Variation in *Fusarium graminearum* isolates and their response to a susceptible and resistant winter wheat.

M. Wanjiru Wanyoike and H. Buchenauer

University of Hohenheim, Institute of Phytomedicine, 70593, Stuttgart.
E-mail: Wanyoike@uni-hohenheim.de Fax: 0711-459-2408.

Abstract

Fusarium graminearum causes Fusarium head blight (scab) in wheat and it is of economic importance. Fifteen isolates of *F. graminearum* originating from Germany and the United States of America were examined regarding their cultural characteristics and their virulence on the resistant wheat cultivar 'Arina' and on the susceptible wheat cultivar 'Agent' after a single spikelet inoculation. The variation in cultural characteristics was mainly on the pigmentation on potato dextrose agar (PDA). Sub-culturing the fungal strains on SNA media for six generations or more decreased their ability to sporulate. In the outdoor pot experiments, all the isolates used were pathogenic on both the tested wheat cultivars but varied in their ability to cause scab. The time required for symptoms to appear varied for the various isolates but on average it was longer in the resistant cultivar 'Arina' (5-11 days) than in the susceptible cultivar ''Agent'` (4-9) days. The same trend was found in the movement of symptoms from the inoculated spikelet to the non-inoculated spikelet (10-18 days in 'Arina' and 8-15 days in 'Agent'). However for the trichothecene-nonproducing mutant (isolate GZT40), symptoms never moved to the next spikelet. The sudden brightening of the top half of the spike also varied depending on the isolates and the cultivar. Isolates that were slow in inducing symptoms showed low virulence and vice versa. Head blight rating appeared to be a more stable and reliable parameter of measuring virulence of *Fusarium graminearum* as compared to one thousand corn weight. The experiments demonstrated that scab symptoms move down the rachis and then spread to the corresponding spikelets.

Key words: Fusarium graminearum, virulence, wheat (Triticum aestivum)

Introduction

The fungus *Fusarium graminearum* Schwabe (telemorph *Gibberella zeae* (Schwein.) Petch) has been shown to be the most predominant species causing the *Fusarium* head blight (scab) of wheat (Parry *et al.,* 1995 , Snijders, 1990). *Fusarium* head blight (scab) has attained an overwhelming attention because the fungus causes not only quantitative yield losses and deterioration of the quality of seeds but also produces mycotoxins which contaminate the grains. The mycotoxins (trichothecenes and zearalenon) are toxic to animals and human (Marasas *et al.,* 1984).

This work was undertaken to investigate the variation of virulence of *Fusarium graminearum* isolates using a resistant and a susceptible wheat cultivar. The information obtained will be essential for better understanding of the scab disease and hence its proper management and control.

3.0 Materials and methods

The resistant cultivar ‘Arina’ and the susceptible cultivar ‘Agent’ were grown in outdoor pot experiments. Fifteen isolates were used: 12 collected from Germany and 3 from the USA (a trichodiene synthase negative mutant, the revertant and the wild type isolate). At mid anthesis (Gs 65), a suspension of 100,000 conidia per ml was injected into the cavity between the lemma and palea of the central spikelet of a spike.

Spreading of the disease from the inoculated spikelet was determined by counting the diseased spikelets at different time intervals after inoculation. Disease severity was calculated as the percentage of diseased spikelets per spike. An area under the Fusarium progress curve (AUFPC) for each plant was derived from the proportion of diseased spikelets in a spike at each observation date (Shaner & Finney, 1977). The time required for appearance of scab symptoms on non-inoculated spikelets was calculated as the average number of days from inoculation to when symptoms appeared on non-inoculated spikelets.

To relate spreading of symptoms in the spike to colonisation of the fungus within the spike, single spikelets of the varieties 'Arina' and 'Agent' were inoculated with the various isolates of the fungus. Fifteen days after inoculation, infected spikes were cut from the peduncle, halved, placed on two layers of wet filter paper in petri dishes, and incubated under constant light at room temperature. After 4 days of incubation, the spikes were examined and kept for another 4 days to determine if the characteristic *Fusarium* colour appeared.

Tukeys Studentized range test was used to compare means and test for normality followed by analysis of variance using SAS (Dufner *et al.,* 1992).

Results

Cultural characteristics of isolates. F. graminearum isolates when placed on fresh PDA medium and incubated under near UV light, they covered the agar surface with dense mycelium within 5 - 7 days. There was no significant differences between the isolates in their growth rates. Light reddish pigment or orange colour or yellowish or reddish yellow were observed from the bottom of the petri-dishes, which later changed colour over time - a characteristic of *Fusarium* isolates. But this character was not found to be stable for each particular isolate. Subculturing the fungal strains on SNA media for 6 times or more decreased their ability to sporulate. However, since it was not clear whether this would affect the virulence of the isolates in outdoor pot experiments, first generations were used for inoculations.

Virulence of the F. gramineaarum isolates. Results of the studies over two years show that all the isolates used were pathogenic on both wheat cultivars tested. However their virulence differed significantly (Table 1). Those isolates that were considered to be most virulent in the first year tended to remain in that group also in the second year and

likewise for those considered to be least virulent. The greatest variability was observed in those isolates that showed intermediate virulence.

Table 1. Area under *Fusarium* progress curve (AUFPC) of *Fusarium* head blight for two wheat cultivars inoculated with 15 isolates of *Fusarium graminearum* over two years.

	1997/98		1998/99	
Isolates	'Arina'	'Agent'	'Arina'	'Agent'
GZT40	119 f	--	97 g	108 h
GZ3639	709 abcde	1177 a	434 bcd	556 bc
Fg20.3	742 abcde	1108 ab	285 ef	582 b
Fg16.8	451 def	997 ab	257 f	432 d
Fg8.2	523 cdef	428 d	129 g	279 g
Fg18.7	1045 a	1100 ab	579 a	673 a
Fg27.4	541 cdef	977 ab	508 b	549 bc
Fg25.7	849 abcd	870 abc	305 ef	355 ef
Fg4.3	593 bcde	489 cd	138 g	423 de
Fg69057	944 abc	1024 ab	458 bc	443 d
Fg210	325 ef	701 bcd	233 f	344 fg
GZT12	1001 ab	--	505 b	555 bc
Fg19.2	737	697 bcd	445 bc	494 cd
Fg20.1	--	908 ab	369 cd	672 a
Fg18.9	--	991 ab	360 de	547 bc

Means followed by the same letter in a column are not significantly different (P=0.5) by Tukey test.
-- Not tested

Effect on thousand grain weight. Various isolates reduced significantly the thousand corn weight of the harvested seeds. This was more

apparent in the susceptible cultivar 'Agent' (F, 9 P>F .0001) than in the resistant cultivar 'Arina' (F, 5 P>F .0001).

Symptoms. Disease symptoms caused by the isolate GZT40 differed from those caused by the other isolates. The symptoms on both the resistant and the susceptible cultiva were confined to the inoculated floret and did not spread to non-inoculated florets. A dark brown discoloration could be seen on the inoculated floret, or in some cases, there was only a small spot on the lemma. Seeds did still develop in the inoculated floret and the non-inoculated florets remained green over a long time and there was no sudden desiccation in the spike above the inoculated spikelet.

However, for the other isolates there were some differences in the symptoms caused on the two different wheat genotypes. On the inoculated spikelet of the susceptible cultivar 'Agent' the seeds did not develop at all or when they developed, they were very small, shrivelled, discoloured, malformed and light. These grains currently termed as ''*Fusarium* damaged kernel'' (FDK) or "scabby kernels'' were lost during threshing while those retained contribute to quality losses. About 15 - 28 days after inoculation the spike was often completely blighted with bleached spikelets and a dark brown rachis and culm. On the resistant cultivar 'Arina', no seeds developed at all on the floret which was inoculated but on the opposite side the seeds developed, however they were shrivelled. The spikes slowly blighted up to the time of yellow ripening.

Symptoms on the top half of the spike were usually characterised by blighted spikelets while as the bottom half was characterised with the characteristic water soaked to dark-brown colour. There was sudden blighting of the top half of the susceptible cultivar 'Agent' which was not common on the resistant cultivar. Occasionally on the susceptible cultivar symptoms were observed on the rachis although the corresponding spikelets showed no symptoms, however, sudden desiccation of the whole spike soon followed thereafter. The movement

of symptoms down the rachis was faster in susceptible cultivar than in the resistant cultivar.

When a blighted spike was incubated on wet filter paper in a petri dish for 4 days, mycelium grew abundantly over the bottom half of the spike (from the point of inoculation) but did not grow from the top part of the spike, although this part of the spike was also blighted. The fungus grew out from the spikelets, the rachis and even the culm when these tissues showed disease symptoms, but less mycelium grew out from the infected tissue of the resistant cultivar than from that of the susceptible cultivar.

Time. The time required for the symptoms to appear was longer in the resistant cultivar 'Arina' (5-11 days) than in the susceptible cultivar 'Agent' (4-9 days) (Fig 1). It was evident that for the more virulent isolates (from AUFPC table 1) it took less time for the symptoms to appear than for the less virulent isolates (Fig 1).

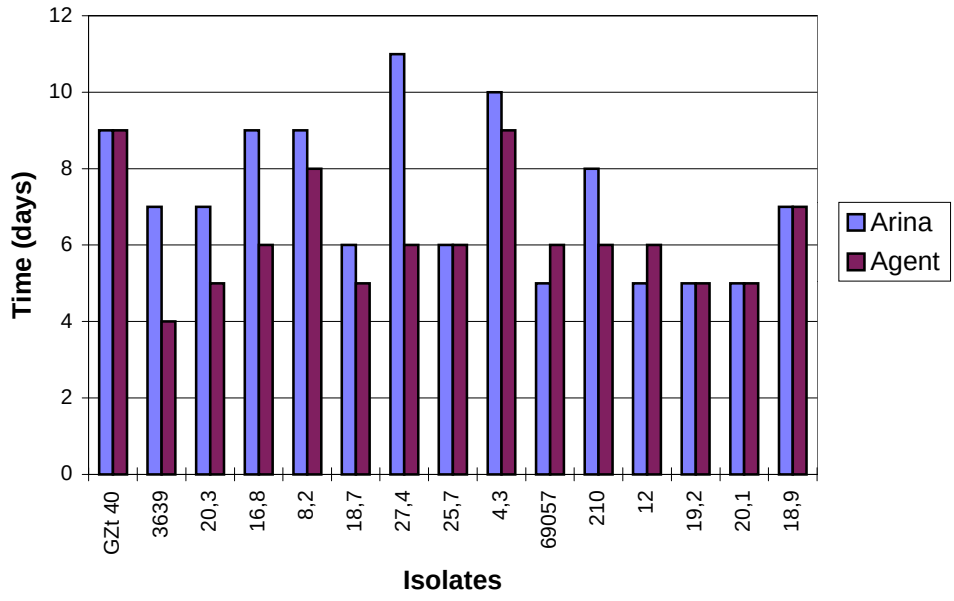
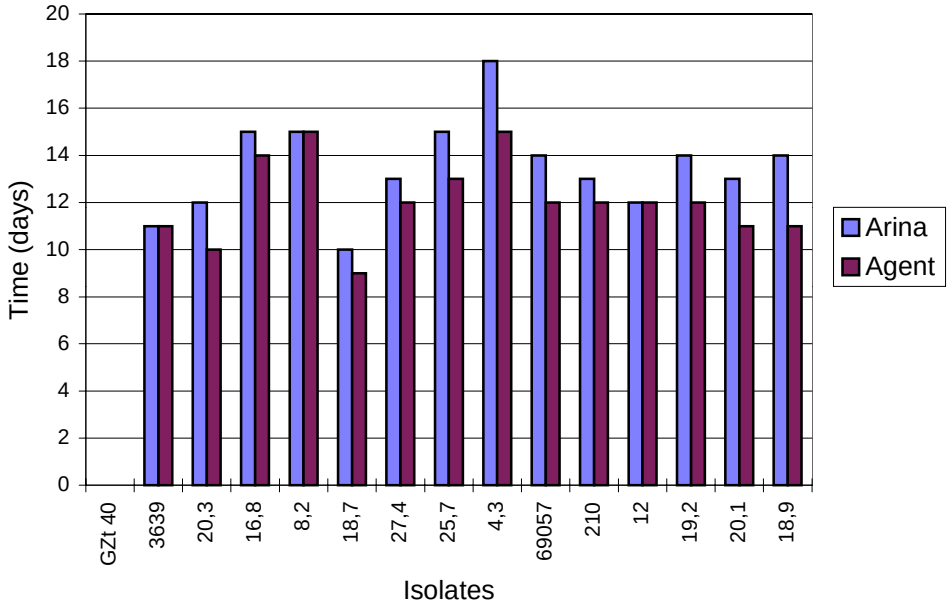


Fig. 1 Time (days) until appearance of scab symptoms on two cultivars of winter wheat cultiva 'Arina' and 'Agent' after single spikelet inoculation with various isolates of *F. graminearum.*

The time required for scab symptoms to appear on non-inoculated spikelets was also longer in the resistant cultivar 'Arina' (10-18 days) than in the susceptible cultivar 'Agent' (9-15 days) (Fig 2). For the



GZT40 isolate, the scab symptoms did not spread to the next spikelet. The proportion of plants that showed spread of disease symptoms from the inoculated spikelet to the other spikelets was low in the resistant cultivar 'Arina' as compared with the susceptible cultivar 'Agent'.

Fig. 2 Time (days) until appearance of scab symptoms on non-inoculated spikelets of two winter wheat cultiva 'Arina' and 'Agent' after single spikelet inoculation with various isolates of. *F. graminearum.*

Discussion

Variation in cultural characteristics is a common phenomenon in the genus *Fusarium* (Oswald, 1949). In this study, isolates showed differences in colour. Subculturing the fungus for 6 times and more on SNA media reduced the ability of the fungus to sporulate. While Bai and Shaner (1996) reported that subculturing *F. graminearum* isolates for 8 times did not decrease the ability of the fungus to cause scab we did not know whether the reduction in spore production would affect their pathogenecity in outdoor pot experiments. Furthermore it has been

reported that older cultures lose their aggressiveness because of mutations during subculturing (Burgess, 1988 and Tu, 1929).

Symptoms on the top half of the spike were usually characterised by blighted spikelets while the bottom half showed characteristic water soaked symptoms to dark-brown colour. This may indicate that scab symptoms spread downward the rachis in both cultiva and then the symptoms spread to the adjacent spikelets. The blighted top symptoms may be due to shortage of water and nutrients resulting from vascular dysfunction, rather than by direct invasion by the fungus (Bai and Shanner, 1996). These results are supported by the fact that mycelium was not found in the top half of the blighted spike after incubation on wet filter paper. The movement of symptoms downward the rachis was faster in the susceptible cultivar 'Agent' than in the resistant cultivar 'Arina'. This may indicate that the resistant cultivar may have some constituents in the spike tissue that suppress the spread of the disease in the primary inoculated spikelet and in the rachis. This corresponds especially with the type II resistance described by Schroeder and Christensen (1963).

The fact that symptoms of the mutant isolate (GZT 40) appeared later in the primary inoculated spikelet and did not spread to other parts of the spike might suggest that DON is less a component of pathogenicity (ability to cause disease) but may be more a component of virulence (extent of disease).

All the *F. graminearum* isolates tested caused head blight symptoms when inoculated on winter wheat at anthesis. However they differed significantly in their virulence on both the resistant and susceptible winter wheat tested. These results indicate yet another factor to be considered in host-pathogen relations.

REFERENCES

Bai GH, Shaner G (1996) Variation in *Fusaruim graminearum* and cultivar resistance to wheat scab. Plant Dis. 80:975-979.

Burgess LW, Klein TA, Bryden WL, Tobin NF (1987) Head blight of wheat caused by *Fusarium graminearum* group I in New South Wales in 1993. Aust. Plant pathol. 16: 72-78.

Dufner J, Jensen U, Schumacher U (1992) Statistik mit SAS. B.G. Teubner. Stuttgart. 396pg.

Marasas WFO, Nelson PE, Toussoun TA (1984) Toxigenic *Fusarium* species. Pennsylvania State University Press, University Park, London.

Oswald JW (1949) Cultural variation, taxonomy and pathogenecity of *Fusarium* species associated with cerial root rots. Phytopathology 39:359-379.

Parry DW, Jenkinson P, Mcleod L (1995) *Fusarium* ear blight (scab) in small grain cereal-a review. Plant Pathology: 44:207-238.

Schroeder HW, Christensen JJ, (1963) Factors affecting resistance to wheat scab caused by *Gibberella zea*. Phytopathology 53:831-838

Shanner G, Finney RE (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67: 1051-1056.

Tu C (1929) Physiologic specialisation in *Fusarium* spp. causing head blight on small grains. Pytopathology 19:143-154.