University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

SCANNING ELECTRON MICROSCOPY OF *FUSARIUM CULMORUM*-INFECTED KERNELS OF ANCIENT WHEAT SPECIES

D. Packa, T. Kulik and M. Hościk

Abstract

Kernels of ancient hulled wheat species, *Triticum monococcum, T. dicoccon, T. spelta,* and threshable wheat species, *T. polonicum, T. turanicum* ('Kamut®'), were studied. *Fusarium culmorum*-infected kernels, classified as FDKs (*Fusarium* damaged kernels), were smaller, shrunken, wrinkled and lighter in colour than healthy kernels. The seed coats of the former were usually damaged, and white or pinkish-white mycelium formed on their surface. FDKs showed different fungal infection levels. Characteristic structural changes were noted in endosperm cells, including loosely arranged starch granules, a partial or even complete absence of the protein matrix enveloping starch granules, the disappearance of small starch granules and the presence of damaged large starch granules. The lowest degree of damage was observed in emmer and spelt kernels.

Key words: *Fusarium culmorum, Triticum* spp., infected kernels, SEM

Introduction

The ancient forms of wheat provide valuable breeding material due to their high quality and resistance traits. Under natural infection conditions, they are infected by *Fusarium* pathogens to a lower degree than common wheat, and smaller amounts of toxins accumulate in their grain (Suchowilska et al. 2010, Wiwart et al. 2011). Following artificial inoculation, symptoms typical of *Fusarium* infection can be observed on spikelets and kernels. The infection and colonization of host plants by necrotrophic pathogens of the genus *Fusarium* involves the production of extracellular hydrolytic enzymes and phytotoxic compounds. *Fusarium culmorum,* one of the causal agents of Fusarium head blight (FHB) in north-eastern Poland, is

Phytopathologia 63: 7–19

ISSN 2081-1756

capable of producing cell wall degrading enzymes – cellulases, xylanases and pectinases, as well as proteases and amylases that break down storage compounds in the endosperm of infected wheat kernels (Kang and Buchenauer 2000). The consequent damage to the endosperm may be observed using a scanning electron microscope (SEM). Damage to common wheat kernels, in particular endosperm cells, caused by *F. culmorum* is well documented in literature (Meyer et al. 1986, Jackowiak et al. 2005). A similar type of kernel damage occurs also in the endosperms of barley and wheat infected by *F. graminearum* (Nightingale et al. 1999, Schwarz 2003) and winter triticale infected by *F. culmorum* (Packa et al. 2008). A different type of damage is observed in oat endosperm cells which are composed of compound starch granules (Packa 2005). Having collected extensive experimental materials, we launched a study to investigate and document damage to the kernels of ancient hulled wheats – *T. monococcum, T. dicoccon, T. spelta* and naked, threshable wheats – *T. polonicum, T. turanicum* ('Kamut®'), resulting from *F. culmorum* infection.

Materials and methods

The experimental plant materials comprised three spring hulled wheat species: *Triticum monococcum* (2n = 14), *T. dicoccon* (2n = 28) and *T. spelta* (2n = 42), and two spring threshable wheat species: *T. polonicum* (2n = 28) and *T. turanicum* (2n = 28). The hulled wheats *T. monococcum* (einkorn), *T. dicoccon* (emmer), *T. spelta* (spelt) and the threshable wheat *T. polonicum* (Polish wheat species) were represented by three accessions each. *Triticum turanicum* was represented by one cultivar, 'Kamut®' (King Tut's wheat). The common wheat *T. aestivum* (2n = 42) was represented by two cultivars, 'Parabola' (group A, quality wheat) and 'Frontana' (used as a Fusarium head blight resistance source). The studied wheat species and accessions are presented in Table 1. Kernel samples were collected from a field experiment conducted at the Experimental Station of the University of Warmia and Mazury in Bałcyny near Ostróda (53°36'N, 19°51'E). Single spikelets used as sowing materials were sown manually at 10 × 20 cm in plots of 6 m^2. No chemical plant protection was applied. The infection material was prepared from DON producing *F. culmorum* isolate originating from the author's own collection. The *F. culmorum* isolate was grown at 22°C on PDA medium for 14 days. Inoculation was carried out by spraying an aqueous spore suspension of 5×10^5 per 1 ml onto the heads at the full flowering stage (65 BBCH). Inoculation of all tested accessions was carried out from 28 June to 10 July 2008. The weather conditions during flowering, ripening and harvest of spring wheat in the growing season 2008 are shown in Figure 1. Inoculation was performed before the evening, twice within two consecutive days using knapsack sprayer with 2 × 600 per 1 ml of spore suspension on each plot. Non-inoculated plants served as control. The presence of *F. culmorum* in infected kernels was confirmed with the use of species-specific molecular markers (Kulik and Hościk, unpublished data). FDKs and control kernels showing no symptoms of infection were examined under a scanning microscope. The

Table 1

Wheat species and accessions

Accession	Species	Accession No.	Origin
K-1	*Triticum monococcum*	PL 020790	NCPGR
K-15	*Triticum monococcum*	PI 584654	NGRL
'Terzino'	*Triticum monococcum* einkorn, German cultivar, organically-grown grain		
K-25	*Triticum dicoccon*	PI 191390	NGRL
K-30	*Triticum dicoccon*	PI 94621	NGRL
K-46	*Triticum dicoccon*	TRI 18117	IPK
K-18	*Triticum spelta*	TRI 17506	IPK
K-19	*Triticum spelta*	TRI 17513	IPK
K-21	*Triticum spelta*	TRI 982	IPK
Pol-2	*Triticum polonicum*	PL 21802	NCPGR
Pol-4	*Triticum polonicum*	PL 22479	NCPGR
Pol-6	*Triticum polonicum*	PL 20770	NCPGR
'Kamut®'	*Triticum turanicum* grain purchased in Austria		
'Parabola'	*Triticum aestivum*	Cultivar	PBC
'Frontana'	*Triticum aestivum*	Cultivar	PBAI

NCPGR – National Centre for Plant Genetic Resources (Radzików, Poland), NGRL – National Germplasm Resources Laboratory (Beltsville, USA), IPK – The Leibniz Institute of Plant Genetics and Crop Plant Research (Gatersleben, Germany), PBC – Plant Breeding Company (Strzelce, Poland), PBAI – Plant Breeding and Acclimatization Institute (Radzików, Poland).

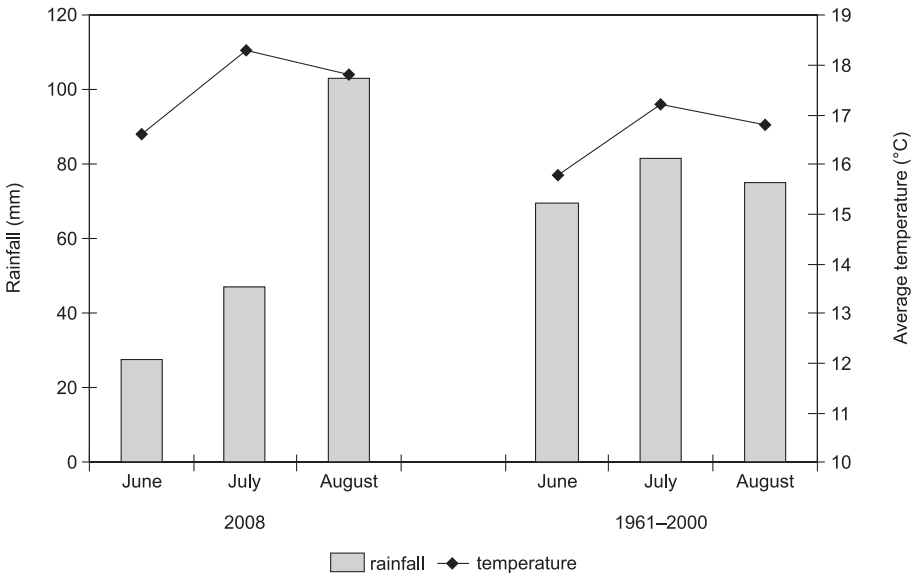
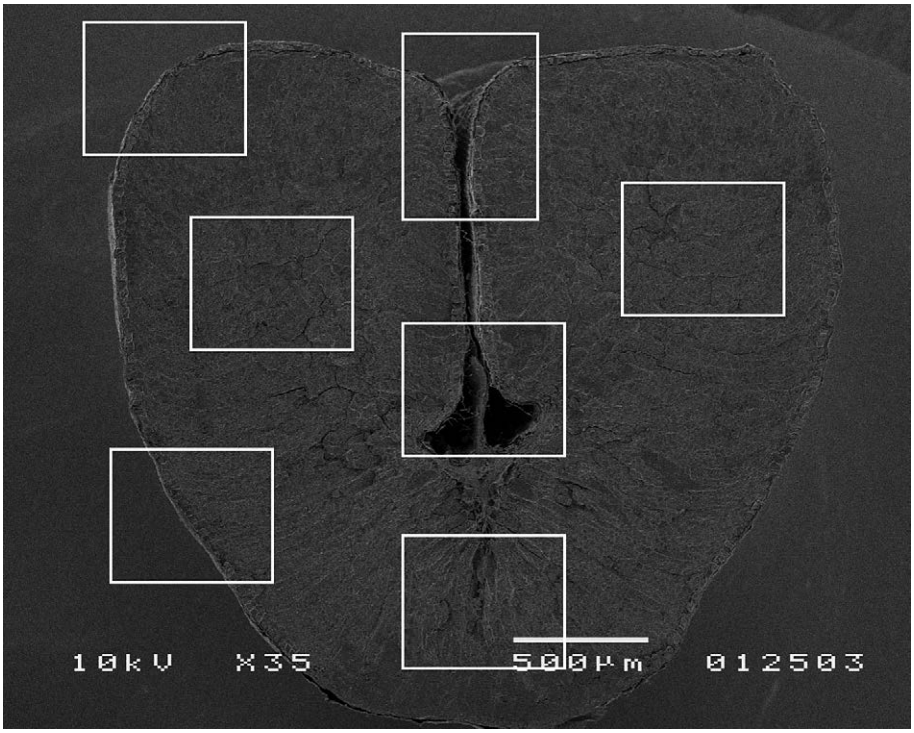


Fig. 1. Weather conditions during flowering, ripening and harvest of spring wheat in the growing season 2008 in Bałcyny

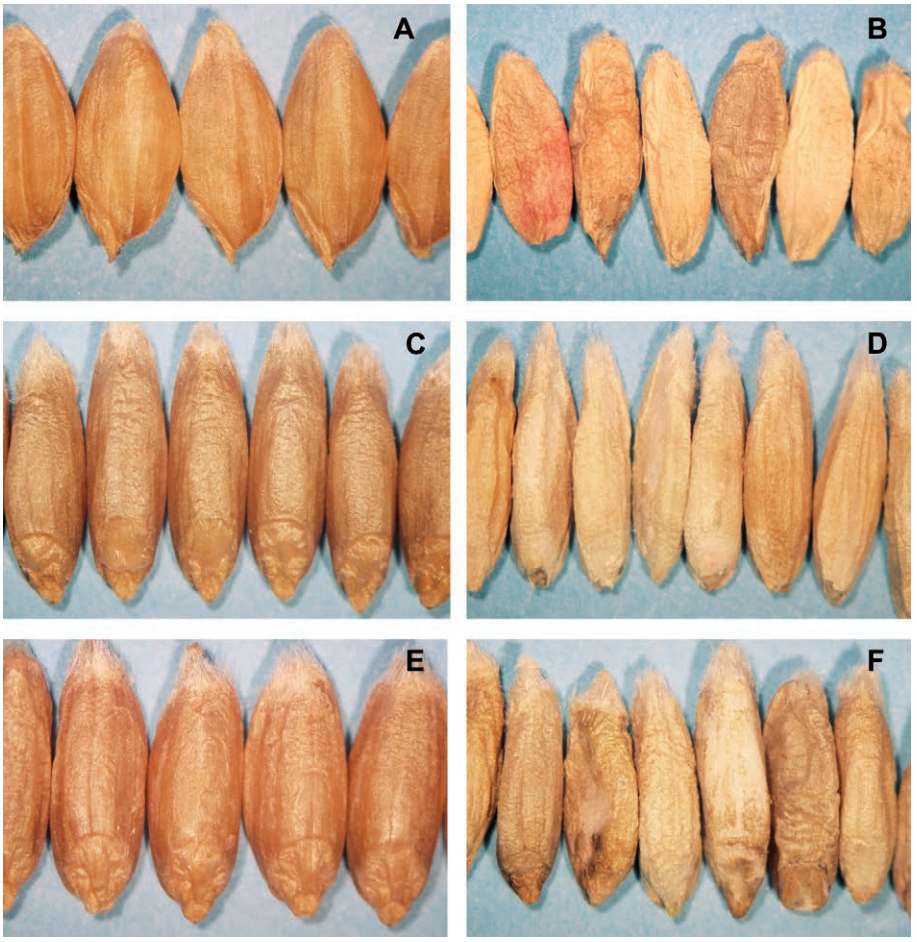


Phot. 1. Examination sites of cross-sections of ancient wheat kernels (photo by D. Packa)

cross-sections of kernels were analysed to determine the most common locations of mycelium (at the base and along the crease, in the seed coat, between the seed coat and the endosperm) and endosperm microstructure (Phot. 1).

Results

Fusarium culmorum-infected kernels, classified as FDKs (*Fusarium* damaged kernels), were smaller, shrunken, wrinkled and lighter in color than healthy kernels. The seed coats of the former were usually damaged, and white or pinkish-white mycelium formed on their surface (Phots. 2 and 3). The kernels of all studied accessions from the FDK fraction had deformed structure (Phots. 4 and 5). The *F. culmorum* mycelium was most frequently found at the base and along the crease, in the seed coat, between the seed coat and the endosperm (Phot. 6). FDKs showed different fungal infection levels, from the colonization of the seed coat to the invasion of endosperm cells. Characteristic structural changes were noted in endosperm cells, including loosely arranged starch granules, a partial or even complete absence of the protein matrix enveloping starch granules, the disappearance of

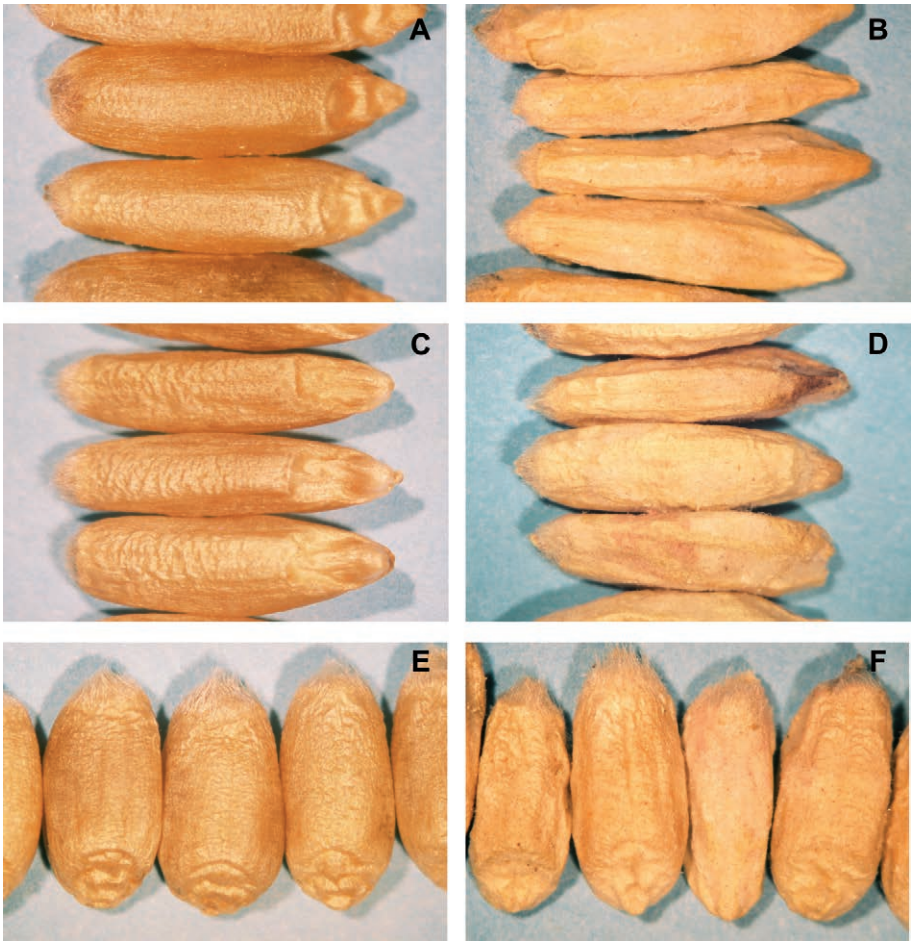


Phot. 2. Healthy and *Fusarium culmorum*-infected kernels of hulled wheats. A, C, E – healthy kernels, B, D, F – *Fusarium*-damaged kernels. A, B – kernels of *Triticum monococcum* K-1, C, D – kernels of *T. dicoccon* K-30, E, F – kernels of *T. spelta* K-18. All photographs are in the same scale. Nikon SMZ-2T stereo microscope (photo by D. Packa)

small starch granules and the presence of damaged large starch granules (Phots. 7 and 8). The lowest degree of damage was observed in emmer and spelt kernels.

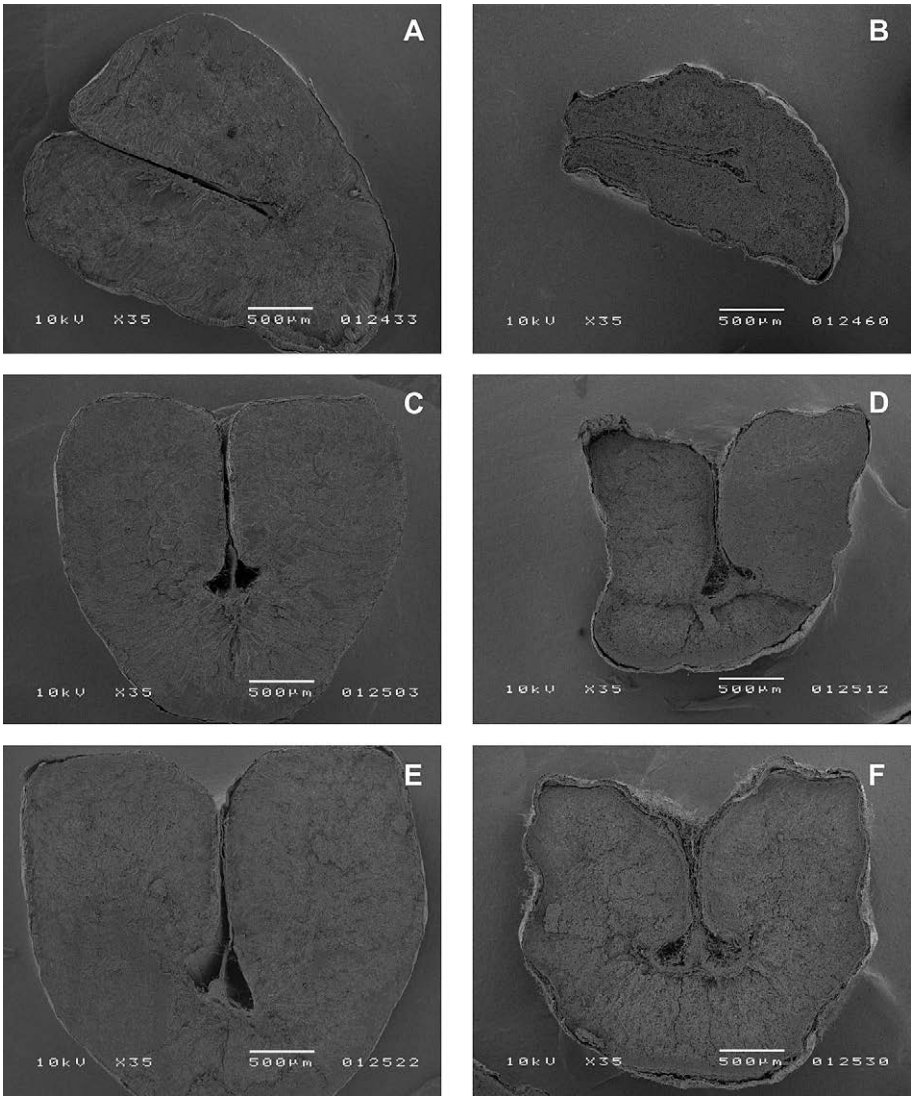
Discussion

In cereal crops, FHB (Fusarium head blight) develops as a result of flower infection by pathogens of the genus *Fusarium*. The spikes in the flowering stage are at the highest risk of infection, but infection also occurs in the later stages of kernel development. Infections that occur during the flowering stage are most dangerous



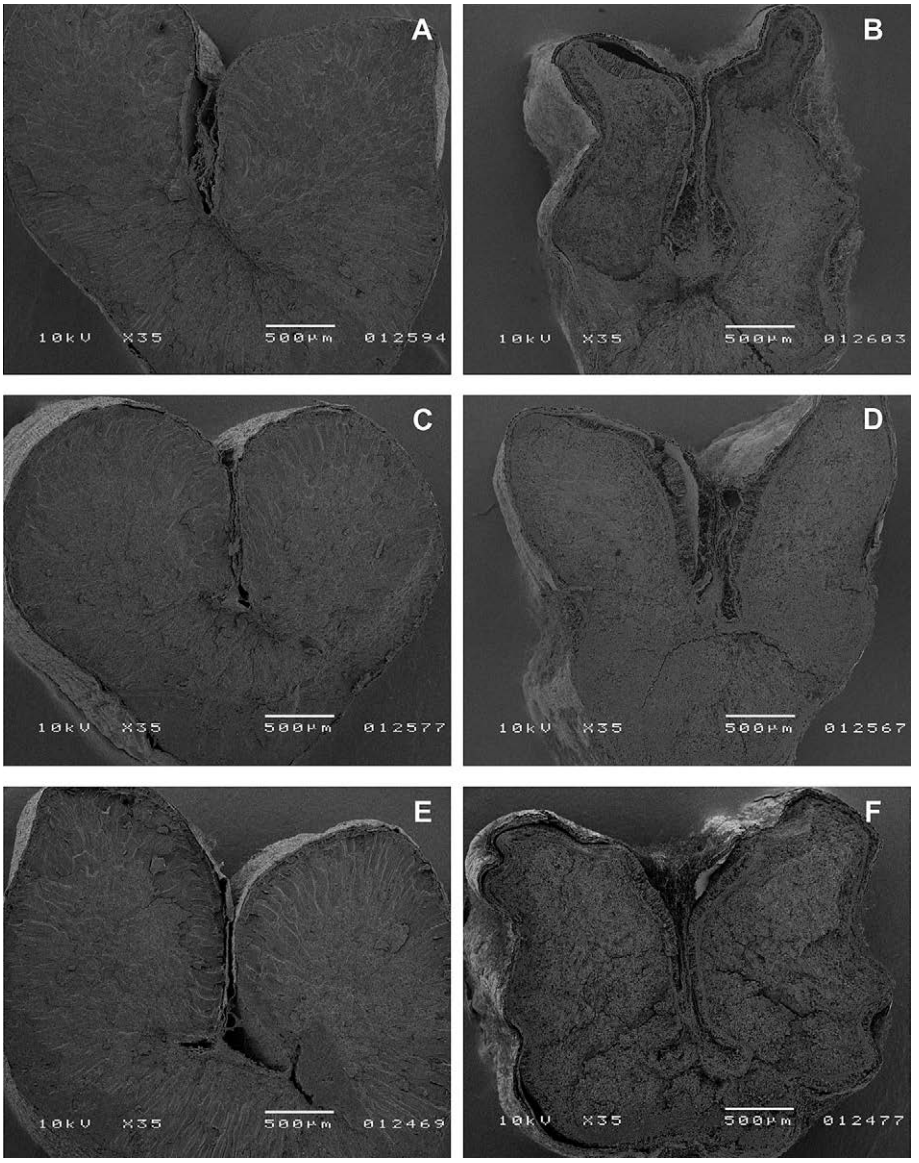
Phot. 3. Healthy and *Fusarium culmorum*-infected kernels of threshable wheats. A, C, E – healthy kernels, B, D, F – *Fusarium*-damaged kernels. A, B – kernels of cv. 'Kamut®', C, D – kernels of *Triticum polonicum* Pol-6, E, F – kernels of *T. aestivum* cv. 'Parabola'. All photographs are in the same scale. Nikon SMZ-2T stereo microscope (photo by D. Packa)

to plants (Parry et al. 1995). Microscopic examinations of different cereal species supported the identification of *Fusarium* hyphae on the surface and inside kernels. In the FDKs of the studied wheats, hyphae were found over the entire surface of kernels, inside the seed coat, between the seed coat and the endosperm, and in the inner endosperm layers of severely infected kernels. Similar locations of *Fusarium* hyphae have been reported for other cereal species, including winter wheat (Srobar and Srobarova 1978, Srobarova 1987, Chełkowski et al. 1990), spring wheat (Jackowiak et al. 2005), winter triticale (Koczowska and Packa 1993, Packa et al. 2008) and oat (Packa 2005). SEM enabled to visualize endosperm ultrastructure in ancient wheat kernels which accumulate larger amounts of protein than common



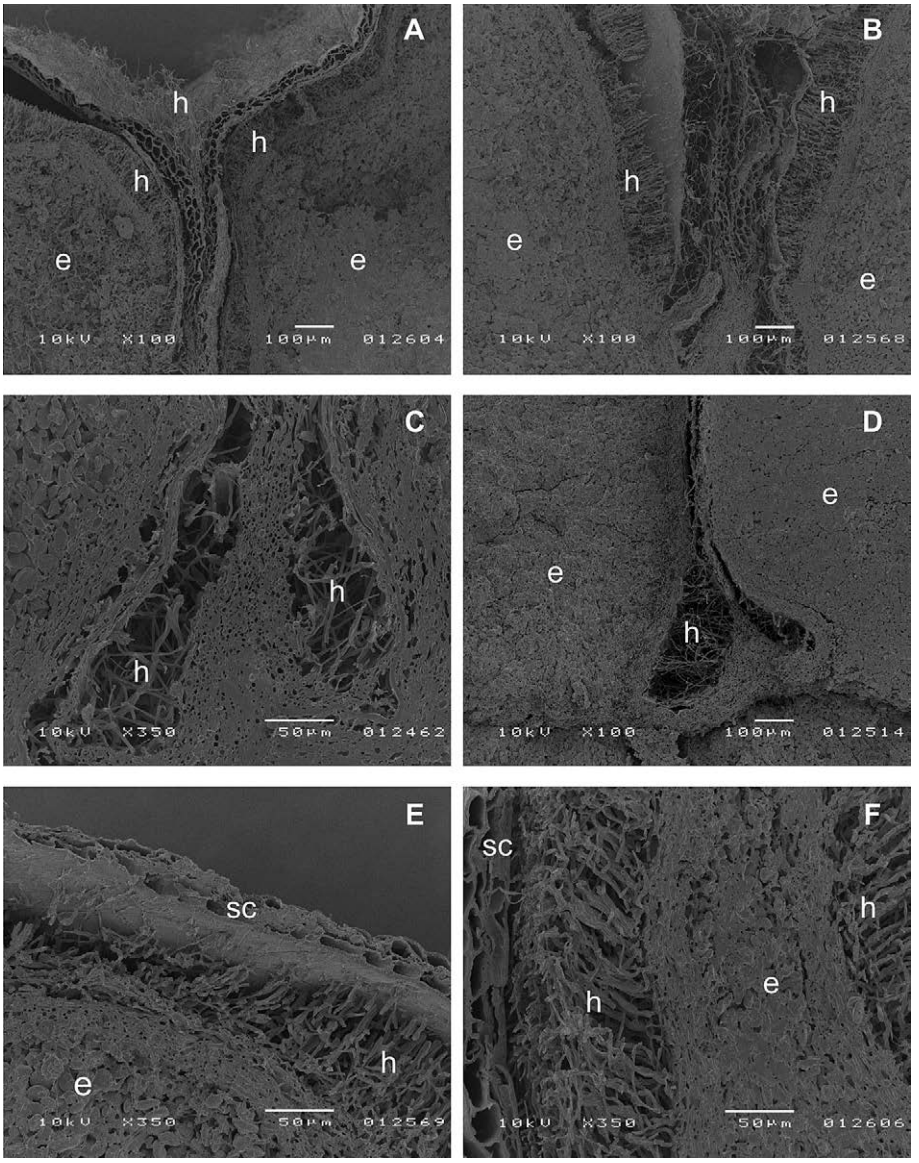
Phot. 4. Cross-sections of healthy and *Fusarium*-damaged kernels of hulled wheats. A, C, E – cross-sections of healthy kernels, B, D, F – cross-sections of *Fusarium culmorum*-infected kernels. A, B – kernels of *Triticum monococcum* K-1 and 'Terzino', C, D – kernels of *T. dicoccon* K-30, E, F – kernels of *T. spelta* K-18 and K-19. SEM (photo by D. Packa)

wheat kernels. Large and small starch granules were enveloped in a protein matrix. In healthy wheat kernels, starch granules were densely packed in cells. Infected kernels were characterized by loosely arranged starch granules, a partial or even complete absence of the protein matrix, and a decrease in the number of small granules. Damage to large starch granules was also observed, in the form of fun-



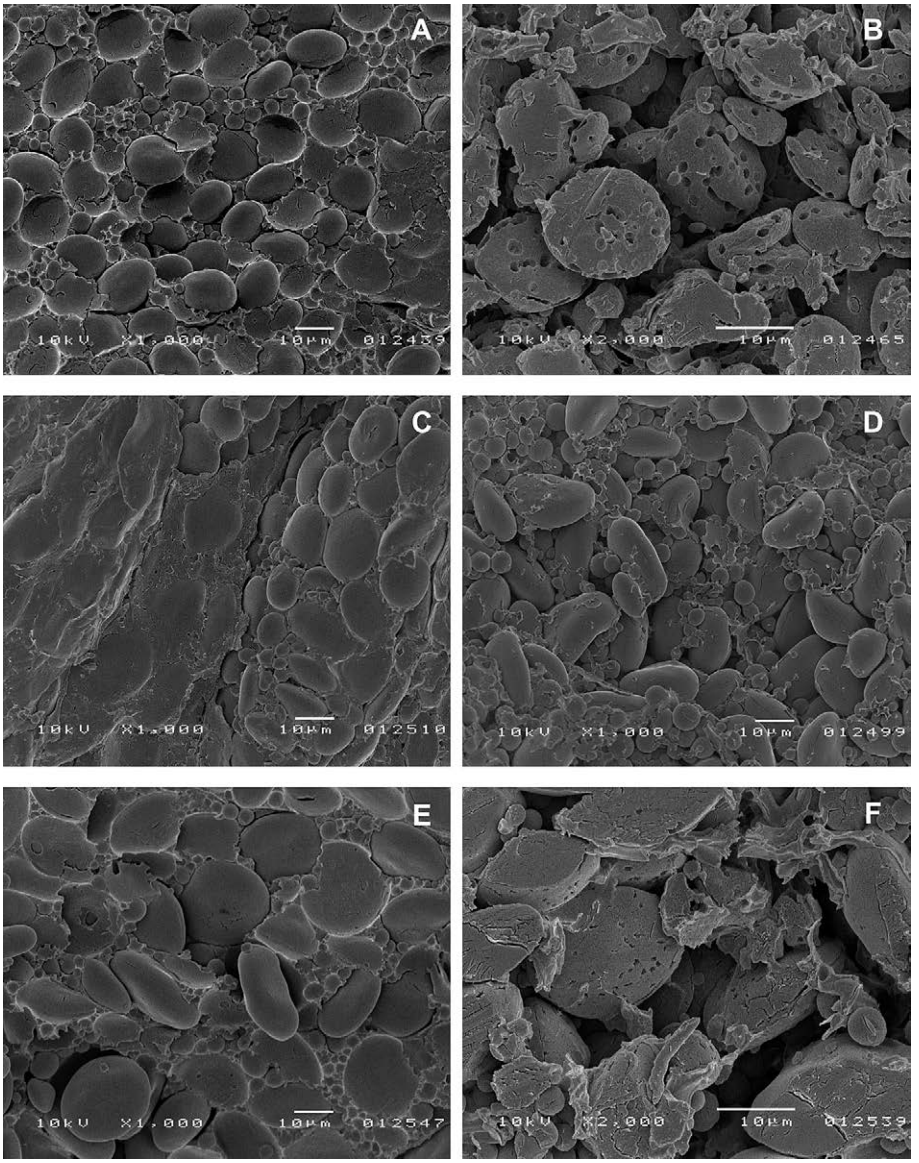
Phot. 5. Cross-sections of healthy and *Fusarium*-damaged kernels of threshable wheats. A, C, E – cross-sections of healthy kernels, B, D, F – cross-sections of *Fusarium culmorum*-infected kernels. A, B – kernels of cv. 'Kamut®', C, D – kernels of *Triticum polonicum* Pol-6 and Pol-4, E, F – kernels of *T. aestivum* cv. 'Parabola'. SEM (photo by D. Packa)

nel-like holes located in the equatorial plane and in lateral surfaces. It is believed that the degree of endosperm damage reflects the rate of fungal infection. The type of damage noted in the analysed wheat species was the same as that reported for

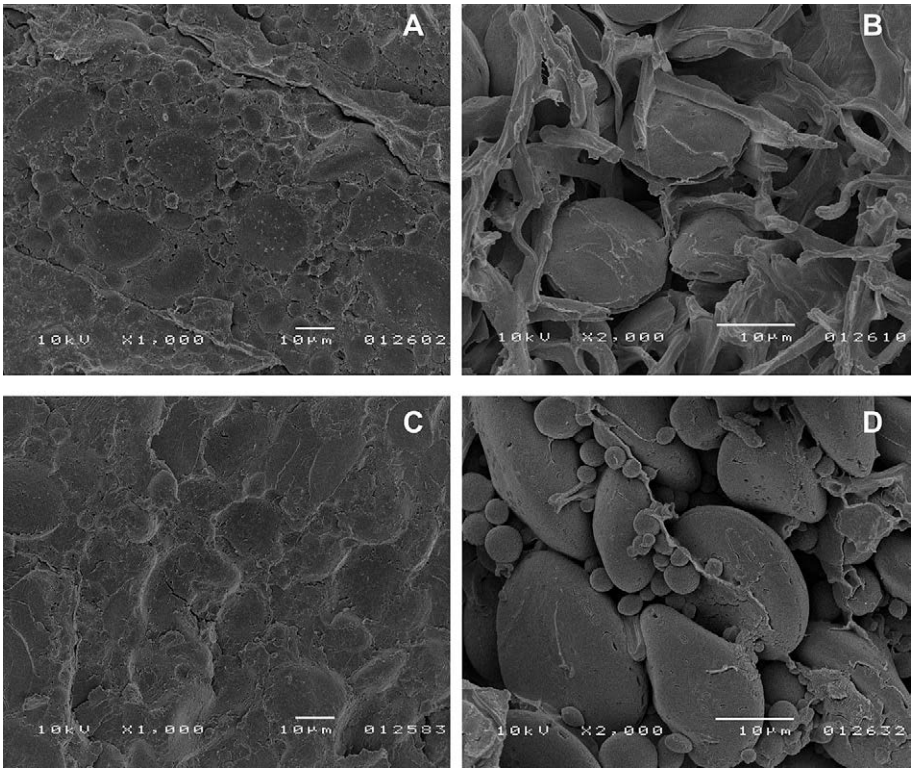


Phot. 6. Common locations of *Fusarium* mycelium in ancient wheat kernels.
A, B – hyphae in the crease and under the seed coat (A – cv. 'Kamut®', B – Pol-4),
C, D – hyphae at the base of the crease (C – cv. 'Terzino', D – K-30), E, F – a thick layer of hyphae between the seed coat and the endosperm, damaged aleurone layer (E – Pol-4, F – cv. 'Kamut®');
h – hyphae, e – endosperm, sc – seed coat. SEM (photo by D. Packa)

common spring wheat and winter triticale, infected by *F. culmorum* (Jackowiak et al. 2005, Packa et al. 2008). The microscopic image of endosperm damage confirms that *F. culmorum* is able to produce extracellular enzymes which facilitate the colo-



Phot. 7. Endosperm microstructure in healthy and *Fusarium*-damaged kernels. A, C, E – endosperm microstructure in healthy kernels, large and small starch granules in the protein matrix, B, D, F – endosperm microstructure in *Fusarium culmorum*-infected kernels, loosely arranged starch granules, a decreased number of small granules (B, F) and damaged large granules (B, F). A, B – *Triticum monococcum* K-1 and 'Terzino', C, D – *T. dicoccon* K-30 and K-25, E, F – *T. spelta* K-21 and K-19. SEM (photo by D. Packa)



Phot. 8. Endosperm microstructure in healthy and *Fusarium*-damaged kernels. A, C – endosperm microstructure in healthy kernels, large and small starch granules in the protein matrix, B, D – endosperm microstructure in *Fusarium culmorum*-infected kernels. B – numerous hyphae between damaged large starch granules, absence of small granules, D – loosely arranged starch granules, damage to large starch granules in the equatorial plane and in lateral planes. A, B – cv. 'Kamut®', C, D – *Triticum polonicum* Pol-6 and Pol-4. SEM (photo by D. Packa)

nization of host plants. Our findings regarding α-amylase activity in the kernels of common wheat and ancient wheats infected by *F. culmorum* were presented at the 11th European *Fusarium* Seminar (Hościk et al. 2010).

Streszczenie

BADANIA Z UŻYCIEM SKANINGOWEGO MIKROSKOPU ELEKTRONOWEGO ZIARNIAKÓW DAWNYCH PSZENIC PORAŻONYCH PRZEZ *FUSARIUM CULMORUM*

Badano ziarniaki dawnych pszenic oplewionych: *Triticum monococcum, T. dicoccon, T. spelta* i wymłacalnych: *T. polonicum*, *T. turanicum* ('Kamut®'). Ziarniaki porażone przez *Fusarium culmorum* kwalifikowane jako FDK (*Fusarium* damaged kernels)

były mniejsze, drobniejsze, pomarszczone, jaśniejszej barwy niż ziarniaki zdrowe, zwykle z uszkodzoną okrywą owocowo-nasienną i białą lub biało-różową grzybnią na powierzchni. Poszczególne ziarniaki w obrębie frakcji FDK wykazywały zróżnicowany stopień inwazji grzybowej. W komórkach bielma obserwowano charakterystyczne zmiany strukturalne, jak: luźny układ ziaren skrobiowych, częściowy lub całkowity brak białkowej matrycy spajającej ziarna skrobi, zanik małych ziaren skrobi oraz uszkodzenia dużych ziaren skrobi. Najmniejsze uszkodzenia komórek bielma obserwowano w ziarniakach pszenicy płaskurki i orkiszu.

Literature

Chełkowski J., Cierniewska A., Wakuliński W., 1990: Mycotoxins in cereal grain: Part 14. Histochemical examination of *Fusarium*-damaged wheat kernels. Die Nahrung 34, 4: 357–361.

Hościk M., Graban Ł., Lajszner W., Packa D., 2010: α-amylase activity in the kernels of diploid, tetraploid and heksaploid spring wheat after head inoculation with *Fusarium culmorum* (W.G. Smith) Sacc. In: 11th European *Fusarium* Seminar, Radzików, September 20–23, 2010. Eds. E. Arseniuk, E. Czembor, T. Góral. Plant Breeding and Acclimatization Institute – NRI, Radzików: 275–276.

Jackowiak H., Packa D., Wiwart M., Perkowski J., 2005: Scanning electron microscopy of *Fusarium* damaged kernels of spring wheat. Int. J. Food Microbiol. 98: 113–123.

Kang Z., Buchenauer H., 2000: Ultrastructural and cytological studies on cellulose, xylan and pectin degradation in wheat spikes infected by *Fusarium culmorum*. J. Phytopathol. 148: 263–275.

Koczowska I., Packa D., 1993: Susceptibility of some winter triticale cultivars to *Fusarium culmorum* (W. G. Smith) and *Fusarium avenaceum* (Corda ex Fr.) infection. Plant Breed. Seed Sci. 37, 5/6: 29–36.

Meyer D., Weipert D., Mielke H., 1986: Effects of *Fusarium culmorum* infection on wheat quality. Getreide Mehl Brot 40: 35–39.

Nightingale M.J., Marchylo B.A., Clear R.M., Dexter J.E., Perston K.R., 1999: *Fusarium* head blight: effect of fungal proteases on wheat storage proteins. Cereal Chem. 76, 1: 150–158.

Packa D., 2005: Fitotoksyczna aktywność patogenów nekrotroficznych z rodzaju *Fusarium*. Rozpr. Monogr. UW-M 106.

Packa D., Jackowiak H., Góral T., Wiwart M., Perkowski J., 2008: Scanning electron microscopy of *Fusarium*-infected kernels of winter triticale (× *Triticosecale* Wittmack). Seed Sci. Biotechnol. 2, 1: 27–31.

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

Schwarz P.B., 2003: Impact of *Fusarium* head blight on malting and brewing quality of barley. In: *Fusarium* head blight of wheat and barley. Eds. K.J. Leonard, W.R. Bushnell. APS Press, St. Paul, MN: 395–419.

Srobar S., Srobarova A., 1978: Mikroskopicke pozorovanie obiliek ozimnej psenice infikovanych hubou *Fusarium culmorum* (W.G. Sm.) Sacc. Biologia (Bratisl.) 33, 7: 583–589.

Srobarova A., 1987: Vyvoj huby, *Fusarium culmorum* (W.G. Smith) Sacc. na psenice. Ochr. Rostl. 23: 179–187.

Suchowilska E., Kandler W., Sulyok M., Wiwart M., Krska R., 2010: Mycotoxin profiles in the grain of *Triticum monococcum*, *Triticum dicoccum* and *Triticum spelta* after head infection with *Fusarium culmorum*. J. Sci. Food Agric. 90: 556–565.

Wiwart M., Perkowski J., Budzyński W., Suchowilska E., Buśko M., Matysiak A., 2011: Concentrations of ergosterol and trichothecenes in the grains of three *Triticum* species. Czech J. Food Sci. 29, 4: 430–440.

Authors' addresses:
Dr. hab. Danuta Packa, **Michał Hościk, M.Sc.**, Department of Plant Breeding and Seed Production, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3, 10-724 Olsztyn, Poland, e-mail: packa@uwm.edu.pl
Dr. Tomasz Kulik, Department of Diagnostics and Plant Pathophysiology, University of Warmia and Mazury in Olsztyn, pl. Łódzki 5, 10-727 Olsztyn, Poland

Accepted for publication: 24.04.2012