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Phytopathogenic Fungus *Fusarium cerealis* in Russia

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Abstract—The fungus *Fusarium cerealis* is described, which had not been previously identified in Russia. *F. cerealis* was isolated from wheat and barley roots and grains, as well as from the leaves of thistle collected in the Far Eastern, North Caucasus, and Central regions. *F. cerealis* strains may be misidentified as *F. culmorum* or *F. graminearum*, since the morphological characteristics of these fungi are similar.
The work was aimed at comparative characterization of *F. cerealis, F. graminearum*, and *F. culmorum* strains by their morphology and pathogenicity to seedlings of Moskovskaya 39 wheat. Differences in the shape and average length of macroconidia were demonstrated for *F. cerealis, F. graminearum,* and *F. culmorum* grown on carnation–leaf agar. While all investigated strains were pathogenic to wheat seedlings, *F. cerealis* was less aggressive than *F. graminearum* and *F. culmorum*. The geographic range of *F. cerealis* in Russia and its range of host plants require further specification.

Key words: fungi, pathogen, *Fusarium cerealis*, morphology, occurrence.

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The fungus *Fusarium cerealis* (Cooke) Sacc. (1886) (syn. *F. crookwellense* L.W. Burgess, P.E. Nelson et Toussoun (1982)), a pathogen of many plant species, has not been previously described in the Russian territory. *F. cerealis* isolates are probably often identified as *F. culmorum* (W.G. Smith) Sacc. (1895) or *F. graminearum* Schwabe (1839), since they are morphologically similar to these species. Unfortunately, no Russian-language description of the diagnostic characteristics of *F. cerealis* exists. However, the broad worldwide distribution of *F. cerealis* and its pathogenicity to various plant species should attract attention to this species.

F. crookwellense was originally described as a result of the investigation of *Fusarium* strains isolated from potato tubers in 1971 in Australia, in the vicinity of Crookwell [1]. In the taxonomic system of Marasas et al. [2], *F. crookwellense* belongs to the *Discolor* section. Description of this species is not included in the *Fusarium* pictorial atlas by Gerlach and Nirenberg [3]. However, Nirenberg [4] described *F. crookwellense* as a species identical to *F. cerealis* and recommended the use of its original name. In the earlier taxonomic system of Wollenweber and Reinking [5], the name *cerealis* was suggested for the *F. culmorum* variety, with the description fitting the modern concept of *F. cerealis* species. Since many mycologists insist on the priority use of the original name, *F. cerealis* [6], we will use it when citing publications, even if the name *F. crookwellense* was used in the original.

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The species *F. cerealis* is known as a pathogen of various plant species. It causes, for example, stalk rot of cotton [7] and rot of hop cones [8] and avocado fruit [9]. This is a dangerous pathogen of cereal crops, which have caused root rot and seedling blight of cereals. As a pathogen causing seedling blight of cereals, *F. cerealis* occurs in North America, Canada, many European countries, South Africa, Australia, New Zealand, and China [10–13]. In Japan, this species was identified in the complex of wheat blight pathogens in 1991 [14].

In Europe, *F. cerealis* often occurs in regions of moderate climate; in a complex of *Fusarium* head blight pathogens, it is second only to the species *F. graminearum, F. culmorum*, and *F. avenaceum* (Fr.) Sacc. (1886). Moreover, together with *F. graminearum, F. culmorum, F. avenaceum*, and *F. subglutinans* (Wollenw. & Reinking) P.E. Nelson, Toussoun et Marasas (1983), this pathogen is in the group of dominant species which are responsible for corn red rot and constitute 90–95% of all *Fusarium* isolates [15]. In New Zealand, *F. cerealis* is the second most widespread pathogen of corn heads and leaves [16].

Treatment of wheat seedlings with *F. cerealis* culture extract decreased their length by 57% [17]. Scanning electron microscopy of wheat heads did not reveal differences between infection processes caused by *F. graminearum* and *F. cerealis*. However, *F. graminearum* strains were more aggressive at 22 and 24.6°C, while aggression of *F. cerealis* occurred at 13.8°C [18]. Japanese researchers had demonstrated that, after spraying of wheat and barley heads with a

suspension of *F. cerealis* conidia in a greenhouse at 25°C, the first symptoms of infection occurred on the 11th–15th day, while, in the case of *F. graminearum*, the symptoms appeared on the 7th day [14]. Australian and South African researchers also reported lower pathogenicity of *F. cerealis* for wheat heads, compared to *F. graminearum* [19]. *F. cerealis* strains produced the symptoms of rootrot in artificially infected wheat [19, 20]. *F. cerealis* strains isolated from corn stems were less aggressive against corn seedlings than *F. graminearum* and *F. culmorum* [21]. *F. cerealis* strains are highly pathogenic for oat seedlings under laboratory conditions [22]. Inoculation of ten varieties and four lines of oat with a suspension of fungal conidia under field conditions resulted in a decrease in yield and amount of grain by 32 and 24%, respectively [23].

Strains of *F. graminearum, F. culmorum*, and *F. cerealis* are known to produce trichothecene mycotoxins of group B and belong to two chemotypes differing in their ability to produce deoxynivalenol (DON) or nivalenol (NIV) [24]. *F. cerealis* strains produce NIV and do not produce DON [15, 25–27]. In Japan, *F. cerealis*, together with *F. poae*, are considered the major sources of grain contamination with NIV [14]. *F. cerealis* strains may also produce fusaric acid [28], zearaleone [27], fusarin C [29], and diacetoxyscirpenol [26]. Differences in capacity for toxin production were demonstrated for *F. cerealis* strains from North America, Europe, and Australia [26].

RAPD-PCR revealed significant genetic similarity between *F. graminearum, F. culmorum*, and *F. cerealis* [30]. The species *F. cerealis, F. graminearum*, and *F. culmorum* share a gene cluster responsible for the biosynthesis of trichothecene mycotoxins [31]. Analysis of isozyme profile polymorphism [32], nucleotide sequences of the ribosomal DNA ITS regions [33], β-tubulin genes [34], and 1α elongation factor [35] revealed close relations between the species, with *F. cerealis* strains always comprising a separate group.

Although the teleomorph of *F. cerealis* has not been observed in nature, by its sexual stage it belongs to the genus *Gibberella*. Since *F. cerealis* strains have different mating types, MAT-1 or MAT-2 [36], this species is heterothallic.

In our studies, *F. cerealis* was found in the North Caucasus, Central, and Far East regions. Fungal strains identified as *F. cerealis* were isolated in 2003 from wheat grain in North Ossetia. Among *F. culmorum* cultures isolated from wheat and barley grain in 2002–2003 in Primorskii krai, Russia (Ussuriisk, Kamen'-Rybolov), and in Heilongjiang, China, strains were detected that differed from this species and were subsequently classified as *F. cerealis*. *F. cerealis* was identified on wheat grain in Krasnodar krai in 2004 and in North Ossetia in 2004–2005; its frequency of occurrence was 1–4%. This species was also isolated from wheat grain grown in Orel and Moscow oblasts in 2004. Moreover, *F. cerealis* was also identified among fungal isolates obtained from wheat roots on the fields of the Far Eastern Institute of Plant Protection (Kamen'-Rybolov, Primorskii krai). *F. cerealis* was isolated from thistle leaves with necrotic spots. Species-specific DNA primers Fg11f/r and 175f/430r were used to confirm the morphological identification of *F. graminearum* and *F. culmorum*, respectively [37]. Identification of *F. cerealis* strains in a fungal collection by genetic typing of single-nucleotide replacements was also carried out [38]. Thus, we maintain that *F. cerealis* is widespread in Russia.

The goal of the present work was to characterize the morphological features of *F. cerealis* and its pathogenicity for Moskovskaya 39 wheat seedlings in comparison to *F. graminearum* and *F. culmorum* strains.

MATERIALS AND METHODS

Single-spore isolates of *Fusarium* fungi from the collection of the Laboratory of Mycology and Phytopathology, All-Russia Institute of Plant Protection, were used (Table 1). For determination of cultural characteristics, strains were grown on potato–sucrose agar (PSA) for 1 week in the dark at 23–25°C. Morphological characteristics were described for cultures grown on carnation–leaf agar (CLA) for 10–14 days in the dark at 23–25°C. The length and width of conidia were measured under a Carl Zeiss microscope using the AxioVision software package. At least 25–50 conidia with a specified number of septa were measured for three strains of each species. Works [2, 3] were used for identification of *Fusarium* fungi.

Pathogenicity of the strains was determined by the modified Chelkowski and Manka method [39]. Grains of Moskovskaya 39 wheat were surface-sterilized with 70% ethanol and soaked for 24 h in sterile water. The grains with swollen germs were then placed over the fungal culture grown for a week on PSA (10 grains per plate) in triplicate. In the control, grains were placed on the agar medium. After a week of incubation in the dark at 23–25°C, the length of seedlings was measured and their state scored by a four-grade scale: 0, healthy seedling; 1, dotted tissue necroses; 2, necrosis of about 50% of the area; and 3, complete death. In every variant, the length of each seedling was determined and the average value for each experimental variant was calculated. The decrease in seedling length caused by the effect of fungi was assayed as a percentage of the average length in the control. The experiments were repeated in duplicate.

RESULTS AND DISCUSSION

Comparative analysis of colony morphology of *F. cerealis, F. culmorum*, and *F. graminearum* on PSA revealed high similarity between *F. cerealis* and *F. culmorum*. Both species had flocculent, loosely or densely fuzzy, felted aerial mycelium of intense dark red, reddish-brown, or yellowish color. The reverse color of

Table 1. Origin and pathogenicity of *Fusarium cerealis*, *F. culmorum*, and *F. graminearum* strains used in the present work

Species	Strain no.	Year of isolation	Origin	Host plant	Pathogenicity	
					Seedling length, %	Necrosis score
F. cerealis	37031	2003	China, Harbin	Wheat, grain	38.4	2.7
"	41727	2004	North Ossetia	Thistle, leaf	19.3	3.0
"	56050	2005	"	Wheat, grain	17.7	2.8
"	64722	2006	Far East, Khabarovsk krai	Wheat, head	25.7	3.0
"	64902	2006	Far East, Primorskii krai	Barley, grain	28.2	3.0
Average					25.90 ± 3.0	2.9 ± 0.04
F. culmorum	20021	2002	Arkhangel'sk oblast	Potato, tuber	26.2	2.9
"	70505	2003	Belarus	Wheat, head	16.0	2.8
"	20300	2004	Rostov oblast	Thistle, leaf	20.2	3.0
"	58802	2005	Moscow oblast	Wheat, grain	25.1	2.6
"	61916	2005	Bashkortostan	Wheat, root	5.8	3.0
"	70552	2007	Leningrad oblast	Hemp, stem	21.3	2.9
Average					19.1 ± 3.3	2.9 ± 1.06
F. graminearum	15001	2003	Far East, Primorskii krai	Wheat, grain	9.0	3.0
"	54148	2003	Kaliningrad oblast	"	5.8	3.0
"	50002	2004	Far East, Primorskii krai	"	3.0	2.9
"	48900	2004	Tula oblast	"	3.8	3.0
"	48706	2004	Bryansk oblast	"	16.7	2.7
"	49601	2004	Leningrad oblast	Barley, grain	17.3	3.0
"	56050	2005	North Ossetia	Wheat, grain	14.7	2.8
"	65206	2006	Far East, Primorskii krai	"	19.0	2.7
"	64720	2006	Far East, Khabarovsk krai	Wheat, head	8.3	3.0
"	70725	2006	Orel oblast	Wheat, grain	15.0	2.9
Average					12.9 ± 1.7	2.9 ± 0.04

F. and *F. culmorum* was also similar, red to reddish-brown. In *F. cerealis*, the aerial mycelium was lighter than in *F. culmorum* and had more ochre-yellow tints. In *F. graminearum*, the aerial mycelium was fuzzy, flocculent, whitish-pink, and pink, with yellow tints in older cultures. The reverse was pink, crimson-red, wine-red, and considerably lighter than in *F. cerealis* and *F. culmorum*.

On rich media (PSA and others), the differences in morphology between *F. graminearum, F. culmorum*, and *F. cerealis* were difficult to determine [14, 40]. Agarized nutrient media with low carbohydrate content are most suitable for the purpose. For *F. graminearum, F. culmorum*, and *F. cerealis*, the length of macroconidia with five septa from the sporodochia on CLA was 47.2, 36.7, and 41.3 μm, respectively (Table 2). While the differences in the average length of conidia were sufficiently significant, variability of their size prevented practical differentiation between *F. cerealis* and morphologically close species. The shape of macroconidia provides more information. In *F. culmorum*, most of the macroconidia have three to four septa, while macroconidia with five septa predominate in *F. cerealis* (Fig. 1a) and *F. graminearum* (Fig. 1b); in *F. graminearum*, however, the diameter of macroconidia remains more or less constant along their length. Macroconidia of *F. cerealis* are longer than those of *F. culmorum* (Fig. 1c), with a more dorsoventral, rather than ventral, curvature, with the maximal diameter at the center of the conidium. In *F. culmorum*, macroconidia are uniform and relatively broader than conidia of other two species. The apical cells of *F. cerealis* conidia taper gradually, unlike the short, abruptly tapering cells of *F. culmorum*. The basal cells of *F. cerealis* conidia are pedicellate, while in *F. culmorum* they are not always clearly pedicellate and may have a papillar shape. Macroconidia of *F. graminearum* are narrower and mostly lighter than conidia of *F. cerealis* and *F. culmorum*. All three species form similar conidiophores with monophialid conidiogenic cells (Figs. 2a, 2b). However, under the same growth conditions,

Table 2. Size of macroconidia in *Fusarium cerealis*, *F. culmorum*, and *F. graminearum* strains used in the present work

Species	Number of septa in macroconidia	Average size, μm	Size variation, μm	Length to width ratio
F. cerealis	5	41.3 × 5.9	31.6–58.1 × 4.8–7.2	7.0
	4	38.2 × 5.9	25.4–48.1 × 4.9–7.0	6.3
	3	30.0 × 5.5	23.5–33.6 × 4.3–7.0	5.2
F. culmorum	5	36.7 × 6.5	30.2–44.9 × 5.1–7.5	5.5
	4	34.7 × 6.2	28.4–39.4 × 5.0–7.6	5.6
	3	28.7 × 6.0	21.5–34.8 × 4.2–7.6	5.6
F. graminearum	5	47.2 × 5.7	39.4–57.5 × 4.6–6.7	8.3
	4	44.2 × 5.4	37.4–50.0 × 4.7–6.5	8.2
	3	41.9 × 5.5	40.5–42.6 × 4.7–6.5	7.5

Note: Strains were grown for 2 weeks on CLA at 23°C.

sporulation in *F. cerealis* and *F. culmorum* usually begins earlier than in *F. graminearum*. Colored, well-visible chlamydospores in the hyphae and macroconidia are usually also formed earlier by the first two species than by *F. graminearum*.

Analysis of pathogenicity of the strains of different origin demonstrated that, while *F. cerealis* strains were able to cause plant diseases, their aggressiveness to wheat seedlings was lower than in *F. graminearum* and *F. culmorum* (Table 1). *F. graminearum* and *F. culmorum* strains caused a more pronounced decrease in seedling length (12.9 and 19.1% of the control) than *F. cerealis* (25.9%).

It was previously demonstrated that all *F. graminearum* strains from different regions of Russia belonged to the DON chemotype [41]. Genetic typing of single-nucleotide replacements (SNP) of allele-specific primers for over 250 strains of Russian origin producing group B trichothecene mycotoxins enabled us to characterize their chemotype composition [37]. The results demonstrated that *F. graminearum* in Russia was represented by the DON chemotype producing 3- and 15-DON acetates, *F. culmorum* belonged to the DON chemotype with production of 3-DON acetate, and *F. cerealis* belonged to the NIV chemotype.

According to the information from the All-Russian Research Institute of Veterinary Sanitary, Hygiene, and Ecology [42], in the *F. graminearum* population responsible for wheat blight in Krasnodar krai in 1985–1986, the DON and NIV chemotypes constituted 91.2 and 8.8% of isolates, respectively. While it may be suggested that the NIV chemotype isolates possibly belonged to *F. cerealis*, this assumption requires experimental confirmation.

Future specification of the area of distribution of *F. cerealis* in Russia and determination of its host plant range are necessary. Accurate diagnostics of any plant disease requires precise identification of the infecting agent and its biological characteristics.

Descriptions of three *Fusarium* species are presented below.

Description of *Fusarium cerealis* Burgess, Nelson, Toussoun (1982). The colonies rapidly grow; aerial mycelium is flocculent, loosely or densely fuzzy, velvety, of intense dark red, reddish-brown, or ochre-yellowish color. The reverse color is intense, red to reddish-brown, with ochre tints in older cultures.

Conidiophores initially appear laterally on the hyphae of the aerial mycelium, and then branch extensively. Conidiogenic cells are monophialides. Sporodochia are formed rapidly, as a brick-red or reddish-brown mass of conidia in the center of the culture. Macroconidia are of spindly sickle shape, elliptically curved, and thick-walled, with the dorsoventral side more curved than the ventral one, with the greatest diameter in the middle, usually with five septa (three to six). The apical cell is gradually tapering, conical, and slightly curved. The basal cell is pedicellate. Macroconidia with five septa are on average 41.3 × 5.9 μm (31.6–58.1 × 4.8–7.2 μm). Microconidia are absent. Chlamydospores are intercalary; form usually in the hyphae, in chains or clusters, relatively rapidly; and are colored (Fig. 2c). Chlamydospores may also be formed in macroconidia.

Description of *Fusarium culmorum* (W.G. Smith) Sacc. (1895). The colonies are rapidly growing; aerial mycelium is flocculent, loosely or densely fuzzy, velvety, of intense dark red, reddish-brown, or ochre-yellowish color. The reverse color is intense, red-brown to reddish-brown, with ochre tints in older cultures.

Conidiophores initially appear laterally on the hyphae of the aerial mycelium, and then branch extensively. Conidiogenic cells are monophialides.

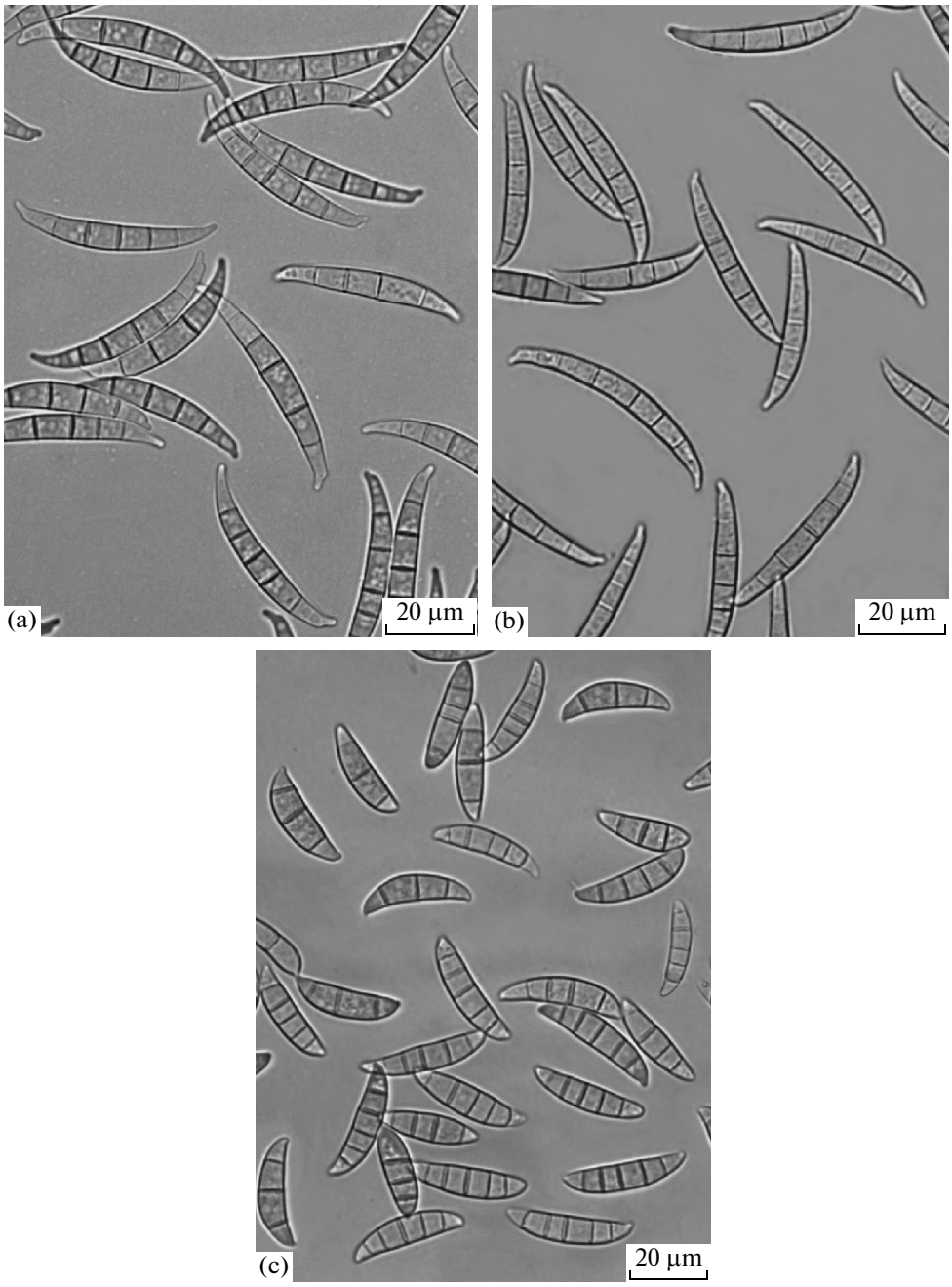

Fig. 1. Macroconidia of *F. cerealis* (a), *F. culmorum* (b), and *F. graminearum* (c).

Sporodochia are formed rapidly, as a brick-red or red-brown mass of conidia in the center of the culture. Macroconidia are of spindly sickle shape, and thick walled, with the dorsoventral side more curved than the ventral one, with the greatest diameter in the middle, usually with three to five septa. The apical cell is sharply tapering, short, and not pointed. The basal cell is pedicellate with papilla. Macroconidia with five septa are on average 36.7 × 6.5 μm (30.2–44.9 × 5.1–7.5 μm). Microconidia are absent. Chlamydospores are intercalary, form rapidly in the hyphae and marconidia, single, in chains or clusters, colored.

Description of *Fusarium graminearum* Schwabe (1839). The colonies are rapidly growing; aerial mycelium is well-developed, flocculent, fuzzy, white-pink or pink, with yellow tints in the center of older cultures. The reverse is pink, crimson, or wine-red, often with radial rays.

Conidiophores initially appear laterally on the hyphae of the aerial mycelium and then branch extensively. Conidiogenic cells are monophialides. Sporo-

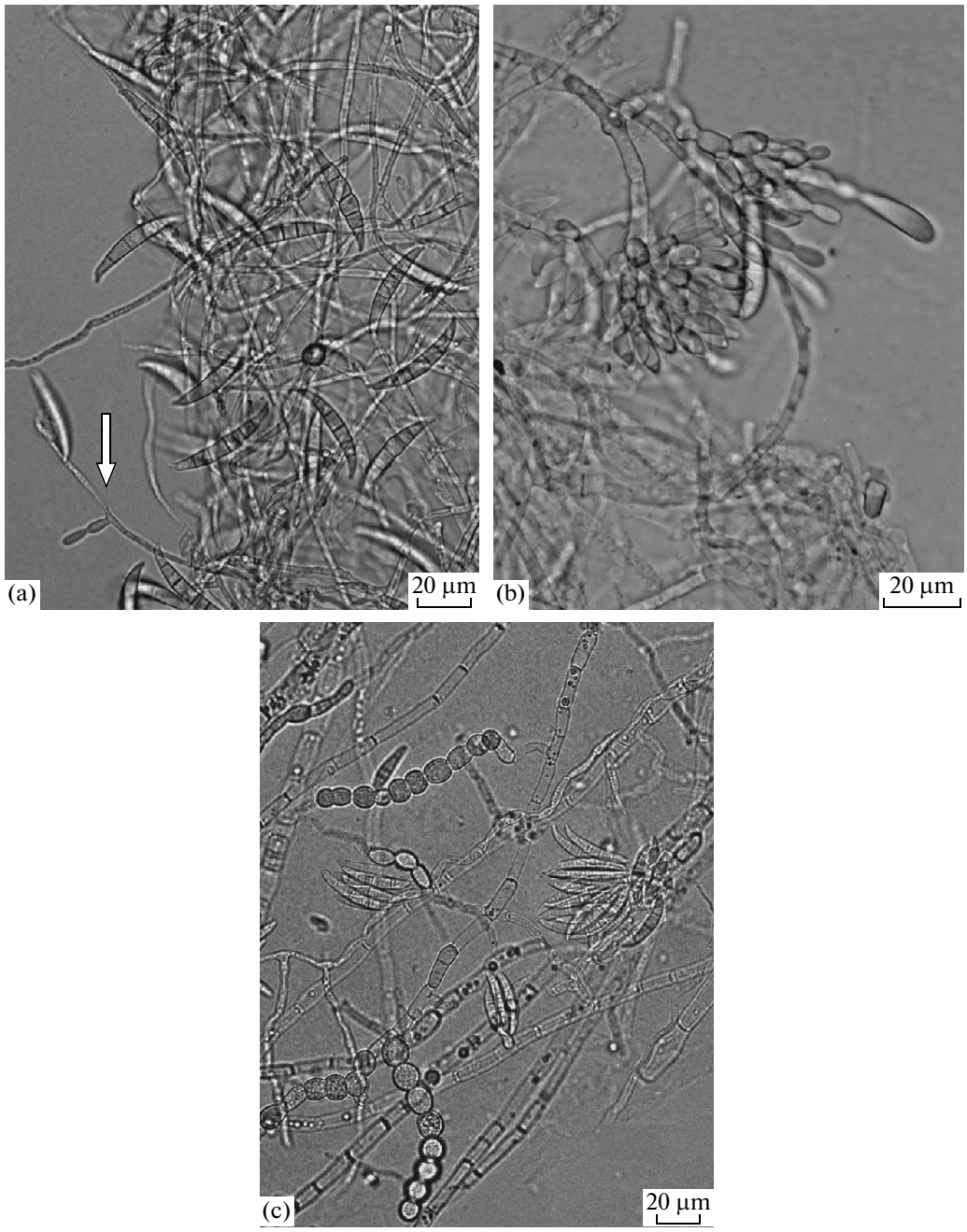

Fig. 2. Unbranched (a) and branched (b) conidiophores bearing monophialidic conidiogenic cells, and chlamydospores (c) of *F. cerealis*.

dochia are formed with age, brick-red or orange. Macroconidia are of spindly sickle shape, elliptically curved, usually with the same diameter along the length, mainly with five septa (three to six). The apical cell is gradually tapering, conical, slightly curved. The basal cell is pedicellate. Macroconidia with five septa are in average 47.2 × 5.7 μm (39.4–57.5 × 4.6–6.7 μm). Microconidia are absent. Chlamydospores are intercalary, form usually in the hyphae and macroconidia, single, in chains or clusters, colored. Chlamydospores are often absent.

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