

EVALUATION OF MAIZE (*ZEA MAYS* L.) INBREDS BY INFECTION WITH *SPORISORIUM REILIANUM*

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Topicality. The development and selection of maize genotypes resistant to head smut (*Sporisorium reilianum*) is a topical issue in modern breeding research, which helps prevent yield losses caused by the pathogen and ensure high-quality grain. **Purpose.** To evaluate the infection rate with *S. reilianum* of promising maize inbreds on natural and artificial infection backgrounds and identify resistant and susceptible genotypes among them. **Materials and methods.** Ten promising maize inbreds adapted to conditions of the Steppe of Ukraine were selected as the research material. The research was conducted in 2021 and 2024. The research methods were as follows: field method, statistical analysis. **Results.** The viability of the head smut spores used in the artificial infectious background was tested and confirmed by cultivating the spores on an artificial nutrient medium in vitro before inoculation of maize seeds. A two-year field testing of maize inbreds showed that the average infection rate with *S. reilianum* was 0.90 % on the natural infection background during the study years, while it was 10.28 % on the artificial infection background. Plant infection with head smut depended on the maize genotype and agrometeorological conditions of the year. The highest rate of pathogen infection was observed in the P354 inbred on an artificial infectious background and was 41.18 % and 59.26 % for two years of the study, respectively. No cases of plant infection in the inbreds DK3044, DK267 and Mo17 with head smut were observed either on natural or artificial infectious backgrounds during the years of research. **Conclusions.** Ten promising maize inbreds adapted to conditions of the Steppe of Ukraine were evaluated in terms of resistance to *S. reilianum*. A high level of resistance to the pathogen was established at the studied inbreds. Inbreds DK315, DK3044, DK267, PLS61, DK3008 and Mo17, which had less than 5% of plants infected with *S. reilianum* on an artificial infectious background for two years, were identified as resistant. They have the potential as donors of resistance to head smut. Inbreds P354, DK3023 and DK7575, which had 20 % or more of plants infected on an artificial infection background, were identified as susceptible to the disease.

Key words: maize (*Zea mays* L.), resistance to biotic factors, fungal diseases of grain crops, head smut, donors of resistance, artificial infectious background

Introduction. The development and selection of maize genotypes resistant to diseases and pests is one of the priority areas of modern plant breeding and biotechnology research. Global losses in maize harvest due to phytopathogens average 9.4 %, while in Ukraine they are 19–25 %, and in years with widespread disease, they reach 50 % [1].

Along with diseases such as blister smut, Fusarium ear blight, seed mould, stem and root

rots, and bacteriosis, head smut is one of the most common and dangerous diseases of maize [2]. In Ukraine, the infection rate of maize plants with head smut is 0.2–3.0 %, and in some areas this rate is 4–6 %. In case of severe disease infection, the grain yield losses can reach 15–20 % or more [3].

The pathogen of head smut is the biotrophic fungus *Sporisorium reilianum*. The disease affects tassels and ears of maize. The panicles

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turn into a black dusty mass, and the cobs turn into a black cone-shaped ball of fibres and fungal spores. Difference in appearance between head and blister smut consists in the fact that the spore galls are dry and have no shell. Under appropriate conditions, *S. reilianum* spores germinate in the promycelium, which forms basidiospores. The latter, after copulation, infect the young maize seedling through contact with it. Infection occurs through the root after seed germination. The fungal mycelium penetrates the seedlings through the mesocotyl. The development of smut on ears and tassels depends largely on whether the fungus reaches the shoot meristem. If the fungal hyphae do not reach the stem growth point, only the ears will be affected, while the tassels will remain healthy. Infected plants often retard in development, lose apical dominance, and form many tillers. After spores mature in smut galls, they are spread out, settle on the soil and on maize post-harvest residues, and get on the ears and kernels during harvesting and threshing [4–8].

The infection rate of head smut in maize plants depends on genotypic characteristics, the method of creating an infectious background, agrometeorological conditions, etc. [9–11].

There are several approaches to the immunological assessment of resistance of maize genotypes to smut. A. V. Balian et al. define the following groups of resistance to smut: 1 – very low resistance (>50 % of infected plants), 3 – low resistance (26–50 % of infected plants), 5 – medium resistance (11–25 % of infected plants), 7 – high resistance (6–10 % of infected plants), 9 – very high resistance (0–5 % of infected plants) [12]. G. V. Grisenko and E. L. Dudka recommend determining resistance groups of maize genotypes based on the infection rate of plants grown on an artificial infectious background. The infectious background is created by direct introduction of pathogen spores into the soil. The authors identify highly resistant forms with 0–10 % of plant infection, resistant forms with 10–25 %, medium resistant forms with 25–50 %, and susceptible forms with more than 50 % of plant infection [9]. T. M. Pedash et al. also use this scoring scale when evaluating maize hybrids grown on a natural infectious background [11]. Analysing the infection of maize plants grown on an artificial infectious background (created by seed inoculation),

V. Yu. Cherchel et al. define the following classes of genotypes resistant to head smut: 1 – resistant (have 0 % of infection), 2 – low sensitivity (up to 10 % of plant infection), 3 – medium susceptibility (up to 20 % of plant infection), 4 – non-resistant to the disease (more than 20 % of plants infected) [13]. However, for some studies, such as the search for associations between molecular genetic DNA polymorphisms and pathogen resistance, a more simplified scale of breeding instead of immunological assessment of resistance to smut is relevant. In this case, maize genotypes should be divided into two groups – resistant and non-resistant (susceptible to the pathogen) with a third group of genotypes with medium resistance to the pathogen.

Agrotechnological methods are often costly and not always environmentally friendly to control smut. An important area of *S. reilianum* control is the selection and development of maize genotypes resistant to the pathogen. Therefore, the aim of our work was to evaluate the resistance to *S. reilianum* infection of breeding promising maize lines adapted to the Steppe zone of Ukraine against natural and artificial infectious background and to identify resistant and non-resistant genotypes of maize among them. The study was aimed at checking the viability of head smut spores before inoculation of maize seeds, breeding testing of maize lines in the field on natural and artificial infectious backgrounds, identification of resistant and non-resistant (susceptible to the pathogen) genotypes.

Materials and Methods. The research material was breeding promising maize lines adapted to the Steppe of Ukraine: DK267, DK315, DK3008, DK3044, DK3023, DK7575, DK633266MV, PLS61, Mo17, and P354. The Mo17 line with resistance to head smut [14] was selected as a positive control. Line P354, identified as susceptible to this pathogen [15], was selected as a negative control. The maize lines were tested for resistance to head smut in the field against natural and artificial infectious backgrounds for two years (2021 and 2024). The artificial infectious background was obtained by inoculating maize seeds with *Sporisorium reilianum* spores using methyl cellulose [13]. Smut spores for inoculation of maize seeds in 2021 were picked from affected field plants in the previous 2020. Smut spores for inocula

tion of seeds in 2024 were picked in 2021. In both cases, the spores were stored in an unheated room with sufficient ventilation.

Before seed inoculation, the viability of the head smut spores was tested. For this purpose, the spores were cultivated on nutrient medium in Petri dishes. The nutrient medium contained 30 g/l of sucrose and 6 g/l of agar-agar. Cultivation was performed at a temperature of 27–29 °C. Spores (5 g) from the galls were shaken into a sterile vial and mixed with 5 ml of sterile distilled water. The prepared suspension was streaked onto the nutrient medium. Spore germination analysis was performed on the fourth and eighth day of cultivation.

The infected plants were recorded at full maturity, during the harvesting period. For each genotype, we analysed 11–71 plants in all variants. The infection rate of head smut was calculated as a percentage of the number of plants infected to the total number of plants grown.

To determine resistance to *S. reilianum*, a simplified scale for breeding evaluation of maize genotypes was used. Against the infectious background, lines with less than 5 % of plants infected were considered resistant to *S. reilianum*, and lines with more than 20 % of plants infected were considered non-resistant (susceptible to *S. reilianum*). Lines with 5–20 % of plants infected were defined as medium resistant lines.

Statistical analysis of the experimental data was performed using the Student's t-test at a significance level of 0.05 [16].

Results and Discussion. The germination of head smut spores stored for more than two years (from September 2021 to April 2024) on the nutrient medium confirmed their viability. Spore germination was observed already in the first four days after the start of their cultivation on the medium, with active hyphal formation on the eighth day (Fig. 1). The hyphae were used for further inoculation of maize seeds to create an artificial infectious background.

In the field trials, maize plants infected with head smut had characteristic traits of the disease (Fig. 2). The infection rate of maize breeding promising lines adapted to the Steppe of Ukraine depended on the genotype and year of the study (Table 1). The average percentage of infected plants with *S. reilianum* on the natural infectious background was 0.90 % over the years of research, while this indicator was high-

er on the artificial infectious background and amounted to 10.28 %, indicating a high level of resistance of maize lines to *S. reilianum*, and also demonstrating the effect of the artificial infectious load.

The highest percentage of plant infection with head smut was observed in P354 line (negative control), namely, 41.18 % in 2021 and 59.26 % in 2024. In the resistant line Mo17 (positive control), no plants infected with head smut were observed either under natural or artificial infectious background. The lines DK3044 and DK267 were also not infected by the pathogen during the two years of research. They were identified as resistant lines. The lines DK315, PLS61 and DK3008 were infected at the level of 0–4.62 %, i.e. less than 5 %. These genotypes were included in the resistant group. The DK633266MV line had an infection rate of 17.86 % in 2021 and 9.09 % in 2024. This line was included in the medium resistance group of genotypes. Since DK7575 had a hundred-percent resistance in 2021, and in 2024, the infection rate of the line was 28.57 %, we classified this line as a non-resistant genotype. The percentage of infected plants of DK3023 line in 2021 was 24.24 %, while in 2024 it was 12 %. This genotype is also classified as non-resistant. In 2021, seven lines out of the ten studied were included in the group of resistant genotypes, for which the infection rate did not exceed 5 %, namely: DK315, DK3044, DK267, PLS61, DK3008, DK7575 and Mo17. In 2024, this group included six lines: DK315, DK3044, DK267, PLS61, DK3008 and Mo17. In 2021, two lines P354 and DK3023 were susceptible to the disease. The infection rate of these genotypes exceeded 20 %. In 2024, lines P354 and DK7575 were susceptible to head smut; however, line DK7575 was resistant in 2021. During the two years of the study, DK633266MV line had medium resistance to head smut.

The highest coefficient of variation on the natural infectious background was observed in the DK633266MV line (141 %, which corresponds to significant variability). This indicator was 36 % (significant variability) in the P354 line, and 15 % (medium variability) in the DK3008 line. Against the artificial infectious background, the coefficient of variation for PLS61, DK7575 and DK3008 lines was 141 %, for DK3023 – 48 %, DK633266MV – 46 %,

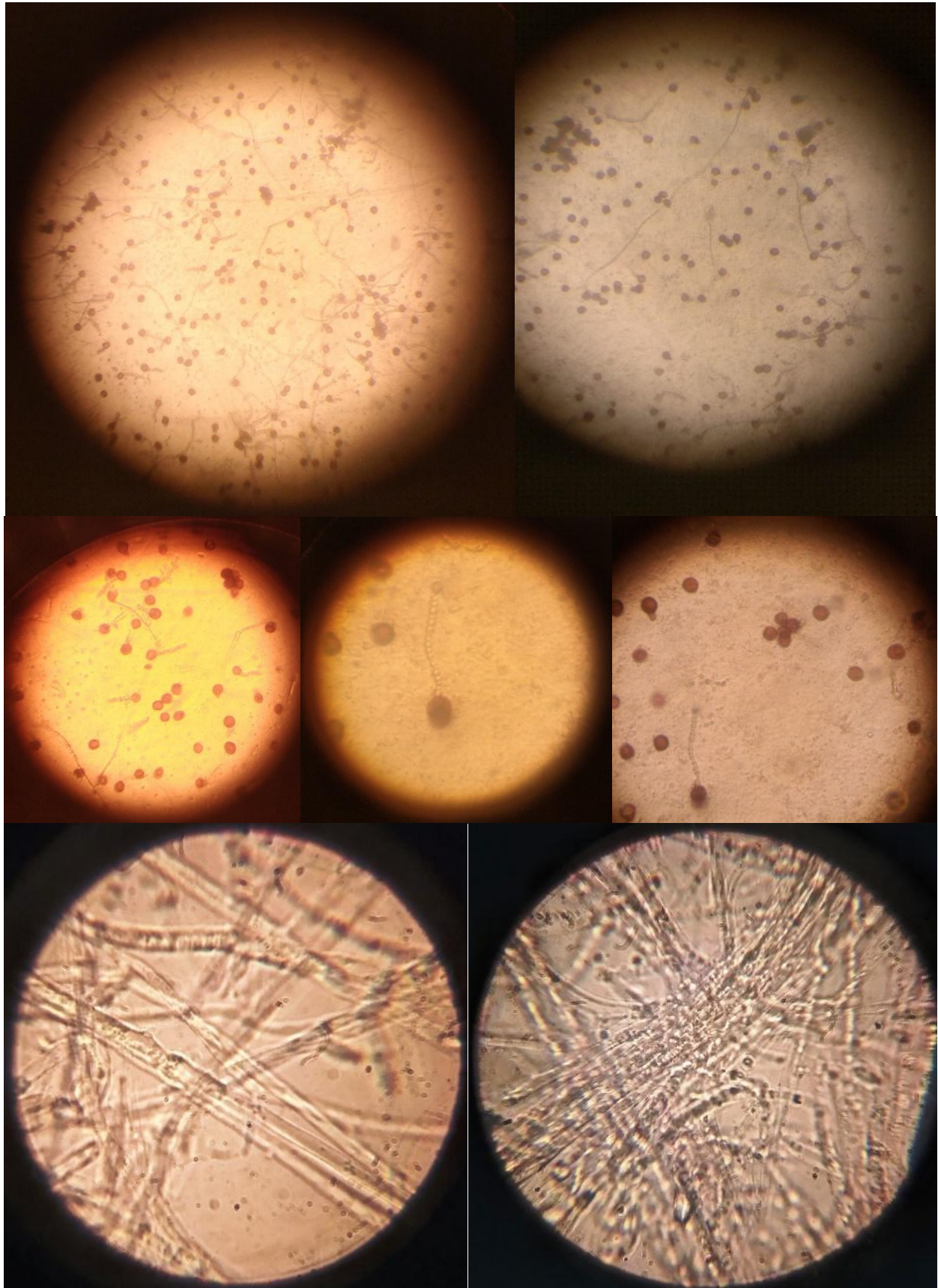


Fig. 1. Spore germination of head smut on the nutrient medium on the 4th day of cultivation (top and middle rows) and 8th day (bottom row).



Fig. 2. Characteristic traits of head smut disease in maize plants in field trials.

Table 1. Resistance of maize lines to *Sporisorium reilianum*

Line	Natural infectious background					Artificial infectious background				
	2021		2024		Average percentage of infected plants, %	2021		2024		Average percentage of infected plants, %
	infected plants, %	n	infected plants, %	n		infected plants, %	n	infected plants, %	n	
P354	4.55	22	2.70	37	3.63	41.18	17	59.26	27	50.22
DK315	0	26	0	27	0	3.45	29	3.57	28	3.51
DK633266MV	0	23	7.14	14	3.57	17.86	28	9.09	11	13.48
DK3044	0	51	0	40	0	0	32	0	36	0
DK267	0	66	0	30	0	0	71	0	31	0
PLS61	0	64	0	31	0	4.62	65	0	31	2.31
DK3023	0	45	0	42	0	24.24	33	12.00	25	18.12
DK7575	0	52	0	25	0	0	48	28.57	14	14.29
DK3008	1.56	64	1.92	52	1.74	1.75	57	0	32	0.88
Mo17	0	50	0	19	0	0	50	0	14	0
Average	0.61±0.72	46.3	1.18±1.25	29.8	0.90±1.02	9.31±2.81	43.0	11.25±4.01	24.9	10.28±3.46

Note. The data in the table is calculated by the formula $x \pm m \cdot t_{0.05}$, where x – arithmetic mean of the indicator, m – standard deviation, $t_{0.05}$ – Student's test at the significance level 0.05, n – number of plants studied, pcs.

P354 – 25 %, which corresponds to significant variability, while DK315 line demonstrated low variability with a coefficient of variation of 2.4 %. According to the coefficients of variation determined by the years of the study, there was significant variability in resistance to the disease among maize genotypes.

Thus, the lines are resistant to *S. reilianum* infection (less than 5 % of plants affected on the infectious background during two years of the study): DK315, DK3044, DK267, PLS61, DK3008, and Mo17. These lines can be donors of resistance to head smut. P354, DK3023, DK7575 lines are unstable, susceptible to infection (infection rate of 20 % or more). DK633266MV line has medium resistance to the disease.

The results on the grouping of maize lines into resistance groups will be used in further studies to find associations between molecular genetic DNA polymorphism and resistance to the pathogen.

The results of our study confirm the results obtained by other scientists. Thus, studies conducted by T. M. Pedash et al. showed that the average infection rate of 52 analysed maize hybrids against a natural infectious background was 0.9–5.9 % over three years, at the same time some genotypes had no infection at all, and some had infection rate of 25.3 %. In our study, the two-year average for the infection rate of lines on the natural infectious background was

0.61–1.18 %, while the infection rate of some lines reached 7.14 %. The authors also observed fluctuations in the infection rate of head smut depending on the agrometeorological conditions of the study year [11].

The maize line Mo17, known for its high resistance to head smut, demonstrated a 100 % resistance on the artificial infectious background in our study. According to some researchers, the infection rate of the Mo17 line was 0–2 % [14] and did not exceed 2.5 % [8].

An interesting aspect is the maximum infection rate of maize genotypes with head smut. According to the results of our research, the highest infection rate (41.18 % in 2021 and 59.26 % in 2024) was predictably observed in the P354 line (negative control) against the artificial infectious background. In 2018 and 2019, the same line had an infection rate of 68.7 % and 53.9 %, respectively [13]. G. Márquez-Liconab et al. used Az 41801 as a negative control, which showed 42.3 % and 70.7 % infection rates similar to our results, with maximum infection rates in maize populations of 22.2 % and 28.8 %, respectively, during the two years of the study [17]. The infection rate of other lines - negative controls (Huangzao4, Jing7 and Chang7-2) against the infectious background was 92.31 %, 89.58 % and 73.81 %, respectively, which significantly exceeded the data obtained for the most susceptible line P354 among the studied lines [14].

Conclusions. The resistance of breeding

promising maize lines adapted to the Steppe zone of Ukraine to the infection by *Sporisorium reilianum* was evaluated in our research. The studied lines were generally characterised by a high level of resistance to the pathogen. It was found that the lines DK315, DK3044, DK267, PLS61, DK3008, and Mo17 were resistant to *S. reilianum* with less than 5 % infection during two years. These lines can be used as donors of resistance to head smut. Lines P354, DK3023

and DK7575, which had infection rate of 20 % and more on the infectious background, are non-resistant to maize head smut. This feature should be considered in selecting these genotypes for the breeding process.

The obtained results on the classification of maize lines into resistance groups will be used in further studies to find associations between molecular genetic DNA polymorphism and resistance to the pathogen.

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Актуальність. Створення і добір генотипів кукурудзи, стійких до ураження летючою сажкою *Sporisorium reilianum*, є актуальним напрямом сучасних селекційних досліджень, адже дозволяє запобігти втратам урожаю, пов'язаним із ураженням патогеном, і отримати зерно високої якості. **Мета досліджень.** Оцінити ступінь ураження летючою сажкою *S. reilianum* селекційно перспективних ліній кукурудзи на природному і штучному інфекційному фонах та ідентифікувати серед них стійкі і нестійкі до ураження генотипи. **Матеріали і методи.** За матеріал дослідження обрали десять селекційно перспективних, адаптованих до зони Степу України, ліній кукурудзи. Дослідження проводили у 2021 і 2024 рр. Використовували методи: польовий, статистичного аналізу. **Результати.** Було перевірено і підтверджено життєздатність спор летючої сажки, використаних для створення штучного інфекційного фону, перед заспорюванням насіння кукурудзи шляхом культивування їх на штучному живильному середовищі *in vitro*. Проведене тестування ліній кукурудзи у польових умовах впродовж двох років показало, що на природному інфекційному фоні за роки дослідження середнє значення ураження рослин *S. reilianum* становило 0,90 %, тоді як на штучному інфекційному фоні – 10,28 %. Ураження рослин летючою сажкою залежало від генотипу і агрометеорологічних умов року дослідження. Найвищий показник ураження патогеном спостерігався у лінії П354 на штучному інфекційному фоні і складав для двох років досліджень 41,18 % і 59,26 %, відповідно. Лінії ДК3044, ДК267 та Мо17 за роки досліджень не продемонстрували жодного випадку ураження рослин летючою сажкою ні на природному, ні на штучному інфекційному фонах. **Висновки.** Оцінено стійкість до ураження летючою сажкою *S. reilianum* десятих селекційно перспективних, адаптованих до зони Степу України, ліній кукурудзи. Встановлено в цілому високий рівень стійкості до патогену у досліджених ліній. Лінії ДК315, ДК3044, ДК267, PLS61, ДК3008 і Мо17, які мали відсоток ураження *S. reilianum* рослин на штучному інфекційному фоні впродовж двох років менше 5 %, визначені як стійкі. Вони можуть виступати донорами стійкості до летючої сажки. Лінії П354, ДК3023 і ДК7575, які мали ураження на штучному інфекційному фоні 20 % і більше, ідентифіковані як нестійкі до ураження.

Ключові слова: кукурудза, стійкість до біотичних факторів, грибні хвороби зернових культур, летюча сажка, донори стійкості, штучний інфекційний фон