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Examinations of Take-all, Foot - rot and Sharp eyespot diseases of winter wheat, their causal organisms and possibilities for monitoring symptomless infections

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Cereals are of major importance in Hungary; wheat and maize are the the two principal crops. Although there are excellent Hungarian wheat varieties available which are grown on approximately 1.0 million ha, there are no satisfactory and effective resistance sources against foot-rot diseases caused by *Fusarium* species among current breeding lines. In spite of their widespread occurrence in Hungary, stem-base diseases have not generally been considered sufficiently important to warrant detailed investigation. However, depending on season and climate factors, losses may reach 20 to 40 per cent in Hungary (12).

In the early 1960's, although the importance of *Bipolaris sorokiniana* (= *Helminthosporium sativum*) was stressed nationally, imported wheat varieties were mainly damaged by eyespot disease (*Pseudocercosporella herpotrichoides*) (5, 6).

There were heavy epidemics of *Fusarium* ear diseases during the early 1970's, causing serious mycotoxicosis outbreaks. Since then these pathogens have remained important especially when weather conditions are wet during anthesis.

Kükedi & Szabóné (12) showed that yield losses due to take-all (*Gaeumannomyces graminis* var. *tritici*) might reach 50 per cent. Losses are higher if irrigation has been applied in the absence of any fungicide sprays.

Although since the middle of the 1980's in Hungary there have been frequent drought conditions during the heading stage of wheat growth, the significance of stem-base diseases has increased, especially as caused by *Fusarium* species.

During the years 1988/89 - 1990/91 crops were examined for the frequency of disease, the role of crop rotation and the effects of stem-

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base diseases on quality and quantity of yields in Hungary. The role of previous plants was negligible on the incidence of *Fusarium* species but the infection rate was significantly higher of *Gaeumannomyces graminis* (8 - 11 %) and *Pseudocercosporella herpotrichoides* (10 - 18 %) if grain preplant was followed by wheat (1).

A large number of *Fusarium* species can occur on wheat. Nierenberg (14) listed 18 *Fusarium* spp.: *Fusarium acuminatum, F. anthophilum, F. avenaceum* var. *avenaceum, F. culmorum, F. dimerum, F. equiseti* var. *equiseti, F. graminearum, F. sacchari* var. *subglutinans, F. merismoides* var. *merismoides, F. nivale* var. *nivale* and var. *majus, F. oxysporum* var. *oxysporum, F. poae, F. sambucinum* var. *sambucinum, F. solani* var. *solani, F. sporotrichoides* var. *sporotrichoides, F. tricinctum* and *F. verticilliodes.* Burgess *et al.* (2) described a further *Fusarium* species which may contribute to cereal diseases: *Fusarium crookwellense* Burgess, Nelson & Toussoun. All *Fusarium* species can be seed-borne and cause pre- and post-emergence seedling disease (9, 10). The symptoms, which can range from death soon after germination to superficial stem lesions on the emerged plants, are similar for all the pathogens.

Colhoun & Park (4) do not regarded *Fusarium poae* as a pathogen of seedlings. They found that *F. graminearum* and *F. culmorum,* causing pre- and post-emergence death, was most severe in dry soils and increased with increasing temperature (in the range 8 - 23 ºC). *Fusarium culmorum* and *F. graminearum* can cause brown lesions on stems without causing plant death, but under favourable conditions cause post-emergence death about 14 days after sowing. Seeds infected with *Fusarium nivale* result in severe pre-emergence death (up to 80 per cent) often after the germination of seed. Later symptoms include a browning of the coleoptile which may be deformed and, in contrast to the other *Fusarium* species, the first leaf may be attacked causing it to lie on the soil surface. The first and second leaves may also bear lens-shaped pale brown or auburn lesions.

The principal sources of *Fusarium* diseases of cereals are seed and soil. Seed is commonly affected by *Fusarium,* though normally at low levels, and is an obvious means of spreading the disease from one place to another. Because *Fusarium* species are often pluryvorous and can survive on plant debris and organic matter, the role of plant rotation is of fundamental importance. Hewett (10) points out a difficulty in testing naturally infected seed samples, in the field since both *Fusarium* spp. and *Septoria nodorum* are usually present (the latter often more common) and cause similar emergence problems.

Colhoun (3) recorded yield losses of up to 18 and 20 per cent in plots when plants were grown from seed inoculated with *F. nivale* and *F. culmorum* respectively. Rennie *et al.* (16) found that seed with high levels of *F. nivale* infection had higher levels of seedling infection than untreated seed, though emergence was not affected. According to Perry's (15) experiments with artificially infected seeds, there was no effect on emergence, crop growth or grain yield. Seed treatments give some control of seed-borne and soil-borne *Fusaria.*

Some reports do not consider seed treatments necessary for certified seed sown to produce a non - seed crop, unless loose smut is known to be present (17).

Recently, reports of symptomless infection and the detection of systemic stem vascular colonisation by *Fusarium* species have been made. Richardson *et al.* (18) used the single tiller method in winter wheat to relate dark lesions on sheaths just above the nodes, normally associated with *Fusarium nivale,* to yield. Disease assessments were made just after ear emergence from the sheaths. Richardson *et al.* (18) found, from isolations of stem-base tissues, that *F. avenaceum* and/or *F. culmorum* were also present in affected stems. Some "healthy" stems yielded *Fusarium* spp. indicating that some infections were not causing symptoms at the time of assessment. No lodging or whiteheads were associated with the lesions but the affected tillers yielded 20 per cent less than the unaffected tillers, with 75 % of the loss due to a reduction in seed number and 25 % to seed size.

Hutcheon & Jordan (11) found that infection of wheat ears through systemic colonisation of stem vascular tissues by *Fusarium avenaceum, F. culmorum, F. graminearum* and *F. nivale* resulted in significant reductions in grain number, thousand grain weight and yield losses of between 5 % and 19 %, all in the absence of visible ear symptoms.

Materials and methods

1. Isolation of stem-base disease pathogens from wheat plant debris collected in Western Hungary

Plant samples (stem pieces with roots) with visible stem base symptoms were collected from different areas of Western Hungary (Table 1).

Sites used for the collection of diseased plant material

Table 1

Samples	Area of collection
I.	Lovasberény Felső
II.	Lovasberény Alsó
III.	Váli Erdő
IV.	Alcsút (near 811 Road)

The samples were sterilized in 5 per cent NaOCl solution for 15 minutes and after rinsing with sterilized tap water put into Petri dishes containing wetted filter papers in a dark incubator of 25 ºC temperature for 10 days. The grown fungi were transferred into potato -dextrose - agar (PDA) plates to get pure cultures. Pure fungal isolates were transferred to agar plates with low nutrient content (SNA medium) to improve sporulation. These cultures were grown according to the method of Nierenberg (13, 14) in an incubator with continuous black light at 17 ºC for 14 days.

2. Seed pathological assay

Four seed samples, of UK origin, heavily infected with *Fusarium*, together with a sample of certified seed, were tested to determine the extent of seed infection caused by *Fusarium* species. The grain was harvested from different areas of the UK. Information supplied with the seed samples is shown in the Table 2.

The seed was surface sterilized in 70 % ethanol solution and after flamming, 20 seeds were placed on SNA medium. Five replicates of each sample were examined. Incubation was in the dark at 25 ºC for 12 days. Identification of *Fusarium* species was based on Nierenberg`s (14) methods, using morphological characteristics.

3. Effects of seed dressing on emergence, vigour and early symptoms of Fusarium diseases

To test the efficacy and phytotoxic effects of the selected fungicides, five wheat seed samples were examined (see Table 2). Seeds were coated with Baytan Flowable - triadimenol 187.5 FS + fuberidazol 23 or Cerevax - carboxin 360 FS + thiabendazole 25. Seed treatments were carried out in Erlenmeyer flasks with a dosage of 2.5 - 3.0 litre/t seeds. Seventeen seeds of each treatments were sown in pots filled with compost and five replicates were used. Randomized pots were placed under a polythene tunnel with a controlled temperature of

24 - 27 ºC. Germination and seedling vigour was assessed 21 days after sowing.

Seed health data supplied with heavily infected and certified wheat seed

Table 2

Sample	Cultivar	Harvest date	Infection	Amount of disease (%)
I	Maris Huntsman	1992	Certified	-
II	N.A.*	1991	*Fusarium avenaceum*	N.A.*
III	Apollo	1991	*Fusarium nivale*	90
IV	N.A.*	1991	*Fusarium nivale*	34
V	Admiral	N.A.*	*Fusarium nivale*	34-47

* Data not available

4. Monitoring symptomless infections by Fusarium species at an early stage of wheat seedlings development

During the project a method was developed for monitoring symptomless seedlings infection. Seedlings emerging from heavily infected seed samples were gently removed from the soil with roots intact. After thoroughly rinsing the roots in tapwater, leaves were excised near to the leaf sheaths. Surface sterilization of plant material was carried out in two steps involving immersion in 1.5 % NaOH solution for 15 minutes followed by rinsing with sterile distilled water, and immersion in 70 % ethanol solution for 10 minutes.

Plants were placed in sterile Petri dishes and 1 mm segments were excised from roots and from the middle of each leaf sheath. Plant samples were transferred to SNA medium and incubated at 25 ºC for 12 days.

Results

1. Isolation of stem-base disease pathogens from wheat plant debris collected in Western Hungary

The isolated and identified fungi are listed in Table 3.

In many cases a complex of disease infection was observed on the plant material. However, the most frequent occurrence of *Fusarium* species was evident. *Fusarium culmorum* was isolated from three samples; Sample III showed infection of *Fusarium graminearum, F. sporotrichoides* and *Pseudocercosporella herpotrichoides.*

The above results confirm the importance of *Fusarium* species as stem base pathogens on wheat in Western Hungary.

Isolated fungi from infected plant samples from Hungary

Table 3

Fungi	Number of samples
Fusarium culmorum	I, II, IV
Fusarium graminearum	I, III
Fusarium avenaceum	II
Fusarium sporotrichoides	I, III
Pseudocercosporella herpotrichoides	III
Bipolaris sorokiniana	II
Rhizoctonia solani	IV

2. Seed pathological assay

The results of the seed health test are shown in Table 4.

These observations suggest that the seed samples, including the certified seed, were heavily infected by *Fusarium* species. Some samples were contaminated more than one *Fusarium* species.

The generally accepted advice for the usage of *Fusarium* infected seeds is:

when seed contamination rises to 10 per cent a fungicide seed treatment is advisable,
when infection is 10 - 20 % seed dressing is essential and
when infection is more than 20 % seed should not be used as planting material.

Experimentally derived seed health data of four contaminated and one certified sample of wheat seed

Table 4

Fusarium species		Sample number	Infection rate (%)
Fusarium	*avenaceum*	II	17
F.	*avenaceum*	IV	3
F.	*nivale*	III	86
F.	*nivale*	IV	36
F.	*nivale*	V	42
F.	*nivale*	I	4
F.	*culmorum*	I	3
F.	*culmorum*	V	5

3. Effects of seed dressing on emergence, vigour and early symptoms of Fusarium diseases

Results are shown in Table 5.

The effect of fungicide treatment on emergence and vigour of seedlings arising from *Fusarium* infected wheat seed

Table 5

Sample number	Control		Baytan Flowable		Cerevax	
	Healthy	Disordered	Healthy	Disordered	Healthy	Disordered
I	94.1	1.2	37.7	3.5	49.4	2.4
II	81.2	5.9	56.5	7.1	81.2	5.9
III	84.7	3.5	38.8	24.7	76.5	4.7
IV	82.4	2.4	44.7	14.1	80.0	5.9
V	42.4	8.3	25.9	15.3	38.8	8.3

The seedling emergence data showed that both fungicide treatments decreased the vigour of seedlings. Baytan Flowable caused an approximately ten day delay in rate of emergence. The number of normal seedlings was also decreased with a significant growth rate reduction observed: plant height being only 1/3 of control values. Furthermore, leaf necrosis symptoms were evident on leaves; an indication of heavy phytotoxic effects.

Some phytotoxic effects could be observed from the Cerevax treatments but these were less severe than with the Baytan trial. There was (approximately) a one week delay in emergence; however after three weeks this delay was no longer evident. Untreated seedlings had no symptoms of phytotoxicity. The introduction of the product Baytan in the late 1970's offered improved control of *Fusarium* diseases. However Garmashov *et al.* (8) reported phytotoxic side effects of Baytan. Furthermore, observations on resistance of some *Fusarium* strains against MBC (methyl benzimidazole carbamate) type fungicides (benomyl, carbendazim, fuberidazole, which is a component of Baytan) have been made.

It is considered necessary to make further trials to determine the possible cause(s) of the phytotoxic effects observed which may have resulted from fungicide overdosage, high soil and/or air temperature and/or differences in susceptibility of wheat varieties.

4. Monitoring symptomless infections by Fusarium species at an early stage of wheat seedlings development

Results indicated that not only the symptom-bearing seedlings were infected but 50 % of healthy seedlings were also infected with one or two *Fusarium* species. *Fusarium nivale* and *F. avenaceum* were isolated from different exceeded segments of wheat seedlings.

Summary

The significance of stem-base diseases has increased, especially as caused by *Fusarium* species. Plant samples with visible stem-base symptoms were collected from different areas of Western Hungary. We isolated and identified four *Fusarium* species (*F. culmorum, F. graminearum, F. avenaceum, F. sporotrichoides*), *Pseudocercosporella herpotrichoides, Bipolaris sorokiniana* and *Rhizoctonia solani.* In many cases a complex of disease infection was observed on the plant material. However, the most frequent occurrence of *Fusarium* species was evident and it was confirmed the importance of *Fusarium* species as stem-base pathogens on wheat. Four seed samples, of UK origin, heavily infected with *Fusarium,* together with a sample of certified seed, were tested in seed pathological assay to determine the extent of seed infection caused by *Fusarium* species.These observations suggest that the seed samples, including the certified seed, were heavily infected by *Fusarium* species. Some samples were contaminated more than one *Fusarium* species. To test the efficacy and phytotoxic effects of two selected fungicides, Baytan Flowable and Cerevax were examined on five wheat seed samples.The seedling emergence data showed that both fungicide treatments decreased the vigour of seedlings. Baytan Flowable caused an approximately ten day delay in rate of emergence. The number of normally seedlings was also decreased with a significant growth rate reduction observed: plant height being only 1/3 of control values. Furthermore, leaf necrosis symptoms were evident on leaves; an indication of heavy phytotoxic effects.Some phytotoxic effects could be observed from the Cerevax treatments but these were less severe than with the Baytan trial.A method was developed for monitoring symptomless seedling infection. Results indicated that not only the symptom - bearing seedlings were infected but 50 % of healthy seedlings were also infected with one or two *Fusarium* species.

Összefoglalás

A szártőbetegségek jelentősége - különösen a *Fusarium* fajok által okozottaké - megnövekedett. Tüneteket mutató őszi búza növényi szártő-mintákat gyűjtöttünk Nyugat-Magyarország különböző területeiről. Négy *Fusarium* fajt (*F. culmorum, F. graminearum, F.*

avenaceum, F. sporotrichoides), a *Pseudocercosporella herpotrichoides*-t, *Bipolaris sorokiniana*-t, valamint a *Rhizoctonia solani* fajokat izoláltuk és identifikáltuk. A mintákon sok esetben komplex fertőzés volt megfigyelhető. A leggyakoribb a *Fusarium* fajok károsítása volt, amely igazolja a búza szártőbetegségek előidézésében betöltött jelentőségüket. Négy - az Egyesült Királyságból származó, erős *Fusarium* fertőzöttségű, valamint egy fémzárolt - magtétel kórtani vizsgálata során meghatá: ,ztuk a magvak *Fusarium* fertőzöttségét. Ezek a megfigyelések bizonyítják, hogy a magtételek - ide értve a fémzároltat is - erős *Fusarium* fertőzöttségűek voltak. Néhány mintánál egynél több *Fusarium* faj is okozott fertőzést. Két kiválasztott fungicid: a Baytan Flowable és a Cerevax hatékonyságát és fitotoxicitását öt búzafajta magvain teszteltük. A csíranövények kelési adatai azt mutatják, hogy mindkét fungicid kezelés csökkentette a csíranövények vigorát. A Baytan Flowable mintegy tíz napos késést okozott a kelésben. A normális csíranövények száma szintén lecsökkent és jelentős növekedési ráta-csökkenés volt megfigyelhető: a csíranövények magassága csak 1/3-a volt a kontrollénak. Ezen túlmenően nekrotikus levéltünetek voltak megfigyelhetők, melyek súlyos fitotoxikus hatás jelzői. Kisebb fitotoxikus hatást a Cerevax kezeléseknél is meg lehetett figyelni, de ez sokkal kevésbé volt súlyos, mint a Baytan kísérletben. Módszert fejlesztettünk ki a tünetmentes csíranövények látens fertőzésének ellenőrzésére. Az eredmények azt mutatják, hogy nem csupán a tüneteket mutató csíranövények voltak fertőzöttek, hanem az egészségesek mintegy 50 %-a is fertőzött volt egy vagy két *Fusarium* fajjal.

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