results) and the suggested inhibitory effect of DON on kernel filling (Snijders and Perkowski, 1990) are possibly due to the effects of DON on protein synthesis.

Another criterion to assign the toxin to be a factor in pathogenesis is the correlation of toxin biosynthetic rate with pathogenicity and aggressiveness, where pathogenicity is defined as the ability to cause disease, while aggressiveness means the amount of disease caused. Quantitative correlations between quantity of toxin produced *in vitro* and aggressiveness are not easily interpreted because toxin production *in vitro* is influenced by the composition of the medium and by the physical environment (Miller and Greenhalg, 1985; Yoder, 1980). The quantity of toxin produced *in vivo* cannot easily be interpreted in terms of cause and effect: does a highly aggressive isolate cause more disease because it produces more toxin, or does it produce more toxin because it causes more disease (Yoder, 1981). However, qualitative correlations between toxin production and pathogenicity or aggressiveness can provide a very persuasive line of evidence (Yoder, 1980). In Snijders and Perkowski (1990) for three out of eight *F. culmorum* strains studied intensively, ranks for pathogenicity, *in vivo* DON production and *in vitro* DON production were the

same, in descending order IPO 39-01, IPO 436-01 and IPO 348-01. Not reported was that the five remaining strains showed pathogenicity in the order of IPO 348-01, and that their *in vitro* DON production was below the detection limit, similar to IPO 348-01. Apparently DON has no relation with pathogenicity, the ability to cause disease, but it does have with aggressiveness, the amount of disease caused.

Although the above criteria in combination have increased the number of indications for a causal role for a toxin in disease, the evidence is still far from persuasive. According to Yoder (1980) the most powerful and convincing technique to test whether toxins are involved in disease development is the use of genetic analysis, which includes the manipulation of genetic variation in both the pathogen and the host: segregation ratios in progenies from intraspecific crosses of the pathogen are analyzed for pathogenicity and aggressiveness to both resistant and susceptible host genotypes, and for ability to produce toxin. Based on genetic variation for *Fusarium graminearum*, Adams and Hart (1989) concluded that DON and 15-ADON were not pathogenicity or aggressiveness factors on maize. However, only susceptible corn cultivars were used for this study, so interaction of the pathogen strains with host genotypes with different levels of resistance was not studied. Therefore this study fails in this respect. Several other studies focussed on the host. Resistant wheat cultivars inoculated with *F. graminearum* gave much higher fungal biomass to DON ratios than susceptible cultivars, suggesting that resistant cultivars had factors that prevented synthesis or promoted degradation of DON (Miller *et al.*, 1985). Snijders and Perkowski (1990) observed that for 10 wheat genotypes the Fusarium head blight rating to DON ratio varied by a factor of 10, which was not correlated with Fusarium head blight resistance. Besides the resistance mechanisms which determined the head blight severity, a second type of mechanism was suggested which influenced kernel DON content. There seem to be at least two responses to the toxin: (1) degradation or conjugation of DON. It was possible to demonstrate *in vitro* the conversion of DON by a resistant cultivar (Miller and Arnison, 1986); (2) Tolerance to DON. Using a coleoptile tissue assay, cultivars known to be resistant to Fusarium head blight were more tolerant to DON compared to susceptible cultivars (Wang and Miller, 1988). This might be based on a modification of the peptidyl transferase, rendering it insensitive to DON inhibition (Miller, 1989).

The fact that (*i*) active (induced) resistance mechanisms are inhibited in infected plant tissues in the presence of DON, and (*ii*) the strong suggestion that there are resistance mechanisms based on prevention of interaction with DON by degradation of the toxin or alteration of the receptor site, indicates that there is a role of DON in pathogenesis, particularly in colonization in penetrated plant tissue.

The DON produced with head blight can also have physiological effects in other parts of the plant. DON is water soluble and distribution through the plant is conceivable. DON was found in tisues of the infected corn plant which were not invaded by *F. graminearum* (Miller *et al.* 1983; Young and Miller, 1985). As *F. culmorum* can infect wheat also in the seedling stage, booting stage or shooting stage (Snijders, 1990b),

DON production too would start during these growth stages and will have its physiological effects in these plant stages.

If DON plays a role in pathogenesis/aggressiveness, it is possible that one of the resistance mechanisms is directed at neutralizing this DON: plants tolerant to DON should have a higher level of resistance to Fusarium head blight than those sensitive to DON. The practical significance of pathologically important toxins is that they can act as reliable surrogates for the pathogens that produce them. If DON is causally involved in disease, DON could facilitate screening for resistance among populations of whole plants (Snijders, 1988b), population of cells, population of pollen, and protoplasts. Selection for resistance mechanisms preventing DON-effects could select for a resistance component which prevents or retards extension of colonization within plant tissue (component II).

Resistance

The variation for resistance to Fusarium head blight appeared to be very large (chapter 8). Reactions of different genotypes ranged from 100% affected field plots with completely white heads to only a dozen infected spikelets in 2.00 × 0.75 m field plots. The resistance is inherited quantitatively (Snijders, 1990d). However, the estimated number of resistance genes in the studied winter wheats was small (≤ 6 in the total population), the individual genes had large effects on resistance and inherited mainly additive. The response to selection in F_2 generations showed that it was possible to select plants on a single plant base. These findings illustrate that there is a large potential for breeders to develop cultivars with a high level of resistance to Fusarium head blight. There is an analogy with quantitative resistance in other host-pathogen relationships. The quantitative resistance to *Septoria tritici* in spring and winter wheat cultivars was controlled by a small number of genes (usually 2), with mainly additive effects (Danon and Eyal, 1990). The partial resistance to wheat leaf rust (*Puccini recondita* f.sp. *tritici*) in several spring wheat genotypes was based on only a few genes (≤ 3), some of the genes with relatively large effects (Broers and Jacobs, 1989; Jacobs and Broers, 1989). A study of the quantitative resistance to bacterial blight (*Xanthomonas campestris* pv. *oryzea*) in rice indicated the presence of at least five resistance genes, each with a small effect. A moderate level of resistance was achieved when at least two resistance genes were present (Koch, 1990). The results of the aforementioned studies of quantitative resistance indicate that the 'polygenic' resistance is in fact an oligogenic resistance. Transmission of quantitative resistance from unadapted germplasm to high yielding cultivars should therefore be possible with relative small effort.

In this study no indications were found for *Fusarium* strain-specific resistance. However, only strains from the Netherlands were used. An evaluation of the standard set used in this study for resistance to strains from other countries will have to prove

if interactions exist between wheat genotypes and strains from geographically separated populations.

Components of resistance

In Schroeder and Christensen (1963), Snijders (1990b, 1990c) and Snijders and Perkowski (1990), resistance to Fusarium head blight was described in two components: I resistance to initial penetration, and II, resistance to spread of the pathogen in host tissue. In Schroeder and Christensen (1963) component II was studied by single-spikelet inoculation, "in which a drop of spore suspension was introduced in a spikelet with a hypodermic syringe, thus penetrating the glumes". This method was not appropriate for the large number of plants in the studies of this thesis. In Snijders (1990c) component II was studied by analyzing the area under the disease progress curve for each infected head. However, the conclusions were similar to those of analysis of Fusarium head blight ratings determined at the right time (Snijders, 1990c). The Fusarium head blight rating is in fact the resultant of component I and II. This would mean that either secondary infections had masked component II, or there was no genetic variation of component II. Yet, in Snijders (1990b) it was demonstrated that there was genetic variation for systemic fungal growth in wheat stems, which belongs to component II resistance.

With respect to epidemiology two components of resistance were studied: (*i*) incubation period (IP), the period from inoculation to the appearance of first symptoms, and (*ii*) latent period (LP), the period from inoculation to sporulation. In Snijders and Perkowski (1990) is was demonstrated that there was a significant negative correlation between head blight and incubation period: the longer the incubation period, the more resistant the genotype. This means that IP can be used for indirect selection for Fusarium head blight resistance. In fact IP also belongs to component II, as the time needed to produce symptoms is related with the rate of colonization by the pathogen of host tissue. Under the field conditions of 1986, the latent period was at least 11 days (Snijders, 1990c). As in many combinations of resistant genotypes and strains of low pathogenicity sporulation was not visible, datasets for LP were incomplete. In addition to the high interaction of LP with relative humidity, it was decided that LP could not be analyzed (Snijders, unpublished). IP, which covers the first period of LP, should therefore be used as an indication for LP. With respect to head blight, secondary infections usually do not play an important role. By the time the sporulation of infected heads takes place, the susceptible stage of the head is far behind and important yield reductions due to these secondary infections will not occur. However, secondary infections still can lead to toxin contamination and seed infection. Also regarding the Fusarium infections in seedling and shooting stages, a long LP, and thus IP, can reduce the build-up of an epidemic which finally might lead to Fusarium head blight.

In addition to the components of resistance to Fusarium head blight mentioned

above, a component of resistance based on resistance to DON might help to prevent a high level of Fusarium head blight infection. How many, if any, of the resistance genes in the studied winter material (Snijders, 1990d; 1990e) or exotic wheat material (Snijders, 1990f) are based on this type of resistance mechanism is not known.

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SUMMARY

In the Netherlands, Fusarium head blight in wheat (*Triticum aestivum*) is predominantly caused by *Fusarium culmorum*. Inoculum is seed-borne, soil-borne, and is produced on infected debris of cereals and maize, weeds and certain grasses. Besides head blight, *F. culmorum* causes seedling blight, and root, crown and foot rot. The first symptoms of Fusarium head blight consist of light brown, watersoaked spots on the glumes. Soon, infected spikelets lose the water-soaked appearance, dry up, and assume the colour of the ripe heads. There are no practical chemical treatments that are effective in preventing this disease. The only and best way to eliminate this problem is to introduce adapted cultivars that are resistant to Fusarium head blight. The purpose of this thesis was to investigate the host-pathogen relationship between winter wheat and *Fusarium culmorum*, to identify sources of resistance, to evaluate the nature and genetic aspects of resistance, and to study the relation between disease incidence and damage.

Mycotoxins

Various *Fusarium* species including *F. culmorum* produce mycotoxins. These toxins have a range of chronic and acute effects on humans and animals that consume infected grain. Ten winter wheat genotypes were inoculated with three strains of *Fusarium culmorum*. The mycotoxin deoxynivalenol (DON) was detected in seed samples in concentrations ranging from 0 to 48 mg kg^{-1}. The mycotoxins 3-acetyldeoxynivalenol, nivalenol, fusarenon-X and zearalenone were not detected. The three strains ranked the same for pathogenicity, DON production and *in vitro* DON production. For each strain a high correlation was found between DON content and yield reduction. Infection by the highly pathogenic strain IPO 39-01 reduced yield mainly in terms of kernel number reduction. In the case of two moderately pathogenic strains (IPO 348-01 and IPO 436-01) yield loss was ascribed to lower kernel weight. A phytotoxic effect of DON on kernel filling was suggested.

Natural *Fusarium* epidemics and mycotoxin contamination

In the years 1979-1986, Fusarium head blight epidemics of wheat in the Netherlands averaged 1.7% infection of all spikelets, with a maximum of 4.6% in 1982. Epidemics were influenced by the level of epidemic of the preceding year and the precipitation in the period around anthesis. Based on a linear regression of DON contamination (mg kg^{-1}) on head blight (%) with a regression coefficient of 0.54 and constant equal to 0, DON contamination of the grain was estimated. Estimates for DON contamination averaged 0.9 mg kg^{-1}. Taking a guideline level for DON of 2 mg kg^{-1}, the wheat produced in 1979 and 1982 had estimated DON concentrations above the

limit of tolerance. Human and animal exposure to mycotoxins in the Netherlands appears to be small but chronic.

Nature of Fusarium head blight resistance

Fusarium head blight values were assessed as the product of the 'percentage infected heads' and the 'proportion of infected spikelets' per infected head. The percentage yield reduction depended on the square root of percentage head blight with an average regression coefficient of 6.6. Heritability estimates indicated that for selection for Fusarium head blight resistance, visually assessed Fusarium head blight was a better selection criterion than yield reduction.

Resistance of Fusarium head blight can be distinguished into two components: I, resistance to initial penetration, and II, resistance to spread of the pathogen in host-tissue (colonization). Component II was studied separately in three ways: (*i*) the area under the disease progress curve (AUDPC) for each infected head. Conclusions from analysis of AUDPC data of parental lines, F_1 and F_2 generations of a 10×10 diallel were similar to those for a single observation of Fusarium head blight at the right time; (*ii*) systemic fungal growth in wheat stems. Existence of genetic variation for resistance to spread of *F. culmorum* in stems was demonstrated among 15 genotypes. This resistance was not correlated with resistance to Fusarium head blight; (*iii*) the incubation period (IP), the period from inoculation to the appearance of first symptoms. Genetic variation for IP was observed among ten studied winter wheat genotypes. IP was negatively correlated with Fusarium head blight.

Besides components I and II, which determine the head blight severity, there may be a second type of mechanism which influences kernel DON content. With respect to a suggested phytotoxic action of DON on kernel filling, this type of mechanism might be important for breeding purposes.

Based on a study of 17 winter wheats inoculated by four strains in three consecutive years, the Fusarium head blight resistance was described as horizontal resistance in terms of Vanderplank, with the exception of three genotypes selected from one particular cross which showed a 'strain-year combination' dependent resistance which was ineffective in one year.

Genetic aspects of Fusarium head blight resistance

The inheritance of resistance to *Fusarium culmorum* was studied based on F_1 and F_2 generations of crosses among 10 winter wheat genotypes representing different levels of resistance. A major role for general combining ability effects was indicated. The level of head blight resistance of the parental line appeared to be a good indicator of the potential resistance of its crosses. The additive-dominance model adequately described the inheritance of Fusarium head blight resistance, with additive gene action being the most important factor of resistance. With respect to non-additive effects, dominance of resistance predominated over recessiveness. The

number of independently segregating resistance genes in the studied populations was estimated to be at least six. The Fusarium head blight resistance genes differed between parents and affected resistance differently. The most resistant parents differed in one or two resistance genes. Transgression for resistance of selected F_3 lines proved the possibility of accumulation of resistance.

Sources of Fusarium head blight resistance

Based on evaluation of Fusarium head blight resistance of 258 winter and spring wheats after experimental inoculation with *F. culmorum* strain IPO 39-01 in one to four years, it was concluded that genetic variation for resistance was very large. Resistant spring wheats which had been reported to be resistant to Fusarium head blight caused by *Fusarium graminearum* were also resistant to *F. culmorum*. The resistant germplasm could be divided into three gene pools: winter wheat genotypes from Eastern Europe, spring wheat genotypes from China/Japan and spring wheat genotypes from Brazil.

SAMENVATTING

Aaraantasting door *Fusarium* spp. in tarwe (*Triticum aestivum*) wordt in Nederland voornamelijk veroorzaakt door *Fusarium culmorum*. Infectie vindt plaats vanuit het zaad, de bodem en door inoculum geproduceerd op resten van granen, maïs, en op akkeronkruiden en grassen. Behalve aarziekte veroorzaakt *Fusarium culmorum* ook aantastingen van kiem en kiemplant, voetziekte en stengelaantasting. De eerste symptomen van *Fusarium* aaraantasting bestaan uit scherp begrensde vlekken op de kafjes. Dit wordt gevolgd door het verbleken van hele pakjes, afzonderlijk of in groepjes gelijktijdig, terwijl de rest van de aar nog groen is. Op de aangetaste delen van de aar kan zich schimmelpluis ontwikkelen, bij vochtig weer vergezeld van sporodochia met roze-oranje conidiosporen. Hieraan is de naam (oranje of) rode kafschimmel ontleend. Er bestaan geen chemische bestrijdingsmiddelen die deze ziekte effectief kunnen bestrijden. De enige manier om deze ziekte in tarwe te voorkomen is door de teelt van resistente rassen. Het in dit proefschrift beschreven onderzoek had als doel (*i*) de waardplant-pathogeen relatie voor wintertarwe en *Fusarium culmorum*, (*ii*) bronnen van resistentie, (*iii*) componenten van de resistentie, (*iv*) de genetische basis van de resistentie en (*v*) de aantasting-schade relatie te bestuderen

Mycotoxinen

Veel *Fusarium* soorten, waaronder *F. culmorum*, produceren mycotoxinen. Consumptie door mens of dier van met mycotoxinen besmet graan kan leiden tot verschillende chronische en acuut toxische effecten. In een experiment werden tien wintertarwe genotypen geïnoculeerd met drie stammen van *Fusarium culmorum*. In het zaad werd het mycotoxine deoxynivalenol (DON) aangetoond in concentraties tot 48 mg/kg. De toxinen 3-acetyldeoxynivalenol, nivalenol, fusarenon-X en zearalenon werden niet aangetoond. De rangordes van de drie *F. culmorum*-stammen voor pathogeniteit, DON-produktie en *in vitro* DON-produktie waren identiek. Voor elke stam werd een hoge correlatie gevonden tussen DON-gehalte in het zaad en opbrengstreductie. Infectie met de zeer pathogene stam IPO 39-01 leidde tot een opbrengstreductie voornamelijk veroorzaakt door een verminderde zaadzetting. Twee middelmatig pathogene stammen (IPO 348-01 en IPO 436-01) veroorzaakte een lagere opbrengst door een verminderd korrelgewicht. De resultaten suggereren een mogelijk fytotoxisch effect van DON op korrelvulling.

Fusarium epidemieën en natuurlijke toxinen in graan

Fusarium epidemieën in Nederland in de jaren 1979-1986 hebben geleid tot een aantasting van pakjes van tarwearen van gemiddeld 1.7%, met een maximum van 4.6% in 1982. De ontwikkeling van de epidemieën werd beïnvloed door de ernst van

de epidemie van het voorgaande jaar en de regenval rond de datum van bloei. Uitgaande van een lineaire regressie van DON besmetting (mg kg^{-1}) op *Fusarium* aaraantasting (%) met een regressiecoefficiënt van 0.54 en een constante gelijk aan 0, werd een schatting gegeven van de concentratie DON in Nederlandse tarwe. De schattingen voor de jaren 1979-1986 bedroegen gemiddeld 0.9 mg DON per kg graan. Rekening houdend met de herkomst en verwerking van tarwe blijken in Nederland zowel in dierlijk als menselijk voedsel lage concentraties DON chronisch voor te komen. Op basis van een maximaal toelaatbare dagelijkse dosis DON van 3 $\mu g\ kg^{-1}$ lichaamsgewicht werd de dagelijkse opname van DON in het jaar volgend op de oogst van 1982 geschat op nauwelijks toelaatbaar.

Resistentie tegen *Fusarium* aaraantasting

Fusarium aaraantastingen werden geschat door het product van het percentage aangetaste aren en het aandeel aangetaste pakjes van de aangetaste aren. Het verband tussen opbrengstreductie (y) en *Fusarium* aaraantasting (x), beiden uitgedrukt in percentage, kan worden weergegeven door de regressievergelijking $y=6.6\sqrt{x}$. Alhoewel aaraantasting en opbrengstreductie hoog gecorreleerd waren, suggereerde de erfelijkheidsgraad h^2 dat aaraantasting een beter criterium is voor selectie op resistentie.

Resistentie tegen *Fusarium* aaraantasting kan worden beschreven in twee componenten: I resistentie tegen penetratie, en II resistentie tegen kolonisatie. Component II werd op drie wijzen bestudeerd: (*i*) de oppervlakte onder de curve van het aantastingsverloop (AUDPC) van individuele aren. De conclusies uit een analyse van ouders, F_1 en F_2 van een 10×10 halve diallel voor AUDPC waren identiek aan die van analyse van de aaraantasting waargenomen op één (differentiërend) tijdstip; (*ii*) systemische schimmelgroei in de stengel. In twee experimenten met in totaal 15 genotypen werd genetische variatie voor resistentie tegen systemische schimmelgroei aangetoond. Deze resistentie was echter niet gecorreleerd met resistentie tegen aaraantasting; (*iii*) de incubatieperiode (IP), de periode van inoculatie tot het verschijnen van de eerste symptomen. In een collectie van 10 wintertarwe genotypen werd genetische variatie aangetoond. IP was negatief gecorreleerd met de mate van *Fusarium* aaraantasting.

Behalve component I en II die tezamen de mate van aaraantasting bepalen, bestaat er waarschijnlijk ook nog een tweede type mechanisme dat de hoeveelheid DON in het zaad beïnvloed. Gezien het mogelijke fytotoxische effect van dit toxine op korrelvulling, kan dit type mechanisme van belang zijn voor resistentieveredeling.

Op basis van een studie van 17 wintertarwe genotypen en kunstmatige inoculatie met vier stammen van *Fusarium culmorum* in drie opeenvolgende jaren kon worden geconcludeerd dat de resistentie tegen *Fusarium* aaraantasting horizontaal is volgens het Vanderplank concept. Een uitzondering vormden drie zusterlijnen waarvan de resistentie in één jaar niet werkzaam was.

De genetische basis van de resistentie

De overerving van de resistentie tegen *Fusarium* aaraantasting werd bestudeerd aan F_1 en F_2 generaties van een halve diallel van 10 ouders met verschillend resistentieniveau. Het resistentieniveau van de ouder kon worden beschreven in de termen van algemene combinatiegeschiktheid en bleek aldus een goede indicator voor het resistentieniveau in de nakomelingschappen. De overerving van de resistentie bleek te kunnen worden beschreven met het additief-dominantiemodel, waarbij additieve geneffecten domineerden. Bij de niet-additieve geneffecten bleek dominantie van de resistentie vaker voor te komen dan recessiviteit. Het geschatte aantal onafhankelijk uitsplitsende resistentiegenen in de onderzochte populatie was minstens zes. De resistentiegenen in de ouders bleken verschillend te zijn en de resistentie in verschillende mate te beïnvloeden. De twee meest resistente genotypen bleken in één à twee genen te verschillen voor *Fusarium* aarziekte resistentie. Transgressie van geselecteerde F_3 lijnen toonde de mogelijkheid van stapeling van resistentiegenen aan.

Bronnen van resistentie tegen *Fusarium* aaraantasting

In vier jaar tijd werden in totaal 258 winter- en zomertarwe genotypen getoetst op resistentie na inoculatie met *F. culmorum* stam IPO 39-01. De genetische variatie voor resistentie was zeer groot. Genotypen met een bekende resistentie tegen *Fusarium gramineareum* bleken ook resistent tegen *F. culmorum* te zijn. Het resistente materiaal is in te delen naar drie herkomsten: winter tarwes uit Oost-Europa, zomertarwes uit China/Japan en zomertarwes uit Brazilië.

CURRICULUM VITAE

Charles Henri Arnold Snijders werd op 17 juli 1960 geboren te Geleen, Limburg. Hij behaalde zijn Atheneum-B diploma in juni 1978 aan het St.-Michiel lyceum te Geleen. Van september 1978 tot mei 1985 volgde hij aan de Landbouw Hogeschool te Wageningen de studie Plantenveredeling, met als doctoraalvakken Plantenveredeling, Tropische Plantenteelt, Erfelijkheidsleer en Entomologie. Hij bracht zijn praktijktijd door op het 'Institut de Recherches sur le Caoutchouc' (IRCA) in de Ivoorkust. Van augustus 1985 tot heden was hij werkzaam als onderzoeker bij de Stichting voor Plantenveredeling (SVP) te Wageningen: van augustus 1985 tot januari 1986 als tijdelijk medewerker, van januari 1986 tot januari 1989 in dienst van de Stichting Nederlands Graan-Centrum (NGC), en van januari 1989 tot januari 1990 als vaste medewerker in de functie van onderzoeker tarwe. Op 1 januari 1990 fuseerde de SVP met het IVT, ITAL en CGN tot het Centrum voor Plantenveredelingsonderzoek (CPO). Sinds deze fusie is hij hoofd van de afdeling Granen.