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Detection of *Gibellina cerealis* infection on winter common wheat in Ukraine

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Abstract

False eyespot disease caused by *Gibellina cerealis* (Pass.) Pass. was first detected in Ukraine during field inspections of wheat canopies in 2017–2018. Symptoms were detected at different plant development stages (from booting to soft dough) on stems, sheaths and leaf blades. The severely infected plants showed reduced tillering and did not form a head. The causal agent was successfully isolated from symptomatic tissue and identified using morphological methods as *Gibellina cerealis*. Identification was confirmed with molecular methods using two sets of species-specific primers. Changes in climate and farming practices are suspected to contribute to spread and increase of the disease.

Keywords Winter wheat · False eyespot · Disease detection using polymerase chain reaction · Molecular markers

Introduction

Until recently, false eyespot of wheat (synonyms: white foot rot or white straw disease) has not been diagnosed or wrongly identified in Ukraine (Golosna 2013). The disease is caused by the ascomycete fungus *Gibellina cerealis* (Pass.) Pass. It also infects barley, rye and triticale (Glynne 1985).

The first symptoms could be observed on a coleoptile as light- or dark-brown prolonged (2–3 mm) spots darkened in the center; in 2–5 days the spot size increases up to 5–7 mm, and the clear border and the dark stroma in the center of the spots appear; in the case of simultaneous infestation by multiple ascospores of a small tissue area, the described symptoms are not observed, and necrosis areas overlap covering the stem (Gorkovenko et al. 2013). Under field conditions at the early stages of plant development, the symptoms of

false eyespot might be mistaken for eyespot (*Oculimacula yallundae* (Wallwork & Spooner) Crous & W. Gams) or sharp eyespot (*Rhizoctonia cerealis* E.P. Hoeven, *Rhizoctonia solani* J.G. Kühn), at the later stages for powdery mildew (*Blumeria graminis* (DC.) Speer) (Zhalieva 2007). At the tillering stage, the lower leaves can be infected; the infestation is followed by development of oval, sand-colored spots 5–7 mm in length with the light brown border (Kuznetsov 2010). In this case, the symptoms are similar to those caused by the pink snow mold pathogen *Microdochium nivale* (Fr.) Samuels & I.C. Hallett. In case of intense disease development, the spots may encompass the stem. They form a felty layer of mycelium: the white mycelium on fresh spots (this accounts for its other names–white foot rot and white straw disease) and the gray mycelium on the old ones (Glynne et al. 1985; Gorkovenko et al. 2013). Mycelium can reach ears. Symptoms were observed on the scales and spikes.

The source of infection is plant residues (Gasich et al. 2016). A certain amount of ascospores (10–20%) can germinate immediately after harvest, the rest of the ascospores require a long period of maturation (Gasich et al. 2016).

The infection at the early growth stages is considered to be the most dangerous, in particular at the seedling stage (first leaf though coleoptile), resulting in plant death. Infection at the tillering stage causes loss of productive tillers or inhibits heading (Kuznetsov 2010). Intense infections at the later growth stages may result in fragile straws and

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empty heads (Shutko et al. 2012). False eyespot is reported to decrease wheat head length and number of spikelets (Zhalieva 2007) as well as plant height (Zazimko et al. 2006). For instance in case of three or more spots per stem, the reduction of grain weight per spike of 31–70% has been reported (Shutko et al. 2012). Under favorable conditions, the disease can lead to a 10–40% decrease in yield (Gasich et al. 2016).

The disease was first described on wheat near Parma by G.Passerini in 1886 and later in the northern regions of Italy (Glynne et al. 1985). In the 1930s, the disease was detected at the Rothamsted research station in Great Britain (Glynne 1968; Glynne et al. 1985). An episodic occurrence of the disease was also reported from France, Bulgaria, Romania, Georgia and North China (Glynne 1968). In the last decades, false eyespot became widespread in some regions of Russia, in particular in the Northern Caucasus region (Kuznetsov 2010; Gorkovenko et al. 2018). In this study, we provide the first evidence for the occurrence of *G. cerealis* on wheat in Ukraine.

Materials and methods

Observations of common wheat fields were performed during 2017–2018 in the Artsyzsk, Sarata and Ismail districts in the south-eastern part of the Odessa region, Ukraine. The area is a part of the Black Sea Lowland. The climate is humid, temperate continental. Annual year temperature ranges 8.2–10.8 °C. Total annual precipitation is 340–470 mm per year. In total, 18 sites were monitored. Assessments were conducted on cultivars 'Nebokrai', 'Smuglyanka', 'Sailor' 'Kuyalnyk', 'Bumer', 'Mudrist', 'Hylux'.

Observations started after snow thawing (February to March) and continued on a monthly basis. In total, 5 observations per site were performed. The common wheat cultivar 'Nebokrai' at the booting stage (37 on BBCH-schale) was sampled in two sites in 2017, the cultivars 'Kuyalnyk' (two sites) and 'Bumer' (one site)–in 2018. The sampling was repeated when the plants reached the soft dough stage (85 on BBCH-schale). In total, 10 samples per 50 plants were taken from the wheat fields where disease symptoms were detected.

To study the fungal morphology in the laboratory, microscopy techniques were used for the assessment of symptoms detected on plant parts and for fungal colonies obtained from the isolation. The sampled material was assessed using microscopy before isolation assays were performed. Stem tissue showing lesions was washed with tap water, surface sterilized with 96% ethanol and rinsed twice with sterile water. To obtain pure cultures of *G. cerealis*, the disinfected plant parts were placed onto potato sucrose agar medium containing Triton X-100 1 ml/l (Bio-Rad, USA) and gentamycin sulfate–10 mg/l («Gentamicin-zdorovye», Ukraine) and incubated at 24 °C (Gasich et al. 2016). After the appearance of signs of mycelium, small portions from colony edges were transferred onto a triton-free potato sucrose agar medium.

DNA was extracted from the pure culture, infected parts of the wheat stem, as well as unaffected wheat stems with the use of standard silica-based kits of the Neogene company (Ukraine) according to manufacturer's recommendations. Two *G. cerealis*-specific PCR primer pairs developed by Pilshchikova and Gannibal (2015) were used to test symptomatic plant tissue as well as pure cultures: Gib-F/Gib-R (Gib-F 5′-CCG GAG GTA CCA AAC TCT AAG-3′; Gib-R 5′-GCT GGA ACC CGA CTG GAG-3′) and GibC-F/GibC-R (GibC-F 5′-GCG CCC TCT TCT CCA TCT CA-3′; GibC-R 5′-TAG ACG CTC ATG CGC TCC AG-3′). The PCR protocol was the following: initial denaturation at 94 °C for 7 min (due to the hot start Taq polymerase used) following by 30 cycles with denaturation at 94 °C for 40 s, annealing at 65 °C for 30 s and alongation at 72 °C for 30 s following by final alongation at 72 °C for 7 min (Pilshchikova and Gannibal 2015). The Gib-F/R primer pair yields a 368-bp amplicon and GibC-F/R primer pair–a 325-bp amplicon with the samples containing *G. cerealis* DNA (Pilshchikova and Gannibal 2015). PCR results were visualized on 2% agarose gels with ethidium bromide.

Results

False eyespot symptoms were detected in canopies of the common wheat cultivars 'Nebokrai', growing in the Sarata (2017), 'Kuyalnyk' growing in the Sarata, Artsyzsk (2018) and 'Bumer' growing in the Izmail (2018) districts of the Odessa region, Ukraine. In foci, the proportion of plants with clearly distinguishable symptoms made up, on average, 61.7%. The plants showed reduced tillering. They did not form a spike or it barely emerged from the leaf sheath.

In 2017, samples were taken from 'Nebokrai' plants in the booting and soft dough stage. Stem lesions on younger plants were characterized by prolonged depressed overlapping light spots with dark-brown edges on the first–second internodes (Fig. 1a, b, Fig. 2). Additionally, the symptoms were detected on leaf sheaths and blades (Fig. 3). The stems of older plants looked rough because of numerous fruiting bodies (Fig. 4). Microscopic examination of symptomatic tissues revealed perithecia with unripe ascospores (Fig. 5a, b, c). Their size ranged 380–560 μm. The asci (105–127 μm) contained eight spores. The ascospores were of honey color with thick walls and one partition (28–34×7–9 μm, see Fig. 5c, d). The observed
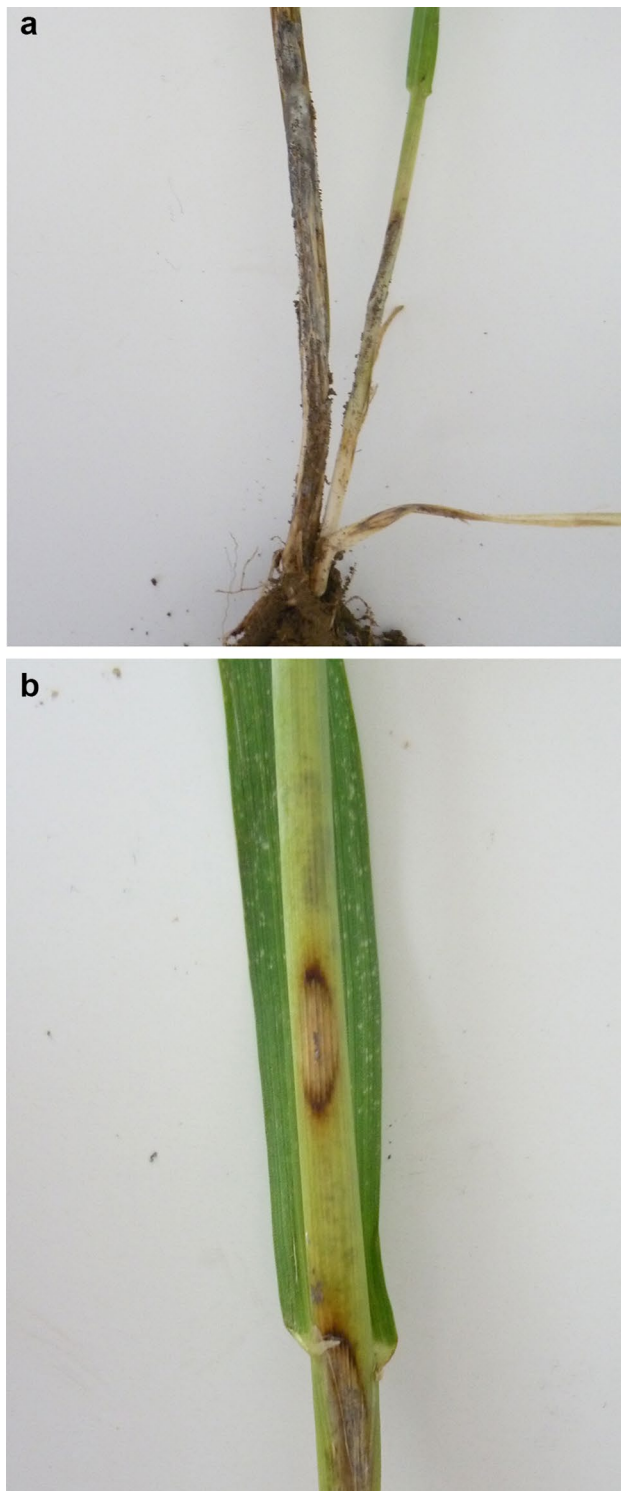
Fig. 1 False eyespot symptoms on wheat stem: **a** wheat stem infected with *Gibellina cerealis* (booting stage); **b** close-up of false eyespot symptoms on wheat stem (booting stage)

Fig. 2 Mycelium of *Gibellina cerealis* on the affected wheat stem tissue (booting stage)

symptoms and detected fungal structures were identical to the descriptions of *G. cerealis* (Glynne et al. 1985).

In 2018, 'Kuyalnyk' and 'Bumer' plants (tillering stage) were found to exhibit false eyespot indicative symptoms characterized by lens-like oval spots of sand color with light brown edges and a visible black layer in the center. Subsequent microscopic examination of collected plant material also suggested the infection with *G. cerealis*.

Colonies of *G. cerealis* isolates obtained from plating assays were fluffy velvet. The air mycelium was white with a shade of gray (Fig. 6a, b). The reverse side of the plated colony was dim gray. On week 6–7 of culturing, the formation of perithecia with asci and ascospores was observed.

To confirm the results obtained using morphological methods, *G. cerealis*-specific PCR assays were used to test symptomatic plant tissue as well as pure cultures. Both primer pairs Gib-F/Gib-R and GibC-F/GibC-R yielded in DNA fragments with the expected length (Fig. 7).

Fig. 3 False eyespot symptom on wheat leaf blade (booting stage)

Discussion

False eyespot symptoms were detected in Ukraine in 2017 and 2018. This is in line with the observation that the disease spread during the last decades in Russia, where it was detected in 1986 in the Stavropol region (Monastyrnaya 1990), and the area of the disease spread was reported to be about 62% of the territory under surveillance in 2011 (Savchenko 2012). The disease was also detected in the Rostov and Volgograd regions (Gasich et al. 2015). According to the research of false eyespot distribution during 1992–2014 performed in Kuban State University, the disease epiphytotics were observed during 12 vegetation seasons (Gorkovenko et al. 2015).

In the current study, the symptoms were found at different plant development stages and on different plant parts. On the first and second internodes, they are described as prolonged, depressed, overlapping spots with light inner part and dark-brown edges (Fig. 1). Layering of dirty-white mycelium with dark fruiting bodies was observed on the large spots covering the stem diameter almost entirely (Fig. 2). Additionally, the symptoms were detected on leaf sheaths and blades (Fig. 3). The spots were lens-like with the pupil-like darkening in the center. At the soft dough stage,

Fig. 4 Perithecia of *Gibellina cerealis* on an infected wheat stem (soft dough stage)

the fruiting bodies were formed and the stem look dark and rough (Fig. 4). Therefore, they match the symptoms caused be *G. cerealis* as described in the literature (Glynne et al. 1985; Gorkovenko et al. 2013; Zhalieva 2007).

Microscopic assessment and isolation assays are necessary to detect relevant morphological characters (e.g., perithecia) as in-field symptoms often are not distinct enough to differentiate false eyespot from other diseases. For example, oval sand-colored spots with the light brown border on lower leaves may look like pink snow mold; at the early stages the symptoms of false eyespot on stems are similar to eyespot (*O.yallundae*) or sharp eyespot (*R. cerealis*, *R. solani*); at the later stages, white mycelium on lesions could be attributed to powdery mildew (*B. graminis*).

Infection with *G. cerealis* causes the following problems: loss of productive tillers, stem fragility, absence of spike or short empty not emerged spike, and even plant death when infection happens at the seedling stage.
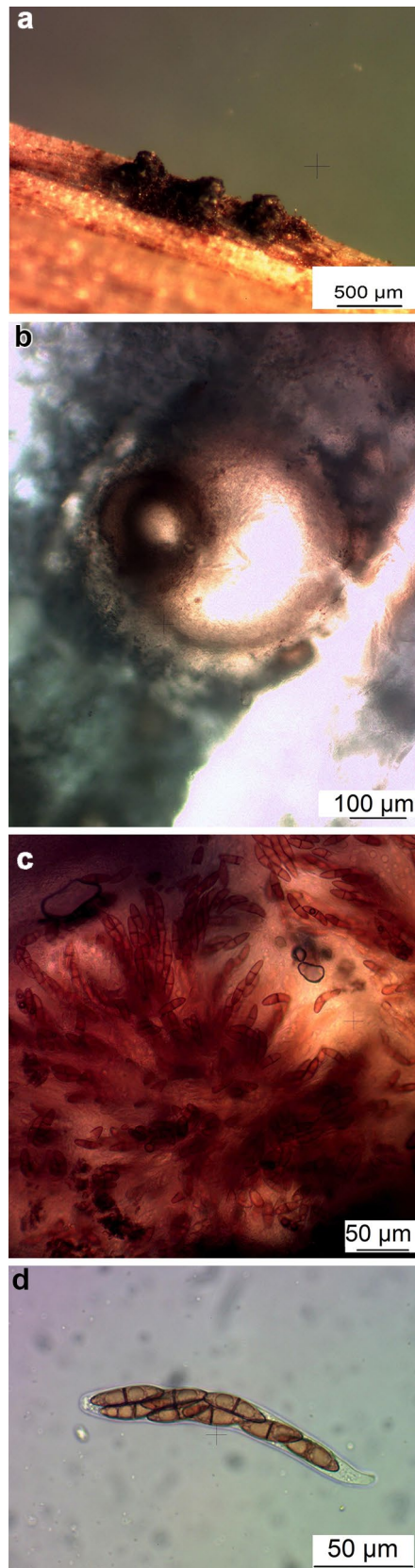
Fig. 5 Microscopic images of *Gibellina cerealis*: **a**, **b** perithecia extruding from symptomatic plant tissue; **c** asci with ascospores in a perithecium; **d** ascus with 8 ascospores
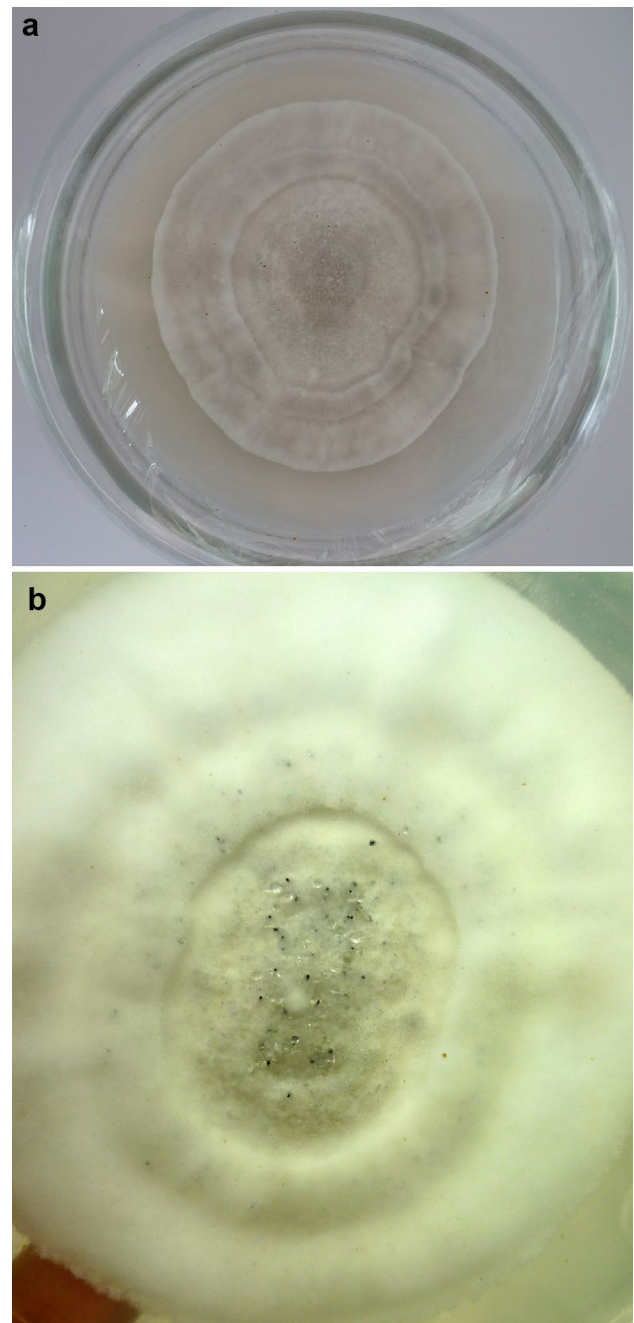
Fig. 6 Colony of *Gibellina cerealis*: **a** colony of *Gibellina cerealis* on potato sucrose agar (50 days of cultivation); **b** close-up of a *Gibellina cerealis* on potato sucrose agar (50 days of cultivation) with black perithecia

In addition, cereals might be also affected by other pathogens. In the current study at the soft dough stage, it was observed that plants in some cases were simultaneously infected with *Gaeumannomyces graminis* var *graminis* (Sacc.) Arx & D.L. Olivier. *G.graminis* caused necrotic dark-brown spots on crown and stem base covered with a black coating, a shiny black discoloration of the basal stem

Fig. 7 PCR amplification products using the two different primer pairs Gib-F/Gib-R (1–4) and GibC-F/GibC-R (5–8) to test DNA extracted from isolated *G. cerealis* cultures (2, 6), infected plant tissues (3, 7), and uninfected plant tissues (4, 8); 1 and 5: H$_2$O (negative control); M: Thermo Scientific™ GeneRuler™ 100 bp DNA Ladder
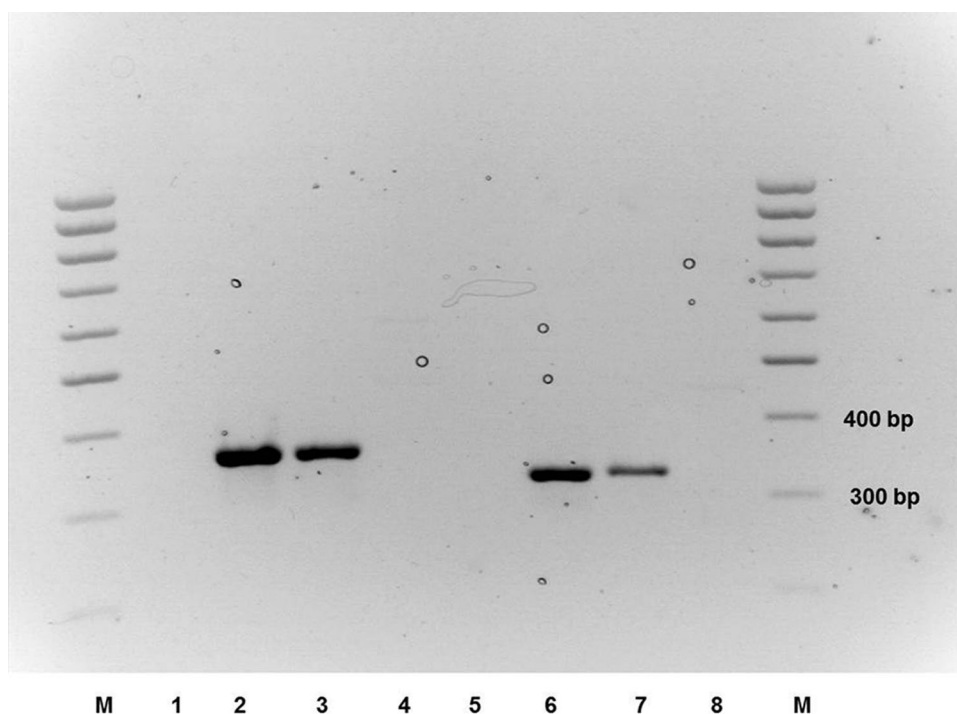
is a main effect that used for disease diagnostic. Unlike it, *G. cerealis* forms prolonged, depressed, overlapping light spots with dark-brown edges covered by dirty-white mycelium and the stems look rough due to numerous fruiting bodies. Plants infected with *G. cerealis* usually did not form ears or they barely emerged from the leaf sheath, and in case of *G. graminis,* empty discolored heads were formed. Additionally *G.graminis* cause dark-brown to black lesions on roots and severe infected plants are pulled up easily because the root system is brittle. *G.graminis.*

A PCR-based method for *G. cerealis* identification is available. It uses two primer pairs Gib-F/Gib-R and GibC-F/GibC-R for species-specific amplification of *G. cerealis* ITS regions and the β-tubulin gene, respectively (Pilshchikova and Gannibal 2015). In our study, they were found to work for identification of the pathogen in pure culture, but more importantly to detect the pathogen directly from symptomatic plant material.

Changes in agricultural practices (Deppermann et al. 2018) and climate conditions (Boychenko et al. 2016; Pautasso et al. 2012; Zazimko et al. 2006) are considered as the main factors contributing to the disease distribution. It has been reported that *G. cerealis* is the most active during years with mild winters and humid springs (Zazimko et al. 2006). Such conditions have been observed in the South of Ukraine during the last 5 years (Boychenko et al. 2016). Changes in soil cultivation, implementation of energy saving approaches might also have resulted in accumulation of crop residue in fields, which is source of diseases (Krupinsky et al. 2002). As a result, pathogens that occurred occasionally and had no

economic effect are propagated to form a significant infectious pool in certain areas and cause outbreaks.

Therefore, the climate changes and other conditions are rather favorable for *G. cerealis*. The high harmfulness and speed of spreading of false eyespot under appropriate climate conditions suggest that there is a significant risk of its spreading to the South of Europe, in particular to France, Bulgaria and Romania where the disease was previously detected (Glynne 1968).

Conclusion

After two decades of its identification in the in the Northern Caucasus region of Russia, false eyespot has been detected in the South of Ukraine with similar climate conditions. Agricultural practices and climate factors are expected to contribute to an increase in false eyespot disease in Ukraine, and there is a risk of its westward spread. Early detection and accurate identification of *G. cerealis* infections using visual symptoms as well as morphological and molecular identification techniques are considered as decisive in order to develop control strategies.

Declaration

Conflict of interest The authors declare that they have no conflict of interest.

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