Effect of plant growth regulators on the induction of peach resistance to leaf curl

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Abstract. The article presents data from studies of the effect of growth regulators when applied for three years in peach plantations in the humid subtropical zone of the Black Sea coast of Russia. The influence of drugs (Zerebra® Agro (colloidal silver + polyhexamethylenebiguanide hydrochloride), Epin-Extra® (24-epibrassinolide) and Biosil® (triterpene acids)) on the degree of development of peach leaf curl (pathogen – Taphrina deformans (Berk) Tul.) was analyzed. The preparations were used in pure form and in combination with fungicides, the dosage of which was two times lower than in the standard. Already in the first year of the experiment, an increase in the resistance of the peach to leaf curl was noted. The maximum biological efficiency (up to 80.3%) in relation to leaf curl was observed when using Zerebra Agro in a tank mixture with fungicides. An inverse correlation was established between the degree of development of leaf curl and the level of antioxidant system enzymes activity (catalase and guaiacol peroxidase), which confirms the immunity-inducing effect of the growth regulators use.

1. Introduction

Peach cultivation faces the threat of intensive development of leaf curl, the causative agent of which is the ascomycete Taphrina deformans (Berk.) Tul. [1-4]. The harmfulness of leaf curl is in the violation of photosynthesis and deformation of leaves, their premature abscission, the death of annual shoots, which leads to a strong weakening of trees. In the absence of protective measures, diseased plants do not bear fruit at all [5-7]. In some years in the humid subtropics of Russia, the loss of peach yield from the disease is more than 90% [8].

Protection of fruit plants from diseases can increase the yield by 10-30% or more. The applied chemical fungicides, if used irrationally, can accumulate in the soil and have an adverse effect on the agrocenosis as a whole [9].

The introduction of plant immunity inducers into plant protection systems, the action of which is based on the activation of nonspecific resistance mechanisms, is one of the new ecologically safe areas of plant protection [10-12]. Many plant growth regulators of the existing assortment have an immunity-inducing action, activating the natural defense mechanisms of plants. This helps to reduce crop losses from diseases and, as a result, increase crop yields by 15-30% with the use of fewer fungicides used [4]. The use of growth regulators on agricultural crops leads not only to stimulation of growth processes, productivity and product quality, but also in a number of cases increases the degree
of plant protection from stresses of various nature. The effectiveness of plant growth regulators may be superior to the effect of conventional pesticides [13-14].

Enzymes of the antioxidant system (catalase and peroxidases) play a decisive role in the system of plant defense reactions [15-19]. An increase in their activity allows plants to resist oxidative stress, which is an integral part of the infectious process [20-21]. A direct correlation has been established between the activity of peroxidases in plant tissues and resistance to pathogens [22]. In connection with the above, the study of oxidative system reaction to the use of certain drugs is a confirmation or refutation of its immunity-inducing properties.

Until now, in the humid subtropics of the Black Sea coast of the Caucasus, tests of Zerebra® Agro, Epin-Extra® and Biosil® have not been carried out, therefore, the study of their effectiveness in peach agroecoses as the most common stone fruit crop in the region is very important.

2. Materials and methods
The research was carried out in peach gardens (cv. Red Haven) on the basis of the Federal Research Centre the Subtropical Scientific Centre of the Russian Academy of Sciences in Sochi in 2018-2020. The experiment was carried out in accordance with generally accepted methods [23].

Zerebra® Agro (colloidal silver, 500 ml/l + polyhexamethylenebiguanide hydrochloride, 100 ml/l) is a growth stimulant with fungicidal, bactericidal and elicitor effects [24]. The mechanism of Zerebra Agro action is complex and is implemented in two directions – direct (biocidal effect of silver on pathogens) and indirect (immunizing) [25].

Epin-Extra® (24-epibrassinolide, 0.025 g/l) is natural phytogromone, growth regulator. It increases the plants resistance to stress and phytopathogens, the yield and quality of fruits [26].

Biosil® (triterpene acids, 100 g/l) is obtained on the basis of an extract from Siberian fir [27].

In the experiment, ten variants were laid (each of them in three repetitions). Experiment scheme:

- Control (water treatment, without fungicides and growth regulators);
- Standard (production treatment) – Delan® (dithianon, 700 g/kg) (0.7 kg/ha) first treatment; Scor® (diphenconazole, 250 g/l) (0.2 l/ha) second and third treatments;
- Zerebra® Agro (colloidal silver, 500 ml/l + polyhexamethylenebiguanide hydrochloride, 100 ml/l) 150 ml/ha with half doses of fungicides: Delan® (0.35 kg/ha) – first treatment; Scor® (0.1 l/ha) – second and third treatments;
- Epin-Extra® (24-epibrassinolide, 0.025 g/l) 200 mg/ha with half doses of fungicides: Delan® (0.35 kg/ha) – first treatment; Scor® (0.1 l/ha) – second and third treatments;
- Biosil® (triterpene acids, 100 g/l) 100 ml/ha with half doses of fungicides: Delan® (0.35 kg/ha) – first treatment; Scor® (0.1 l/ha) – second and third treatments;
- Zerebra® Agro 150 ml/ha, without fungicides three treatments;
- Epin-Extra® 200 mg/ha, without fungicides three treatments;
- Biosil® 100 ml/ha, without fungicides three treatments.

Prior to the experiment, the plantings were treated once with a Bordeaux mixture (3%) during the period of bud swelling. Diagnosis of diseases was carried out on the 7th day after treatment.

The activity of catalase and guaiacol peroxidase in peach leaves was determined in 2020, in the third year of the experiment (in the first and third ten days of June, after the end of all treatments) according to generally accepted methods [28-30].

The obtained research results were processed by methods of statistical analysis in the program MSExcel.

3. Results
During the years of the experiment, in the control variant, a moderate degree of development of peach leaf curl was observed (table 1). Treatment with fungicides in the standard reduced the degree of
development of curl by 2-2.5 times. When Zerebra Agro, Epin-Extra and Biosil, together with fungicides, were included in the protection system, a decrease in the degree of development of the disease compared to the standard was observed, despite a two-fold decrease in the rate of consumption of fungicides. Experimental variants with Zerebra Agro, Epin-Extra and Biosil, together with fungicides, reduced the degree of leaf curl development more efficiently than in the standard. This fact was noted despite a two-fold decrease in the rate of consumption of fungicides.

| Experience variant | Peach leaf curl development (M±m, %) |
|--------------------|------------------------------------|
|                    | 2018 April | 2018 May | 2018 June | 2019 April | 2019 May | 2019 June | 2020 April | 2020 May | 2020 June |
| Control            | 20.1 ±1.5  | 19.7 ±1.5 | 16.2 ±1.4  | 23.1 ±1.6  | 20.0 ±1.0  | 12.6 ±1.8  | 19.3 ±0.8  | 13.6 ±0.4  | 10.2 ±0.4  |
| * Standard         | 9.9 ±1.1   | 7.3 ±0.9  | 6.0 ±0.8   | 9.4 ±1.3   | 8.8 ±1.2   | 7.8 ±1.3   | 8.6 ±1.0   | 7.1 ±0.6   | 5.8 ±0.5   |
| ** Zerebra Agro + fungicides | 7.0 ±0.9 | 4.0 ±0.7 | 4.8 ±1.2   | 7.1 ±1.3   | 5.4 ±0.8   | 3.8 ±1.0   | 5.2 ±0.8   | 5.2 ±1.0   | 6.0 ±1.5   |
|                    | 7.7 ±2.6   | 8.7 ±1.1  | 6.4 ±1.3   | 4.8 ±0.6   | 7.2 ±1.1   | 5.5 ±1.4   | 6.1 ±1.0   | 8.5 ±1.4   | 6.0 ±1.4   |
|                    | 8.6 ±1.3   | 8.0 ±0.7  | 8.2 ±1.3   | 6.6 ±0.7   | 8.0 ±1.3   | 6.8 ±1.4   | 8.1 ±0.7   | 8.1 ±0.7   | 7.0 ±0.8   |
|                    | 8.0 ±1.3   | 5.6 ±0.7  | 5.0 ±1.3   | 5.8 ±1.3   | 7.5 ±1.3   | 7.6 ±1.4   | 6.5 ±0.5   | 6.7 ±0.5   | 6.7 ±0.8   |
|                    | 8.1 ±1.0   | 9.5 ±0.8  | 7.8 ±2.0   | 5.8 ±0.8   | 7.6 ±0.8   | 6.6 ±1.2   | 9.5 ±0.5   | 7.0 ±0.5   | 7.0 ±0.8   |
|                    | 7.6 ±1.4   | 9.5 ±1.1  | 9.2 ±0.9   | 7.6 ±1.0   | 9.2 ±1.2   | 9.0 ±1.8   | 9.8 ±1.0   | 9.3 ±1.4   | 8.0 ±1.4   |
|                    | 7.6 ±2.0   | 9.5 ±1.7  | 9.2 ±1.6   | 7.6 ±0.9   | 9.2 ±1.2   | 9.0 ±1.7   | 9.8 ±1.0   | 9.3 ±1.4   | 8.0 ±1.4   |
| LSD<sub>0.05</sub> | 0.8 ±0.9   | 0.9 ±0.7  | 1.2 ±1.6   | 0.8 ±1.6   | 0.4 ±1.0   | 0.6 ±0.4   | 0.8 ±1.6   | 0.4 ±1.0   | 0.6 ±1.4   |

Note: * Standard – production treatment: Delan® (dithianon, 700 g/kg) (0.7 kg/ha) first treatment; Scor® (diphenoxaconazole, 250 g/l) (0.2 l/ha) second and third treatments; ** Tank mixture of growth regulators with fungicides at half application rates: Delan® (0.35 kg/ha) – first treatment; Scor® (0.1 l/ha) second and third treatments).

When using growth regulators in their pure form, their protective effect had a character similar to the variants with the combined use of growth regulators in a tank mixture with fungicides. After the third treatment, the minimum degree of leaf curl development was recorded in variants with Zerebra Agro and Epin-Extra both in pure form and in a tank mixture with fungicides. This indicates a high response of peach to the phytopathogen.

In the second and third years of the experiment, the protective effect of the studied drugs was preserved, which indicates the absence of depletion of the peach's immune system.

Protective properties of growth regulators against peach leaf curl reflect indicators of biological effectiveness. In the first year of the experiment, the biological efficiency of the variants with Zerebra Agro after the second and third treatments was higher in comparison with the other variants of the experiment (70.3-79.6 %). The biological effectiveness of the variants with Epin Extra and Biosil ranged from 55.8-60.4%. At the same time, there were no significant differences between the options for using growth regulators in their pure form and with a chemical fungicide.
In the second year of the experiment, a decrease in the values of biological effectiveness was observed from the first to the third treatment. This tendency is noted both in the variants with growth regulators and in the standard. The maximum values of the biological efficiency were noted in April (71.4-79.6 %), during the period of active development of leaf curl. All variants with growth regulators has shown biological efficiency in standard or higher level.

In the third year of the experiment, the biological effectiveness of all variants decreased. We associate this with some decrease in the degree of leaf curl development in the control variant. After the first treatment, the biological efficiency of the standard was only 55.4%, of the variant with Zerebra Agro with half doses of fungicides – 80.3%, of the variant with Epin Extra and Biosil with half doses of fungicides – 68.3 and 64.7%, respectively. The effectiveness of subsequent treatments decreased significantly.

In 2020, the state of the antioxidant system in leaf tissues was studied. It was found that an increase in peach resistance to T. deformans when using growth regulators is accompanied by an increase in the activity of antioxidant system enzymes. The maximum level of activity of catalase and guaiacol peroxidase was observed at the minimum degree of peach leaf curl development (table 2).

| Experience variant | Disease development (R) I decade of June | III decade of July | Disease activity level (CA and GPA) I decade of June | III decade of July | Correlation coefficient, r |
|-------------------|-----------------------------------------|-------------------|------------------------------------------|-------------------|-------------------------|
|                   | R, % | CA* | R, % | CA | R, % | GPA* | R, % | GPA |
| Control           | 10.2 | 32  | 4.2  | 26 | 10.2 | 111  | 4.2  | 102 |
| ** Standard       | 5.8  | 46  | 2.0  | 32 | 5.8  | 136  | 2.0  | 129 |
| *** Zerebra Agro + fungicides | 6.0  | 49  | 2.4  | 34 | 6.0  | 139  | 2.4  | 134 |
| Epin-Extra + fungicides | 6.0  | 46  | 2.8  | 36 | 6.0  | 140  | 2.8  | 130 |
| Biosil + fungicides | 7.0  | 44  | 3.0  | 28 | 7.0  | 138  | 3.0  | 116 |
| Zerebra Agro (without fungicides) | 6.7  | 42  | 3.4  | 32 | 6.7  | 132  | 3.4  | 132 |
| Epin-Extra (without fungicides) | 7.0  | 46  | 3.5  | 32 | 7.0  | 134  | 3.5  | 112 |
| Biosil (without fungicides) | 8.0  | 30  | 3.8  | 30 | 8.0  | 120  | 3.8  | 114 |
| Correlation coefficient, r | -0.8740 | -0.7706 | -0.9256 | -0.8422 |

Note: * Catalase activity (CA), mlO₂·g⁻¹ raw weight; guaiacol peroxidase activity (GPA), units·g⁻¹ secc raw weight; ** Standard – production treatment: Delan® (dithianon, 700 g/kg) (0.7 kg/ha) first treatment; Scor® (diphenaconazole, 250 g/l) (0.2 l/ha) second and third treatments; *** Tank mixture of growth regulators with fungicides at half application rates: Delan® (0.35 kg/ha) – first treatment; Scor® (0.1 l/ha) second and third treatments).

The established relationship of the studied indicators was noted both in the variant with the use of growth regulators in tank mixtures with half doses of fungicides, and when they are used in their pure form. The established dependence is confirmed by high correlation coefficients.

4. Discussion
The results obtained in the experiments show that Zerebra® Agro has an immunity-inducing action. This drug is able to enhance the action of chemical fungicides, which allows significant savings by reducing their dosage. The study of the activity of enzymes of the antioxidant system in different
applications of Zerebra Agro confirmed the ability of the drug to induce oxidative stress (the protective reaction of the plant organism to adverse environmental conditions and phytopathogens). This feature of the drug was pointed out by O.A. Shapoval and I.P. Mozharova [25].

The use of Biosil® and Epin-Extra® for three years has shown a stable result. The effectiveness of this drugs in combination with fungicides was at the standard level, which allows including these preparations in plant protection systems, reducing the pesticide load by half. The effectiveness of the application of the growth regulator Biosil with half the rates of fungicide consumption has also been established when growing cereals [27]. The results of laboratory studies have confirmed the reaction of antioxidant system enzymes to the use of Biosil and Epin-Extra. This indicates the launch of a defense reactions system in plants, and, consequently, the presence of immunity-inducing properties.

5. Conclusion
Based on the analysis of the results, we can talk about the presence of immunity-inducing properties in the drugs Zerebra® Agro (colloidal silver, 500 ml/l + polyhexamethylenebiguanide hydrochloride, 100 ml/l), Epin-Extra® (24-epibrassinolide, 0.025 g/l) and Biosil® (triterpene acids, 100 g/l). The use of these plant growth regulators, both in pure form and in tank mixtures with half dosages of fungicides in peach agrocnoses, makes it possible to control the peach leaf curl development. The maximum biological efficiency in relation to peach leaf curl was noted for Zerebra® Agro. The study of the questions of drugs use frequency with immunity-inducing properties should be continued.

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