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ORIGINAL ARTICLE

Species composition, morphological and biological peculiarities of leaf pathogens of spring wheat

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We concluded that the leaf Septoriosis (pathogens *Septoria tritici* Rob. Et. Desm, *Erysiphe graminis* DC) and brown leaf rust (pathogen *Puccinia recondita* Rob. Et. Desm) are the main diseases of spring wheat leaves in the Eastern Forest-Steppe of Ukraine. We determined that the first visible signs of powdery mildew on the leaves of spring wheat appeared in the form of spots with a white spider web film. After 2–3 days, the film on the leaf blade was compacted and turned into cotton-like pads - conidial sporulation. The interval between the leaf infection to the appearance of the first visible disease symptoms, caused by *Septoria tritici* pathogen, was approximately 10-14 days. The period between the first symptoms and the beginning of sporulation is quite short. The incubation period of brown leaf rust development lasted for 7–21 days. We registered that the telia with teliospores were formed after 12–19 days of uredinia appearance on the mycelium.

Keywords: Septoriosis; Powdery mildew; Brown leaf rust; Life cycle

Introduction

The decrease in spring wheat photosynthetic surface, caused by damage to it leaf apparatus by infectious diseases results to the deep violations of physiological and biochemical processes, early leaves death, which lead to reduced yields and deterioration of grain quality. Powdery mildew, Septoriosis, and brown leaf rust belong to deciduous-stem infections. The life cycle of their pathogens occurs in the air and their infectious structures have an aerogenic and airborne distribution patterns. Pathogen reproductions are carried out by spores of asexual origin, which have several generations with abundant sporulation during the growing season. They have high epiphytic potential (Horiainova, 2017). In addition, the climate changes lead to the transformation of agrocoenoses and therefore to the changes in phytopathocoenoses. Consequently, the identification of the phytopathogenic complex in spring wheat sowings and clarification pathogen biological characteristics contribute to wheat timely protection (Bilyk & Kulieshov, 2006).

Materials and Methods

Experimental studies were performed in accordance with the methods of field experiments, records of the diseases detection of spring wheat leaves and their prevalence according to guidelines and recommendations (Dospekhov, 1973; Ishkova, 2001; Omelyuta, 1986; Khokhryakov, 1984; Chenkin et al., 1994).

We used the data from the Main Department of the State Food and Consumer Service in Kharkiv region and own observations. The constant sowing areas surveys were conducted with the aim of establishing the time of disease onset and further monitoring their development to determine the species composition of wheat diseases. We used the visual method, i.e. the disease was diagnosed according to the external signs and the pathogen was identified by the field guide. Measurements of spring wheat, inquired with powdery mildew disease, were carried out in two replications per 1 ha of field with inspection of 10 plants per field. The actual area of leaves and stems, which was covered with film was determined according to the scale of E.E. Geshele (1971), by eight-point scale (0, 1, 5, 10, 20, 40, 60, and 80%). Brown leaf rust was determined at the stage of milk-wax grain ripeness, we inspected 5 plants in 20 plots of two field diagonals. The degree of disease was determined sequentially on all leaves of the main stem, starting from the top. Leaves with more than 75% of dried part were not considered. The degree of brown leaf rust damage was determined according to the scale of T.D. Strahov, which considered the conditional percentage of the leaf area is covered by pustules - 0, 5, 15, 25, 45, 65, and 100% (Chumakov, 1986) The studies of morphological and biological peculiarities of fungi *Erysiphe graminis* DC, *Septoria tritici* Rob. et Desm. and *Puccinia recondita* Rob. et Desm. f.sp.tritici were performed in accordance with V.I. Bilay (1989). The sporulation organs microscopy was conducted by Biolam C-70 biological microscope with 300x and 600x total magnification. The measurements of pathogen structures were done by the ocular micrometer MOV-1-15x with MFN photomultiplier (Bleyker, 1980).

Results and Discussion

We determined that spring wheat leaves were annually affected by Septoriosis, powdery mildew, brown leaf rust, Pyrenophorosis and mosaic in the Eastern Forest-Steppe of Ukraine. We generalize the average data on their distribution and proportion in the phytopathogenic complex (Figure 1).

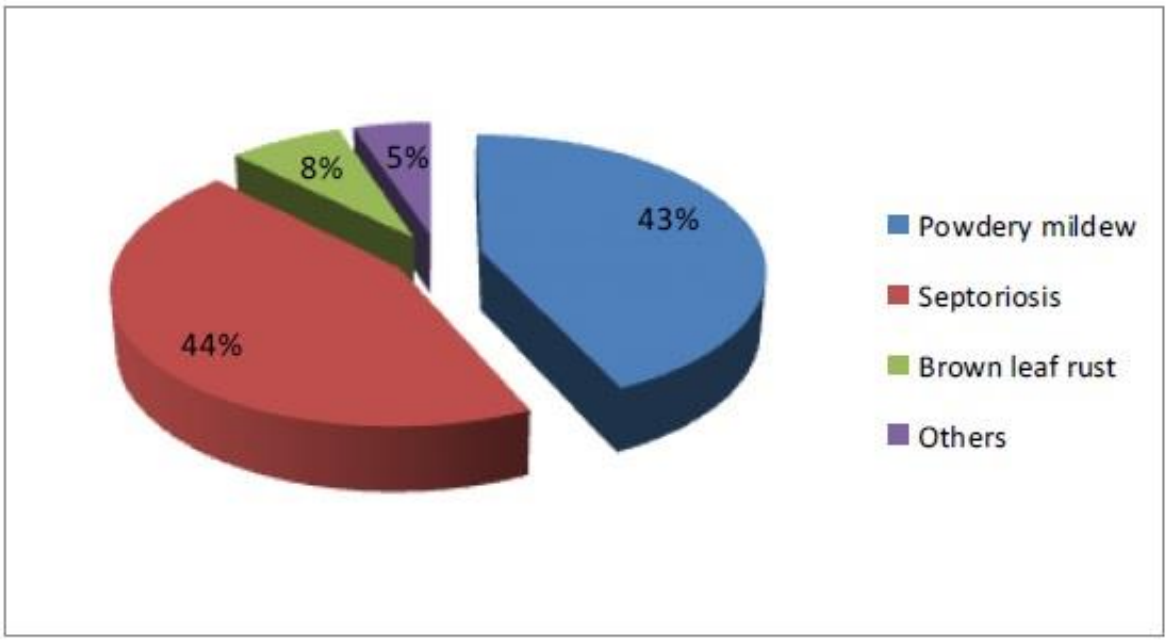

Figure 1. Ratio of spring wheat leaves diseases (Kharkiv region, 2012–2017).

The Septoriosis and powdery mildew prevailed among other leaf diseases. We determined that the prevalence of these diseases was almost at the same level, their shares were 44.2 and 43.2% on average. Brown leaf rust accounted for 7.5%, whereas the share of Pyrenophorosis and mosaic was 5.1%. Of course, this distribution was relative, because there were the signs of plant damage by several diseases on single leaf. This especially was concerned the brown leaf rust and spots, or brown rust and mosaic; it indicated the absence of antagonism among these pathogens. In this case, we considered the degree of dominant disease prevalence.

The determination signs of leaf diseases is presented in Figures 2-5. In general, a reliable diagnosis of these diseases can be visually performed in the field. Thus, when affected by powdery mildew, certain areas of the leaves and axil were first covered with a thin white film, which quickly became floury, and further got gray, which was compacted in pads. Also, the appearance of black dots on the pads - the fungus fruiting bodies – cleistothecia is an important feature (Figure 2).

Figure 2. Diagnostics signs of powdery mildew (photo taken by the authors, 2015).

Diagnosing spots of Septoriosis and Pyrenophorosis can cause complications, as one disease can be mistaken for another. The colour of Septoriosis spots varies from light brown to brown, and they are often bordered. The fact of pathogen sporulation was also taken into account. Pycnidia in the form of black dots were well visible on the spots caused by the fungus Septoria tritici Rob. et Desm. (Figures 3 and 4).

The peculiarity of Pyrenophorosis (yellow spot) is the size of the spots; they are oval, yellow or yellow-brown and smaller than the Septoriosis spots. A brown spot appears in the center, the tissues are necrotized. Over time, the spots grow longwise and cannot be distinguished from the Septoriosis spots, but yellow spot do not form the pycnidia.

Figure 3. Septoriosis on the leaves of spring wheat (photo taken by the authors, 2015).

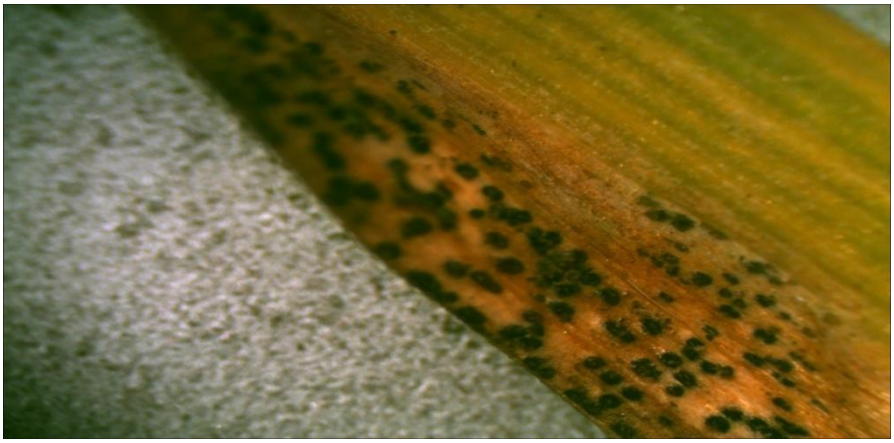

Figure 4. Spring wheat. Leaf segment affected by Septoriosis with the pycnidia of the fungus *Septoria tritici* Rob. et Desm (photo taken by the authors, 2016).

Brown leaf rust development (Figure 5) in the form of oval red-brown urediniopustules (pads), which are always formed inside the leaf tissues, are clusters of spores, initially covered with epidermis, the leaf eventually ruptures under the pressure of sporulation.

Figure 5. Spring wheat affected by brown leaf rust (photo taken by the authors, 2016).

The exact type of fungus, the disease pathogen, can only be determined in the laboratory. As it is mentioned in the literature review, there are evident differences in the symptoms of the same disease. It depends on the species variety of the host plant, the properties of the pathogen and environmental conditions etc. Especially it concerns the spots, or in the case when several pathogens can develop on leaves at one time. Therefore, the microscopic examination of the morphological structures of pathogens to determine the disease etiology must be carried out. The microscopy results of sporulation, which were found on the infected wheat leaves, coincide with their description given in the literature. The author illustrations are presented below (Figure 6).

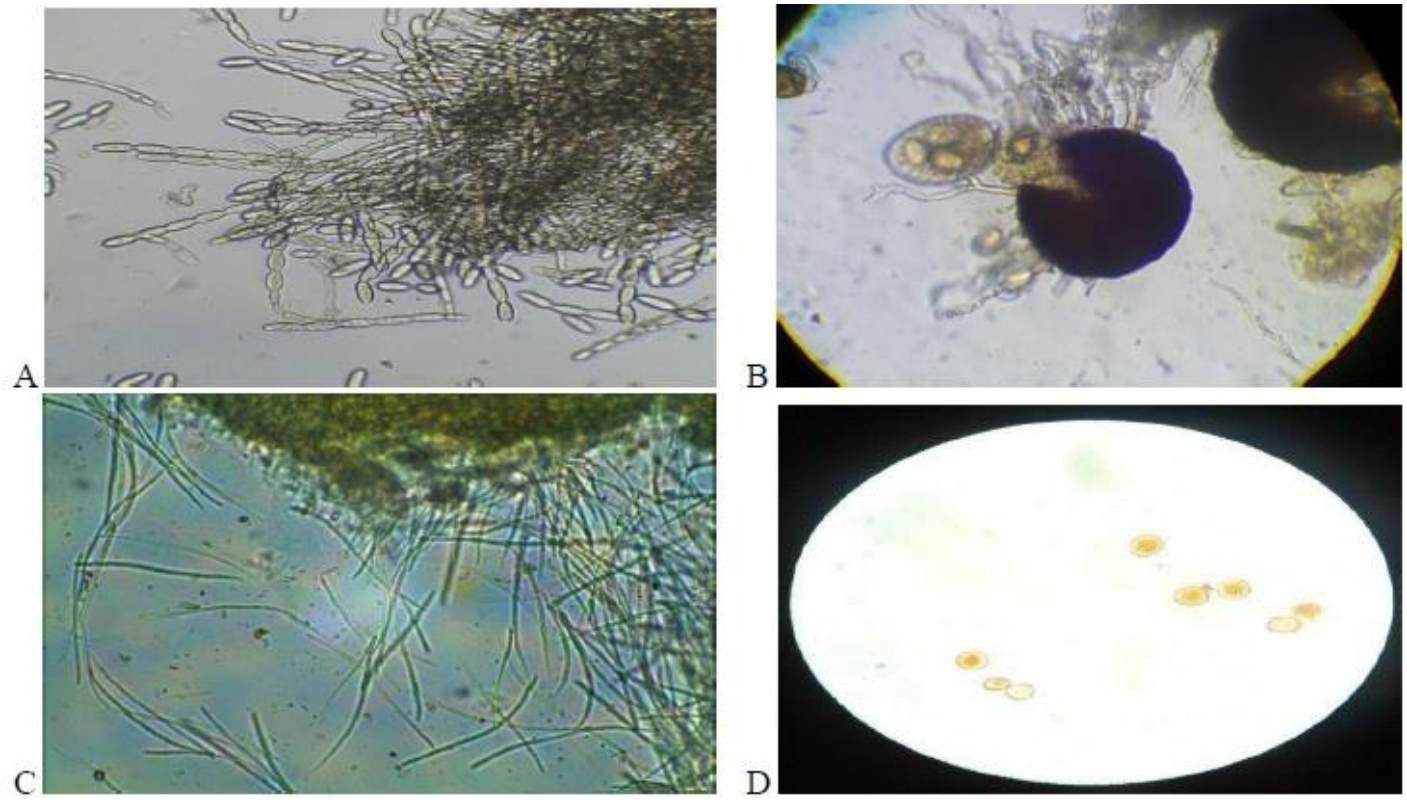

Figure 6. A - conidia of the fungus *Erysiphe graminis* DC; B - Cleistothecia, bags with bag spores of the fungus *Erysiphe graminis* DC; C - pycnospores of the fungus *Zymoseptoria tritici* Rob. et Desm., coming from the pycnidia; D - urediniospores of the fungus *Puccinia recondita* Rob. et Desm (x 200) (laboratory of the Department of Phytopathology of V.V. Dokuchaiev KhNAU, photo by the authors, 2012–2017)

Microscopic studies of pathogen morphological characteristics convincingly demonstrate the correctness of their identifying in the sowings of spring wheat. We determined the sporulation parameters from local populations, which we compared with literature data (Table 1).

Table 1. Biometric indicators of spring wheat pathogens.

Type of fungus sporulation	**Biometric indicators, microns, pcs**	
	data obtained	**literature data**
	Septoria tritici *	
Pycnidia (diameter)	89-183	60-200
Pycnospores	28–83×1.5–3.3	20–98×1.4–3.8
Number of membranes in pycnospores	3-7	3-4
	Erysiphe graminis **	
Cleistostea (diameter)	153-255	135-280
Bag spores	65–100×17–36	70–110×25–40
Number of bag spores in bags	7-26	8-30

* According to Cunfer (1998), ** according to Pidoplichko (1978).

These data indicated that the biometric indicators of pathogen reproductive from the local population are correspond to indicators reported in literature. Thus, our results convincingly testify the reliability of pathogen identification for the spring wheat local varieties. We suggested that the determining of pathogen development cycle is extremely important, because these knowledge allows to prevent, protect and preserve the crop yield quality. Therefore, we were faced with the task of artificially infecting crops of spring wheat with these pathogens.

The plants were inoculated with a suspension of freshly harvested *Erysiphe graminis* conidia at spiking and flowering phase (Figure 7). We determined that the optimum conditions for the spores germination, plants infection and the development of the fungus *Erysiphe graminis* in the Eastern Forest-Steppe of Ukraine are within temperature range of 15-20 ºC and relative humidity of 60-100%. The alternation of dry and wet periods promotes the penetration of infectious hyphae of the pathogen powdery mildew through the leaf epidermis. When the temperature is 28–30ºC, the growth of mycelium stops.

The first visible symptoms of the disease appeared in the form of mat spots, which were covered with a white spider web film. After 2-3 days, the film on the leaf blade was compacted and turned into cotton-like pads - conidial sporulation. According to the results of our research, 3-7 days passed over from the moment of infection to the appearance of the first symptoms.

The period between the appearance of the first symptoms and the beginning of sporulation is rather short. So, one day is passed from tissue yellowing to the formation of conidia. The formed spores caused new infections, giving rise to a new generation of the pathogen. The growth of pathogen one generation took 8 days. The fungus *Erysiphe graminis* developed up to 16 generations during the growing season.

The pathogen of Septoriosis, the fungus *Septoria tritici*, inquired the leaf blade, its spores began to germinate inside the host plant in drip moisture and optimum temperature of +14-16 °C after 1-2 hours. The first visible signs of the disease appeared in the form of elongated light, and later chocolate-brown spots with or without a dark border. There were 10-14 days from the moment of infection to first visible symptoms, caused by the fungus *Septoria tritici*, on average. The interval between the appearance of first symptoms and sporulation is quite shorter.

Figure 7. Insulator for the pathogens in sowings of spring wheat, (June, 2016, photo taken by the authors).

The pathogen of brown leaf rust, the fungus *Puccinia recondita* Rob. et. Desm overwinters with uredomycelium on plant residues. Spherical, yellow-orange urediniospores as they mature reached the healthy leaves, where they germinated. The optimum temperature for germination was 15–25°C. Under this temperature and the presence of dew, the plants became infected in less than 4 hours.

According to our research results, the incubation period depended on the temperature and lasted from 7 to 21 days. Thus, the infection is possible several times during the growing season. 12–19 days after the appearance of uredopustules, the mycelium formed teliopustules with teliospores. Teliopustules were formed more often on the lower side of the leaf in the form of black pads covered with epidermis.

The obtained data show that in those areas where there are winter crops in winter, the cycle of brown leaf rust development is incomplete and can be reduced to one uredinium stage. Under such cycle of development, neither the intermediate host with the ecidial stage nor the teliospores as a source of infection are of practical importance. The reliable diagnosis of diseases can be performed in field conditions and visually. It is primarily due to the type of disease (in the form of pustules). The latter are always formed inside the tissues of the affected organs and are clusters of spores, initially covered with epidermis, which eventually ruptures under the pressure of sporulation. There is a great need for systematic monitoring of the dynamics of progressive diseases, for the assessment of their harmfulness and elaboration of effective protective measures.

Conclusion

Our study proved that the main leaf diseases of spring wheat in the Eastern Forest-Steppe of Ukraine are leaf Septoriosis (pathogen *Septoria tritici* Rob. Et. Desm), powdery mildew (pathogen *Erysiphe graminis* DC), and brown leaf rust (pathogen *Puccinia recondita* Rob. Et. Desm). We found that the first visible signs of powdery mildew on spring wheat leaves appeared in the form of spots with a white spider web film. After 2–3 days, the film on the leaf plate was compacted and turned into cotton-like pads – the conidial sporulation. We registered that there were 10-14 days from the moment of leaf infection to the first visible symptoms of *Septoria tritici* pathogen, on average. The period between the first symptoms and beginning of sporulation was quite short.

The incubation period of brown leaf rust development lasted for 7–21 days. The telia with teliospores were formed in 12–19 days after the appearance of uredinia on the mycelium.

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