Induced plant immunity as a factor of an integrated winter wheat disease protection system

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Abstract. The study deals with the system of integrated protection of winter wheat against phytopathogenic organisms. This includes the use of quaternary ammonium compounds combined with nanosilver in two stages: 1) pre-sowing treatment of seeds with the tank mixture of fungicide (difenoconazole + cyproconazole) and didecyldimethylammonium bromide enriched by nanosilver (0.15 %); 2) spraying of plants with immunity inducer at the end of the tillering – the beginning of booting. The leading role both in terms of protection and yield capacity belongs to early spring spraying with didecyldimethylammonium bromide enriched by nanosilver (0.3 %). This technique allowed obtaining the biological yield of winter wheat equal to 9.52 t/ha.

1. Introduction
Stavropol Krai is one of the leaders among the crop-producing regions of Russia [15]. However, modern farming systems that have formed for the past 20 years are characterized by short crop rotation and the use of resource-saving technologies for soil cultivation. This results in the accumulation of phytopathogenic organisms, pest insects and weed seeds in the soil that significantly harms the farm ecosystem of winter wheat despite a huge volume of fungicide treatment. Thus, it is necessary to use an integrated approach towards plant protection to avoid losses caused by unwanted organisms and obtain high and qualitative yields.

Stavropol Krai is a zone where root rot causes a lot of harm to winter wheat [1]. The branch of Russian Agricultural Center in Stavropol Krai notes that before 2008 winter wheat was mainly infected by Fusarium and Bipolaris genera, while since 2009 more agricultural territory became affected by Fusarium and Cercospora types of rot [9]. Root rot results in significant loss of yield due to blindness in seedlings, reduction in tilling capacity, the nimbler of seeds in a head, and the mass of 1000 seeds, accompanied by a decrease in quality. In the years of significant development of rot, losses can account for 15–40 %. Also, fungi produce mycotoxins that are very harmful to humans and animals.

Septoria blight is a very harmful disease during the entire vegetation period in Stavropol Krai. It can result in 30–40 % of yield loss. If the disease appears on the latter stages of plant development, the yield loss doesn’t exceed 5–7 % usually.

One type of dangerous leaf spot that has recently become dominating is the tan spot of winter wheat. There is an opinion that the territory affected by the tan spot has increased because this disease has occupied a niche of brown rust. In the fields where there is no or minimal soil cultivation, the flag leaf is the only one that remains green, while lower winter wheat leaves dry up due to optimum conditions for rot agent. This results in 30–60 % of losses and a decrease in the quality of crops.
The foundation stone of the integrated plant protection system is the cultivation of resistant varieties. However, the review of literature on phytopathology shows that resistance genes introduced in a plant are in some time surmounted by pathogenic organisms and the plant becomes less resistant to a targeted organism. Moreover, the broad use of chemicals to protect plants results in the formation of resistance in plant disease agents. That’s why the induced plant immunity becomes more practically relevant in the system of integrated plant protection.

This needs new non-traditional approaches to the use of immunogenetic techniques of plant protection, in particular, the creation and use of biocidal protection means that aim at optimizing the physiological state of plants and increasing their immune status. The impact of chemical activators of resistance to plant diseases involves stimulating active forms of oxygen in plants. It also helps to increase resilience to the oxidation process itself by means of creating active oxygen radicals for protection against phytopathogenic organisms. The rate of active oxygen forms formation will depend on which organism (plant or phytopathogenic) will be affected more [14].

Nowadays, there are a few products containing an active substance which is a synthetic disease resistance inducer having considerable practical use in plant protection. The reason for the limited use of these products is the insufficiency of fundamental basis and technologies of effective use in the general system of integrated plant protection. Some disease resistance inducers show high biological effectiveness in laboratory tests (more than 95 %). However, this effectiveness drops in uncontrolled field conditions.

To improve the germination ability of seeds and the development of root system, increase the activity of photosynthesis, and identify pathogenic organisms before symptoms appear it is possible to use nanoparticles of silver, copper, silicon, zirconium, aluminum, magnesium, zinc, titan, etc. both individually and in combination with traditional pesticides [2, 3, 11]. The development of products with nanosilver is of particular importance. Nanoparticles of silver have a small size and a large specific surface. They can destroy fungi, bacteria, and viruses on large surfaces due to close contact [13].

This study aims to improve the technologies for winter wheat cultivation by optimizing phytosanitary conditions of farm ecosystems by means of immunogenetic means of plant protection on leached black soils in the Central Pre-Caucasian region.

2. Methods and materials

The immunogenetic techniques of winter wheat protection against phytopathogenic organisms (root rot, Septoria blight, tan spot, mildew) were studied at the educational and experimental station of Stavropol State Agricultural University over the period between 2014 and 2017 agricultural years against the natural infection background.

The area of an experimental plot is 20 m². The plots are located in several tiers, while the variants are located systematically. The testing of fungicides was carried out with the four-time frequency according to methodological guidelines [12]. The object of research is winter soft wheat of Pysanka variety.

The pre-sowing treatment of seeds was carried out with HEGE 11 liquid seed treater.

In the period between the end of tilling and the start of booting, plants were sprayed by the solution of didecyldimethylammonium bromide enriched with nanosilver (0.3 %) using the technique of a split plot. Thus, the experiment involved two stages with four-time repetition and tiered placement of the plots. The variants were placed systematically.

The first stage (stage A) involved the study of products for the pre-sowing treatment of seeds. The second stage (stage B) dealt with the treatment of plants during vegetation (the end of tilling – the start of booting).

A product containing didecyldimethylammonium bromide at 6.0 % concentration was used as a source of quaternary ammonium compounds. In 2007 it was authorized by the Federal Service for Veterinary and Phytosanitary Surveillance of the Russian Federation and is used for the disinfection of veterinary surveillance objects including animal food. According to the research by the Stavropol
Research Institute of Animal Farming and Fodder Production \[10\], the product has an antiseptic effect on pathogenic and potentially pathogenic fungi, developing in stored fodder, including such genera as Fusarium, Aspergillus, etc. that can, in turn, cause diseases in vegetating plants.

The colloid solution of nanosilver included polyvinylpyrrolidone as a carrier with 8 000 molar mass. The carrier was aggregated with silver nanoparticles having a size of 50 nm. The product was synthesized in the laboratory of the Nanotechnology department in North Caucasus Federal University. The researchers also obtained a new sample of the product based on 100 nm particles of silver, stabilized by a quaternary ammonium compound.

The reference product was represented by a general-purpose systemic two-component fungicide containing difenoconazole (30 g/l) + cyproconazole (6.3 g/l).

The preceding crop is winter wheat. The soil was treated at a depth of 10–12 cm in a combined manner (K-744 + AKM-6.3). The recommended period for sowing winter wheat in zones with unstable humidification is between September 20 and October 15. Planting was done in lines with the interline spacing of 15 cm. The seeding rate was 5 million germinating seeds per 1 hectare. The drilling depth was 5–6 cm. The doses of N124P72K30 fertilizers corresponded to tonal recommendations: N54P72K30 – before seeding, N30 – at the stage of trilling, N20 – at the stage of booting, and N20 – at the stage of heading. The fertilizers included ammophos, potassium chloride, ammonia nitrate, and calurea.

The treatment of vegetating plants by protective means was carried out according to the experimental design. During the period of flag leaf expansion, the experimental plot was treated by Alto super fungicide, (propiconazole – 250 g/l + cyproconazole – 80 g/l. The rate of application was 0.5 l/ha.

The harvesting was done at the full-ripe stage from 1 m$^2$ of each plot.

The phytosanitary state of winter wheat was studied according to the methodology of the All-Russian Institute for Plant Protection (2009). The harvest structure was evaluated according to the methodology of state tasting of agricultural crop varieties (1985). The statistical treatment of the results was carried out by the methodology of B.A. Dospekhov [4].

3. Results

The results show that the occurrence of root rot during the stage between tilling and the beginning of booting varied depending on the pre-sowing treatment of seeds. This indicator varied between 95.0 and 100 % except for the 2017–2018 agricultural year which resulted in 98–100 % occurrence of disease due to weather conditions that were favorable for preserving the stock of Fusarium rot. As for the development of the disease, this indicator hasn’t reached the economic threshold of harmfulness (5–10 %) in the period between the end of tillering and the beginning of booting.

At the stage of maturity, the occurrence of rot in all the variants reached almost 100 % while the development of the disease exceeded the economic threshold of harmfulness (10–15 %). In the case of the control variant and the experiment involving nanosilver (0.3 %), the intensity of the disease exceeded the threshold almost twice. The results of the three-year researches showed the effectiveness of pre-sowing seed treatment with the chemical product containing difenoconazole and cyproconazole.

The use of the product based on quaternary ammonium compound combined with didecyldimethylammonium bromide enriched by nanosilver (0.15 %) has led to a decrease in Fusarium rot development during the maturity stage by 1.2–1.4 times in comparison to the control sample and by 2.17 % less in comparison to a chemical product. This can be explained by the fact that silver nanoparticles interact with fungi, suppressing their growth and providing protection for plants against phytopathogenic organisms which are resistant to antiseptic and antibacterial products, as some researches show.

The analysis of the mutual influence of stages (pre-sowing treatment and vegetating plant treatment) shows that there are no significant differences in respect to the occurrence of winter wheat root rot that could depend on the variants of pre-sowing treatment or the use of early spring spraying. However, there are differences in the development of the disease in the cases of choosing the
combination for pre-sowing treatment and the results of early spring spraying of plants by the quaternary ammonium compounds in combination with nanosilver.

The interaction of two types of protection techniques didn’t show significant differences in the experimental variants ($F_{0.629}<F_{0.5}=1.93$).

The highest biological effectiveness was in the case of pre-sowing treatment of seeds with the product based on quaternary ammonium compounds – didecyldimethylammonium bromide enriched by nanosilver (0.15 %) (at the level or even higher than in the case of using difenoconazole and cyproconazole).

The research showed that Septoria blight appeared on winter wheat annually between the end of tillering and the beginning of booting. Its occurrence in control plots was 40–45 %, but the development of disease wasn’t high, and the infection appeared on lower overwintered leaves. The occurrence of the tan spot was significantly lower (20–25 %). This is because the maturation and dispersion of ascospores of the previous year infection are aligned with a later phase of winter wheat development.

The treatment with quaternary ammonium compounds in combination with nanosilver was done to increase the overall immune status of plants and preventing the development of the disease. The calculations performed 14 days after the treatment showed that the maximum biological effectiveness in comparison with the control sample was in the variants with difenoconazole and cyproconazole, including the combinations with quaternary ammonium compounds and nanoparticle products as immunity inducers. In these variants of the experiment, the highest biological effectiveness against Septoria blight was in the case of the disease development which indicates the presence of protective mechanisms in plants that prevent the occurrence of phytopathogenic organisms in plant tissues. Also, the use of didecyldimethylammonium bromide enriched by nanosilver (0.3 %) for pre-sowing treatment showed a high level of biological effectiveness. It’s obvious that it is the treatment with quaternary ammonium compounds combined with a higher concentration of nanosilver that influences positively on the overall physiological state of plants, their overwintering, and general immune status of plants during vegetation resumption in spring [8].

The biological effect of treatment with quaternary ammonium compounds in combination with nanosilver against tan spot is significantly lower in comparison to Septoria blight. The exception is the variants involving didecyldimethylammonium bromide enriched by nanosilver (0.15 and 0.3 %) used for the pre-sowing treatment of seeds. It’s obvious that this treatment in combination with the chemical treater gives a high level of biological effectiveness. It’s possible that this combination of acting substances that doesn’t have a retardant impact on seeds (the retardant impact of difenoconazole is a fact proven by a lot of research) helps to form plants that are more resistant to tan spot in Autumn, which further influences on their state and responsiveness to early spring treatment. Moreover, our researches show that quaternary ammonium compounds in combination with nanosilver can neutralize this retardant impact.

The analysis of the results in the two-stage experiment with respect to leaf spots showed significant differences in the variants of the experiment related to both pre-sowing treatment and spraying of vegetating plants in terms of the occurrence and the development of the disease.

It’s worth noting that with respect to this group of diseases (Septoria blight and tan spot) the complex use of quaternary ammonium compounds to treat seeds and spray plants showed significant differences in the variants of the experiment.

The use of didecyldimethylammonium bromide enriched by nanosilver (0.15 %) for pre-sowing treatment and its combination with the chemical treater gives a high level of biological effectiveness. It’s possible that this combination of acting substances that doesn’t have a retardant impact on seeds (the retardant impact of difenoconazole is a fact proven by a lot of research) helps to form plants that are more resistant to tan spot in Autumn, which further influences on their state and responsiveness to early spring treatment. Moreover, our researches show that quaternary ammonium compounds in combination with nanosilver can neutralize this retardant impact.
vegetation treatment. The most appropriate use of this immunity inducers is in the form of the tank mixture with the chemical treater. These techniques allow decreasing the occurrence of Septoria blight by 1.7–2.5 times.

The minimum vulnerability of winter wheat to tan spot in terms of occurrence was in the case of treatment by didecyldimethylammonium bromide (0.3 %). The same result was in terms of the development of the disease where the minimum vulnerability was in the same variant and in the case of the tank mixture of the fungicide and didecyldimethylammonium bromide (0.15 %).

The minimum occurrence of the disease when combining pre-sowing and vegetation stage treatment types was in the variants involving the chemical treater and the tank mixture of the treater with nanosilver (2 %). However, the most effective means against the development of the was difenoconazole + cyproconazole (the application rate is 1.0 l/g) + didecyldimethylammonium bromide enriched by nanosilver (0.15 %) with the further treatment of vegetating plants with didecyldimethylammonium bromide enriched by nanosilver (0.3 %).

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We should note that the best indicators of yield structure both in terms of the mass of grains from 1 head and the mass of 1000 grains as well as the highest biological yield of winter wheat (9.52 t/ha) were in the case of pre-sowing treatment of seeds with the tank mixture of difenoconazole + cyproconazole (the application rate is 1.0 l/g) + didecyldimethylammonium bromide enriched by nanosilver (0.15 %) with the further treatment of trilling and booting plants with didecyldimethylammonium bromide enriched by nanosilver (0.3 %) to improve the overall immune status of plants (table).

The analysis of the yield structure depending on the degree of damage by the root rot showed that there were no healthy plants in experimental plots. The occurrence of root rot was 100 %.

At the degree of damage by Fusarium rot equal to 0.1 points, the mass of grains in 1 head was 1.24 g. The pre-sowing treatment of seeds with difenoconazole and cyproconazole as well as the quaternary ammonium compounds, including combinations with nanosilver, improved the health of plants by means of destroying external and internal infections and activating immunologic systems. As a result, the mass of winter wheat grains from one head in all the variants of the experiment has increased by 110–560 mg.

The maximum positive effect was in the case of treatment with the tank mixture of difenoconazole and cyproconazole (the application rate is 1.0 l/g) + the colloid solution of silver (2 %). The increase of the level of damage to 1 point, as a rule, leads to a decrease in the indicators of the yield structure, including the decrease of the mass of grains from one head by 40 to 60 %.

The early spring treatment with didecyldimethylammonium bromide, enriched by nanosilver (0.3 %) as the immunity inducer has positively impacted the formation of grains in all the variants of the experiment. The comparative analysis of indicators, especially the mass of grains from one head, indicates that this indicator has increased in comparison with the pre-sowing treatment of seeds according to the experiment design by 150–320 mg in some variants of the experiment. The biggest rise in this indicator was in the variant of treatment with didecyldimethylammonium bromide (0.15 %).

4. Conclusion
The analysis of phytosanitary state and yield of winter wheat during two-stage experiment (stage A – products for pre-sowing treatment of seeds, stage B – the treatment of plants between the end of tillering and the beginning of booting) showed that the leading role in the system of integrated protection of winter wheat against phytopathogenic organisms (involving the use of quaternary ammonium compounds and their combinations with nanosilver in two stages) belongs to early spring spraying in terms of both phytosanitary character and the formation of yield.
Table 1. The yield of winter wheat depending on the complex use of quaternary ammonium compounds including the combination with nanosilver products by means of pre-sowing treatment and early spring spraying (on average for 2014–2017 agricultural years)

| Product                                                                 | Rate of Application kg(l)/t | Early spring spraying with didecyldimethylammonium bromide enriched by nanosilver (0.3 %) (stage B) |
|------------------------------------------------------------------------|-----------------------------|-------------------------------------------------------------------------------------------------------------|
| Control sample (treatment with water)                                  | –                           | 6.76 8.63                                                                                                   |
| Difenoconazole (30 g/kg) + Cyproconazole (6.3 g/kg) – reference        | 1.0                         | 7.56 9.27                                                                                                   |
| Difenoconazole (30 g/kg) + Cyproconazole (6.3 g/kg) + colloid solution of nanosilver | 0.2                         | 7.32 8.26                                                                                                   |
| Difenoconazole (30 g/kg) + Cyproconazole (6.3 g/kg)                    | 1.0                         | 6.85 8.84                                                                                                   |
| Difenoconazole (30 g/kg) + Cyproconazole (6.3 g/kg) + didecyldimethylammonium bromide | 0.015                      | 7.14 7.65                                                                                                   |
| Difenoconazole (30 g/kg) + Cyproconazole (6.3 g/kg) + didecyldimethylammonium bromide | 0.03                       | 6.59 8.46                                                                                                   |
| Didecyldimethylammonium bromide enriched by nanosilver                | 0.015                       | 6.99 7.99                                                                                                   |
| Didecyldimethylammonium bromide enriched by nanosilver                | 0.03                        | 6.39 7.52                                                                                                   |
| Didecyldimethylammonium bromide                                       | 0.015                       | 6.50 8.42                                                                                                   |
| Didecyldimethylammonium bromide                                       | 0.03                        | 8.86 8.24                                                                                                   |
| Difenoconazole (30 g/kg) + Cyproconazole (6.3 g/kg) + didecyldimethylammonium bromide enriched by nanosilver | 0.015                      | 7.63 9.52                                                                                                   |
| Difenoconazole (30 g/kg) + Cyproconazole (6.3 g/kg) + didecyldimethylammonium bromide enriched by nanosilver | 0.03                       | 6.59 8.46                                                                                                   |

A: $F_3=10.119>F^{0.05}_{3}=2.115$ HCP$_{0.05}=0.415$
B: $F_3=290.404>F^{0.05}_{3}=4.161$ HCP$_{0.05}=0.177$
AB: $F_3=1.548>F^{0.05}_{3}=1.93$ HCP$_{0.05}=0.587$

Thus, the use of quaternary ammonium compounds and their combinations with nanosilver in the tank mixture with chemical treaters followed by the treatment of vegetating plants with didecyldimethylammonium bromide, enriched by nanosilver (0.3 %) increases the immune status of plants and can be recommended for use in a zonal system of integrated winter wheat protection against diseases.

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