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ORIGINAL ARTICLE

Response of winter wheat (*Triticum aestivum* L.) to micronutrient foliar application enriched with silver

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Abstract:
The aim of the research was to analyze the degree of infection of winter wheat by fungal diseases and to evaluate the morphological and physiological parameters of plants depending on varied foliar fertilization (with and without the ionic form of silver) and applied plant protection agents (active ingredients: propiconazole, fenpropidin, azoxystrobin) in the 2016/2017, 2017/2018 and 2018/2019 growing seasons. The results showed that micronutrient fertilizers with silver and pesticides reduced the severity of fungal diseases better than the control. In most cases, foliar fertilizers enriched with the ionic form of silver at a dose of 1 and 2 $l \cdot ha^{-1}$ were the most effective. Moreover, foliar fertilization and pesticides had a positive effect on the morphology of wheat. Combined treatment (micronutrient fertilizer with silver and pesticide at a dose of 1 $l \cdot ha^{-1}$) increased stalk length and weight, ear weight and thousand grain weight to the greatest extent in comparison to the other treatments, while the pesticides stimulated ear length the most. In turn, microelement fertilizers with silver at a dose of 1 and 2 $l \cdot ha^{-1}$ were better in terms of flag leaf length. Wheat treated with foliar fertilizer and pesticide significantly improved the chlorophyll content based on the leaf greenness index (*SPAD*). It was found that the foliar application of microelements with silver is promising for use in agriculture because they controlled fungal diseases and ensured the good condition of plants more effectively than pesticides harmful to the environment.

Keywords: disease control, foliar fertilization, plant growth, silver, winter wheat

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal plants in the world in terms of production and as a food source for humans and animals (Niyigaba *et al.* 2019). While the demands for cereal products continue to increase, their cultivation is threatened by various abiotic and biotic stresses. Among biotic stresses, diseases and pests are the main threats (Singh 2017; Figueroa *et al.* 2018). Global losses caused by pathogens are estimated at 21.5% for wheat, 22.5% for maize and 30.0% for rice (Savary *et al.* 2019). Fungi are one of the most dominant groups of pathogens in cereal crops and are responsible for about 80% of plant disease infections (Shuping and Eloff 2017). Diseases occurring in cereals are caused mainly by fungi *Fusarium* spp., *Puccinia* ssp., *Pyricularia oryzae*, *Blumeria graminis* and *Zymoseptoria tritici* (Dean *et al.* 2012). Phytopathogens inhibit the development and growth of cereals and as a result they reduce seed yield and deteriorate its quality (Doehlemann *et al.* 2017; Newitt *et al.* 2019). The harmfulness of *Fusarium* spp.

also involves the production of mycotoxins – secondary metabolites harmful to both human and animal health (Covarelli *et al.* 2015; Gorczyca *et al.* 2018). According to the Food and Agriculture Organization (FAO) a quarter of the world's crop production is contaminated with mycotoxins, although new data indicate that the global incidence of mycotoxins in crops is much higher (60–80%) (Eskola *et al.* 2020).

The presence of pathogens on plants depends on the mutual relations of many factors that interact with each other throughout the growing season, i.e., climatic conditions, agrotechnical factors (soil cultivation, sowing date, fertilization) and varietal sensitivity (Kiseleva *et al.* 2016; Hýsek *et al.* 2017).

Fertilization is an important aspect of cultivation that affects the growth, development and yield of plants. Plants, apart from the main nutrients (macronutrients), need elements for proper functioning, i.e., zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), boron (B), molybdenum (Mo), chlorine (Cl) and nickel (Ni) – the so-called essential micronutrients (Jat *et al.* 2020). There are two methods of micronutrient application: soil and foliar application. Micronutrients applied in foliar application are 8–20 times more potent than those introduced into the soil, as scarce nutrients go directly to high demand areas (Alshaal and El-Ramady 2017). Many authors have shown that an adequate supply of micronutrients promotes good, stable development and growth of wheat (Rawashdeh and Sala 2013; Pandey *et al.* 2020) and leads to an increase in its yield (Khan *et al.* 2010; Gomaa *et al.* 2015; Esfandiari *et al.* 2016; Kandoliya *et al.* 2018) mainly due to the role of these elements in many important biological processes. Zinc is a cofactor for approximately 300 enzymes involved in cellular metabolism (Singh and Singh 2019). Boron participates in the synthesis of nucleic acids and proteins, regulates carbohydrate metabolism, and also influences root elongation, flowering processes, pollination efficiency, setting and seed formation (Pandey *et al.* 2020). In turn, the role of Mo in plants is related mainly to nitrogen metabolism, which results from its participation in nitrate reductase and nitrogenase (Rana *et al.* 2020). Necessary for the photosynthesis and respiration process are Fe, Cu and Mn, which take part in the photolysis of water in photosystem II (Tripathi *et al.* 2015; Schmidt *et al.* 2020). Moreover, Mn is considered to be a cofactor of many enzymes participating in decarboxylation, hydrolysis and oxidation reactions, and by controlling the metabolism of carbohydrates it influences the energy management of plants. Like Cu, it participates in the detoxification of free radicals (Alejandro *et al.* 2020).

Balanced fertilization not only affects the condition of plants, but also strengthens their defense against pathogens (Bala *et al.* 2018; Zhang *et al.* 2021). Mn, Cu and Zn activate enzymes that produce defense metabolites, i.e., ammonium phenylalanine lyase and polyphenol oxidases, thus increasing plant resistance to diseases. Mn also inhibits the synthesis of aminopeptidase, which produces essential amino acids necessary for fungal growth, and pectin methylesterase – an enzyme that breaks down host cell walls (Gupta *et al.* 2017). Micronutrients can also inhibit pathogen penetration by creating mechanical barriers. Mn, Cu, and B are components of many enzymes important for the synthesis of lignin, which imparts rigidity and strength to the cell wall (Broadley *et al.* 2012). Furthermore, B and Zn are involved in maintaining the integrity of the biomembranes. Plants deficient in these micronutrients show impaired integrity of cell membranes, which results in the leakage of organic compounds from the cell, e.g., carbohydrates and amino acids, which are suitable food substrates for pathogens (Broadley *et al.* 2012; Huber *et al.* 2012). Available literature shows that plant disease management is also possible with the use of silver compounds. Silver has a strong antimicrobial effect both in ionic form and as nanoparticles (NPs) (Ejaz *et al.* 2018; Kędziora *et al.* 2018; Loo *et al.* 2018; Sadoon *et al.* 2020; Gorczyca *et al.* 2021). This metal is effective against 650 different microorganisms (Salomoni *et al.* 2017). Silver ions affect a number of vital functions of microorganisms. They can cause structural and functional changes in the cell membrane, as a result of which the electron transport chain is broken and ultimately the metabolism is disturbed. In addition, it has been found that silver inhibits the expression of proteins associated with ATP production, thereby destroying cell viability (Yamanaka *et al.* 2005).

The aim of this study was to assess the impact of compound foliar fertilizers with and without the ionic form of silver as well as the applied plant protection agents using fungicides on the severity of the occurrence of fungal diseases and selected morphological and physiological features of winter wheat in a strict 3-year field experiment. Due to the oligodynamic action of silver ions, the use of micronutrients with the addition of silver can prove to be effective in controlling crop diseases and improving crops by more targeted and strategic promotion of plant resistance based on proper nutrition.

Materials and Methods

Agronomic conditions

Over 3 years (growing seasons: 2016/2017, 2017/2018 and 2018/2019) a strict field experiment was carried out near Krakow (Southern Poland, 50°06′52″N, 20°04′23″E). The two-factor experiment was carried out in a randomized block design in four replications. The plot area was 10 m^2. The experimental factors were:

1) treatment – the type of foliar fertilization and the plant protection agents,
2) growing season.

Treatments used in the experiment are shown in Table 1. The experimental plant was winter wheat (*Triticum aestivum* L.) of the Ludwig variety.

Wheat was grown on chernozem (bonitation class 1), formed from loess, classified as a very good wheat complex. Soil analysis was performed in an accredited laboratory. The exact agrochemical characteristics of the soil are given in Table 2. Pea (*Pisum sativum* L.) was the forecrop. In the experiment, all agrotechnical cultivation treatments recommended for wheat were used. Plowing at a depth of 20–25 cm and harrowing was done between September 10th and 20th. Pre-sowing mineral fertilization included: superphosphate (P_2O_5) at a dose of 80 kg · ha^{-1} and potassium salt (K_2O) at a dose of 160 kg · ha^{-1}. Then, in order to mix fertilizers with the soil and prepare the upper layer for sowing, aggregate equipment consisting of a cultivator with a string roller was used. Sowing was carried out during the first 10 days of October. Wheat was sown to a depth of 2–3 cm, in the amount of 450 pcs m^2. The first dose of nitrogen fertilizer (80 kg N · ha^{-1}) was sown during the start of vegetation and the second (40 kg N · ha^{-1}) at the beginning of the stem elongation phase. Weeds were controlled using herbicide (active ingredients: florasulam, piroksysulam and aminopyralid) at a dose of 200 g · ha^{-1} with adjuvant at a dose of 0.5 l · ha^{-1}. Moreover, the growth regulator (active ingredient: trinexapac-ethyl) at a dose of 0.4 l · ha^{-1} and the insecticide (active ingredient: lambda-cyhalothrin) at a dose of 0.1 l · ha^{-1} were used in the cultivation of wheat. Harvest fell the between July 10th and 20th.

Table 1. Characteristics of applied treatments in the study

Treatment	Marked	Composition*/Active ingredient**	Dose [l · ha^{-1}]	Application dates (growth stage)
Control – no fertilization and no plant protection treatment	C	–	–	–
Foliar fertilization	FF1	N – 4%, MgO – 5%, S – 4.3%, B – 0.16%, Cu – 0.35%, Fe – 1%, Mn – 0.98, Mo – 0.005%, Zn – 0.9%	1	BBCH 30–32 and BBCH 56–58
Foliar fertilization	FF2	N – 4%, MgO – 5%, S – 4.3%, B – 0.16%, Cu – 0.35%, Fe – 1%, Mn – 0.98, Mo – 0.005%, Zn – 0.9%	2	
Foliar fertilization with the addition of silver ions	FF1+Ag	N – 4%, SO_3 – 11%, MgO – 5%, B – 0.16%, Cu – 0.35%, Fe – 1%, Mn – 0.98, Mo – 0.005%, Zn – 0.9%, Ag – 0.0002%	1	
Foliar fertilization with the addition of silver ions	FF2+Ag	N – 4%, SO_3 – 11%, MgO – 5%, B – 0.16%, Cu – 0.35%, Fe – 1%, Mn – 0.98, Mo – 0.005%, Zn – 0.9%, Ag – 0.0002%	2	
Foliar fertilization with the addition of silver ions + + pesticide	FF1+Ag+P	N – 4%, SO_3 – 11%, MgO – 5%, B – 0.16%, Cu – 0.35%, Fe – 1%, Mn – 0.98, Mo – 0.005%, Zn – 0.9%, Ag – 0.0002%	1	
		propiconazole, fenpropidin	1	
		azoxystrobin	1	
Pesticide	P	propiconazole, fenpropidin	1	
		azoxystrobin	1	

N – nitrogen, MgO – magnesium oxide, S – sulphur, SO_3 – sulphur trioxide, B – boron, Cu – copper, Fe – iron, Mn – manganese, Mo – molybdenum, Zn – zinc, Ag – silver
*in the case of foliar fertilization; **in the case of pesticide

Table 2. Agrochemical characteristics of soil

Growing season	pH_{KCl}	P_2O_5	K_2O	Mg	$S\text{-}SO_4$	N-org.	C-org.	S-org.
			[mg 100 · g^{-1} soil]				[%]	
2016/2017	5.23	9.4	13.0	9.9	0.20	0.098	1.03	0.0170
2017/2018	6.43	15.5	15.0	12.7	0.92	0.098	1.10	0.0200
2018/2019	6.42	14.0	12.0	12.7	0.35	0.112	1.03	0.0178

Climatic conditions of the experiment

Climatic conditions of the region's research seasons are compared to the multiannual data in Figure 1. Weather conditions were monitored by the Delta-T Devices (Cambridge,`UK) automatic weather station located near the experimental field.

Significant variability of humidity and thermal conditions was observed during the three growing seasons of the study. In comparison to multiannual data, precipitation recorded in the growing season 2016/2017 significantly differed. October, April and May were particularly wet months, with significantly higher precipitation than that recorded in the multiannual data, which could have increased the incidence of fungal diseases in the crop. The dry months were December, January and June. In the remaining months, precipitation did not differ significantly from those typical for multiannual data. The autumn of the 2017/2018 season was characterized by higher precipitation than that found in multiannual data. September and July were the most favorable months in this respect, and the most conducive to the development of pathogens. Winter and the beginning of spring were very dry. April especially significantly deviated from the long-term average. In the following months there was similar precipitation. The last season, i.e., 2018/2019, was characterized by a varied amount of precipitation compared to multiannual data. November, February, March and June were dry months. In turn, December, January and April were wet. Particularly abundant precipitation occurred in May – almost three times higher than in multiannual data.

The air temperatures of the first season of research were slightly higher than in multiannual data. The exception was January, which was significantly cooler. The growing season 2017/2018 was characterized by variable temperatures in the autumn. In September the temperature was lower than that recorded in multiannual data, while in November a higher temperature was recorded. Higher temperatures were also recorded in the winter months except February, which was much cooler. Spring months were characterized by higher temperatures, with the exception of March, in which lower temperatures were noted. Summer was particularly warmer than that seen in multiannual data. In the last season of research, significantly higher average daily temperatures than noted in multiannual data occurred, with the exception of May.

Analysis of health

Assessment of symptoms of fungal diseases was made by macroscopic analysis of 40 randomly selected plants from the object on a scale of 4° (the stem-base diseases) and 9° (leaves and ear diseases), where 1 = healthy plants, max scale = symptoms covering

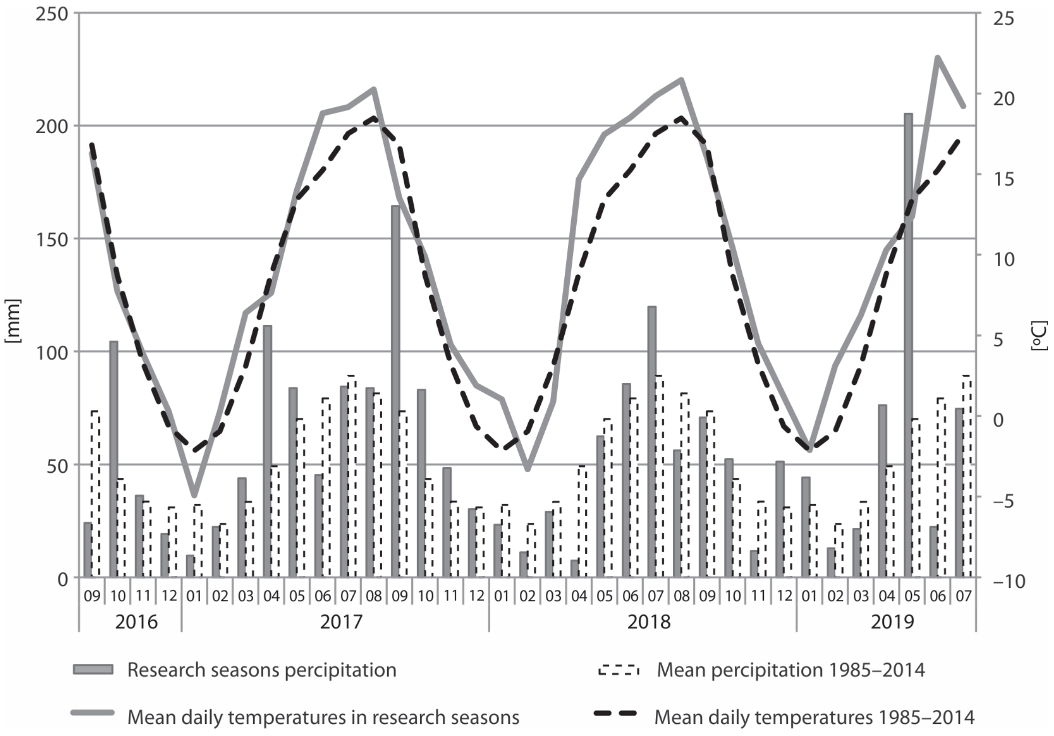

Fig. 1. Weather conditions of the study area observed in the growing seasons 2016/2017–2018/2019 against the background of multiannual data

70–100% of the stem surface at the level of the root collar, as well as symptoms covering 100% of the leaf and ear surface according to a modified scale reported by Koyshibayev and Muminjanov (2016). Observations of the degree of infestation of the stem-base and leaves were carried out at the milky-wax maturity stage (BBCH 71–77), and ear diseases were observed at the stage of full maturity (BBCH 89).

The results obtained on a scale were converted into a Disease Index (*DI*) coefficient taking into account that the significance of the damage increases with the size of the damage, such as the arithmetic sequence with the multiplicity of two according to the formula proposed by Pierre and Regnatul (1982):

$$DI = \frac{\sum_{i=2}^{\text{max scale}}\left[2(i-2)+1\right]n_i}{\sum_{i=1}^{\text{max scale}} n_i},$$

where n_i is the number of plants in category *i*.

Assessment of black dot symptoms after harvest

After a month of storage of the grain, an assessment of the scale of black dot was made, i.e., discoloration of the end of the germ of the grain and the surrounding areas of the wheat kernel. The coated grain of each ear was assessed for discoloration, and then the percentage share of the discoloration was calculated in a given sample.

Analysis of morphological parameters of wheat and thousand grain weight (*TGW*)

Morphological assessment of 40 randomly selected plants from the object was carried out. Length and weight of stalk, flag leaf length as well as ear length and weight were determined. Thousand grain weight (*TGW*) for individual experimental facilities were also analyzed.

Measurements of photosystem II efficiency

Efficiency of photosystem II (PSII) was measured using a Plant Efficiency Analyzer (PEA; Hansatech Ltd., King's Lynn, UK) with an excitation light intensity of 3 mmol $\cdot$ m^{-2} $\cdot$ s^{-1} (peak wavelength 650 nm). The measurements were done on the youngest fully expanded leaves after 30 min of adaptation to darkness in leaf clips (Hansatech), in 40 replications per treatment. The following parameters of PSII efficiency were calculated based on Strasser *et al.* (2000) as follows: Fv/Fm – maximum yield of photosystem II, PI_{ABS} – performance index, φE_0 – quantum yield for electron transport, ψ_0 – probability of electron transport, *OEC* – yield of oxygen evolving complex, RC/CS_0 – active PSII reaction center per excited cross-section. Moreover *ABS* – absorption flux, TR_0 – trapped energy flux, ET_0 – electron transport flux, DI_0 – dissipated energy flux were calculated and expressed per *CS* (cross section of the sample) and *RC* (reaction center).

Leaf greenness index (*SPAD*)

The chlorophyll content based on the leaf greenness index was assessed using a *SPAD* 502DL chlorophylometer (Soil Plant Analysis Development, Minolta Camera Co., Osaka, Japan). Measurements were made on randomly selected, fully developed leaves of wheat in the heading phase (BBCH 50–53). Forty replicates were performed for each object. The principle of the *SPAD* measurement method is to determine the quotient of the difference in light absorption by a leaf at two wavelengths – 650 and 940 nm (Barutçular *et al.* 2016).

Statistical analysis

Statistical analysis was conducted using XLSTAT program (Addinsoft, UK). The obtained results were checked for normality of distribution (Shapiro–Wilk test) and variance homogenity (Levene's test). Analysis of variance was performed by ANNOVA (at a significance determined by Duncan's test) or Kruskal-Wallis test (at a significance determined by Dunn's test with Bonferroni correction) at a significance level of $p < 0.05$. Results were prepared using the means and standard errors (*SE*) for each data point. The relationships between observations were determined by Principal component analysis (PCA) based on Pearson correlation matrix.

Results

Analysis of health

The following stem-base diseases were found in the experiment: Fusarium foot rot (*Fusarium* spp.), eyespot (*Oculimacula yallundae*, *O. acuformis*), and take-all (*Gaeumannomyces graminis*).

The statistical significance of the treatment and the growing season for the Fusarium foot rot index is shown in Figure 2A. Based on the 3-year average, this disease was significantly reduced after foliar fertilization with the addition of silver (FF1+Ag, FF2+Ag), P as well as FF1+Ag+P compared to the control (Fig. 2B). The infection of wheat by pathogens causing this disease was observed in each growing season, but to a different extent, due to the fact that *Fusarium* spp. adapts well to changing conditions (Fig. 2C). Both in the 2016/2017 and 2018/2019 seasons, the most effective treatments were fertilizers with silver (FF1+Ag and FF2+Ag). These seasons were characterized by heavy

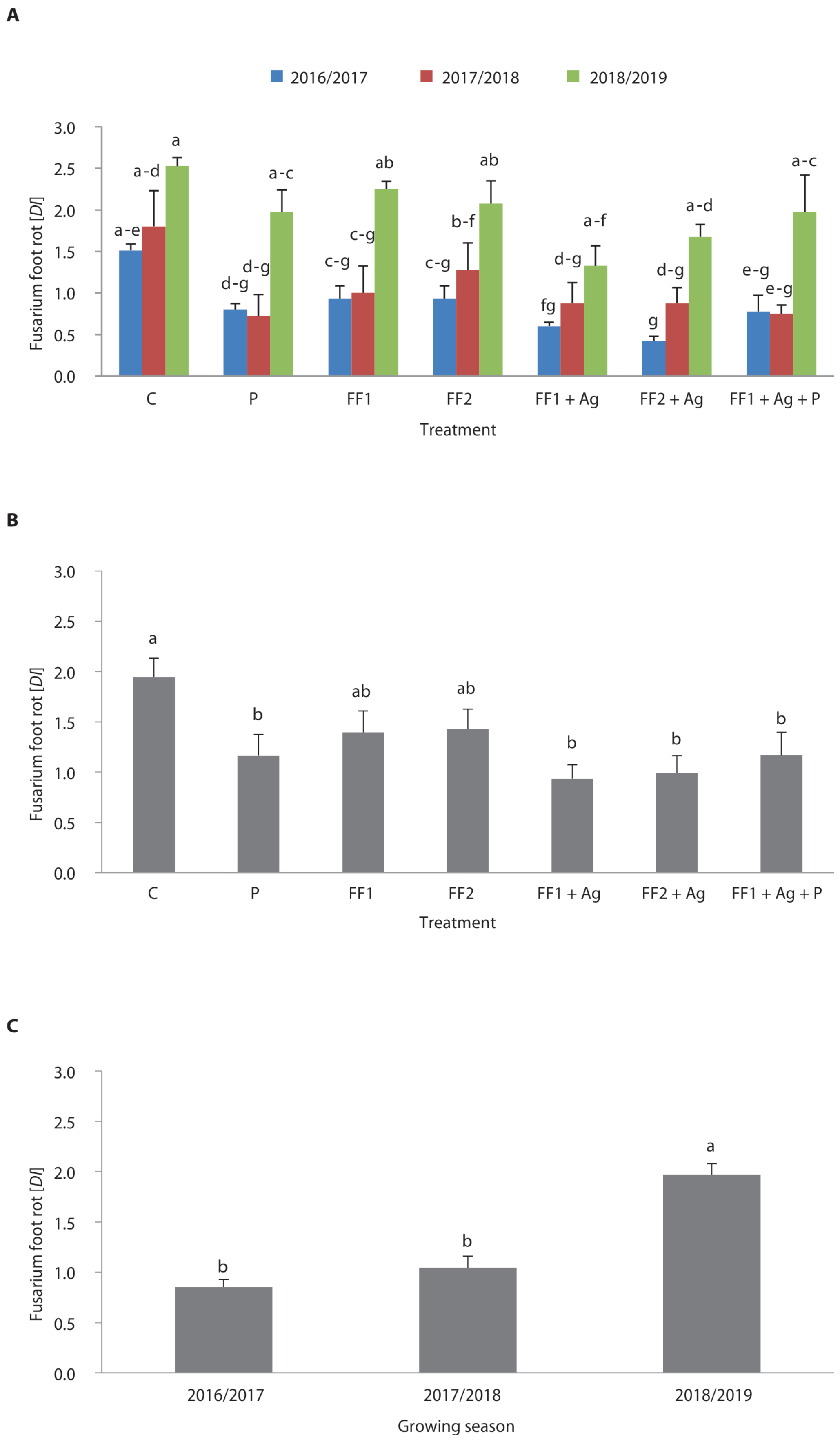

Fig. 2. Index of diseases of winter wheat by Fusarium foot rot depending on foliar fertilization and fungicide treatment, and growing season: interaction effect (A), effect of factors (B, C). Means followed by the same letters are not significantly different at $p < 0.05$. C – control, P – pesticide, FF – foliar fertilization

rainfall (especially in October, April and May), which may have exacerbated the incidence of this disease. Despite this, fertilizers with silver reduced the average intensity of Fusarium foot rot by 66% (2016/2017) and 41% (2018/2019) compared to the control, which indicates its high efficiency even under favorable conditions for the development of pathogens causing this disease. In turn, P and FF1+Ag+P showed the greatest effectiveness in the 2017/2018 season.

Eyespot was the disease of the lowest severity compared to the other two stem-base diseases . The average *DI* was 0–0.53 (Fig. 3A). On the basis of the obtained results, it was found that only FF2+Ag significantly limited the intensity of the disease in comparison to the control (Fig. 3B). In the 2018/2019 season, this disease was not found, while in the remaining seasons, the infestation of plants was at a similar level (Fig. 3C). In the 2016/2017 season, the effectiveness of FF2+Ag was 96%, and in the 2017/2018 season it was 82%. It shows the high potency of the application of this treatment under weather conditions conducive to infection. In October there was heavy rainfall and in autumn the coleoptile is most susceptible to fungi infestation. In addition, in a cool and humid spring this disease develops very quickly.

Figure 4A shows the interaction of the influence of the investigated experimental factors on the occurrence of take-all. Among the applied foliar fertilization and fungicide protection, only FF2+Ag significantly reduced the severity of this disease by 57% as compared to the control (Fig. 4B). Different infections of wheat were noted depending on the growing season. Take-all was the most intense (average *DI* 2.0) in the 2016/2017 season (Fig. 4C). This season was characterized by a humid autumn (especially October), a warm spring, and a hot, dry summer. All of these conditions favor the infestation of wheat by G. *graminis*.

From the diseases occurring on the leaves, symptoms were observed which indicated the presence of septoria nodorum blotch (*Zymoseptoria tritici*), Fusarium leaf blotch (*Fusarium* spp.) and brown rust (*Puccinia recondita*).

Septoria nodorum blotch was the most severe disease of all the diagnosed wheat leaf diseases, with a *DI* of 0.4–7.3 (Fig. 5A). Compared to the control, only P treatment as well as FF1+Ag+P significantly reduced the incidence of this disease, which was present in each of the growing seasons (Figs 5B, C). This demonstrates the great potential of these treatments against pathogens causing Septoria nodorum blotch.

Another identified leaf disease was Fusarium leaf blotch. The degree of infestation by this disease was in the range of 0.3 to 6.0 (Fig. 6A). Foliar fertilizers with the addition of silver (FF1+Ag, FF2+Ag), P and FF1+Ag+P were more effective in reducing the severity of this disease than the control (Fig. 6B). Among these treatments in each of the growing seasons the fewest symptoms on the analyzed plants occurred when FF2+Ag and FF1+Ag+P were used. This disease occurred in each growing season, although with different intensities (Fig. 6C). The 2017/2018 season was most conducive to the occurrence of this disease which could have been caused by air temperatures in the spring and summer which were higher than the multiannual, and intense precipitation in autumn and summer.

The interaction of the investigated experimental factors on the occurrence of brown rust is shown in Figure 7A. In the case of this disease, only FF1+Ag+P contributed to a significant reduction of wheat infection compared to untreated plants (Fig. 7B). This treatment was effective in 2017/2018 and 2018/2019, which were the seasons favorable for the development of disease (Fig. 7C). This could have been caused by warm weather in autumn and high air temperature, especially in the 3 months of April, May and June. The development of pathogens of the genus *Puccinia* spp. is stimulated by warm and humid weather. In the 2016/2017 season, brown rust was not identified.

Septoria glume blotch (*Phaeosphaeria nodorum*, *Parastagonospora nodorum*), Fusarium head blight (*Fusarium* spp.), and black head molds (*Alternaria* spp., *Cladosporium* spp., *Epiccocum* spp., *Ascochyta* spp.) were observed on the ears.

The degree of infection of wheat by septoria glume blotch is shown in Figure 8A. In each growing season the lowest plant infection index occurred after the application of P and FF1+Ag+P (Figs 8A, B). Treatment of P was the most effective in the 2016/2017 and 2017/2018 seasons, while in the following year, FF1+Ag+P was more effective. All growing seasons were favorable for the occurrence of this disease, of which the greatest infection occurred in the 2016/2017 season (Fig. 8C).

Fusarium head blight affected the ears the least with the mean *DI* being in the range of 0 to 1.6 (Fig. 9A). All treatments except FF1 and FF2 significantly reduced the severity of this disease compared to the control (Fig. 9B). In the 2018/2019 season, Fusarium head blight was the most severe, while during the next two research seasons symptoms were present in trace amounts (Fig. 9C). The 2018/2019 season was characterized by a mild autumn and winter, and a warm and wet spring, which favored the development of the disease. Higher temperatures and high humidity are a prerequisite for the development of this disease. Nevertheless, in this season FF1+Ag+P significantly reduced this disease by 83%, indicating that this treatment is effective against the pathogens causing Fusarium head blight even under climatic conditions favorable for their occurrence.

Figure 10A shows the interaction of the influence of the tested experimental factors on the degree of

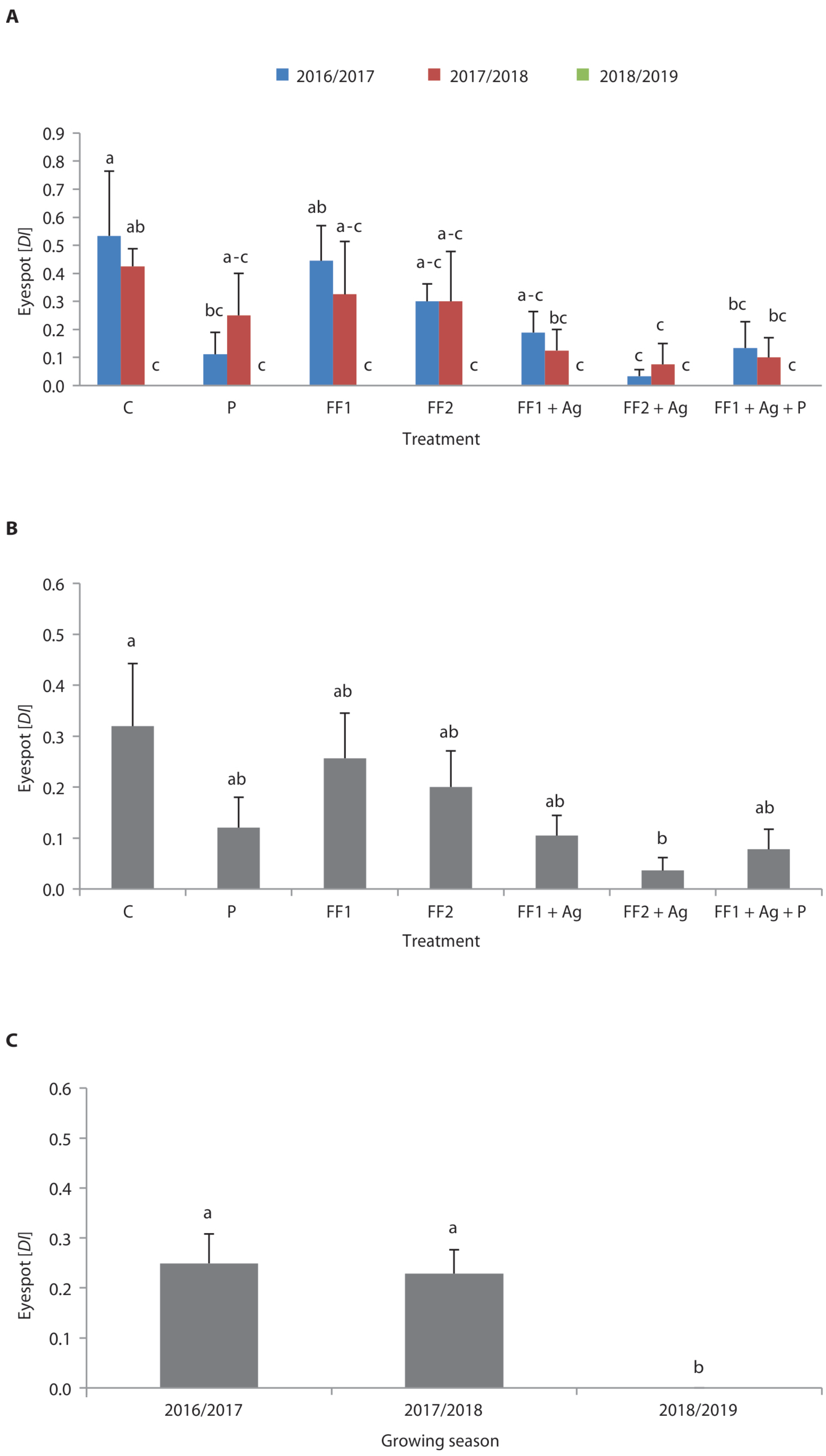

Fig. 3. Index of diseases of winter wheat by eyespot depending on foliar fertilization and fungicide treatment, and growing season: interaction effect (A), effect of factors (B, C). Means followed by the same letters are not significantly different at $p < 0.05$. C – control, P – pesticide, FF – foliar fertilization

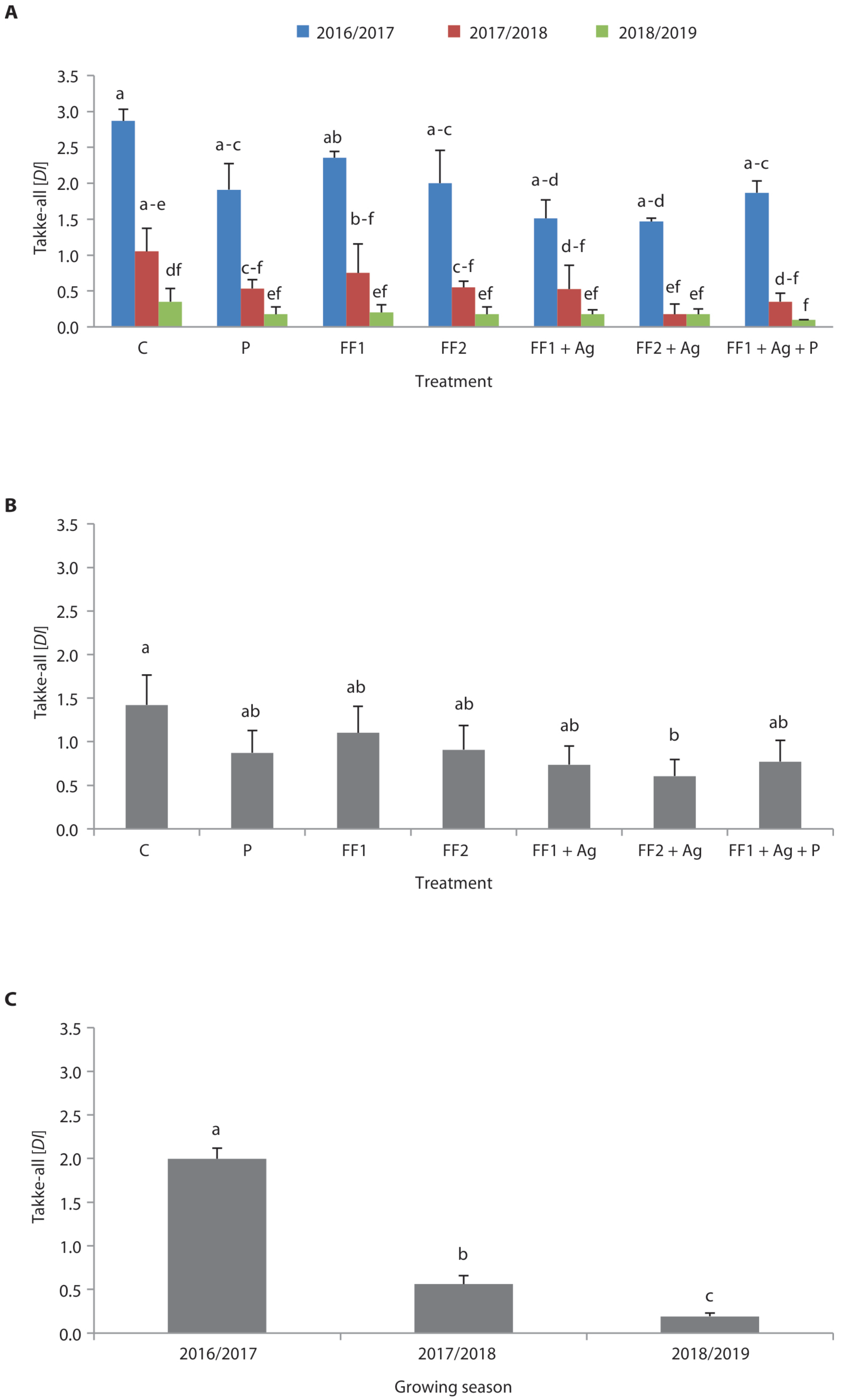

Fig. 4. Index of diseases of winter wheat by take-all depending on foliar fertilization and fungicide treatment, and growing season: interaction effect (A), effect of factors (B, C). Means followed by the same letters are not significantly different at $p < 0.05$. C – control, P – pesticide, FF – foliar fertilization

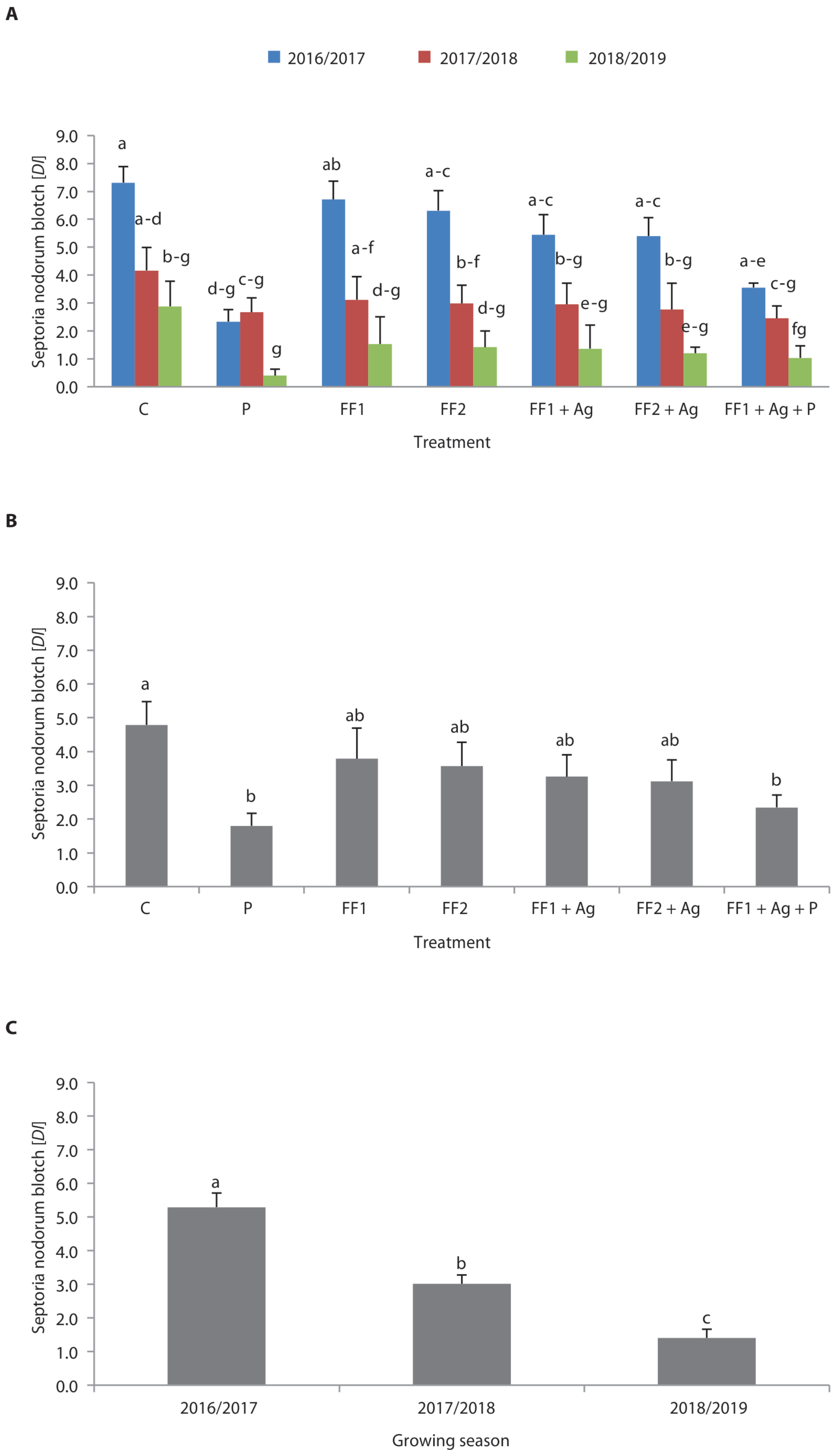

Fig. 5. Index of diseases of winter wheat by septoria nodorum blotch depending on foliar fertilization and fungicide treatment, and growing season: interaction effect (A), effect of factors (B, C). Means followed by the same letters are not significantly different at $p < 0.05$. C – control, P – pesticide, FF – foliar fertilization

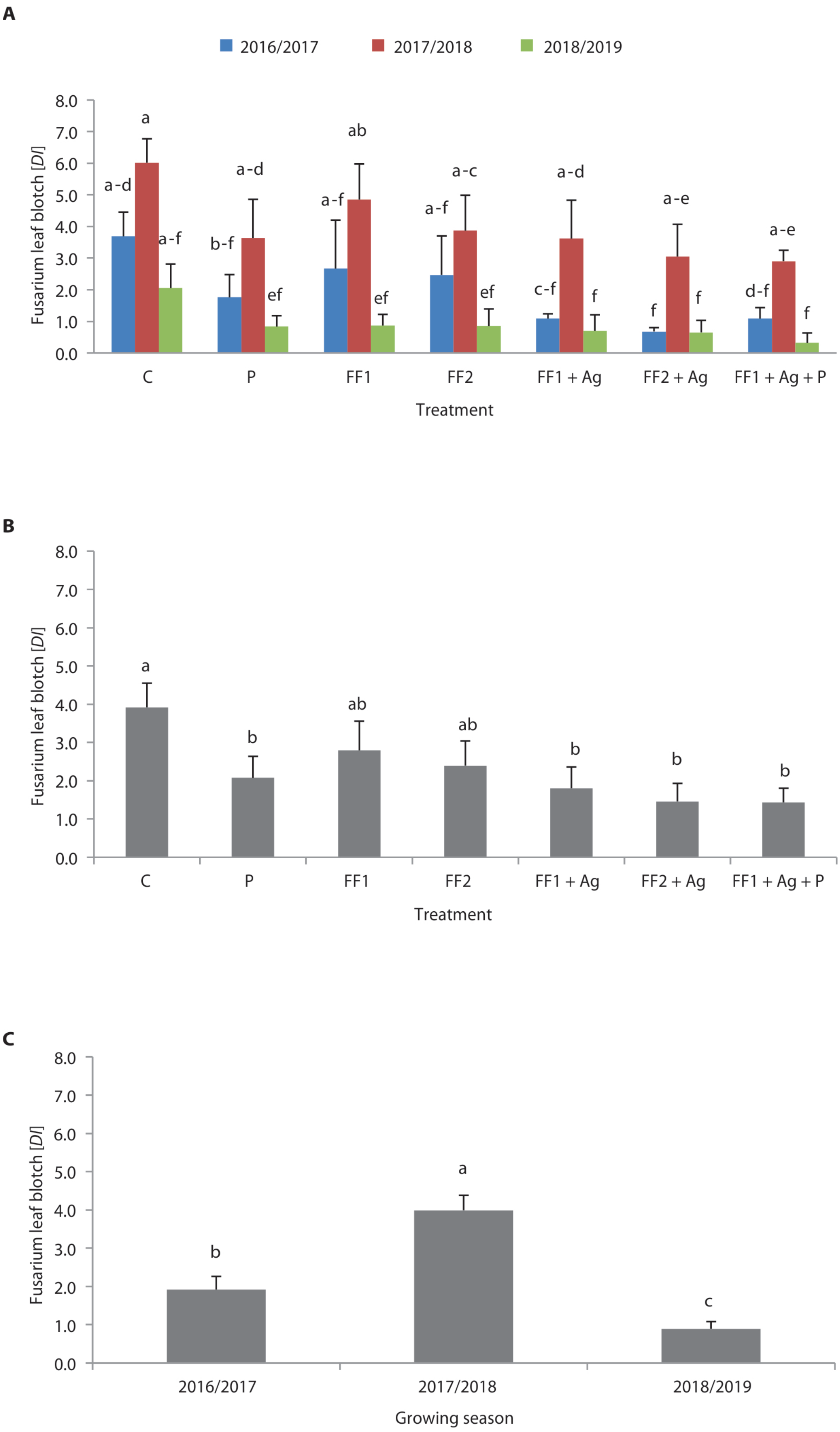

Fig. 6. Index of diseases of winter wheat by Fusarium leaf blotch depending on foliar fertilization and fungicide treatment, and growing season: interaction effect (A), effect of factors (B, C). Means followed by the same letters are not significantly different at $p < 0.05$. C – control, P – pesticide, FF – foliar fertilization

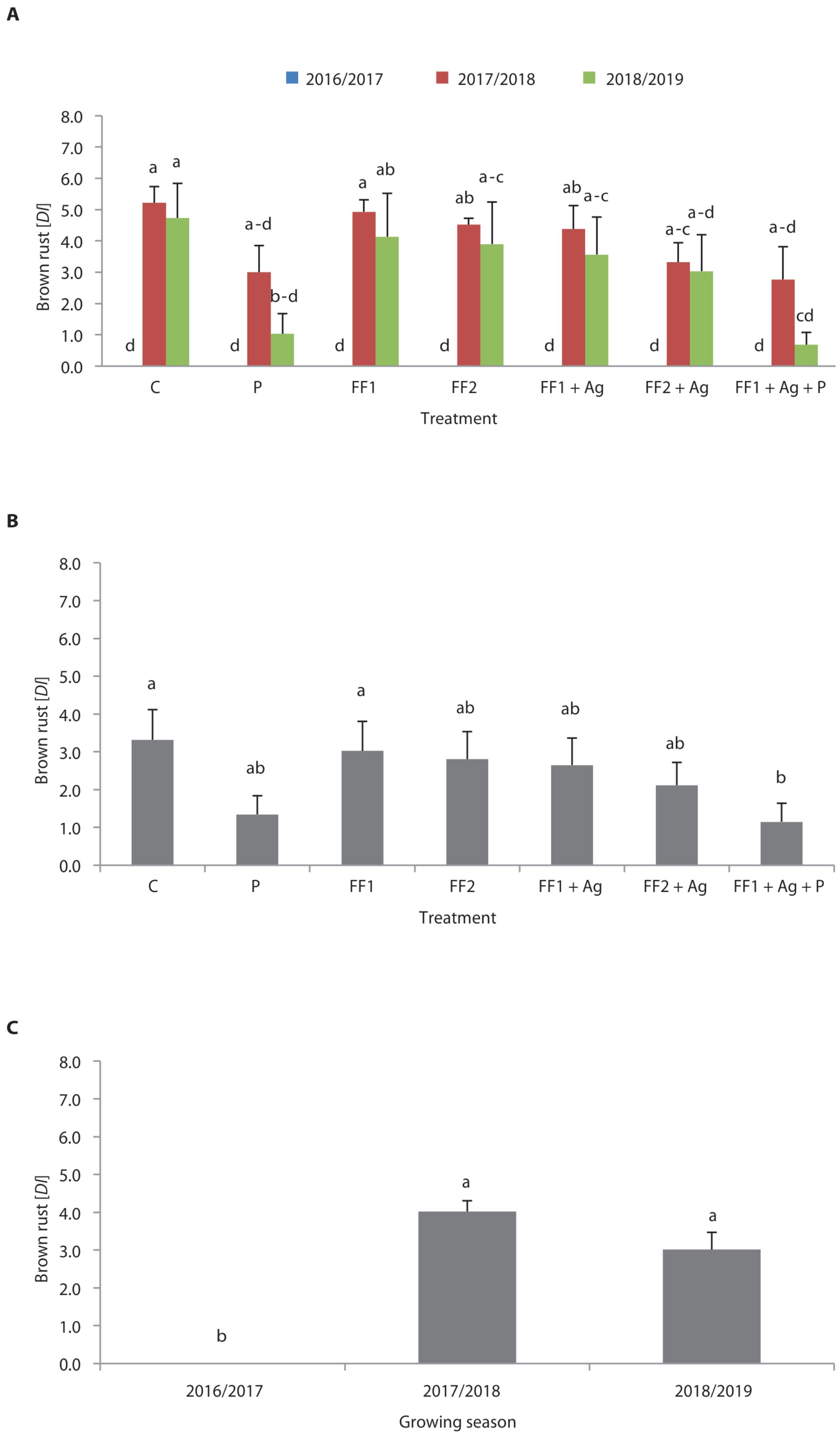

Fig. 7. Index of diseases of winter wheat by brown rust depending on foliar fertilization and fungicide treatment, and growing season: interaction effect (A), effect of factors (B, C). Means followed by the same letters are not significantly different at $p < 0.05$. C – control, P – pesticide, FF – foliar fertilization

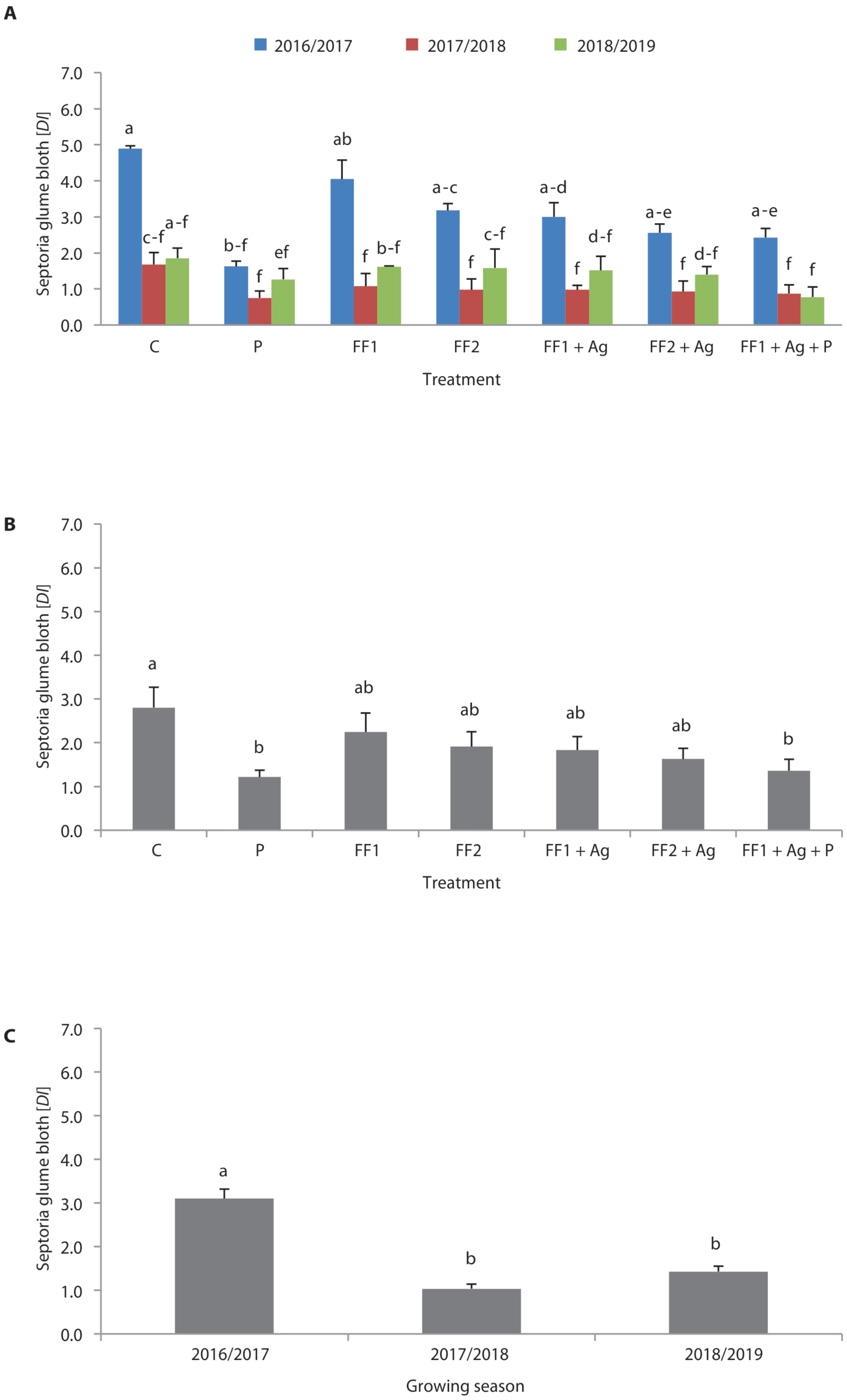

Fig. 8. Index of diseases of winter wheat by septoria glume blotch depending on foliar fertilization and fungicide treatment, and growing season: interaction effect (A), effect of factors (B, C). Means followed by the same letters are not significantly different at $p < 0.05$. C – control, P – pesticide, FF – foliar fertilization

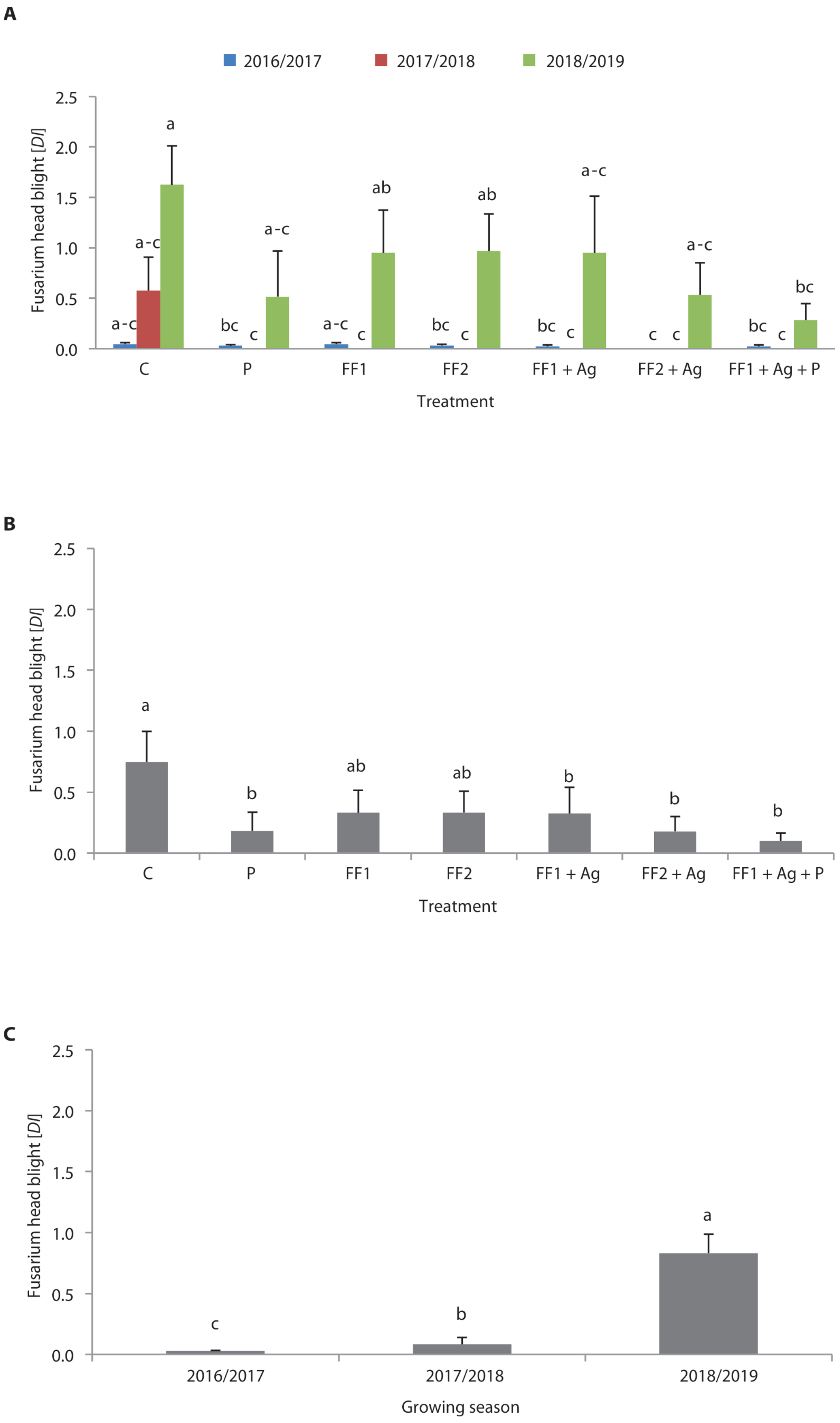

Fig. 9. Index of diseases of winter wheat by Fusarium head blight depending on foliar fertilization and fungicide treatment, and growing season: interaction effect (A), effect of factors (B, C). Means followed by the same letters are not significantly different at $p < 0.05$. C – control, P – pesticide, FF – foliar fertilization

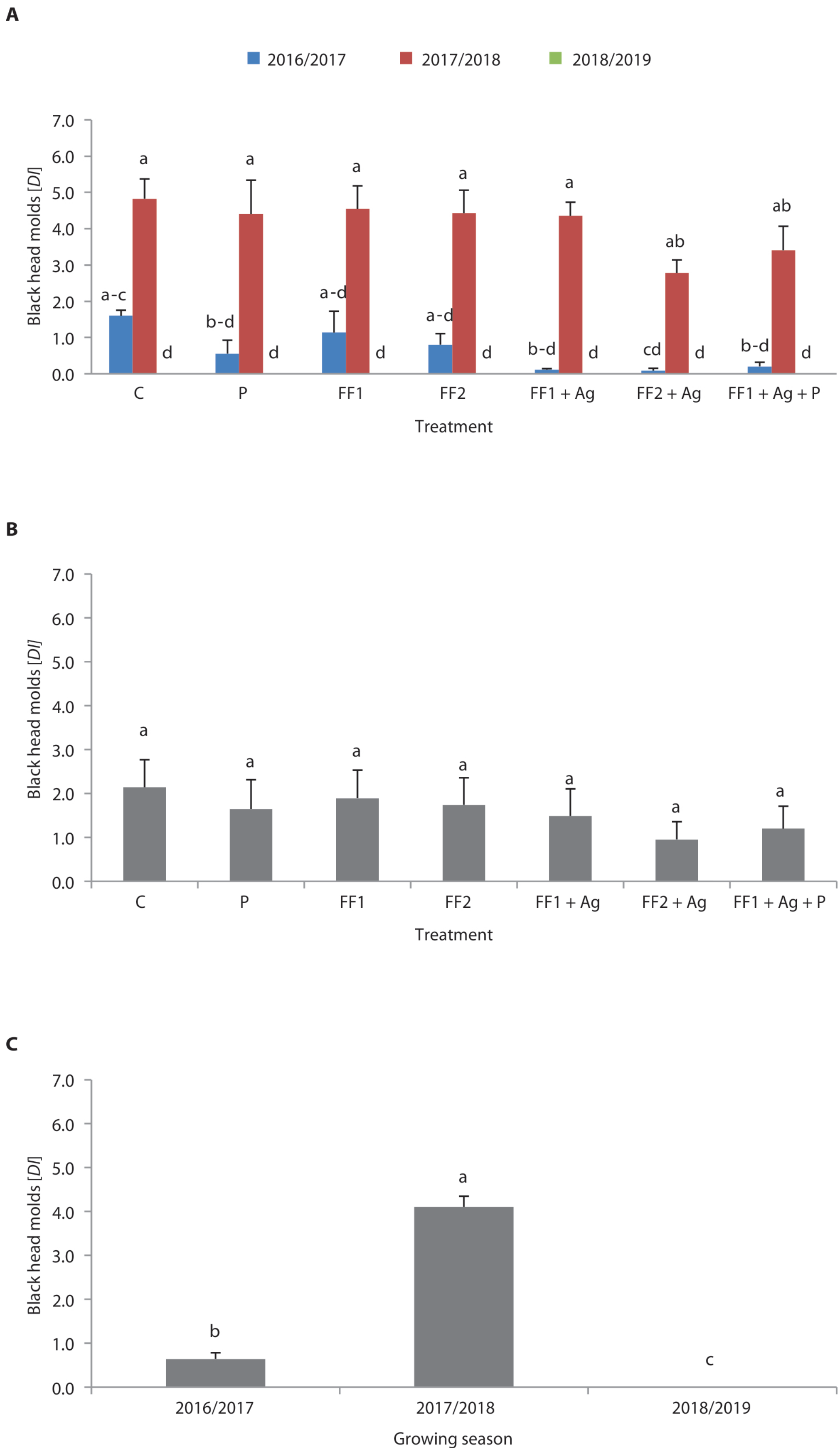

Fig. 10. Index of diseases of winter wheat by black head molds depending on foliar fertilization and fungicide treatment, and growing season: interaction effect (A), effect of factors (B, C). Means followed by the same letters are not significantly different at $p < 0.05$. C – control, P – pesticide, FF – foliar fertilization

Fig. 11. Symptoms of black dot on the kernel

infection of wheat by pathogens causing black head molds. Based on the 3-year average, the infestation of wheat did not significantly vary between the applied fertilization and fungicide protection (Fig. 10B). Only in the 2016/2017 season, fertilizers containing silver significantly reduced the severity of this disease as well as P and FF1+Ag+P, but to a lesser extent. In the 2018/2019 season, wheat was not infected with this disease, while the greatest infection was recorded in the 2017/2018 growing season, because the development of this disease is favored by high air humidity and precipitation during cereal ripening (Fig. 10C).

Fungi that cause black or brown discolorations on kernels can seriously reduce the quality of crops, so it is important to pay close attention to this type of discoloration when examining ears. Figure 11 presents typical symptoms of the black dot observed on kernels. Figure 12A shows the percentage of kernels with black dot symptoms depending on the tested experimental factors. A significant reduction in the percentage of kernels with black dot symptoms was observed with the FF2+Ag and FF1+Ag+P treatments compared to the control (Fig. 12B). The most black dot symptoms were recorded in the 2017/2018 season, while the remaining seasons were characterized by a similar infection of kernels (Fig. 12C).

Analysis of morphological parameters of wheat and thousand grain weight (*TGW*)

The characteristics of the tested morphological parameters of winter wheat and *TGW* are summarized in Table 3. All the experimental factors (treatment and growing season) had a statistically significant influence on the examined parameters. The longest stalks and the highest weight of wheat stalks were recorded in the 2016/2017 season. In the remaining seasons, the parameters examined were very similar. The average stalk was 97 cm, while the average stalk weight was about 3 g. Of the applied foliar fertilization and fungicide protection, the greatest effect was recorded with FF1+Ag+P treatment. Moreover, fertilizers with the addition of silver (FF1+Ag, FF2+Ag) and P effectively influenced the stalk length in comparison to the control.

The longest flag leaves were recorded after the use of fertilizers with silver (FF1+Ag and FF2+Ag), which was reflected in *TGW* that was 7–9% higher than the control. The longest leaves were obtained in the 2016/2017 season.

The average length of ears was in the range of 8.59–10.69 cm. The longest ears were recorded after applying P in the 2018/2019 season. In turn, the highest weight of ears was recorded also in the 2018/2019 and 2016/2017 seasons. FF1+Ag+P, P and FF2+Ag were the most favorable for this parameter compared to the control.

The highest *TGW* was achieved in the 2017/2018 season with an average *TGW* of about 53 g. In the 2018/19 and 2016/2017 seasons, the *TGW* was lower, over 48 g and 45 g, respectively. The highest *TGW* of wheat was recorded with the FF1+Ag+P, P and silver fertilizers (FF1+Ag and FF2+Ag).

Analysis of physiological parameters of wheat

Table 4 presents the characteristics of selected physiological parameters of wheat. On the basis of the obtained results, it was found that the maximum efficiency of photosystem II (Fv/Fm) was increased only with treatment with FF1+Ag+P compared to the control. In contrast, the performance index (PI_{ABS}), phenomenological energy fluxes per excited cross section (*CS*), yield of oxygen evolving complex (*OEC*) and density of reaction centers per cross section (RC/CS_0) showed no significant differences between the treatments. There were also no changes in the parameters φE_0 and ψ_0, which mainly reflect the functioning of the PSII electron acceptor side. Interestingly, from the observed trends, it can be seen that all these parameters were most stimulated by fertilizers with the addition of silver, although the difference was not statistically significant. In turn, the phenomenological energy fluxes per reaction center (*RC*) for wheat grown without fertilization (control) and under the applied foliar fertilization and fungicide protection showed significant differences only in the case of the electron transport flux per reaction center (ET_0/RC). Compared to the control there was a significant reduction of this parameter only under the influence of FF2+Ag. Foliar fertilization and fungicide protection significantly influenced the chlorophyll content based on the *SPAD*. Leaf greenness index was the highest after treatment with FF1+Ag+P.

The efficiency of PSII was more diversified depending on the growing season. The parameters defining phenomenological energy flows per excited cross-section were characterized by the highest values in the 2017/2018 and 2016/2017 seasons, while

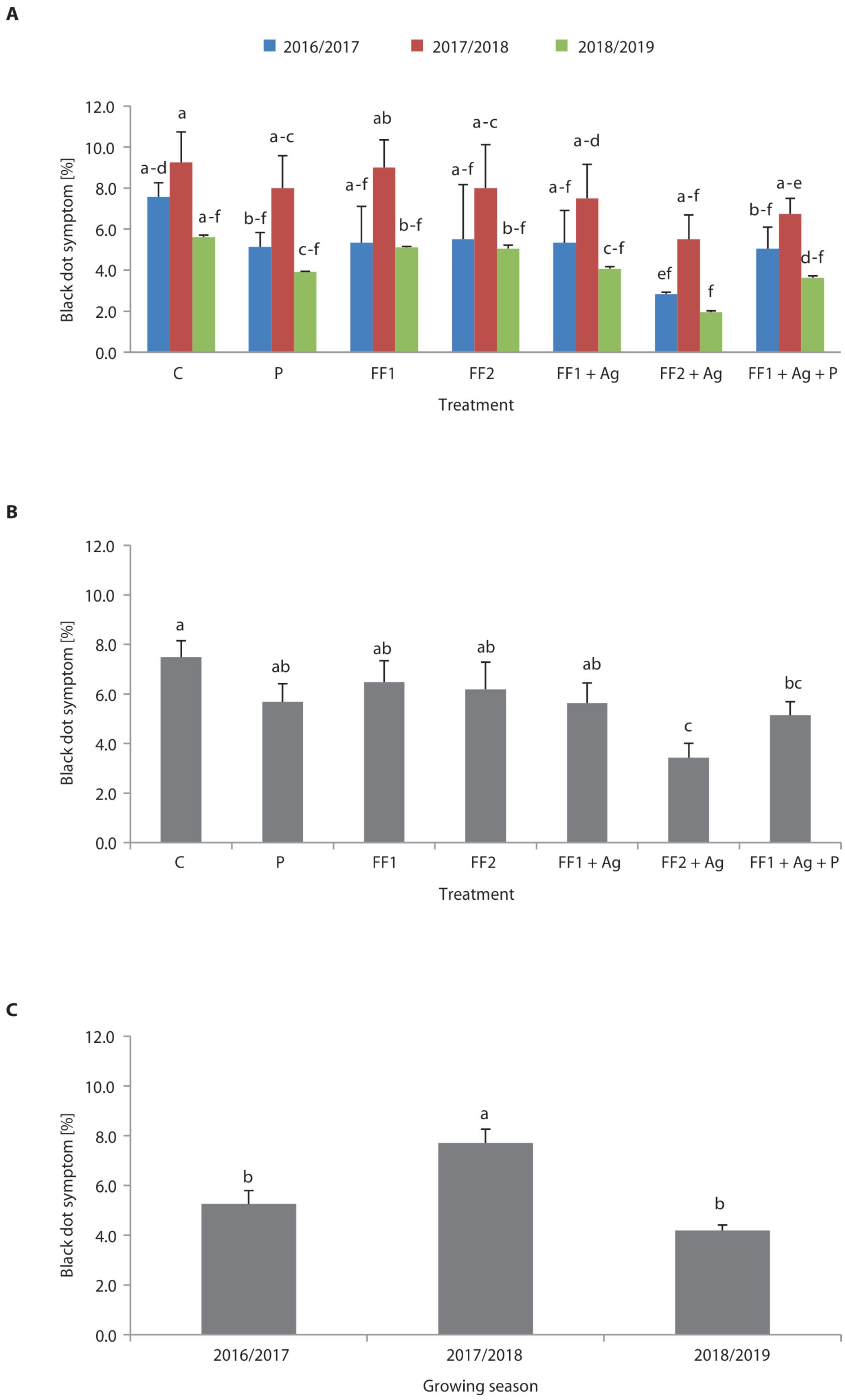

Fig. 12. Percentage of kernels with black dot symptoms depending on foliar fertilization and fungicide treatment, and growing season: interaction effect (A), effect of factors (B, C). Means followed by the same letters are not significantly different at $p < 0.05$. C – control, P – pesticide, FF – foliar fertilization

Table 3. Morphological characteristics of winter wheat and *TGW* in dependence on treatment and growing season

Trait	Means of factor										Level of significance (*p*) for the interactions
	treatment*							growing season*			treatment × growing season
	C	P	FF1	FF2	FF1+Ag	FF2+Ag	FF1+ +Ag+P	2016/ 2017	2017/ 2018	2018/ 2019	
Stalk length [cm]	95.78 c**	100.49 ab	97.50 bc	98.77 bc	100.59 ab	100.65 ab	104.12 a	104.40 a	98.13 b	96.58 b	<0.0001
Stalk weight [g]	4.04 b	4.85 ab	4.54 ab	4.65 ab	4.76 ab	4.74 ab	5.49 a	7.99 a	3.22 b	2.96 b	<0.0001
Flag leaf length [cm]	18.89 b	20.18 ab	19.78 ab	20.02 ab	21.41 a	21.44 a	20.34 ab	24.95 a	17.75 b	18.19 b	<0.0001
Ear length [cm]	9.15 b	10.13 a	9.41 b	9.57 ab	9.67 ab	9.62 ab	9.83 ab	9.34 b	9.27 b	10.27 a	0.000
Ear weight [g]	2.11 c	2.65 a	2.24 bc	2.29 c	2.39 a-c	2.50 ab	2.66 a	2.55 a	2.11 a	2.56 a	0.042
TGW [g]	45.50 c	51.65 ab	47.08 bc	47.34 bc	49.44 ab	48.76 ab	52.89 a	45.14 b	52.94 a	48.77 b	<0.0001

C – control; P – pesticide; FF – foliar fertilization; TGW – thousand grain weight

*means were compared separately for treatment and growing season; **means marked by the same letter for trait and factor are not statistically different at $p < 0.05$

Table 4. Physiological characteristics of winter wheat in dependence on treatment and growing season

Trait	Means of factor										Level of significance (*p*) for the interactions
	treatment*							growing season*			treatment × growing season
	C	P	FF1	FF2	FF1+Ag	FF2+Ag	FF1+ +Ag+P	2016/ 2017	2017/ 2018	2018/ 2019	
Fv/Fm	0.78 b**	0.79 ab	0.79 ab	0.79 ab	0.80 ab	0.80 ab	0.81 a	0.78 b	0.80 a	0.79 b	0.006
PI_{ABS}	2.18 a	2.38 a	2.35 a	2.36 a	2.45 a	2.48 a	2.44 a	2.83 a	2.79 a	1.50 b	<0.0001
ψ_0	0.59 a	0.58 a	0.58 a	0.59 a	0.60 a	0.61 a	0.60 a	0.66 a	0.64 a	0.48 b	<0.0001
φE_0	0.46 a	0.47 a	0.47 a	0.47 a	0.47 a	0.49 a	0.48 a	0.52 a	0.52 a	0.38 b	<0.0001
OEC	0.51 a	0.51 a	0.51 a	0.51 a	0.52 a	0.53 a	0.52 a	0.47 c	0.58 a	0.50 b	<0.0001
ABS/CS	335.52 a	339.88 a	342.38 a	345.09 a	352.07 a	351.10 a	346.18 a	372.25 a	345.49 b	316.07 c	0.006
TR_0/CS	264.84 a	270.31 a	268.30 a	268.74 a	276.71 a	277.41 a	277.04 a	294.79 a	274.80 b	246.12 c	<0.0001
ET_0/CS	147.13 a	150.36 a	148.76 a	149.85 a	151.59 a	153.41 a	153.30 a	165.82 a	166.58 a	119.48 b	<0.0001
DI_0/CS	62.42 a	63.74 a	62.61 a	63.38 a	64.58 a	65.24 a	64.99 a	64.06 a	64.61 a	62.88 a	0.901
RC/CS_0	114.75 a	122.81 a	119.47 a	116.06 a	121.14 a	121.74 a	123.42 a	110.89 b	142.28 a	106.57 b	<0.0001
ABS/RC	2.59 a	2.52 a	2.53 a	2.54 a	2.55 a	2.52 a	2.49 a	2.16 c	2.45 b	2.99 a	<0.0001
TR_0/RC	2.01 a	1.98 a	2.01 a	2.00 a	1.96 a	1.95 a	1.95 a	1.71 c	1.93 b	2.29 a	<0.0001
ET_0/RC	1.18 a	1.16 ab	1.14 ab	1.13 ab	1.12 ab	1.07 b	1.17 ab	1.11 b	1.22 a	1.09 b	0.000
DI_0/RC	0.52 a	0.48 a	0.50 a	0.51 a	0.50 a	0.48 a	0.48 a	0.45 b	0.46 b	0.58 a	<0.0001
SPAD	43.69 c	48.24 b	46.31 b	46.60 b	48.36 b	47.98 b	51.12 a	48.23 a	45.92 b	48.26 a	0.000

C – control; P – pesticide; FF – foliar fertilization

Fv/Fm – maximum yield of photosystem II; PI_{ABS} – performance index; ψ_0 – probability of electron transport; φE_0 – quantum yield for electron transport; *OEC* – yield of oxygen evolving complex; *ABS/CS* – absorption flux/cross section of the sample; TR_0/CS –trapped energy flux/cross section of the sample; DI_0/CS – dissipated energy flux/cross section of the sample; ET_0/CS – electron transport flux/cross section of the sample; RC/CS_0 – active PSII reaction center per exited cross-section; *ABS/RC* – absorption flux/reaction center; TR_0/RC – trapped energy flux/reaction center; DI_0/RC – dissipated energy flux/reaction center; ET_0/RC – electron transport flux/reaction center

*means were compared separately for treatment and growing season; **means marked by the same letter for trait and factor are not statistically different at $p < 0.05$

the phenomenological energy flows to the reaction center were the highest in the 2018/2019 season. The highest *SPAD* values were recorded in 2016/2017 and 2018/2019.

PCA analysis

The principial component analysis (PCA) analysis is shown in Figure 13. PCA analysis showed a high variability of the results (88.87%), which demonstrates the high power of the test performed. The results of the analysis indicate a complete separation of the controls from the objects treated with micronutrient foliar fertilizers with the addition of silver and the applied pesticide. The variant without fertilization (control) was closest to the FF1 and FF2 objects. Control was associated with the highest phenomenological energy flows to the reaction center (*RC*) and wheat diseases. FF1 and FF2 had no global effect on the plant compared to the control. Both FF2+Ag and FF1+Ag had the strongest effect on improving plant photosynthesis parameters, such as phenomenological energy flows per excited cross-section (*CS*), PI_{ABS}, *OEC*, φE_0, ψ_0 and the length of the flag leaf which was inversely correlated with ET_0/RC, TR_0/RC and with almost all wheat diseases. High values of *SPAD*, *Fv*/*Fm*, RC/CS_0, *TGW* and biometric parameters of the ear and stalk were characteristic of FF1+Ag+P and P and were inversely correlated with DI_0/RC, *ABS*/*RC* and with almost all diseases.

Discussion

Factors significantly determining plant health status, morphological and physiological parameters of winter wheat and *TGW* included the weather conditions in the growing season, as well as the applied fertilization and fungicide protection.

The meteorological conditions in a given growing season have a significant impact on the development of fungal diseases in plant crops. Depending on the species of fungus, pathogens can develop in a very wide temperature range. Most fungi require high humidity for their growth and development, therefore longer periods with precipitation increase the symptoms of infection caused by pathogens (Nazari *et al.* 2018; Zayan 2018; Rodríguez-Moreno *et al.* 2020). In the experiment, the atmospheric factors recorded during the study period were quite varied and can be described as atypical in relation to the multiannual data. These conditions favored the development of stem-base, leaf and ear diseases as well as black dot on kernels.

Disease control in advanced cereal development stages becomes virtually impossible, therefore it is important to protect plants at the early stages of development. Bala *et al.* (2018) and Zhang *et al.* (2021) emphasize that the proper management of nutrients affects the condition of plants and is of great importance for resistance to pathogens. In many cases, nutrients are

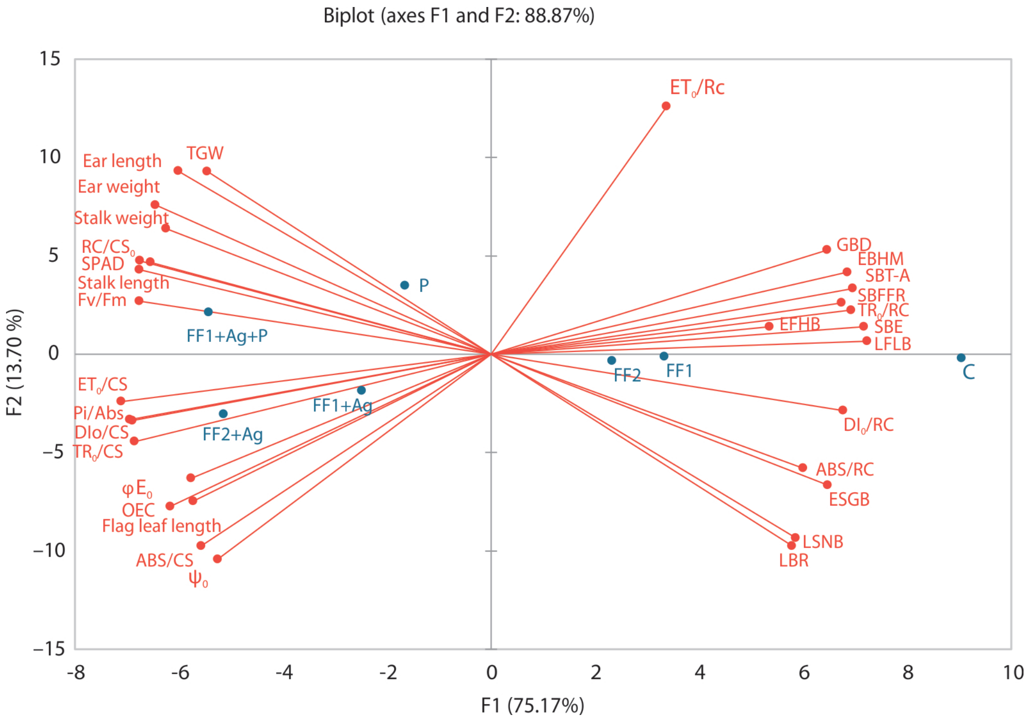

Fig. 13. PCA showing the relationship between the factors and the parameters. SBFFR – Fusarium foot rot; SBE – eyespot; SBT-A – take-all; LSNB – Septoria nodorum blotch; LFLB – Fusarium leaf blotch; LBR – brown rust; ESGB – Septoria glume blotch; EFHB – Fusarium head blight; EBHM – black head molds; GBD – black dot; C – control, P – pesticide, FF – foliar fertilization

the first and foremost line of defense against plant disease. Boron-deficient wheat showed a greater infection with powdery mildew than plants with an adequate B supply (Stangoulis and Graham 2007). The use of Cu and B significantly reduced the infestation of MR219 rice by pathogenic fungi (Liew *et al.* 2012). Franzen *et al.* (2008) showed that wheat leaf rust (*Puccinia recondita* Roberge ex Desmaz), tan spot (*Pyrenophora tritici-repentis* (Died.) Dreschler) and Fusarium head blight (*Fusarium graminearum* Schwabe) were reduced by foliar application of Cu. This experiment also confirmed the significant effect of foliar fertilization on limiting the development of diagnosed wheat diseases. The applied microelement fertilizers with the addition of silver were more effective than the microelement fertilizers themselves, and they also worked better or in a similar way to the fungicides used. FF1+Ag+P fertilization was also highly effective. This effect is an example of synergism, i.e., the total effect of the tested compounds is greater than the sum of the effects of their separate action. Silver ions and related compounds have long been known to be highly toxic against a wide range of microorganisms (Loo *et al.* 2018; Sadoon *et al.* 2020). Silver has much higher antifungal activity than other metals. This was shown by Slade and Pegg (1993) who noted the toxic effect of the tested metals in the order $Ag^+ > Cu^{++} > WO_4^{2-} > Ni^+ > Co^{++} > Zn^+$ against the zoospores of *Phytophthora nicotianae parasitica*. In the same study, silver was also effective against other pathogens, i.e., P. *cryptogea*, *Pythium aphanidermatum*, *Berkeleyomyces basicola* and F. *oxysporum*. Jo et al. (2009) showed that preventive (3 h before inoculation with *Bipolaris sorokiniana* and *Pyricularia grisea* spores) use of silver compounds, i.e., $AgNO_3$, AgNPs and electrochemical silver generated by electrolysis, effectively reduced the severity of leaf spot on perennial ryegrass. On the other hand, 24 h after inoculation, the effectiveness of the antifungal silver was significantly reduced, which suggests that the direct contact of silver with spores is important in inhibiting their viability and thus reducing the development of the disease. The beneficial effect of silver against the phytopathogenic fungi *Aspergillus flavus* in rice plants was also shown by Ejaz *et al.* (2018). Moreover, these compounds positively influenced the root growth and shoot length of plants, fresh and dry weight, leaf area and number of leaves. Al-Kaaby *et al.* (2015) also found that $AgNO_3$ added to the Murashige and Skoog (MS) medium with Gamborg vitamins, especially in high concentrations, caused a significant increase in the fresh weight of the callus, accelerated the regeneration of somatic embryos, as well as increased the number of leaves and roots of seedlings of two varieties of wheat (Fateh and Dor29) compared to control. In this experiment, a positive effect of fertilizers containing silver on the morphological parameters of wheat, i.e., stalk length, ear weight and *TGW* was noted. Moreover, combined foliar fertilization with the addition of silver ions and pesticide resulted in the highest stalk weight and *TGW* compared to the control. In turn, Vishwakarma *et al.* (2017) demonstrated the negative effect of silver compounds on growth parameters, i.e., the length of the roots and shoots as well as the fresh weight of the shoots and roots of mustard. Limiting the growth of mustard seedlings could have affected the total chlorophyll and caused some decrease in photosynthetic parameters. In our research, the performance of wheat photosystem II was not sensitive to silver ions. In addition, foliar fertilization enriched with silver significantly improved the chlorophyll content based on the *SPAD*.

Ensuring the right size and quality of yield is one of the primary goals in the cultivation of agricultural plants. Among all the vegetative organs, the flag leaf has the greatest yield-forming significance, therefore its proper condition is very important (Liu *et al.* 2018; Yan *et al.* 2020). In this experiment, the longest flag leaves were obtained with the use of fertilizers with the addition of silver, which correlated with a higher *TGW* in these variants than the control. Senescence of leaves is a very important process for plants during which cells undergo changes at the structural and functional levels (Lambert *et al.* 2017). Senescence affects many parameters, including the number of grains, seed germination time, yield and grain quality (Liebsch and Keech 2016). Early plant senescence can lead to a 50% decrease in grain yield (Gan 2007; Li *et al.* 2014). Labraña and Araus (1991) discovered that the Ag^+ foliar application delayed the senescence of spring wheat leaves (Kolibri variety) and hindered grain maturation, thereby improving seed yielding. Öktem and Keleş (2018) also demonstrated that $AgNO_3$ slows down the senescence process of wheat (Gün-91). In addition, they showed that changes in some morphological and physiological parameters that occurred when growing plants in the dark and after treatment of plants with indole-1-acetic acid (IAA) at a concentration of 50 and $100\ mg \cdot l^{-1}$, could be limited by using this compound.

Based on the PCA analysis, a negligible or insignificant effect of FF1 and FF2 on the severity of disease incidence and selected morphological and physiological features of wheat compared to the control were found. The direct effect of FF1+Ag and FF2+Ag on the reduction of the intensity of plant disease and the improvement of morphological parameters, i.e., the length of the flag leaves, which is consistent with the results of statistical analysis, indicates the uniqueness of fertilizers with silver. This means that fertilizers with silver have potential as plant protection products, which may determine the level of limiting the use of environmentally harmful fungicides and the reduction of pesticide residues in agricultural crops, with the benefit of consumer health. On the other hand, it should be remembered that the use of silver compounds must be controlled as their effect on organisms has not been clearly established and can cause a lot of damage to the environment.

Conclusions

The study showed that the severity of fungal diseases in wheat was reduced by multi-component foliar fertilizers with the ionic form of silver and the plant protection agents (P). In addition, disease causing pathogens can be further reduced by the simultaneous use of fungicides and micronutrients with the addition of silver. This protection system is especially recommended for the reduction of leaf and ear diseases. In most cases, foliar fertilizers with silver at a dose of 1 and 2 l · ha^{-1} worked better or even similarly to applied fungicides. Improving plant health through the use of silver is promising for agricultural practice due to the reduction or elimination of environmentally harmful chemicals. Moreover, foliar fertilization enriched with silver increased the growth and development of wheat. This fertilization had a positive effect on the length of the flag leaf and chlorophyll content based on the leaf greenness index, stalk length, spike weight and *TGW*.

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