

# Differences between spring wheat cultivars in tolerance to *Fusarium* seedling blight under organic field conditions

Bart G. H. Timmermans · Aart M. Osman ·  
GeertJan H. M. Van der Burgt

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**Abstract** Use of *Fusarium*-infected seed for cereal crops results in a reduced plant density due to seedling blight. This is especially a problem in organic agriculture, for which currently no practical seed disinfection methods are available. In the present paper we investigated whether spring wheat cultivars differ in tolerance to seedling blight *in vivo*, whether the possible differences could be linked to cultivar differences in initial growth rates, and whether differences in weed infestation were related to differences in emergence. Seed six spring wheat cultivars (Melon, Lavett, SW Kungsjett, Epos, Pasteur, Thasos), containing three *Fusarium* infection levels were obtained and sown in two field experiments in 2006 and 2007 and in an outdoor pot experiment in 2007. Results indicated that the six spring wheat cultivars differed in their tolerance to seedling blight, and consequently in the percentage of emergence of their seeds. The relative levels of tolerance to seedling blight of the six cultivars were robust in the three experiments performed. No clear relationship between initial growth rates and tolerance was found. In our experiments, no early and homogenous weed pressure was present, but in the 2007 field experiment a relationship between

initial seedling emergence and weed infestation after anthesis was determined. Based on the presented results we suggest that additional to resistance to *Fusarium* head blight (FHB), differences in tolerance to seedling blight should also be considered during selection of wheat cultivars for organic agriculture.

**Keywords** Emergence · *Fusarium culmorum* · Seed quality · *Triticum aestivum* · Weed infestation

## Abbreviations

FHB      *Fusarium* head blight  
PR/F     the percentage of reduction in plant number  
          per percentage of *Fusarium* spp. in seeds

## Introduction

*Fusarium* head blight (FHB) is caused by one or more *Fusarium* species, including *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, and by *Microdochium nivale*. Apart from yield losses, these pathogens 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).

Seeds obtained from FHB-infected crops are usually a mix of visually scabby kernels, kernels that

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B. G. H. Timmermans (✉) · A. M. Osman ·  
G. H. M. Van der Burgt  
Department of Agriculture, Louis Bolk Institute,  
Hoofdstraat 24,  
3972 LA Driebergen, The Netherlands  
e-mail: b.timmermans@louisbol.nl

are infected but not visually damaged and seeds that are neither damaged nor infected (Jones and Mirocha 1999). In certain years, the availability of asymptomatic seeds may be limited due to the widespread nature of FHB epidemics (Jones 1999). In The Netherlands on average once every 2 years organic wheat seed production is affected by FHB (Osman et al. 2004), probably caused by favourable weather conditions during anthesis. Control options of seedling blight in organic agriculture include hot-water treatments and biological control by micro-organisms (Osman et al. 2004; Johansson et al. 2003; Dal Bello et al. 2002). However, these options are currently not available for large-scale use in practice.

Use of 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 *M. nivale* (Johansson et al. 2003; Hare et al. 1999) also *F. culmorum* (Kahn et al. 2006; Johansson et al. 2003; Hare et al. 1999) and *F. graminearum* (Bacon and 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 speed 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. Wheat cultivars differ in levels of resistance against *Fusarium* infection in the ear (Miedaner 1997; Mesterhazy 1995; Snijders 1990). Furthermore, it was shown that cultivars of winter wheat differ in susceptibility to seedling blight *in vitro* (Browne and Cooke 2005).

The aim of the current project was to investigate if commercially available spring wheat cultivars differ in their tolerance to seedling blight *in vivo*, if differences can be linked to the initial growth rates of the cultivars, and if differences in plant density can result in a different weed infestation level.

## Materials and methods

### Seeds

Seeds of six spring wheat cultivars (Epos, Lavett, Melon, Pasteur, SW Kungsjett, Thasos) were obtained from an experiment on an organic field, that was partly inoculated with *F. culmorum* strain IPO-39 in 2004, and stored at 13°C and 30% relative air humidity from then onwards. In both 2006 and 2007, seeds with three *Fusarium* infection levels (referred to as low, middle and high) were created for all cultivars: first, seeds were tested in a blotter test (four repetitions of 50 seeds on wet filter paper, incubation: 3 days at 10°C, then 3 days at 20°C, no light) for the level of *Fusarium* infection. Seeds from the uninoculated part of the field experiment in 2004 were used as the middle level of *Fusarium* infection. In this seed lot, naturally occurring *Fusarium* species were present, being predominantly *M. nivale*, *F. graminearum* and *F. avenaceum* (Table 1), as measured by TaqMan-PCR (Waalwijk et al. 2004).

Mixing these seeds with seeds from the inoculated part of the field (containing the same species and additional infections with *F. culmorum*) resulted in seed with the high level of *Fusarium* infection, and warm water treatment (45°C for 2 h, after which seeds were dried in warm air at 35°C–40°C) resulted in seeds with the low level of *Fusarium* infection. Shortly before the start of the experiments, the precise *Fusarium* infection levels in the seed were measured in a second blotter test (Table 2). In 2007, 1000 seed weights for all cultivars for the middle and high percentage of *Fusarium* treatments (since the treatment with the low level of *Fusarium* infection originated from the same seed lot as the middle level of *Fusarium* infection) were measured in two repetitions, by taking two samples of 30 g seeds, and counting the number of seeds in each sample.

### Field experiments

The experiments were located at 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, and the fertilisation scheme of the field was based on autumn application of compost (50 tons ha<sup>-1</sup>

**Table 1** The presence and abundance of *Fusarium* species measured with TaqMan-PCR in the seed lot with middle infection level

Species	Abundance (pg mg <sup>-1</sup> dry material)	Standard deviation in measurement
<i>F. avenaceum</i>	15.2	14.2
<i>F.graminearum</i>	20.0	21.9
<i>F. culmorum</i>	1.2	3.7
<i>M. nivale</i>	23.5	18.3
<i>F. poae</i>	0.1	0.4

every two years) without additional fertilisers or manure, resulting in a relative low nitrogen availability. The precrop was onion. Seeds of all six cultivars containing low, middle and high percentages of *Fusarium* infection, were sown on 21 March (2006) and 11 April (2007) with a precision sowing machine. In both years, experimental design was a randomised block design with four blocks and 20 m<sup>2</sup> (2 m × 10 m) plot size. Sowing density was 475 seeds m<sup>-2</sup> in 2006 and 400 seeds m<sup>-2</sup> in 2007, with a row distance of 20 cm. Mechanical weed control was performed when necessary according to the farmer. Plant density counting was conducted at the onset of tillering (BBCH stage 21–24, Lancashire et al. 1991), on 13 April 2006 and on 29 May 2007, by counting 4 (2006) and 6 (2007) transects of 1 m length in each plot.

Measurements of above-ground dry matter were conducted on 8 May 2006 (48 days after sowing) and on 7 June 2007 (57 days after sowing) in the lowest infection treatment for all cultivars, in the exponential growth phase of the crops (i.e. before canopy closure). On these dates, an area of 0.2 m<sup>2</sup> (2006) and 0.4 m<sup>2</sup> (2007) was randomly chosen in each plot

with the lowest *Fusarium* infection level for all cultivars. Plants in these areas were cut at soil surface, and dry weight was measured after drying at 105°C. Measurements of the percentage of light interception (LI) were done in all plots using a Sunscan light interception measurement system (Delta-T Devices, Cambridge, UK) on 4 May 2006 (22 days after sowing) and on 7 June 2007 (57 days after sowing).

In the field experiment in 2007, a high level of weed infestation occurred. Its severity was visually estimated, using an index between 0 (no weeds present in plot) and 10 (>90% of the total LAI weeds), on 7 August, in the early—late milk stage of the crop (BBCH stage 73–77).

In the period from sowing until measurement of the above-ground dry matter, average daily minimum air temperatures measured at 150 cm above ground level, were 8.0°C (2006) and 10.4°C (2007) and average daily maximum temperatures of 14.4°C (2006) and 19.5°C (2007). In 2006, in this period the minimum temperature was below zero (–1°C) on two nights. Precipitation in the period from sowing until the measurement of dry matter was 79 mm in 2006, showing no prolonged dry periods, and 106 mm in 2007, non of which fell in the first 3 weeks after sowing when conditions became rather dry.

#### Pot experiment

Seeds were sown in a sandy soil (originating from a former organic grass field) in 5 l pots at the experimental organic farm ‘Droevendaal’, Wageningen University and Research Centre, Wageningen, The Netherlands (51°59′, 5°39′), on 26 March (2007). The experimental set-up was a randomised block design with four repetitions, and each plot contained 20 pots of 5 l

**Table 2** Infection levels of the seed used, as measured in a blotter test before sowing

Year	2006	2006	2006	2007	2007	2007
Infection level with <i>Fusarium</i>	Low	Middle	High	Low	Middle	High
Spring wheat cultivar						
Epos	1 (0)	11.2 (5.5)	24.5 (17.5)	1 (1)	11.3 (6)	24.7 (20)
Lavett	0.5 (0.5)	12.5 (3)	18.8 (12)	1 (1)	6.3 (2)	24.7 (19)
Melon	0.3 (0)	15 (8)	24.3 (13)	0.7 (0)	11 (6)	25.3 (20)
Pasteur	2.3 (0.5)	18.8 (7.5)	25 (17.5)	1.7 (1)	11 (3)	23.3 (14)
SW Kungsjett	2.7 (1)	<sup>a</sup> -	34.3 (16)	2 (2)	14 (8)	25 (21)
Thasos	1.5 (0.5)	15.5 (3)	25.5 (20)	0.7 (0)	9.7 (4)	26 (18)

<sup>a</sup> Treatment not conducted  
Numbers in brackets indicate heavily and primary infected seeds.

(height 19 cm, diam 11.5–15 cm) with 5 seeds sown in each pot (resulting in 100 sown seeds per plot). Pots were watered regularly to maintain optimal moisture conditions for plant growth. Emergence was counted on 18 April (2007) and above-ground dry matter was measured as described above on 18 April (2007).

Air temperature measured at 150 cm above ground level in the period from sowing until measurement of the above-ground dry matter had an average daily minimum of 8.0°C and an average daily maximum of 17.5°C, and minimum temperature was below zero on two nights (−1.5°C and −0.8°C).

### Calculations and statistics

As an indication of the tolerance for seedling blight of the cultivars, the slope of the linear regression between the percentage of emergence and the percentage of *Fusarium* infections in the seeds was calculated for each plot. This slope, multiplied by minus 1, indicated the percentage of reduction in plant number per percentage of *Fusarium* in seeds (PR/F). Statistics were performed using GenStat Seventh Edition version 9.1.0.147, VSN International Ltd., Rothamsted, and R version 2.4.0 (R Development Core Team 2006).

## Results

### Seed weights

Cultivars differed significantly ( $P < 0.001$ ) in their 1000 seed weights; these ranged from 42.0 g for Pasteur to 35.1 g for Lavett in the seeds with the middle infection level, and from 37.2 for Pasteur to 28.5 for SW Kungsjett for the seeds with the high infection level (Fig. 1). For all cultivars, the seeds with the high infection level had lower 1000 seed weights than those with the middle infection level ( $P < 0.001$ ), but the interaction between infection levels and cultivars was significant, indicating a significant cultivar-specific reduction of seed weight with an increased level of *Fusarium* infection. For cultivar SW Kungsjett the difference in 1000 seed weight was larger (8.9 g) than for the other cultivars, whereas for Lavett it was smaller (3.0 g).

The cultivar-specific differences in 1000 seed weight (expressed in g) between seed lots with middle

and high *Fusarium* infection levels were partly explained by differences in *Fusarium* infection levels ( $P = 0.08$ ,  $r^2 = 0.58$ ) but were not related to differences in percentages of primary and heavily infected seeds ( $P = 0.451$ ,  $r^2 = 0$ ).

### Emergence and plant number: tolerance to seedling blight

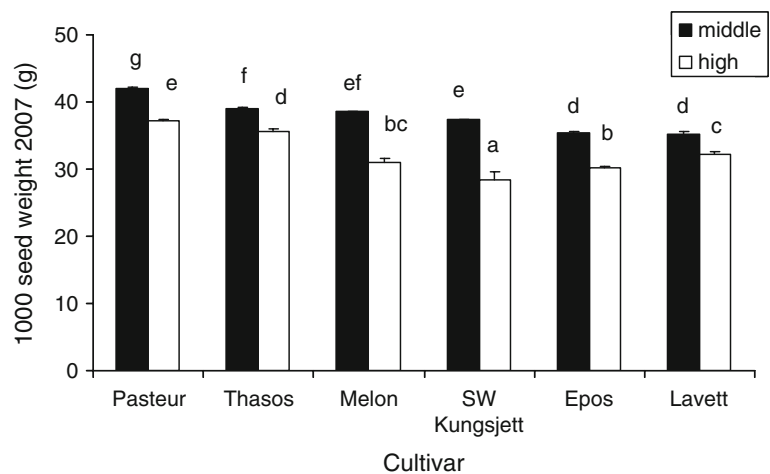
The average emergence in the three experiments was 302 seeds m<sup>2</sup> (76% of sown seeds) in the 2006 field experiment, 164 seeds m<sup>2</sup> (40%) in the 2007 field experiment and 85 seeds per 20 pots (85%) in the 2007 pot experiment. Significant differences in PR/F were found between the six spring wheat cultivars in all three experiments (Fig. 2), revealing on average a high PR/F for WS Kungsjett (0.93) and Lavett (0.79), and a low PR/F for cvs Melon and Epos (0.41 and 0.37), whereas the PR/F for cvs Thasos (0.63) and Pasteur (0.50) were in between. Furthermore, after performing Bartlett's test for homogeneity of variance that showed no significant differences in variance between experiments ( $P = 0.244$ ), analysis of variance showed significant differences between years in the PR/F ( $P < 0.001$ ): the highest PR/F was measured in the 2006 field experiment, whereas in both pot and field experiments in 2007 the PR/F was lower. The cultivar-specific differences in PR/F were robust over the years: the experiment  $\times$  cultivar interaction was not significant ( $P = 0.449$ ) indicating that for the six cultivars, their relative ranking in tolerance was comparable in the three experiments.

### Tolerance for *Fusarium* seedling blight as related to cultivar characteristics

In order to test whether seed size or differences in seed size affected percentage emergence and hence cultivar-specific PR/F, the relationship between PR/F and 1000 seed weights was investigated and was not significant ( $P = 0.67$ ). Also, the difference in 1000 seed weights between seed lots with middle and high *Fusarium* infection levels showed no relationship with the cultivar-specific PR/F ( $P = 0.79$ ).

Next, the relationship between the initial rate of dry matter accumulation and the tolerance for *Fusarium* seedling blight was investigated and was not significant in the 2006 field experiment and in the pot experiment (Table 3). In the 2007 field experiment a

**Fig. 1** 1000 seed weights of cultivars with middle and high *Fusarium* infection levels



weak relationship ( $P=0.07$ , slope=0.03) was found for the PR/F and the measured seedling dry weight, indicating that cultivars with higher initial growth had higher reductions in PR/F.

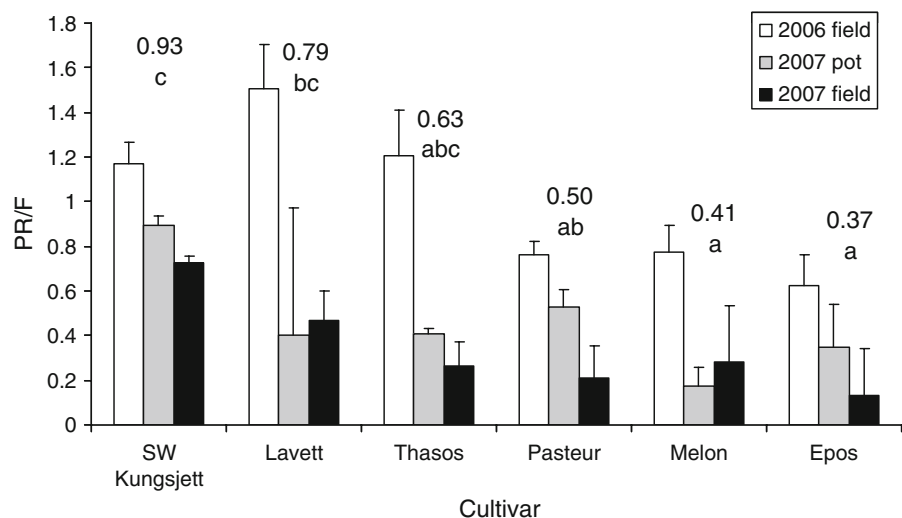
Seedling dry weights in the field experiment in 2006 and in the pot experiment also had no significant relationship with the overall PR/F. The relationship between the seedling dry weight in 2007 and the overall PR/F was significant ( $P=0.02$ , slope=0.04).

Measurements of LI in the young crops had no relationship with PR/F measured in 2006, 2007 or with overall PR/F (Table 4). In the 2007 field experiment a weak relationship ( $P=0.07$ , slope=0.02) was found between PR/F and LI.

#### Weed infestation

In the 2007 field experiment, a high (but not homogenous) level of weed infestation did occur. The weed infestation level differed significantly for the cultivars ( $P=0.014$ , Fig. 3), being highest for Melon and SW Kungsjett (indices of 6.3 and 6.25, respectively) and lowest for cv. Lavett (index 3.5). No significant relationship between PR/F and weed infestation was found. A highly significant relationship ( $P<0.001$ ), with large variation ( $r^2=0.23$ ) was found between reduction in seedling number in each plot and weed infestation (Fig. 4), with a positive slope of 0.16 (SE 0.04), indicating the presence of

**Fig. 2** Differences in PR/F between six spring wheat cultivars in 2006 and 2007 field experiments and in the 2007 pot experiment. Numbers indicate means of three experiments; letters indicate significant differences in means ( $P<0.002$ ). Error bars indicate standard error of the mean. The experiment  $\times$  cultivar interaction was not significant ( $P=0.417$ )



**Table 3** Relationship between measured seedling dry weights and measured PR/F in each experiment and relationship between measured seedling dry weight and overall PR/F

Independent variable Dependent variable	Dry weight 2006		Dry weight 2007		Dry weight pot experiment	
	PR/F 2006	Overall PR/F	PR/F 2007	Overall PR/F	PR/F pot experiment	Overall PR/F
Significance ( <i>P</i> -value)	0.38	0.91	0.07	0.02	0.90	0.57
Slope	NS	NS	0.03	0.04	NS	NS

higher weed infestation in plots with higher reduction in seedling number.

## Discussion

In general, it can be seen from the results presented that the use of spring wheat seeds containing up to 25% *Fusarium* infection can clearly influence the performance of the subsequent crops: in our experiments, plant numbers were reduced by *Fusarium* present on seeds, agreeing with past findings (Gilbert et al. 1997; Bechtel et al. 1985). Interestingly, we showed for the first time that commercially available spring wheat cultivars differed in their tolerance to *Fusarium* seedling blight *in vivo*, and that these differences were robust in three experiments over two years. Lavett, the spring wheat cultivar currently most widely used in organic agriculture in The Netherlands, was one of the two most sensitive to *Fusarium* seedling blight. Between the six cultivars used, differences were of the order of a factor of two, which is quite large. In our study only six genotypes were included, and therefore no general conclusions on seedling blight resistance in spring wheat can be made. However, the results presented here suggest possibilities for future breeding.

The question arises whether it was *Fusarium* that was causing the seeds to be less viable or the effect of seed weight, since seed weights differed significantly between cultivars and treatments and smaller wheat

seeds are known to potentially result in poor stands due to the depletion of seed reserves during the germination process (Bouaziz and Hicks 1990). In principle, such effects may be difficult to separate, since for *F. culmorum* it is known that one effect in the ear is to decrease seed weights (Brennan et al. 2005; Doohan et al. 1999), and that its presence is relatively higher in the lighter seeds of a seed lot (Hare et al. 1999). In our experiments, the seed lot with a high level of *Fusarium* infection had lower seed weights than that with middle and low levels of *Fusarium* infection, and had lower emergence. However, the seeds of treatments with middle and low percentages of *Fusarium* had the same seed weights, and differed in their viability, suggesting that at least some influence on seed viability can be attributed to the effects of *Fusarium*. Interestingly, in our experiments neither 1000 seed weight of the cultivars nor the cultivar-specific difference in 1000 seed weight between the treatments with middle and high *Fusarium* infection levels were related to the differences in tolerance to seedling blight (indicated as difference in PR/F). This indicates that at least the differences in tolerance to seedling blight between the cultivars were not due to smaller seed weight.

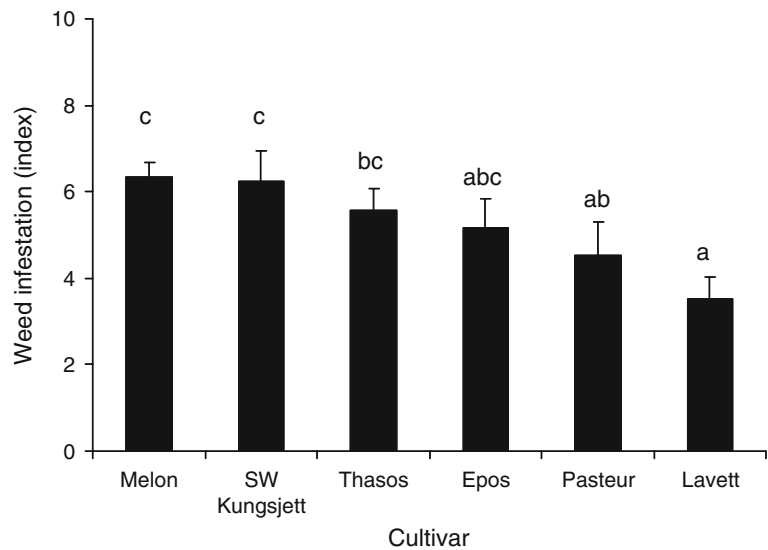
In past experiments, preliminary observations led us to hypothesise that cultivars with faster initial growth were also more tolerant to *Fusarium* and that possibly, the two traits could be linked (Timmermans and Osman 2007). If robust, such a relationship could help in the understanding of the basis of such

**Table 4** Relationship between measured LI and measured PR/F, and relationship between measured LI and overall PR/F in the 2006 and 2007 field experiments

Independent variable Dependent variable	LI 2006		LI 2007	
	PR/F 2006	Overall PR/F	PR/F 2007	Overall PR/F
Significance ( <i>P</i> -value)	0.59	0.72	0.07	0.19
Slope	NS	NS	0.02	NS



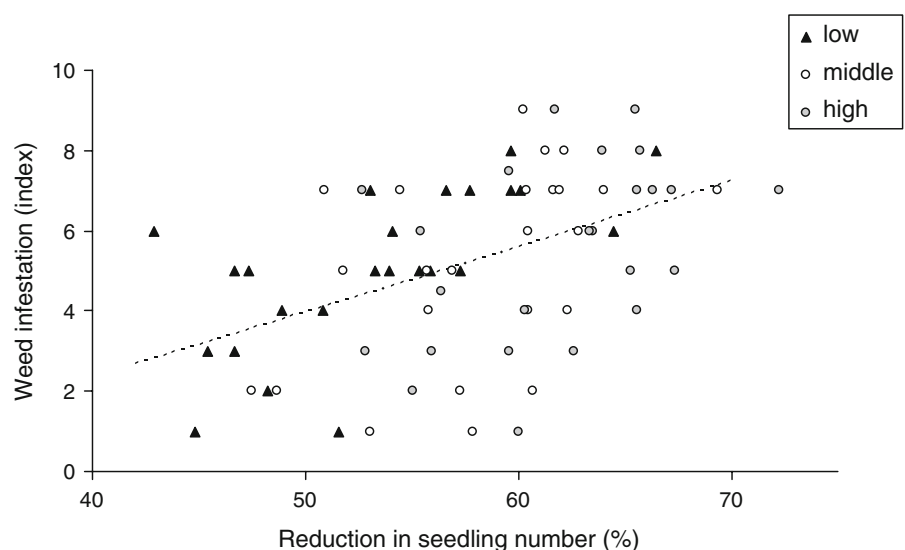
**Fig. 3** Weed infestation index after anthesis for the cultivars. Error bars indicate standard error of the mean, different letters indicate significant differences. Data ( $P=0.014$ ) from the 2007 field experiment



tolerance. Furthermore, breeding for organic wheat cultivars with increased resistance to *Fusarium* seedling blight would be facilitated if the resistance could be linked to a plant trait that is easier to measure than the resistance itself. In the current experiments we did not detect a robust relationship between tolerance and growth rate. Initial growth rates, whether measured as dry matter or LI, were variable for plots, experiments and conditions, and both in the 2006 field experiment and in the pot experiment, no relationship between initial dry matter of the cultivars and tolerance to seedling blight was obtained. Moreover, in the 2007 field experiment a

weak relationship was present and was the converse from our expectation: the cultivars with higher initial growth had lower tolerance to *Fusarium* seedling blight. We can only speculate on the cause of this relationship; it is possible that systemic induced resistance (Van Loon 1997; Heil and Bostock 2002) can result in lower biomass accumulation in young wheat plants (Heil et al. 2000). Another possibility may be that the concentration of mycotoxins, potent inhibitors of protein synthesis, may have been higher in (moderately) resistant cultivars than in susceptible ones, and could have inhibited their growth (Šrobárová and Pavlová 2001).

**Fig. 4** Weed infestation index after anthesis in all treatments plotted against the reduction in seedling number in the 2007 field experiment. Dotted line is the linear regression ( $P<0.001$ ,  $r^2=0.23$ , slope: 0.16 (standard error 0.04)). Different symbols indicate low, middle and high *Fusarium* infection levels



One of the important aspects of tolerance to *Fusarium* seedling blight, especially for organic agriculture, is that it enables better suppression of weeds: for the competitive performance of crops, homogeneous plant densities and a fast canopy closure rate is of high importance (Kruepl et al. 2006, Olsen et al. 2006). The differences in initial plant number resulting from *Fusarium* seedling blight results in a heterogeneous canopy closure in young crops and can enable weeds to settle. In one of the field experiments weed problems occurred, and a significant relationship between seedling reduction and weed infestation was obtained. However, at a cultivar level differences in weed suppression were not related to cultivar differences in *Fusarium* seedling blight. Moreover, Lavett, one of the cultivars least tolerant to seedling blight, was also one of the two cultivars with the lowest weed infestation after anthesis. Therefore in plant breeding it is important to consider the overall performance of a cultivar. The reported differences in tolerance to seedling blight is only one factor that must be considered. Other factors, for example differences in dry matter partitioning to the leaves between spring wheat cultivars, resulting in differences in leaf area ratio or leaf weight ratio (Spitters and Kramer 1986) or differences in canopy architecture (e.g. by early stem elongation), reported to be of importance also for weed competition in barley (Didon 2002), can also be of influence.

It would be interesting to combine tolerance to seedling blight with resistance to FHB. For example, preliminary results of Scholten et al. (2006) have shown that cv. Epos, with the highest tolerance to seedling blight, had a relatively low level of resistance to FHB. If we assume that at least some of the *Fusarium* species that cause FHB also can cause seedling blight, then for cv. Epos, tolerance to seedling blight could be an important trait to prevent emergence problems. Scholten et al. (2006) also showed a high resistance to FHB for cv. Pasteur. This could indicate that for this cultivar, high percentages of infected seed may occur less often and that a high tolerance to seedling blight is not so important. Potentially, there could be cultivars with high resistance to both FHB and seedling blight: Pavlová and Šrobárová (1997) showed that in a group of 21 winter wheat cultivars three with the same common ancestor were both resistant to FHB and tolerant to seedling blight. The type of resistance in

spring wheat cultivars was not yet considered, but could be important in this context, although it was shown that in years with severe disease pressure, resistance to the spread of infection within the head of a plant (Type II resistance) offers little advantage against seedling blight (Argyris et al. 2003).

Following years of widespread FHB epidemics, high seed infection level and subsequent seedling emergence, wheat seeds can be reduced enormously (Jones 1999). We therefore conclude that in wheat breeding, additional to resistance to FHB, tolerance to seedling blight could be considered, especially for organic agriculture, where no alternative measure against seedling blight is available. Differences between the six commercially available cultivars used were present and robust in experiments over two years, indicating good scope for selection. A next step could be to combine data on the level and type of FHB resistance of the cultivars with their tolerance to seedling blight.

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