Fusarium in wheat

Effects of soil fertility strategies and nitrogen levels on mycotoxins and seedling blight

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Preface

Quality plays an important role in further development of organic agriculture. For this reason, the project Quality of Low Input Food was brought into existence, supported by the EU 6th framework. This QLIF project was very extended, and this report only describes a limited research question: the relation between soil fertility strategies and additional nitrogen applications within the strategies at one side, and the presence of Fusarium Head Blight at the other.

Fusarium Head Blight (FHB) is involved in two quality items:

- If present on seeds, it will negatively influence the germination and the seedling stage, thus causing yield losses and weed problems.
- Fusarium on grains can produce mycotoxines, for example DON, being harmful if consumed.

In the Netherlands, the overall nitrogen level in wheat crops is rising, due to the request of bakeries for wheat with a high protein content. Additional nitrogen fertilizer application around flowering of the wheat crop are more and more common. There is some scientific evidence that FHB is enhanced by increased nitrogen levels. Therefore, an experiment was realized to find out whether or not this relation between nitrogen level and FHB plays a role in low input and organic farming.

Content

Summary

In a two-year field experiment in the Netherlands the relation between three soil fertility strategies, additional nitrogen levels and Fusarium Head Blight in wheat are explored. There was a substantial year-effect, as could be expected. The soil fertility strategies showed differences, but were party coinciding with location. Although not consistent over the years and strategies, a significant relation was found between additional nitrogen applications around anthesis and FHB, expressed as presence of mycotoxines (DON) and Total Root Rot from the Blotter test. Higher nitrogen levels from fertilizer applications at anthesis give a higher chance on FHB, with other so far unknown factors playing a role.

Samenvatting

In een tweejarig veldexperiment in Nederland is de relatie onderzocht tussen drie strategieën voor bodemvruchtbaarheid, aanvullende stikstoftrappen en Fusarium in tarwe. Er was sprake van een substantieel jaareffect, wat gezien de aard van Fusarium te verwachten viel. De verschillende strategieën voor bodemvruchtbaarheid lieten verschillen zien, maar die kunnen voor een liggen aan locatieverschillen. Hoewel de resultaten niet consistent zijn over de jaren en de strategieën blijkt er een significant verband te zijn tussen additionele stikstofgiften rond de bloei van het gewas en het optreden van Fusarium, gemeten aan de aanwezigheid van mycotoxinen (DON) en de parameter Wortelrot Totaal van de Blotter test op Fusarium. Hogere stikstofniveaus door additionele bemesting rond de bloei verhoogt de kans op Fusarium, maar andere, onbekende factoren spelen daarbij ook een rol.

1 Introduction

Fusarium head blight (FHB) is a serious problem in wheat cultivation. Fusarium head blight (FHB) is caused by one or more Fusarium species, including F. graminearum (Schwabe), F. culmorum (W.G. Smith) Sacc., F. avenaceum (Fries) Sacc., F. poae (Peck) Wollenw., and by Microdochium nivale (Fries) Samuels and Hallett. Next to slight reductions in yield due to reduced seed weights of infected seeds, FHB infections cause two problems concerning the quality of harvested wheat seeds: firstly, FHB on wheat can produce a variety of mycotoxins, of which deoxynivalenol (DON) is perhaps the most famous (Parry et al., 1995). If present in food or feed, DON can result in serious health problems (D'Mello et al. 1999; Peraica et al. 1999).

Secondly, the seeds infected with Fusarium not only have a lower 1000 grain weight but also the present Fusarium fungi can infect the seedling after sowing, thus causing less dense plant stand due to seedling blight. In certain years, the availability of uninfected seeds may be limited due to the widespread nature of FHB epidemics (Jones, 1999). In the Netherlands on average once every two years organic wheat seed production is affected by FHB (Osman, et al., 2004).

Control options of seedling blight in organic agriculture are focussing on reduction of the pathogen on the seeds before sowing and include hot-water treatments and biological control by micro-organisms (Osman, et al., 2004: Johansson et al. 2003; Dal Bello et al. 2002). Although successful, these options are currently not available for large scale use in practice.

Use of the infected seeds without treatment results in lower plant densities (Gilbert et al. 1997; Bechtel et al. 1985) due to a loss of viability, reduced emergence and post emergence seedling blight (Jones, 1999). Next to Microdochium nivale (Johansson et al., 2003; Hare et al., 1999) also F. culmorum (Khan et al., 2006; Johansson et al., 2003; Hare et al., 1999) and F. graminearum (Bacon & Hinton, 2007; Dal Bello et al., 2002; Chongo et al., 2001) are known to be able to cause these symptoms. In years with favourable weather conditions for wheat production, a reduced plant density does not necessarily affect yield, because plant loss can be compensated by increased tillering (Gooding et al., 2002). However, use of infected seeds may have other effects on spring wheat crops. For example resulting lower plant densities due to seedling blight can reduce the rate of canopy closure and hence make the crop less competitive against weeds. Weed infestation is one of the major constraints in organic cereal production, and the build up of a weed population due to an open crop stand does not only reduce yield of the cereal crop, but also increases weed control costs in subsequent crops in the rotation (Vereijken, 1994; Schotveld en Kloen, 1996).

As said, FHB epidemics occur frequently and cause a general high presence of Fusarium as well as mycotoxins on seeds, but the differences in susceptibility between varieties and locations is not yet well understood. Mesterházi (1995) distinguishes two main groups of types, also called components, of resistance of cereals to Fusarium: active and passive resistance components. In active resistance, physiological processes should be involved, whereas passive resistance is avoidance of the pathogen, involving morphological characteristics of the plant. Influence of plant height, though with a lot of variation, is described by several authors revealing higher FHB infections in lower wheat plants or cultivars (e.g. Lienemann, 2002 ; Buerstmayr et al, 2000 ; Mesterházi, 1995). Other mechanisms mentioned by Mesterházi (1995) are flowering in the boot stage (escape of infection), presence of absence of awns (more awns, higher FHB risk) and spikelet density (higher spikelet density, more FHB).

Relatively new in this context is passive resistance at crop level. Lemmens at el. (2004) measured significant effects of nitrogen fertilization on Fusarium head blight development and DON contamination in wheat. They showed that the type of fertilizer that they used did not have any influence, but the amounts of nitrogen did. Especially at low nitrogen levels (0-80 kg N ha^{-1}) the FHB rate and the DON contamination significantly increased with nitrogen level. However, at higher rates of nitrogen fertilization, relevant to contemporary conventional crop husbandry, no significant effects were measured. Organic agriculture is in general an agronomic system with, relative to conventional agriculture, limited nitrogen input. The nitrogen level at which FHB could be influenced by nitrogen application (Lemmens et al, 2004) might be relevant for organic farming.

At least in the Netherlands, there is a tendency in wheat quality parameters towards higher protein content. As a result, (organic)farmers tend to apply higher basic fertilization rates for the wheat crops, and to apply additional fertilizer applications around the flowering stage of the crop, in order to increase the nitrogen availability during the kernel formation and consequently the protein content. This tendency in fertilization strategy is questioned due to the possibly negative aspects of increased nitrogen application on the presence of FHB, and has lead to the following research questions:

- Can we find any relationship between overall soil fertility management strategies, resulting in different overall plant available nitrogen levels and different nitrogen dynamics, and the presence of FHB and mycotoxines in wheat?
- Can we find any relationship between increased levels of plant available nitrogen during the grain filling stage, realized by additional fertilizer application around flowering, and the presence of FHB and mycotoxines in wheat?

2 Method and materials

Experimental design

In 2006 and 2007, being replicates in time) field trials were done on two locations.

The first location was the experimental farm 'Rusthoeve' on a clayish soil (17% lutum, 2% soil organic matter and >0.8 m potential rooting depth) in Colijnsplaat, The Netherlands (51'35" N, 3'51" E), in the years 2006 and 2007. The experimental field was organic since 2002. In both years the precrop was onion. On this location four different soil fertility management strategies are present, of which two were used in our experiment: compost (C) and Slurry (S).

- S: Each year shortly before sowing or planting a cattle slurry application according to the need of the crop.
- C: Each year in autumn about 30 tons of compost as soil-oriented fertilizer; additionally a crop-oriented fertilizer in the beginning or during the crop growth being Molasse, a N-rich by-product of the sugar industry.

The second location was the farm 'NZ27' on a heavy clay soil (>30% lutum, 4% organic matter and >0.8 m potential rooting depth) near Zeewolde, The Netherlands (52'19" N, 5'25" E). Here the strategy is based on Farm Yard Manure (F) application in autumn with crop-dependant additional applications of slurry and Molasse. For our experiment, in order to have a low general nitrogen level, we skipped the slurry application in spring short before sowing. The precrop was sugar maize for the 2006 wheat crop and French beans for the 2007 wheat crop.

- F: autumn application of about 10 tons of farm yard manure

On both locations and in both years the spring wheat variety 'Lavett' was sown, which was the commonly used variety in The Netherlands by that time.

Within these three systems, with no replicates except the two years, a top dressing of nitrogen fertilizer was applied in four replicates short before flowering using two types of fertilizer (Organic pellets "Monterra Malt" and Molasse) and three nitrogen levels as shown in Table 2-1, with the highest nitrogen level applied in two charges with two weeks in between:

Pellets (kg N ha-1)	Molasse (kg N ha-1)
0	0
65	108
65 + 40	108 + 67

Table 2-1. Nitrogen levels of additional fertilizers

This resulted in five treatments per replicate (only one zero-nitrogen plot) and 20 plots (4 replicates) for C, S and F each. The size was 12 x 2 = 24 m^2 for all plots in 2006 and the F plots in 2007. In 2007, the S and C plots were 12 x 3 = 36 m^2 due to practical reasons related to the harvest. Net harvested surface was 15,75 m^2 (F), 18.9 m^2 (S and C, 2006) and 24.1 m^2 (S and C, 2007). The field design is shown in annex 1, with randomized N-levels within blocks, blocks being replicates.

The molasses, a liquid fertilizer, was spread by hand, and directly afterwards the crop was washed with pure water to clean the plants and to improve soil incorporation of the fertilizer. Due to the ammonium content of the molasse, part of the nitrogen will have been lost by votalisation. This is estimated and taken into account in modelling the nitrogen dynamics.

To enlarge the Fusarium infection risk, chopped maize stems and leaves were spread in the field short before flowering, directly after the first additional fertilizer application (Mesterházy, 1978). For the same reason we wanted the crop to be humid at least three nights in the week after the start of the flowering. If precipitation was absent the crop was sprayed with 1 mm water late in the evening.

Measurements

Temperature and evapotranspiration were obtained from nearby weather stations 'Wilhelminaoord (S, C) and Zeewolde (F). Precipitation was registered at the farm (C,S) and at a nearby farm (F).
Yields of grain and straw were recorded (Grain-Y and Straw-Y) as was nitrogen content of grain (Grain-N) and straw (Straw-N). The grain was dried to 15% moisture content and stored at 18°C. Two month after harvest the seeds were tested in a blotter test (4 repetitions of 50 seeds on wet filter paper, incubation: 3 days at 10°C, then 3 days at 20°C, no light; Limonard, 1966) on the level of Fusarium infection. The amount of mycotoxines was measured: DON (2006 and 2007) and NIV, ADONs, FUS-X, HT-2, T-2 and ZEA (2006). The presence of Fusarium species on the seeds was tested in 2007 by means of the TaqMan-PCR (Waalwijk et al., 2004). In 2007 two more quality criteria for wheat for bread production were measured: the Hagberg-Perten falling number and the 100 litre weight.
The inorganic nitrogen level in the topsoil (0-30 cm) was measured four to five times during the season and the nitrogen dynamics were modelled with the NDICEA model (Van der Burgt et al, 2006). From this modelling the amount of plant available nitrogen (PAN) was calculated, being the sum of inorganic nitrogen at sowing, the direct available inorganic nitrogen in fertilizer and the nitrogen available due to mineralization during crop growth until harvest, minus votalized nitrogen.
In 2007 presence of weed in S and C was registered on a 1-10 scale a few weeks before harvest..
Statistical analysis was done with GenStat 9.1.0.147, Lawes Agricultural Trust.

Course of the experiments

The lay-out and dimensions of the S, C and F experiments was identical in 2006. During harvest, the S and C strategies needed much precision to cut the requested net area. For this reason, the plot size was increased from 2 x 12 m in 2006 to 3 x 12 m in 2007.
The S and C strategies, including the slurry application in S and the strategy-related Molasse application in C, turned out to have high PAN levels. The additional gifts of pellets and Molasse did not result in clearly visible differences between the plots in 2006, contrary to the F location with a very low PAN. Having in mind the findings from Lemmens et al (2004) indicating that a reaction on nitrogen could be expected at low general nitrogen levels, we decided to skip in 2007 the slurry (in S) and system-related Molasse (in C) application. The application of the Organic pellets and Molasse around athesis remained unchanged.

Although the weather in 2006 and 2007 were on average not exceptional, spring 2007 on both SC and F location was characterized by a severe drought from almost eight weeks. In F germination was affected and reduced but continued; in S and C germination was interrupted and resumed almost two months later. On both locations the 2007 yields were low, on S and C even very low.

3 Results

For the analysis of the results we preferred to use plant available nitrogen (PAN) above applied nitrogen This PAN was calculated out of the nitrogen dynamics as presented by the NDICEA model. Our first step was to check the model simulations. The model performance, judged by RMSE (Wallach and Goffinet, 1989), is given inTable 3-1. In 2006 the model performed overall well. In 2007 it was not so good. This may have been caused by a less adequate simulation of mineralization processes during the severe drought in spring. Both PAN and applied nitrogen are used in the further analysis of the experiment.

Year	**2006**			**2007**		
Strategy	C	S	F	C	S	F
RMSE	14,5	20,3	7,3	26,7	13,5	29,8
Judgement	Good	Reasonable	Good	Weak	Good	Weak

Table 3-1 RMSE (Average per Strategy) of inorganic nitrogen, simulated versus measured.

A next step was to check the Fusarium species composition and quantity in the three strategies. This was only done in 2007. There turned out to be no significant interaction between strategy and species composition, so the strategies could be statistically analysed together. In F, the overall amount of pathogens was significantly lower than in S and C (Table 3-2). Fusrium culmorum and F. poae were present in significant smaller quantities than F. avenaceum, F. graminearum and Microdochium nivale (Table 3-3).

	^{10}log (pg mg^{-1} dry material	**(P<0.001)**
C	0,892	b
S	0,98	b
F	0,425	a

Table 3-2 Log-transefered values of Pathogen quantity in the three systems in 2007

	^{10}log (pg mg^{-1} dry material	**P<0.001**
Fusarium avenaceum	0,912	b
Fusarium culmorum	0,418	a
Fusarium graminearum	1,128	b
Fusarium poae	0,259	a
Microdochium nivale	1,11	b

Table 3-3 Log-transferred values of Pathogen quantity in 2007

In both years and in all three strategies there was a significant relation between PAN at one side and Grain-N and Straw-N at the other side, with the highest explained variance related to the Grain-N. This indicates that the crop reacted sufficient on the imposed differences in plant available nitrogen and that the late nitrogen applications increased Grain-N more than Straw-N. In table Table 3-4 is given the percentage of variance of Grain-N and Straw-N which is explained by the parameter PAN.

Year	**2006**				**2007**			
Strategy	**CS**		**F**		**CS**		**F**	
explained variable	**Grain-N**	**Straw-N**	**Grain-N**	**Straw-N**	**Grain-N**	**Straw-N**	**Grain-N**	**Straw-N**
PAN	80	42	90	54	57	39	84	54

Table 3-4. Percentage of explained variance of Grain-N and Straw-N by parameter PAN

PAN in 2006 was significant lower in F compared to S and C (Tabele 3-5). In 2007 the slurry and strategy-related Molasse applications in S and C were skipped in an attempt to reduce nitrogen availability, but the PAN was hardly affected. This was caused by a much higher level of inorganic nitrogen in spring in 2007 compared to 2006.

	2006	**2007**
C	235^{c}	239^{b}
S	191^{b}	182^{a}
F	133^{a}	156^{a}
lsd	24.4	29,29

Tabele 3-5. Average PAN (kg ha^{-1}) of the strategies in 2006 and 2007. Within each column a different letter indicates a significant difference at P=0.05

Straw yield in 2006 was significant different for the three strategies; in 2007 F straw yield was lower than S and C (Table 3-6).

	2006	**2007**
C	4854^{b}	3733^{b}
S	5170^{c}	3479^{b}
F	1971^{a}	1961^{a}
lsd	279.2	453.0

Table 3-6 Straw yield (kg ha^{-1} dm) of the strategies in 2006 and 2007. Within each column a different letter indicates a significant difference at P=0.05

Within the strategies the response to the nitrogen levels differed (Table 3-7). In each year and in each strategy significant differences were found (P=0.05), but only F 2006 is very consistent. F 2007 was less strong in its response to N-levels, and the S and C strategies are inconsistent in their response on N-level although the lowest level of additionally applied N resulted mostly in the lowest straw yield. This weak response is not surprising because of the late nitrogen application, at a moment when leaf and stem formation were almost completed.

	2006					2007				
	0	**65**	**105**	**108**	**175**	**0**	**65**	**105**	**108**	**175**
C	4656^{ab}	5180^{b}	4947^{b}	4481^{a}	5005^{ab}	3708^{ab}	3375^{a}	3688^{ab}	3521^{ab}	4375^{b}
S	4620^{a}	5379^{b}	5446^{b}	5274^{b}	5132^{ab}	2875^{a}	3229^{b}	3667^{b}	3708^{b}	3917^{b}
F	1303^{a}	1900^{b}	1873^{b}	2411^{c}	2369^{c}	1309^{a}	1382^{a}	1814^{a}	2196^{ab}	3106^{b}

Table 3-7 Straw yield (kg ha^{-1} dm) of the strategies as related to N-applied in 2006 and 2007. Within each line and year a different letter indicates a significant difference at P=0.05

Grain yield in 2006 was significant lower in F then in S and C. Only F showed a reaction on applied nitrogen levels (Table 3-8). Grain yield was in 2007 much lower than in 2006 due to the spring drought. Average F Grain yield in 2007 was 3266 kg ha^{-1} and significant higher (at P=0.05) than S (2730 kg ha^{-1}) and C (2833 kg ha^{-1}). Grain yield gave no significant response to N-application levels in 2007.

2006	**0**	**65**	**105**	**108**	**175**
C	7617^{a}	7965^{a}	6984^{a}	7873^{a}	7435^{a}
S	8070^{b}	8017^{b}	8022^{b}	7830^{b}	7328^{a}
F	3605^{a}	4588^{b}	4543^{b}	5838^{c}	4910^{b}

Table 3-8 Grain yield in 2006 of the strategies (kg ha^{-1} at 15% moisture) related to additionally applied nitrogen. Within each line a different letter indicates a significant difference at P=0.05

Significant differences in Total Root Rot (TRR) measured in the blotter test, DON (ppm) are shown together with the PAN for the strategies in Table 3-9 and for the nitrogen levels in Tabel 3-10

	2006			2007		
	PAN	**TRR**	**DON**	**PAN**	**TRR**	**DON**
C	235c	2.20a	146.4a	239b	17.25a	375a
S	191b	2.11a	196.8b	182a	16.85a	482b
F	133a	3.75b	133.9a	156a	16.38a	457ab

Table 3-9 PAN, TRR and DON of the strategies in 2006 and 2007. Within each column a different letter indicates a significant difference at P=0.05

	N-applied	0	65	105	108	175
2006	Additional PAN	0[a]	25,7[b]	39[c]	29,7[b]	46[c]
	DON	108,9[a]	159,2[a]	148,2[a]	159,2[a]	219,4[b]
2007	Additional PAN	0[a]	31,3[b]	49,3[c]	31,7[b]	53,3[c]
	TRR	14,4[a]	16,6[ab]	17,8[b]	16,7[ab]	18,7[b]

Tabel 3-10 Relation between N applied, the increase of PAN due to this application and DON in 2006 and 2008

Although there are significant differences in TRR and DON between the strategies, the pattern is not consistent looking at the systems or the years. When the nitrogen levels are observed the pattern is more clear: with an increase of PAN by added fertilizers, the amount of DON also increased significantly for the highest nitrogen level in 2006, and the total root rot was lowest in the lowest nitrogen level in 2007. Nitrogen levels did not differ significantly for TRR in 2006 and for DON in 2007.

After concluding that there are at least some relationships between DON, and TRR at one side and all nitrogen related parameters at the other, a multivariate analysis was done with TRR, DON and the other pathogens as dependent variables and straw yield, grain yield, straw-N, grain-N, measured N-min level and PAN as independent variables. Some of the regressions were significant.
Tabel 3-11 presents the percentages of variance of DON and TRR which is explained by the parameters PAN, Grain-N and Straw-N for all the significant relations found ($P < 0.05$). In five out of eight situations, there is a significant relation between PAN and DON or TRR, and in five out of eight situations there is a significant relation between Grain-N and DON or TRR. Straw-N has in only one situation a significant relation with DON quantity.

Year	**2006**				**2007**			
Strategy	**CS**		**F**		**CS**		**F**	
explained variable	**DON**	**TRR**	**DON**	**TRR**	**DON**	**TRR**	**DON**	**TRR**
PAN	24		28	28	16	21		
Grain-N	22		30	44		16		21
Straw-N	23							

Tabel 3-11 Percentage of explained variance of DON and TRR by parameters PAN, Grain-N and Straw-N

Weed infestation as was measured in the 2007 field experiment in strategies C and S, in relation to PAN is given in

Figure 3-1. (Note that within each strategy, the differences in PAN are caused by the nitrogen levels applied at anthesis). Differences between S and C, and differences between additional PAN were significant at P=0.05. C has more weeds, but the increase with N-levels is the same is in S. Part of the nitrogen applied at anthesis has been taken op by weeds.

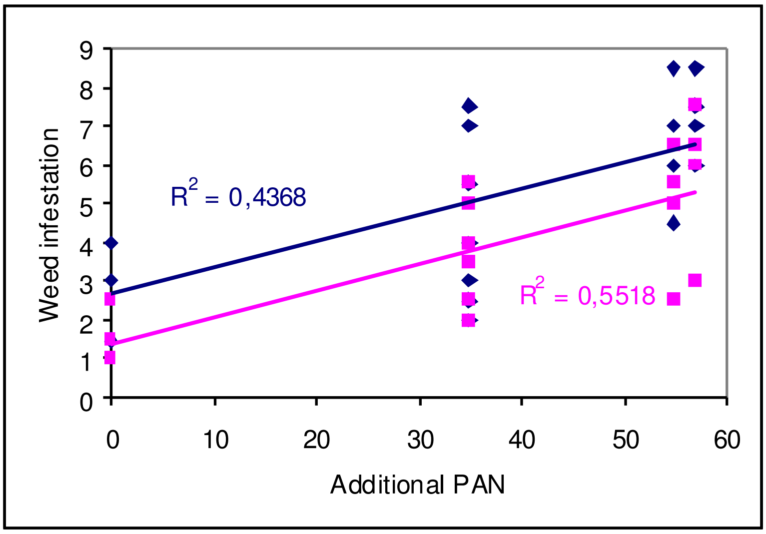

Figure 3-1 Relation between additionally applied PAN (kg ha^{-1}) by fertilizers applied around flowering, and weed infestation. Pink line = S; Blue line = C.

Analysing the data of 2006 and 2007 together (Figure 3-2**Fout! Verwijzingsbron niet gevonden.** up to Figure 3-5**Fout! Verwijzingsbron niet gevonden.**) it can be seen that there was no relation between Straw yield in dry matter and DON (Figure 3-2**Fout! Verwijzingsbron niet gevonden.**). Looking at Straw-N (Figure 3-3),**Fout! Verwijzingsbron niet gevonden.** there was a triangle shaped relation with DON. Although a linear regression could have been slightly significant (with a low percentage of variation accounted for), this is not what we should be interested in. There is a clear pattern in the graph, indicating that for low straw-N there always was also a low DON content, whereas in situations with high straw-N, there was either a low or a high DON content, depending on other factors.

Going to grain yield (Figure 3-4**Fout! Verwijzingsbron niet gevonden.**), the graph can be explained by a combination of year-effect and location but there is no significant relation with DON. To finish with Grain-N (Figure 3-5**Fout! Verwijzingsbron niet gevonden.**) we can see the same pattern as for straw-N: at low levels of Grain-N, the DON content is also low. At high levels of Grain-N the DON content can be low, high and everything in between.

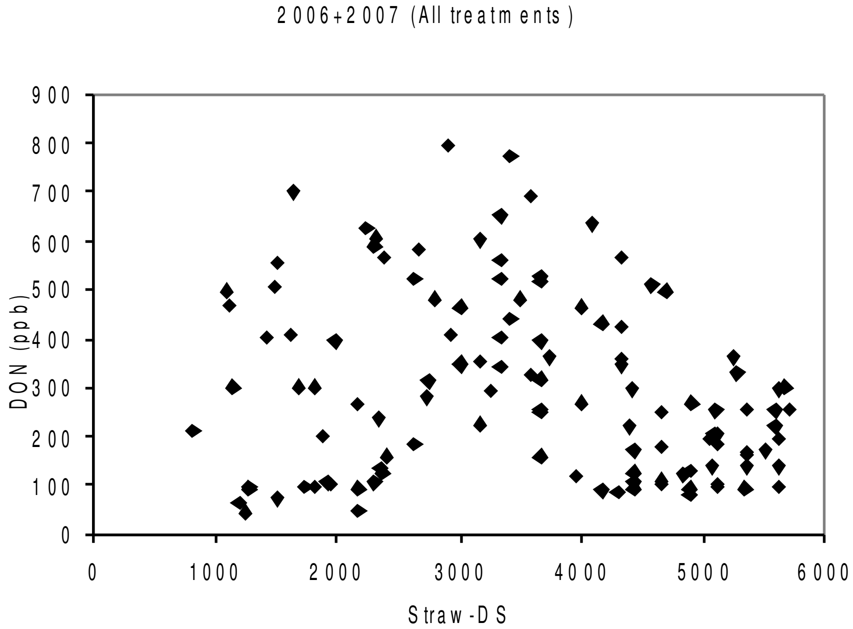

Figure 3-2 Relation between Straw yield (dry matter) and DON, all treatments and two years

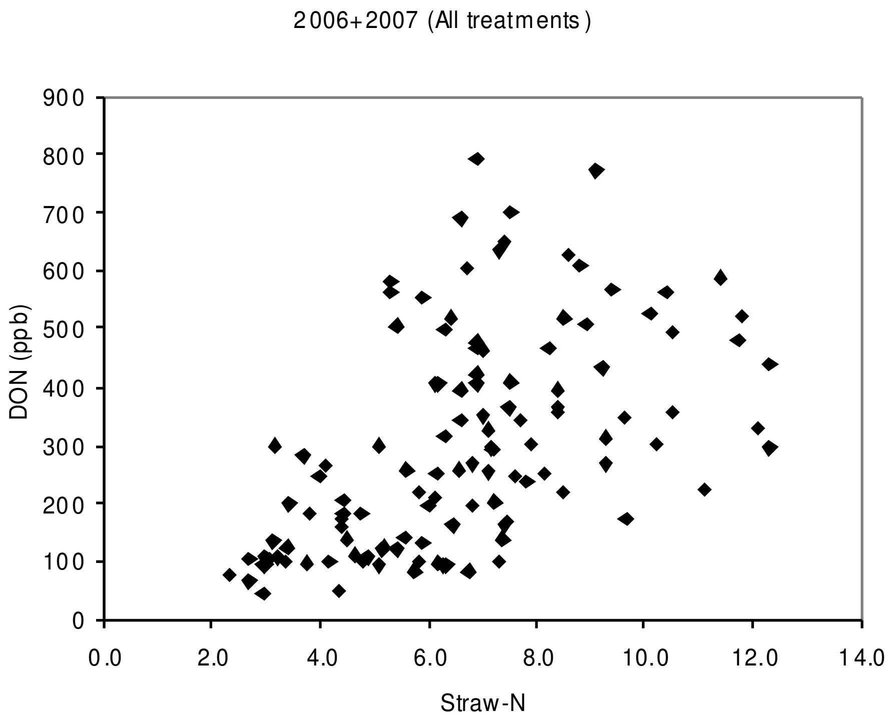

Figure 3-3 Relation between Straw-N and DON, all treatments and two years

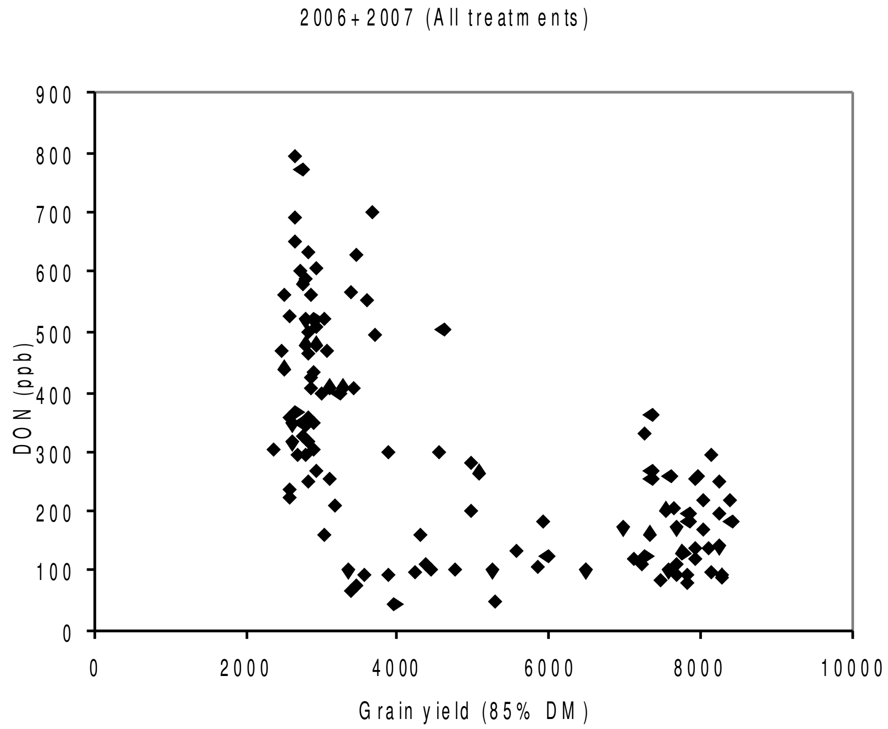

Figure 3-4 Relation between Grain yield and DON, all treatments and two years.

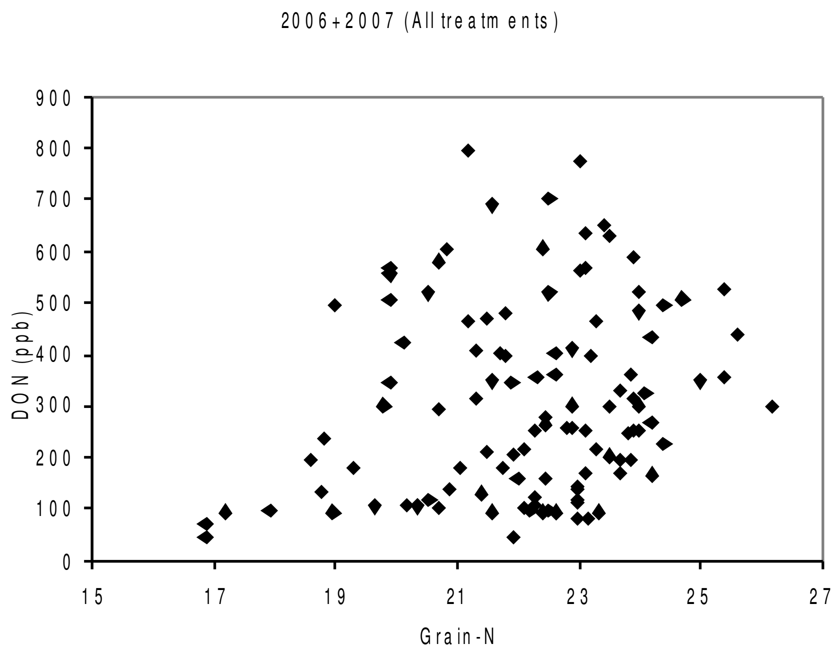

Figure 3-5 Relation between Grain-N and DON, all treatments and two years.

4 Discussion

In our experiments, significant correlations between soil nitrogen (PAN) and plant nitrogen (Grain-N, Straw-N) at one side and the quality parameters DON and TRR at the other side were present, but they were not strong and not consistent over the years and the strategies. On the first of our research questions, whether the basic fertility strategy can influence the FHB infection, we must therefore answer that we cannot conclude this based on our results. There were significant differences between the F strategy on one hand and the C and S strategies on the other hand, but F was located on another field, with less Fusarium present, and probably many more differences that only the fertility strategy. However, for DON, we saw significant differences between the C and S strategies located on the same farm, in both years, indicating a higher DON content for the slurry treatment (S) in both years.
Secondly, we have also shown that there are relations between straw-N and grain-N contents, increased by the nitrogen applications at anthesis, indicating that higher nitrogen application rates tend to increase the level of DON present, although other factors influence this too. In other words: at low Grain-N levels the *chance* to find high DON levels is low; at high Grain-N levels the *chance* to find high DON levels is higher. So there is a relation between DON and Grain-N, but there must be other factors besides grain-N to explain the found DON levels.
Our experiments also show that overall nitrogen availability in 2006, C and S was very high and there was no response to nitrogen application in grain yield and straw yield. Nevertheless there was a significant relation between applied nitrogen levels and DON. In only two situations (2006; C, S) straw-N was significantly related to DON whereas grain-N was in seven situations related to DON or TRR. Straw dry matter yield was significantly increased by nitrogen application in F (2006 and 2007) and much less pronounced in S and C (2007 only) but is less significant than Grain-N in explaining DON. This all supports the idea that it is (at least partly) Grain-N and not microclimate or plant structure as influenced by straw and grain quantity, that causes an increase in FHB when nitrogen levels are increased.

For judgement of the model performance an arbitrary, praxis-oriented maximum RMSE of 20 kg N ha-1 is suggested (Van der Burgt et al, 2006). The 2006 model performance is good (Table 3-1; C and F) and almost good (S); the performance in 2007 was weaker. The effect of a long period of drought after sowing in 2007 is probably not modelled correctly. However, the 2007 S model performance was good. The parameter Plant Available Nitrogen (PAN), derived from the model, was used for further correlation with quality parameters. With a less adequate model performance, this model-derived parameter might however be less adequate.

There is a strong relation between PAN and nitrogen in the plant (Table 3-4; Grain-N and Straw-N). In 2007 the percentage explained variance is lower than in 2006, which might be the result of the weaker model performance. Grain-N is to a higher degree explained by PAN than Straw-N. This is expected, due to the late additional fertilizer application when stem and leaves have almost completed their development. Overall we conclude that the plant N-content and mainly the grain N-content indeed expresses the differences in available N.

Farmers are paid for their wheat in dependence of three main criteria:

- Yield
- Quality criteria, in which protein content is an important factor

- Absence of mycotoxines

In general, yield is related to overall PAN. In this experiment, yield responded to additional N-fertilizers in 2006 in the F strategy only. This F strategy is an overall low-N strategy. At overall higher N strategies, like S and C in 2006, the additional N fertilizers have their effect mainly on Straw-N and Grain-N.
Grain-N content is, just as yield, related to overall PAN, but can be increased by late N applications, increasing the PAN in the plant phase when the grains are filled. This late additional N application is practiced more and more in the Netherlands in order to fulfil the request from the bakeries. If the requested protein content is not reached, the harvest can only be sold as feed, not as human food, and prices are much lower.
The third criterion, absence of mycotoxines, is seldom checked at field or farm level, contrary to protein content. The test on mycotoxines is usually done in a bulk container, containing several charges of farmers and/or fields. This means that the individual farmer doesn't bear the consequences of a too high level of mycotoxines.
In this experiment protein content was influenced by additional fertilizer much more than DON or TRR. For farmers it makes no sense to reduce plant available nitrogen levels in order to reduce DON or TRR. Presence of Fusarium and FHB is partly a year-effect beyond farmer's influence. Reducing the overall nitrogen level or leaving the additional nitrogen application to minimize the risk of Fusarium presence is no option because the effect on mycotoxines is limited, other unknown factors play a considerable role, and the negative effect on Grain-N is dominant.

Some of the additionally applied nitrogen is taken up by weeds, increasing the weed infestation at higher N-levels (**Fout! Verwijzingsbron niet gevonden.**). This was only shown in 2007 (no weed measurements in 2006) in the S end C crops which had a very open stand due to the drought and bad germination. Although this result is well understood, it may play only a minor role in well-developed crops. We conclude that the current results show that there is something going on between FHB, DON contents and plant available nitrogen in the soil: at lower soil nitrogen availability, lower Fusarium infestation and DON contents can be expected. Due to the current payment system this relation is not strong enough to be of any consequence. However, in future research it could be interesting to re-optimize the spring wheat nitrogen application taking the found relations into account and using weather, weed and Fusarium conditions of many years, to design a best practice.

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Disclaimer

References

Bacon, C.W., & Hinton, D.M. (2007**). Potential for control of seedling blight of wheat caused by Fusarium graminearum and related species using the bacterial endophyte Bacillus mojavensis**. Biocontrol Science and Technology 17, 81-94.

Bechtel, D. B., Kaleikau, L. A., Gaines, R. L., & Seitz, L. M. (1985). **The effects of Fusarium graminearum infection on wheat kernels**. Cereal Chemistry 62, 191-197.

Buerstmayr, H., Steiner, B., Lemmens, M. and Ruckenbauer, P. (2000). **Resistance to Fusarium Head Blight in winter wheat: heritability and trait associations**. Crop science 40, 1012-1018

Dal Bello, G. M., Monaco, C. I., & Simon, M. R. (2002). **Biological control of seedling blight of wheat caused by Fusarium graminearum with beneficial rhizosphere microorganisms**. World Journal of Microbiology and Biotechnology 18, 627-636.

D'Mello, J. P. F., Placinta, C. M., & Macdonald, A. M. C. (1999). **Fusarium mycotoxins: a review of global implications for animal health, welfare and productivity**. Animal Feed Science and Technology 80, 183-205.

Gooding, M. J., Pinyosinwat, A., & Ellis, R. H. (2002). **Responses of wheat grain yield and quality to seed rate**. The Journal of Agricultural Science 138, 327331.

Gilbert, J., Tekauz, A., & Woods, S. M. (1997). **Effects of storage on viability of Fusarium head blight-affected spring wheat seed**. Plant Disease 81, 159-162

Hare, M.C., Parry, D.W., & Baker, M.D. (1999). **The relationship between wheat seed weight, infection by Fusarium culmorum or Microdochium nivale, germination and seedling disease**. European Journal of Plant Pathology 105, 859-866.

Johansson, P. M., Johnsson, L., & Gerhardson, B. (2003). **Suppression of wheat-seedling diseases caused by Fusarium culmorum and Microdochium nivale using bacterial seed treatment**. Plant Pathology 52, 219-227.

Jones, R. K. (1999**). Seedling blight development and control in spring wheat damaged by Fusarium gramenearum group 2**. Plant disease 83, 1013-1018.

Khan , M. R., Fisher, S., Egan, D., Doohan, F. M. (2006). **Biological control of Fusarium Seedling Blight Disease of Wheat and Barley.** Phytopathology 96, 386-394.

Lemmens, M., Haim, K., Lew, H. and Ruckenbauer, P. (2004). **The effect of fertilization on Fusarium head blight development and deoxinivalenol contamination in wheat**. Journal of Phytopathology 152, 1-8.

Lienemann, K. (2002). **Auftreten von Fusarium-Arten am Winterweizen im Rheinland und Möglichkeiten der befallscontrolle unter besonderer berücksichtigung der Weizensorte**. Ph.D Thesis, Institut für Pflanzenkrankheiten der Rheinischen Friedrich-Wilhelms-Universität Bonn, Germany.

Limonard, L. (1966**). A modified blotter test for seed health**. Neth. J. Pl. Path. 72, 319-321

Mesterházy, A. (1978). **Comparative analysis of artificial inoculation methods with Fusarium spp. on winter wheat varieties**. Phytopath. Z. 93, 12-25

Mesterházi, A. (1995). **Types and components of resistance to Fusarium head blight of wheat.** Plant breeding 114, 377-386

Osman, A., Groot, S., Köhl, J., Kamp, L., & Bremer, E. (2004). **Seed treatments against Fusarium in organic spring wheat.** In E. Lammerts van Bueren, R. Ranganathan, & M. Sorensen (Eds.), The first world conference on organic seed: challenges and opportunities for organic agriculture and the seed industry (pp. 133-137). International Federation of Organic Agriculture Movements (IFOAM), Bonn.

Parry, D. W., Jenkinson, P., & McLeod, L. (1995). **Fusarium ear blight (scab) in small grain cereals: a review.** Plant Pathology 44, 207–238.

Peraica, M., Radić, B., Lucić, A., & Pavlović, M. (1999). **Toxic effects if mycotoxins in humans**. Bulletin of the World Health Organization 77, 754-766.

Schotveld, E. en Kloen, H. (1996). **Onkruidbeheersing in een multifunctionele vruchtwisseling: innovatieproject voor ecologische akkerbouw en groenteteelt in samenwerking met 10 voorhoedebedrijven in Flevoland**. Rapport AB-DLO 74, Wageningen, Netherlands, 31 pp.

Van der Burgt, G.J.H.M., Oomen G.J.M., Habets A.S.J. & Rossing W.A.H. (2006). **The NDICEA model, a tool to improve nitrogen use efficiency in cropping systems**. Nutrient Cycling in Agroecosystems 74: 275-294.

Vereijken, P. (1994). **Designing prototypes. Progress reports of research network on integrated and ecological arable farming systems for EU and associated countries.** Conference proceedings, AB-DLO Wageningen, Netherlands, 87 pp.

Waalwijk, C., van der Heide, R., de Vries, I., van der Lee, T., Schoen, C., Costrel-de Corainville, G., Häuser-Hahn, I., Kastelein, P., Köhl, J., Lonnet, P., Demarquet, T., & Kema, G. (2004). **Quantitative detection of Fusarium species in wheat using TaqMan**. European Journal of Plan Pathology 110, 481-494.

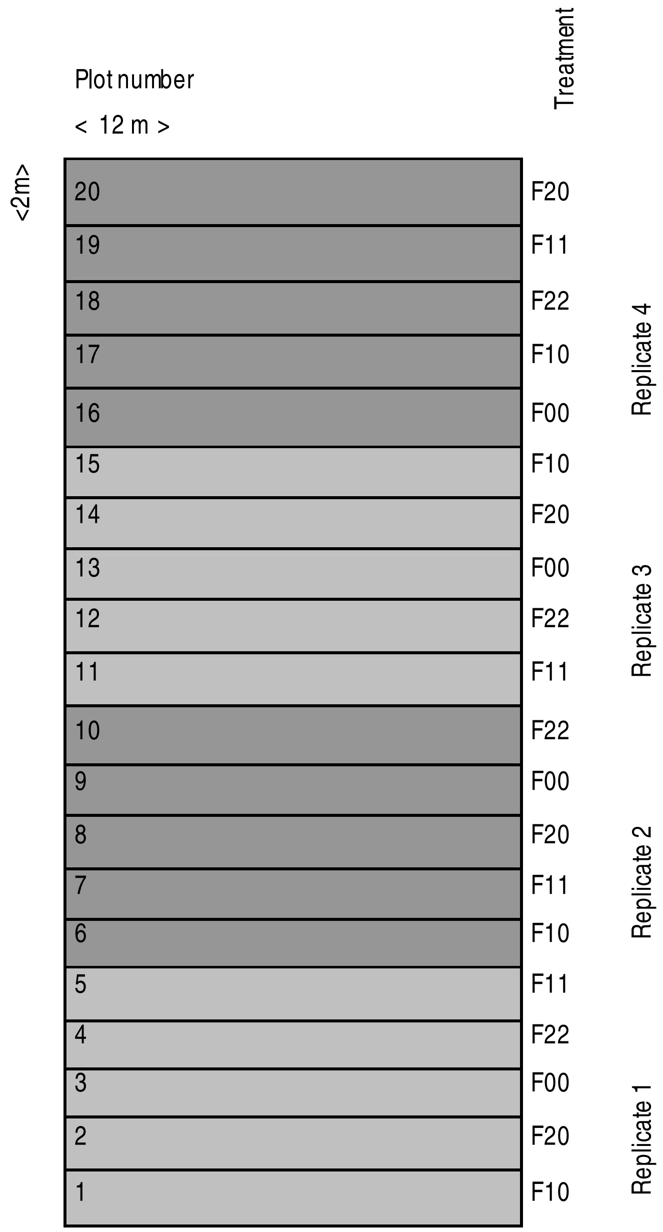
Apendix1: Field lay-out

Example: field lay-out F, 2006.

Blocks are replicates; within the blocks the treatments are randomized.

The S and C lay-out was exact the same in 2006, with C plot 1 adjacent to S plot 1 etc, with an other (randomized) order within the replicates.

The S and C plots in 2007 were 3 instead of 2 meters wide.



F = Farm Yard Manure

00 = no additional fertilizer

10 = Molasse 108 kg ha^{-1}

11 = Molasse 108 + 67 kg ha^{-1}

20 = Pellets 65 kg ha^{-1}

22 = pellets 65 + 40 kg ha^{-1}